Neurobiology of Learning and Memory 166 (2019) 107101

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Facilitation of fear extinction by novelty is modulated by β-adrenergic and 5-HT_{1A} serotoninergic receptors in hippocampus

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ARTICLE INFO

Keywords:
Hippocampus
Extinction
Fear conditioning
Behavioral tagging
Modulatory systems

ABSTRACT

Extinction is the learned inhibition of retrieval of a previously acquired memory and is a major component of exposure therapy, which has attracted much attention because of the use in the treatment of drug addiction, phobias and particularly fear disorders such as post-traumatic stress disorder (PTSD). Exposure to a novel environment before or after extinction training can enhance the extinction of contextual fear conditioning, however the cellular and molecular substrates are still unclear. Here, we investigated the participation of H2-histaminergic, β-adrenergic and 5-HT_{1A}-serotonergic receptors of the hippocampus on the enhancement of extinction memory caused by novelty. The infusion into the CA1 region of the serotonin 5-HT_{1A}-receptor agonist, 8-OH-DPAT and the β-adrenergic blocker, Timolol, after the exposure to the novelty hindered the enhancement of extinction by novelty, while Timolol also hindered the extinction consolidation when infused post-extinction. These impairments were abolished by the coinfusion of 8-OH-DPAT plus the 5-HT_{1A} receptor antagonist, NAN-190 and Timolol plus β-adrenergic agonist, Isoproterenol. However, Dimaprit and Ranitidine blocked the retrieval of CFC, but did not prevented the extinction learning. Here we elucidated some of the molecular mechanisms that are involved on the enhancement of extinction by novelty, demonstrating that the β-adrenoreceptors and 5-HT_{1A} serotonergic receptors participate on this process alongside with dopaminergic D1 receptors previously described, while histamine H2 receptors, so ubiquitous in learning-related functions in hippocampus are not involved.

1. Introduction

Extinction is the learned inhibition of retrieval of a previously acquired memory (Fiorenza, Rosa, Izquierdo, & Myskiw, 2012; Izquierdo, Furini, & Myskiw, 2016; Pavlov, 1927) and can involve neuroanatomical, cellular and molecular substrates similar to those initially recruited for the consolidation of the original memory, besides protein synthesis to stabilize the memory trace again (Szapiro, Vianna, McGaugh, Medina, & Izquierdo, 2003; Vianna, Szapiro, McGaugh, Medina, & Izquierdo, 2001). Pavlovian classical conditioning is the paradigm most used to study extinction memory, where the absence of reinforcement results in a decline or even disappearance of the conditioned response (Furini et al., 2017; Furini, Myskiw, & Izquierdo, 2014; Izquierdo et al., 2016; Pavlov, 1927).

Extinction is a major component of exposure therapy and has attracted much attention because of the use of the latter in the treatment of drug addiction, phobias and particularly fear disorders such as post-traumatic stress disorder (PTSD) (Milad & Quirk, 2012; Milad, Rosenbaum, & Simon, 2014). However, the conventional extinction protocols applied to exposure therapy are not entirely satisfactory (Singewald, Schmuckermair, Whittle, Holmes, & Ressler, 2015), so the improvement of these procedures by behavioral or pharmacological means is important. Recently, our group has shown that exposure to a novel environment at 1 or 2 h before or 1 h after extinction training enhanced contextual fear extinction (De Carvalho Myskiw, Benetti, & Izquierdo, 2013; De Carvalho Myskiw, Furini, Benetti, & Izquierdo, 2014), which opens a new approach to strengthening extinction learning and a potential clinical application, since it makes use of simple procedures.

The enhancement of extinction induced by novelty can be explained by the synaptic tagging and capture hypothesis (Ballarini, Moncada, Martinez, Alen, & Viola, 2009; De Carvalho Myskiw et al., 2013;

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https://doi.org/10.1016/j.nlm.2019.107101
Received 14 December 2018; Received in revised form 21 August 2019; Accepted 16 October 2019
Available online 17 October 2019
1074-7427/

Moncada & Viola, 2007), whose application to behavior became known as behavioral tagging (Almaguer-Melian et al., 2012; De Carvalho Myskiw et al., 2013; Moncada, Ballarini, Martinez, Frey, & Viola, 2011), and depends on plasticity-related proteins (PRPs) in the hippocampus. The synaptic tagging and capture process was first described by Frey and Morris (1997). It takes place in the hippocampus and relies on a mechanism whereby relatively "weak" hippocampal long-term potentiation (LTP) or long-term depression (LTD) lasting only a few minutes can "tag" the synapses involved with PRPs synthesized *ad hoc*, so that other PRPs produced at other sets of synapses by other LTPs or LTDs can be captured by the tagged synapses and strengthen their activity to promote longer-lasting LTPs or LTDs lasting hours or days (Frey & Morris, 1997, 1998).

As with synaptic tagging, behavioral tagging requires *de novo* protein synthesis, dopamine D1/D5 receptor activation, and the occurrence of two different events within a temporal time window (Moncada & Viola, 2007). It is known that classical modulatory neurotransmitters play an important role on both consolidation and extinction memory. Histaminergic, serotoninergic and adrenergic receptors have been widely studied on hippocampal processes related to learning and memory (Fiorenza et al., 2012; Izquierdo & McGaugh, 2000; Zhang & Stackman, 2015). However, the molecular mechanisms of the tagging-and-capture process on the extinction memory have only recently begun to be studied. Evidence suggests the participation of H2-histaminergic, β-adrenergic and 5-HT_{1A} receptors in the regulation of learning processes in the hippocampus (Bauer, 2015; Izquierdo et al., 2016; Passani et al., 2017); therefore it seems reasonable to investigate whether those systems could participate in hippocampal processes of tagging-and-capture mediated enhancement of fear extinction by novelty (De Carvalho Myskiw et al., 2014; Singewald et al., 2015). Thus here we investigate the participation of these tree classes of monoamine receptors of the CA1 region of the hippocampus in novelty-induced enhancement of contextual fear extinction.

2. Materials and methods

2.1. Animals

Male Wistar rats (CrlCembe:WI; 3 months-old; 300–330 g) purchased from the Centro de Modelos Biologicos Experimentais (CeMBE) of this university, were used. The animals were housed four to a cage with water and food *ad libitum*, under a 12-h light/dark cycle (lights on at 7:00 AM) and the room's temperature maintained at 22–23 °C. All experimental procedures were approved by the Animal Committee on Ethics in the Care and Use of Laboratory Animals of PUCRS and were in compliance with National Institutes of Health guidelines for the Care and Use of Laboratory Animals.

2.2. Surgery

Under deep anesthesia (75 mg/kg ketamine and 10 mg/kg xylazine - both administered intraperitoneally) the animals underwent stereotaxic surgery for implantation of bilateral 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) according to the coordinates of the Atlas by Paxinos and Watson (1986). Animals were allowed 7 days to recover from surgery before behavioral procedures. All animals were handled daily during 5 min for 3 consecutive days before the behavioral experiments.

2.3. Contextual fear conditioning

The Contextual Fear Conditioning (CFC) task was performed in a conditioning chamber with aluminum walls (35 × 35 × 35 cm) and a floor made of stainless-steel grid bars connected to a shock source for the delivery of foot shock. This conditioning chamber was placed inside another larger box with soundproof walls to attenuate external sounds. The chamber was cleaned with 70% ethanol before and after each use.

Animals were conditioned and extinguished as previously described (De Carvalho Myskiw et al., 2013, 2014). Briefly, on the CFC training session, animals were allowed to freely explore the apparatus and after 2 min three electrical foot shocks 0.7 mA/2 s separated by 30 s intervals were delivered. Animals were left in the conditioning chamber for another 30 s and then placed back into their home cages. Basal freezing behavior was registered prior to the administration of the foot shocks. After 24 h, the animals were placed in the same apparatus for a 10-min extinction training session (Ext), without the foot shocks. The 3-min retention test (Test) occurred 24 h later. The percentage of time that the animals spent freezing (i.e., with no movement) in the apparatus was measured in all sessions (De Carvalho Myskiw et al., 2013, 2014; Fiorenza et al., 2012).

2.4. Exposure to an open field (OF)

The OF was a 50 × 50 × 40-cm black acrylic box. The animals were exposed to the OF for 5 min 2 h before the extinction training session. Because the animals had never seen the apparatus before, this represented an exposure to a novel environment (De Carvalho Myskiw et al., 2013, 2014; Singewald et al., 2015).

2.5. Pharmacological treatments

The drug administration occurred immediately after the exposure to the OF or the extinction training session. To this, a 10 µl Hamilton microsyringe coupled to a polyethylene tube with an infusion needle (0.05 mm diameter) was used. Drugs and their respective vehicle were infused bilaterally into the CA1 region of the hippocampus (1 µl per side). At the end of the infusion, the needles were held inside the guide cannulae for another 60 s in order to prevent backflow and ensure the perfusion of the drug. The drugs used, at the doses stated in each case, were the H2-receptors agonist, Dimaprit (Dima; 2.3 µg per side); the H2-receptors antagonist, Ranitidine (Rani; 17.5 µg per side); the β-adrenergic receptors agonist, Isoproterenol (Iso; 200 µg per side); the β-adrenergic receptors antagonist, Timolol (Tim; 1.0 µg per side); the 5-HT_{1A} serotonin receptors agonist, 8-OH-DPAT (8-OH; 6.25 µg per side), and the 5-HT_{1A} serotonin receptors antagonist, NAN-190 (Nan; 1.25 µg per side). The doses were chosen among those found to be effective in previous reports from our group or others (Benetti et al., 2015; Cavalcante et al., 2017; Garrido Zinn et al., 2016).

2.6. Correct cannula placement

Correct cannula placement was verified by infusing a 4% (wt/vol) methylene blue solution over 30 s into the CA1 region of the dorsal hippocampus (1 µl per side) at the coordinates mentioned above at 2 d after the last behavioral procedure. The spread of the dye was taken as an estimate of that of the drug infusions in the same animals. Placements were considered correct when the spread was 1 mm^3 or less from the intended infusion sites (De Carvalho Myskiw et al., 2013; Fiorenza et al., 2012) (Fig. 1).

2.7. Statistical analyses

GraphPad Prism software was used for statistical analysis. The data were analyzed by One-Way analysis of variance (ANOVA) followed by the Newman-Keuls test. All data were presented as a mean ± standard error of the mean (SEM) and a p-value less than 0.05 were considered statistically significant.

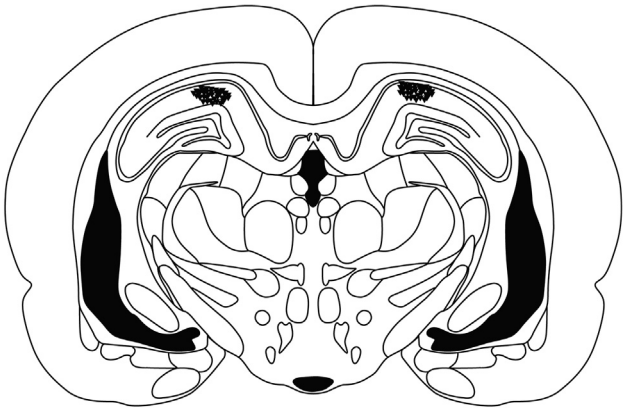

Fig. 1. Schematic representation of infusion location in the CA1 region of the dorsal hippocampus. Histological reconstruction of coronal section of the rat brain showing the infusion sites (black spots) in the CA1 region of the hippocampus (A −4.2 mm, adapted from Paxinos & Watson, 1986).

3. Results

3.1. Intra-CA1 infusion of H2 histamine receptors agonist and antagonist hindered the retrieval of CFC, but did not prevent the extinction learning or the consolidation of extinction

As previously described (De Carvalho Myskiw et al., 2013, 2014) all animals were trained in the CFC, and 24 h latter exposed to a novel OF for 5 min. After two hours, animals were underwent a 10-min extinction training session (Ext). After another 24 h, they were subjected to a 3-min retention test (Test).

Figs. 2–4 show the freezing behavior during the first 2 min (baseline) of CFC training, the first 3 min (0–3 min) of Ext that reflect the effect of the CFC training from the previous day, the last 3 min (7–10 min) of Ext that reflect the effect of Ext learning and, the retention test that reflect the extinction memory (De Carvalho Myskiw et al., 2013, 2014; Fiorenza et al., 2012; Furini et al., 2017).

The Figs. 2A, 3A and 4A show the effect of intra-CA1 infusion of Veh and the drugs immediately after the OF on the enhancing effect of OF on extinction learning. The Figs. 2B, 3B and 4B show the effect of intra-CA1 infusion of Veh and the drugs immediately after the Ext on the consolidation of extinction (De Carvalho Myskiw et al., 2013, 2014; Fiorenza et al., 2012).

As can be seen in Fig. 2A, at the doses used, Dima (2.3 μg per side) and Rani (17.5 μg per side) blocked the retrieval of CFC, but did not prevent the extinction learning. One-way ANOVA showed significant differences between groups ($F_{9,123} = 52.17$; $P < 0.0001$) and Newman-Keuls test revealed that animals that received Dima or Rani displayed significantly less freezing behavior during the first 3 min (0–3 min) of Ext (Dima $P < 0.05$; Rani $P < 0.0001$) than the Veh-treated animals. However, the freezing behavior during the last 3 min (7–10 min) of Ext was not different between drugs treatment and Veh group. Comparison between the last 3 min (7–10 min) of Ext and the retention test revealed that the drugs treatment has no effect on extinction learning (Veh 7–10 min *vs.* Veh test [$P < 0.0001$]; Dima 7–10 min *vs.* Dima test [$P < 0.0001$]; Rani 7–10 min *vs.* Rani test [$P < 0.001$]). Additionally, all animals expressed similar levels of freezing behavior during the retention test.

Furthermore, Dima and Rani also have no effect on the consolidation of extinction (Fig. 2B). One-way ANOVA revealed significant differences between groups ($F_{9,86} = 28.88$; $P < 0.0001$) and Newman-Keuls test showed no differences between groups during the CFC Ext before drugs were administered but revealed that animals that received Veh, Dima or Rani expressed less freezing behavior during the retention test than during the last 3 min (7–10 min) of Ext (Veh 7–10 min *vs.* Veh test [$P < 0.001$]; Dima 7–10 min *vs.* Dima test [$P < 0.001$]; Rani 7–10 min *vs.* Rani test [$P < 0.05$]). No differences between groups were observed during the retention test.

This and the following figures show the percentage of time spent freezing in the first 2 min of the CFC training (Tr), in the first 3 min (0–3 min) and last 3 min (7–10 min) of the Ext and in the Test. (A) Infusion of Dima or Rani into the CA1 immediately after the OF hindered the retrieval but not the extinction learning (n = 10–12 animals per group). (B) Infusion of Dima or Rani intra-CA1 immediately after the Ext had no effect on the consolidation of extinction (n = 8 animals per group). Data are presented as mean ± SEM of the percentage of time spent freezing. *$P < 0.05$, ***$P < 0.0001$ *vs.* first 3 min of Veh groups in the Ext; $^{\#}P < 0.05$, $^{\#\#}P < 0.001$, $^{\#\#\#}P < 0.0001$ retention test *vs.* last 3 min of Ext for respective group, Newman–Keuls test after one-way ANOVA. All other comparisons were not significant. (Upper) Schematic representation of the behavioral protocol used.

3.2. Intra-CA1 infusion of β-adrenoreceptors antagonist blocks the novelty effect and the consolidation of fear extinction

Fig. 3 shows that the intra-CA1 infusion of Tim (1.0 μg/side) immediately after the OF (Fig. 3A) or immediately after the Ext (Fig. 3B) inhibited the enhancing effect of the previously exposure to the OF on extinction. However, Iso (200 μg per side) had no effect by itself on the consolidation of extinction or on the influence of the OF upon it, but its infusion simultaneously with Tim blocked the effect of Tim.

As can be seen in Fig. 3, one-way ANOVA showed significant

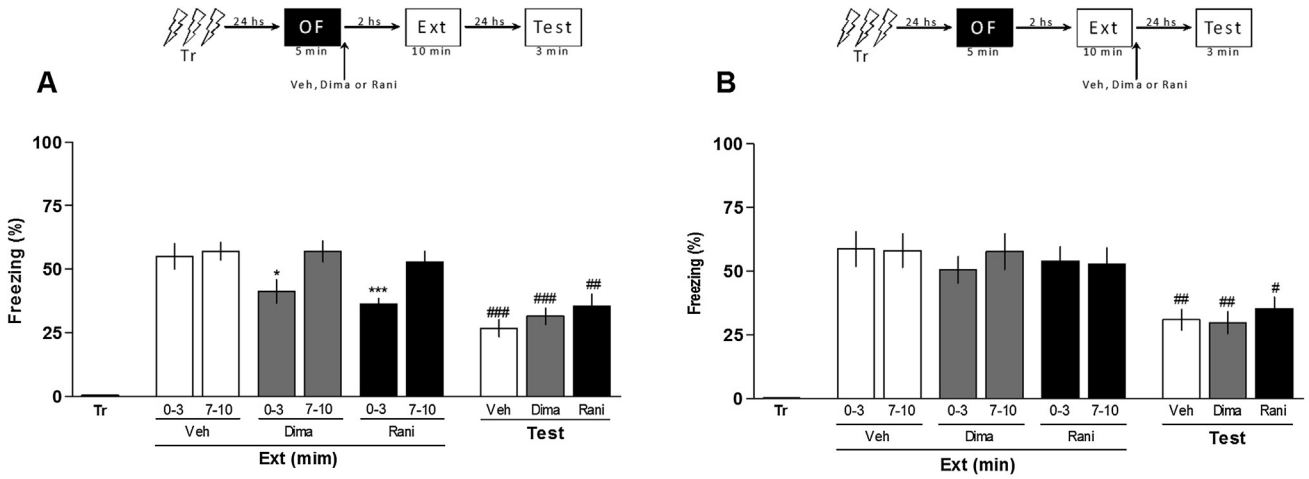

Fig. 2. Effect of H2 histamine receptors agonist and antagonist given into the hippocampus in the enhancement of fear extinction by exposure to novelty.

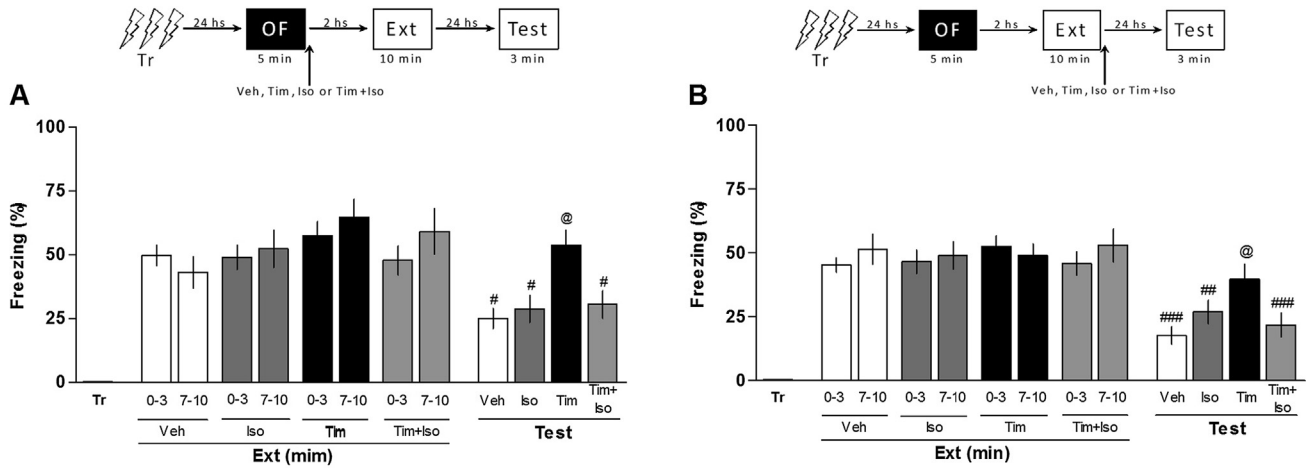

Fig. 3. Effect of β-adrenoreceptors agonist and antagonist given into the hippocampus in the enhancement of fear extinction by exposure to novelty. (A) Tim blocked the enhancement caused by the novelty. (B) Tim impaired the effect of OF on the consolidation of extinction. The effect caused by Tim was blocked when coinfused with Iso on both variables (A and B). Data are presented as mean ± SEM of the percentage of time spent freezing. $^{\#}P < 0.05$, $^{\#\#}P < 0.001$,$^{\#\#\#}P < 0.0001$ retention test *vs.* last 3 min of Ext for respective group; $^{@}P < 0.05$ *vs.* all groups in the retention test, Newman–Keuls test after one-way ANOVA; n = 8–11 (A) and *n* = 9–10 (B) animals per group. (Upper) Schematic representation of the behavioral protocol used.

differences between groups (Fig. 3A: $F_{12,147} = 26.78$; $P < 0.0001$; Fig. 3B: $F_{12,145} = 32.67$; $P < 0.0001$). Newman-Keuls test revealed that animals that received Veh, Iso or Tim + Iso, but not Tim, expressed less freezing behavior during the retention test than during the last 3 min (7–10 min) of Ext (Fig. 3A: Veh 7–10 min *vs*. Veh test [$P < 0.05$]; Iso 7–10 min *vs*. Iso test [$P < 0.05$]; Tim + Iso 7–10 min *vs*. Tim + Iso test [$P < 0.05$]; Fig. 3B: Veh 7–10 min *vs*. Veh test [$P < 0.0001$]; Iso 7–10 min *vs*. Iso test [$P < 0.001$]; Tim + Iso 7–10 min *vs*. Tim + Iso test [$P < 0.0001$]). During the retention test animals that received Tim expressed higher levels of freezing behavior than Veh (Fig. 3A: $P < 0.001$; Fig. 3B: $P < 0.001$), Iso (Fig. 3A: $P < 0.05$; Fig. 3B: $P < 0.05$) and Tim + Iso (Fig. 3A: $P < 0.05$; Fig. 3B: $P < 0.001$). Moreover, when compared with the Veh group drugs-treated animals displayed similar levels of freezing during the first 3 min and also during the last 3 min of Ext.

3.3. Intra-CA1 infusion of 5-HT$_{1A}$-serotoninergic receptors agonist injured the enhancement of fear extinction by exposure to novelty

As shown in Fig. 4, the intra-CA1 infusion of 8-OH inhibited the enhancing effect of exposure to the OF on extinction but not the consolidation of extinction itself. However, Nan had no effect by itself on the consolidation of extinction or on the influence of the OF upon it, but its intra-CA1 administration simultaneously with 8-OH blocked the effect of 8-OH. In Fig. 4A, one-way ANOVA showed significant differences between groups ($F_{12,159} = 38.09$; $P < 0.0001$) and Newman-Keuls test revealed that animals that received Veh, Nan (1.25 µg per side) or 8-OH + Nan, but not 8-OH (6.25 µg per side), expressed less freezing behavior during the retention test than during the last 3 min (7–10 min) of Ext (Veh 7–10 min *vs*. Veh test [$P < 0.0001$]; Nan 7–10 min *vs*. Nan test [$P < 0.001$]; 8-OH + Nan 7–10 min *vs*. 8-OH + Nan test [$P < 0.001$]). During the retention test animals that received 8-OH expressed higher levels of freezing behavior than Veh ($P < 0.0001$), Nan ($P < 0.05$) and 8-OH + Nan ($P < 0.05$). Additionally, when compared with Veh group, drugs-treated animals

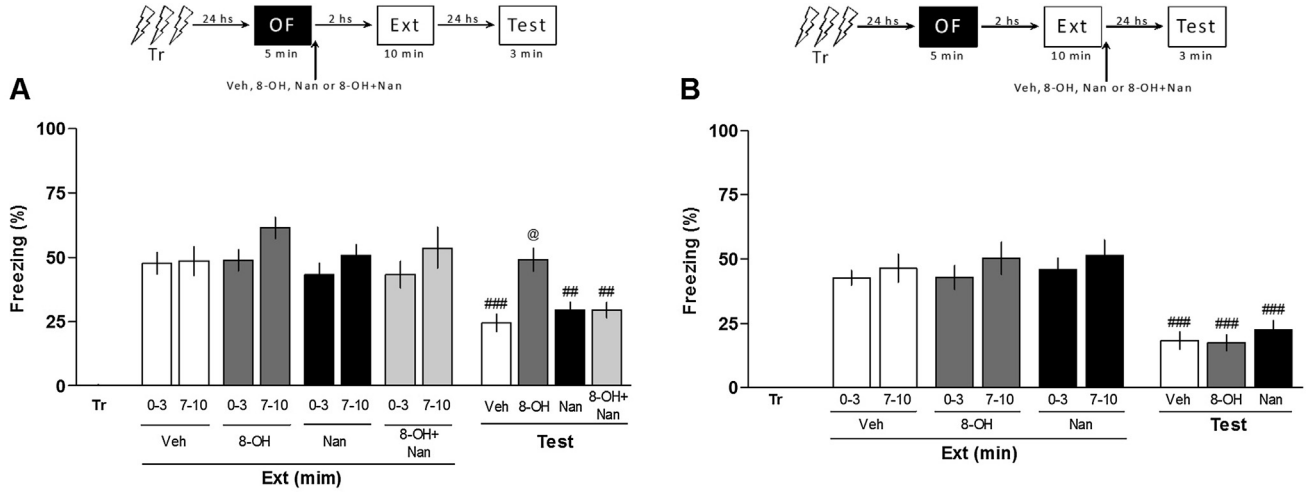

Fig. 4. Effect of 5-HT$_{1A}$-serotoninergic receptors agonist and antagonist given into the hippocampus in the enhancement of fear extinction by exposure to novelty. When given immediately after novel OF into the CA1, 8-OH (A) blocked the Ext enhancement caused by the OF. Additionally, the 8-OH effect was blocked by the coinfusion of Nan (A and B). Data are presented as mean ± SEM of the percentage of time spent freezing. $^{\#\#}P < 0.001$, $^{\#\#\#}P < 0.0001$ retention test *vs.* last 3 min Ext for respective group; $^{@}P < 0.05$ *vs.* all groups in the retention test, Newman–Keuls test after one-way ANOVA; n = 10–12 animals per group (A and B). (Upper) Schematic representation of the behavioral protocol used.

displayed similar levels of freezing during the first 3 min and during the last 3 min of Ext. In Fig. 4B, one-way ANOVA showed significant differences between groups ($F_{9,122} = 34.75$; $P < 0.0001$) and Newman-Keuls test revealed that animals that received Veh, 8-OH or Nan expressed less freezing behavior during the retention test than during the last 3 min (7–10 min) of Ext (Veh 7–10 min *vs.* Veh test [$P < 0.0001$]; 8-OH 7–10 min *vs.* 8-OH test [$P < 0.0001$]; Nan 7–10 min *vs.* Nan test [$P < 0.0001$]). There is no difference between groups during the retention test.

4. Discussion

Here we showed that Tim and 8-OH were able, at the doses used, to block the enhancing effect of the exposure to the OF, which is presumably due to a tagging-and-capture process, and Tim, but not the other drugs, were capable of blocking memory consolidation of the extinction. However, the retrieval of CFC, but not the extinction learning or the consolidation of extinction, was injured by Dima and Rani. The present findings enlighten the understanding of the molecular mechanisms that regulate the behavioral tagging process of extinction memory, demonstrating that the enhancement of extinction by novelty is hindered by the activation in the hippocampus of serotonin-5-HT_{1A} receptors and by the blockade of β-adrenergic receptors and unaffected by drugs acting on H2 histamine receptors.

Synaptic plasticity is a physiological phenomenon whereby specific patterns of neural activity lead to changes in synaptic efficacy and neural excitability. This is required for initial encoding and memory trace establishment (Martin, Grimwood, & Morris, 2000). Events that occur before or after stimuli that induce memory formation may influence synaptic plasticity and memory storage (Govindarajan, Kelleher, & Tonegawa, 2006; Diego Moncada, Ballarini, & Viola, 2015). The theory of synaptic tagging and capture (STC) predicts that a weak stimulus may activate synapses and define a "tag" that subsequently captures the PRPS synthesized from a strong stimulus in a given period of time (Frey & Morris, 1997, 1998). Studies have shown that behavioral tasks that initially depend on weak stimulus unable to induce learning, with the exposure to a novelty, such as, an open field, can induce synthesis of PRPS that will be used by the tag and induce a strong learning (Ballarini et al., 2009; De Carvalho Myskiw et al., 2013, 2014; Menezes et al., 2015; Moncada & Viola, 2007). As corroborated here, the exposure to novelty facilitates the formation of extinction memory of CFC and this can be explained by the STC hypothesis. This result indicates that the weak extinction session was not able to form a LTM, however, when animals are exposed to the OF for 5 min, 2 h before the extinction session, they are able to express a LTM. This probably occurs because exposure to OF induced PRP synthesis that was later captured and, endorses the results of De Carvalho Myskiw et al. (2013, 2014) who demonstrated for the first time that the synaptic tagging process occurs on extinction memory.

Some of the signaling pathways that were already demonstrated to be involved on the enhancement of extinction by novelty are the D1 dopamine receptors, NMDA receptors, Src kinases, calcium/calmodulin-dependent protein kinase II (CaMKII) and L-voltage dependent calcium channels (De Carvalho Myskiw et al., 2014; Menezes et al., 2015; Wang et al., 2016) (Fig. 5).

The histaminergic system has been related to the modulation of different memories (Benetti & Izquierdo, 2013; Benetti et al., 2015; Bonini et al., 2011; Cavalcante et al., 2017; Da Silveira, Furini, Benetti, Monteiro, & Izquierdo, 2013; Fabbri et al., 2016; Fiorenza et al., 2012; Garrido Zinn et al., 2016). This is supported by the fact that the intra-CA1 infusion of histamine and H2 receptors agonist, Dimaprit, enhanced the consolidation and extinction of step-down inhibitory avoidance (IA) (Bonini et al., 2011; Da Silva, Bonini, Bevilaqua, Izquierdo, & Cammarota, 2006) while the blockade of H2 receptors impaired the extinction memory of CFC and IA (Fiorenza et al., 2012). There is also evidence that histaminergic system is important for retrieval memory, since the depletion of brain histamine by α-fluoromethylhistidine, a suicide inhibitor of histidine decarboxylase, blocked the retrieval of IA memory (Fabbri et al., 2016) and this impairment was restored by the intra-CA1 infusion of histamine 10 min. before the retention test. Intra-CA1 infusions of selective H1 or H2 receptor agonists showed that histamine exerted its retrieval-restoring effect by activating hippocampal H1 receptors, but not by the H2 receptors (Fabbri et al., 2016). So, despite the extensive involvement of histaminergic system on memory processes, the regulation of extinction memory induced by novelty seems not to be regulated by the H2 receptors, as occurs to the retrieval of IA memory. More studies, related to other histamine receptors may help to elucidate in more details the involvement of this modulatory system on behavioral tagging. It appears that at some receptors and in some brain areas, histamine enhances memory consolidation of certain tasks, and at other receptors and in other areas or tasks it may have different effects. For example, memory facilitation of IA has been described on one hand with histamine given into i.c.v. (de Almeida & Izquierdo, 1986) or into BLA (Benetti & Izquierdo, 2013; Benetti et al., 2015) and on the other with pharmacological inhibition of the tuberomammilary nucleus (Frisch et al., 1999) of extinction learning.

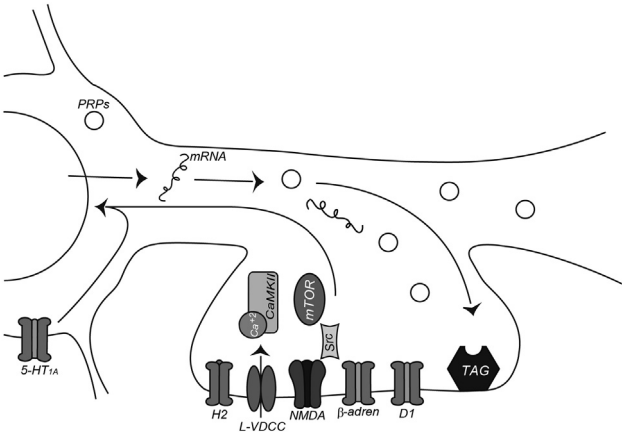

Fig. 5. Schematic representation of the molecular mechanisms that regulate the behavioral tagging process of extinction memory. The exposure to novelty facilitates the formation of extinction memory of CFC and this can be explained by the STC hypothesis. Some of the molecular mechanisms that regulate this process are the β-adrenoreceptors and 5-HT_{1A} serotonergic receptors alongside with dopaminergic D1 dopamine receptors, NMDA receptors, Src kinases, calcium/calmodulin-dependent protein kinase II (CaMKII) and L-voltage dependent calcium channels, while histamine H2 receptors are not involved.

In general, serotonin (5-HT) receptors have different effects on behavior depending on the receptor subtypes present and the behavioral tests used. In the present study the enhancement of extinction by novelty, was hindered by the agonist of 5-HT_{1A} receptors, 8-OH-DPAT. Several studies have shown that 5-HT_{1A} receptor agonists impair CFC when given systemically (Li, Lindenberger, & Sikström, 2001; Nakamura & Kurasawa, 2001), the impairment is also seen with the intra-hippocampal infusion of 8-OH-DPAT pre-training (Stiedl, Misane, Spiess, & Ögren, 2000) or pre-testing on the CFC as well as fear-potentiated startle (Almada, Borelli, Albrechet-Souza, & Brandão, 2009). This effect is observed in other forebrain structures, amygdala, insular cortex, prefrontal cortex and different memories, such as, social recognition and IA memory (Bauer, 2015; Borelli, Gárgaro, dos Santos, & Brandão, 2005; Cavalcante et al., 2017; Garrido Zinn et al., 2016; Gomes da Silva et al., 2012; Li et al., 2001; Mello e Souza et al., 2001). These inhibitory effects may be explained by the fact that activation of 5-HT_{1A} receptors inhibits neuronal activity, since they are mainly located in inhibitory interneurons (Corradetti, Ballerini, Pugliese, & Pepeu, 1992; Tada, Kasamo, Suzuki, Matsuzaki, & Kojima, 2004).

Moreover, we demonstrated that the β-adrenoreceptors of the CA1

region of the dorsal hippocampus are necessary to the learning of extinction memory induced by novelty. Noradrenaline and the activation of β-adrenergic receptors, respectively, were reported to be crucial to the enhancement of LTP and memory by emotional arousal (Cahill & McGaugh, 1998; Griffin & Taylor, 1995; Seidenbecher, Reymann, & Balschun, 1997), in addition; the β-adrenergic-receptor antagonist, propranolol, blocked the novelty-induced LTP reinforcement and application of the β-adrenergic agonist isoproterenol 30 min before LTP induction was sufficient to transform early- into late-LTP (Straube, Korz, Balschun, & Uta Frey, 2003). Moncada et al. (2011) demonstrated that the promoting effect of novelty on consolidation of IA-LTM is prevented by intra-hippocampal administration of propranolol around the time of the OF exposure and that systemic administration of a β-adrenergic receptor agonist, dobutamine, mimics the action of novelty. Also, the infusion of propranolol into the CA1 before the OF, in a protocol with an effective extinction session, inhibited the enhancement of extinction by novelty (Liu et al., 2015). Noradrenergic neurons of the *Locus Coeruleus* (LC) have been shown to fire in bursts upon exposure of a rat or a mouse to a novel OF as the used in behavioral tagging experiments (Roullet & Sara, 1998; Takeuchi et al., 2016; Vankov, Hervé-Minvielle, & Sara, 1995) and, LC stimulation promotes IA-LTM through a mechanism dependent on noradrenaline release and synthesis of PRPs in the hippocampus (Moncada, 2017). So, as occurs at LTP and memory consolidation, the results obtained here, demonstrate that β-adrenergic receptors are also necessary to the enhancement of extinction memory induced by novelty.

Here we elucidated some of the molecular mechanisms that are involved on the enhancement of extinction by novelty, demonstrating that the β-adrenoreceptors and 5-HT_{1A} serotonergic receptors participate on this process alongside with dopaminergic D1 receptors previously described (Menezes et al., 2015), while histamine H2 receptors, so ubiquitous in learning-related functions in hippocampus (Passani et al., 2017) are not involved. These data provide important information to the knowledge of modulation of extinction, which is important in the treatment of fear disorders and memories, such as those of post-traumatic stress disorder, since the treatments of choice for such conditions are based on extinction procedures.

To summarize, these data, together with others (Menezes et al., 2015) indicate that the enhancement of fear extinction by novelty, previously described (De Carvalho Myskiw et al., 2013; Moncada & Viola, 2007; Myskiw, Furini, & Izquierdo, 2017) and indeed recently reproduced in humans (Dunsmoor, Murty, Davachi, & Phelps, 2015), and potentially useful in its application to exposure therapy, involves the modulatory influence of at least three major monoamine sets of synapses in the hippocampus: 5-HT_{1A}-serotoninergic and β-adrenergic, as shown here, and D1-dopaminergic, as shown elsewhere (Menezes et al., 2015) (Fig. 5).

Acknowledgments

This work was supported by research grants from the CNPq - National Council for Scientific and Technological Development (CNPq; 402123/2016-6), CAPES - Coordination of Improvement of Higher Education Personnel (CAPES Brazil; Finance Code 001) and the State Foundation for science support (FAPERGS).

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