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PII: S0091-3057(23)00081-3

DOI: https://doi.org/10.1016/j.pbb.2023.173594

Reference: PBB 173594

To appear in: *Pharmacology, Biochemistry and Behavior*

Received date: 27 March 2023

Revised date: 23 June 2023

Accepted date: 26 June 2023

Please cite this article as: V. Pastor, J.F. Dalto and J.H. Medina, α7 nicotinic acetylcholine receptors in the medial prefrontal cortex control rewarding but not aversive memory expression in a dopamine-sensitive manner, *Pharmacology, Biochemistry and Behavior* (2023), https://doi.org/10.1016/j.pbb.2023.173594

α7 nicotinic acetylcholine receptors in the medial prefrontal cortex control rewarding but not aversive memory expression in a dopamine-sensitive manner

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Running Title: Prefrontal nicotinic control of memory retrieval

Keywords: Cocaine, emotional learning, fear conditioning, place conditioning

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Abstract

Emotional learning involves the association between sensory cues and rewarding or aversive stimuli, and this stored information can be recalled during memory retrieval. In this process, the medial prefrontal cortex (mPFC) plays an essential role. We have previously shown that the antagonism of α7 nicotinic acetylcholine receptors (nAChRs) by methyllycaconitine (MLA) in the mPFC blocked cue-induced cocaine memory retrieval. However, little is known about the involvement of prefrontal α7 nAChRs in the retrieval of aversive memories. Here, by using pharmacology and different behavioral tasks, we found that MLA did not affect aversive memory retrieval, indicating a differential effect of cholinergic prefrontal control of appetitive and aversive memories. Despite being shown that acetylcholine modulates dopamine release in the mPFC, it remains unknown if those modulatory systems act together to control reward-based behavior. We examined that question and found that dopamine type 1 receptor (D1R) activation prevented MLA-induced blockade of cocaine CPP retrieval. Our results suggest that α7 nAChRs and D1R signaling interact in the mPFC to modulate cocaine-associated memory retrieval.

Abbreviations

ACh: acetylcholine

CFC: contextual fear conditioning

COC: cocaine

CPA: conditioning place aversion

CPP: conditioning place preference

DA: dopamine

MLA: methyllycaconitine

mPFC: medial prefrontal cortex

nAChR: nicotinic acetylcholine receptor

1. Introduction

The medial prefrontal cortex (mPFC) controls both appetitive and aversive memory processing (Burgos-Robles et al., 2017; María C Gonzalez et al., 2014; Milad & Quirk, 2002; Otis et al., 2017; T. M. Tzschentke, 2000). This is an essential function for encouraging approach towards potential rewards and avoidance of potential threats, two behavioral choices that promote survival. While both behavioral choices are adaptive, the impairment of their control may be associated with neuropsychiatric diseases including anxiety, posttraumatic stress disorder, or substance use disorder (Ball & Gunaydin, 2022; Milton & Everitt, 2012; Rich & Torregrossa, 2018).

Emotional learning involves the association between sensory cues and rewarding or aversive stimuli, and this information can be recalled during memory retrieval (Berke & Hyman, 2000). Neuronal systems which control the retrieval of rewarding and aversive memories are not completely known, though neuromodulatory systems projecting to the mPFC are likely to be involved (Gritton et al., 2016; Vander Weele et al., 2018). Furthermore, it is not known if neuromodulatory systems are specific for the control of behavior in response to stimuli with different emotional value.

There is evidence for the involvement of mPFC cholinergic system through α7 nAChRs in the control of cocaine-associated memory retrieval (Pastor et al., 2021). However, little is known about the role of prefrontal α7 nAChRs in the retrieval of aversive memories. Here, we asked if the blockade of the cholinergic tone acting on α7 nAChRs, which is necessary for cocaine-associated memory retrieval, is specifically involved in the processing of this kind of appetitive memory or if it also affects aversive memories. Thus, we tested the involvement of mPFC α7 nAChRs in different aversive behavioral tasks.

There is still a gap of knowledge of how different neuromodulators interact in the mPFC to drive behavioral outputs. We then asked what the cholinergic system is controlling through α7 nAChRs. Previous reports point out the dopaminergic system as a good candidate due to its involvement in emotional memory processing through activation of dopamine type 1 receptors (D1Rs). For example, cocaine-associated cues induce dopamine (DA) release in the prefrontal cortex in rodents (Kawahara, Ohnishi, Ohnishi, Kawahara, & Nishi, 2021) and humans (Milella et al., 2013), and DA signaling through D1Rs is necessary for cocaine-associative memory retrieval (Shinohara,

Kamii, Minami, & Kaneda, 2017). Moreover, the infusion of a selective agonist of α7 nAChRs in the mPFC induces an increase in DA release which can be blocked by the local administration of an antagonist of α7 nAChRs (Livingstone et al., 2009), indicating that α7 nAChRs modulate DA release in the mPFC. Thus, we hypothesized that cholinergic signaling through α7 nAChRs is needed in the mPFC to allow cue-induced cocaine memory retrieval by interacting with D1Rs activation. Our study shows that mPFC α7 nAChRs are differentially involved in reward and aversion memory retrieval and suggests that cholinergic and dopaminergic systems interact in the mPFC to control the retrieval of cocaine-associated memory.

2. Methods

2.1. Animals

All procedures agreed with the standards for the care of laboratory animals as outlined in the ARRIVE Guidelines, followed NIH Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978) and were approved by the Institutional Animal Care and Use Committee (CICUAL, School of Medicine, University of Buenos Aires). Male Wistar adult rats aged 7 weeks and weighting 180-200g on arrival were used throughout this study (School of Pharmacy and Biochemistry, University of Buenos Aires). Rats were housed 4 per cage with *ad libitum* access to standard rat chow and water. A constant light/dark cycle (12:12) with lights on at 07:00h and a room temperature of 22–24°C were maintained. Animals were handled once a day for three days before behavioral tasks to avoid emotional stress. Care was taken to minimize the number of animals used and their suffering.

2.2. Drugs

For conditioning place preference (CPP) experiments, a dose of 20 mg/kg cocaine hydrochloride (based on the molecular weight of the freebase; Verardo Laboratory, Buenos Aires, Argentina) was dissolved in saline solution (NaCl 0.9%) and administered intraperitoneally (i.p.) in a volume of 1 ml/kg body weight. For conditioning place aversion (CPA) experiments, a dose of 150 mg/kg LiCl was dissolved in NaCl 0.9% and administered i.p. in a volume of 1 ml/kg body weight. An equal volume of NaCl 0.9% was injected to control groups in place conditioning experiments. 1/2-inch

30-G needles were used to minimize possible discomfort associated with systemic injections. The α7 nAChR selective antagonist Methyllycaconitine citrate (MLA; Abcam #ab120072) and/or the D1/D5 receptor agonist SKF-38393 hydrochloride (SKF; Sigma-Aldrich #S101) were dissolved in sterile saline solution and administered bilaterally intra-mPFC (MLA: 5µg/µl/hemisphere; SKF: 6.25µg/µl/hemisphere). The same volume of vehicle (1µl/hemisphere) was used in control rats. Cocaine, MLA and SKF doses were determined based on previous reports from our group and others (Castillo Díaz, Kramar, Hernandez, & Medina, 2017; Chan, Tsun-Hon Wong, & Sheu, 2007; Pastor et al., 2021; Shinohara et al., 2017).

2.3. Stereotaxic surgeries, intra-mPFC infusions and histological verification.

Surgery was performed as previously described (Pastor et al., 2021). Briefly, at postnatal day 60 (P60) rats were anesthetized with a mix of ketamine (80 mg/kg) and xylazine (8 mg/kg) and placed on a stereotaxic frame. The skull was exposed and leveled and 22-G guide cannulae were bilaterally implanted, aimed to the mPFC: AP +3.20 mm/LL±0.75 mm/DV−3.20 mm from Bregma. Cannulae were protected with modified 30-G needles and fixed to the skull with dental acrylic. Immediately after surgery, animals were injected with meloxicam (0.2 mg/kg) as analgesic and gentamicin (2 mg/kg) as antibiotic and were allowed to recover from surgery for 10 days before behavioral experiments.

For intra-mPFC infusions, 30-G needles connected to 10µl Hamilton syringes were employed. The infusions were always bilateral (1 µl/min/hemisphere). The needle was left in place for an additional minute after infusion to prevent reflux. At the end of each behavioral experiment, 1 µl infusions of 4% methylene blue in saline were used for histological verification of cannulae placement with binocular magnifying glasses. According to the anatomical boundaries defined in the rat brain atlas (Paxinos & Watson, 2007), the extension of the dye infused was taken as indicative of the diffusion of the drugs (Figure 1g). The observed diffusion agrees with published results from our group, which has been estimated to be approximately 1,5 mm (María C Gonzalez et al., 2014; Pastor et al., 2021; Tomaiuolo, Gonzalez, Medina, & Piriz, 2014). Only animals with both cannulae in the correct place were included in the analysis.

2.4. Behavioral experiments

2.4.1 Aversive and appetitive place conditioning

Place conditioning experiments were performed as previously described (Castillo Díaz, Hernandez, Capellá, & Medina, 2019; Pastor et al., 2021). Place conditioning boxes exhibited two large compartments (30 cm long × 25 cm wide × 30 cm high) separated by a small grey compartment (12 × 25 × 30 cm) with sliding doors. One of the large compartments was black with white square patterns and a bar-grid floor, and the other was white with vertical black lines and a perforated floor. For conditioning place aversion (CPA) experiments, conditioning boxes also had pine shavings below the floor in the black compartment and cedar shavings below the floor in the white compartment. These boxes are considered biased because animals show a significant preference for the black compartment over the white one prior to conditioning (Thomas M. Tzschentke, 2007). Conditioning place preference (CPP) and CPA were performed in the same conditioning boxes and consisted of three phases: pretest (day 1), conditioning (days 2-3) and test (day 4). The first day, during a 15-min pretest, animals were allowed to explore the entire box and the time spent in each compartment was used to determine each animal's initial preference. The second day, animals were injected with 1 mL/kg of saline solution and restricted during 30-min to the non-preferred white compartment (for CPA experiments) or to the preferred-black compartment (for CPP experiments). The third day, animals were injected with 150 mg/kg of LiCl and restricted to the black compartment (for CPA experiments) or with 20 mg/kg of cocaine and restricted to the white compartment (for CPP experiments) for 30 minutes. The fourth day, a 24h test was performed in a drug-free state, where animals were allowed to explore the entire box for 15 minutes. Intra-mPFC infusions were made 30 minutes before the 24h test. A 48h test was performed the next day for CPP experiments with no additional infusion. A change on place preference in the test compared to pretest was used to assess associative memory retrieval. A CPA or CPP score was also calculated as the difference between the time spent in the drug-associated chamber in the test minus the time spent in that chamber during the pretest.

2.4.2. Contextual Fear Conditioning (CFC)

CFC was performed in a plexiglas box (25 cm long x 30 cm wide × 25 cm high) with a metallic grid floor. CFC consisted of two phases: conditioning (day 1) and test (day 2). The first day, animals were placed in the CFC box for 2 minutes and then 3 consecutive shocks (0.8 mA, 2 seconds each) were applied with a 30 second interval. Animals were left in the CFC box for another 15 seconds and then returned to their home cages. The second day, animals were placed again in the CFC chamber and freezing behavior was recorded for 5 minutes. Intra-mPFC infusions were made 30 minutes before the test. Freezing behavior was expressed as the percentage of time during which the animal was freezing (Freezing-Panlab software, v1.3).

2.4.3. Inhibitory Avoidance (IA)

IA experiments were performed in an opaque acrylic box (47 × 25 × 30 cm3) with a grid floor and a platform placed on the left of the box (5 cm high, 9 cm wide). A one-trial conditioning protocol was used as previously described (Bekinschtein et al., 2007; Rossato, Bevilaqua, Izquierdo, Medina, & Cammarota, 2009). The first day rats were placed on the platform, and they received a 3-s scrambled mild foot shock (0.5 mA) as they stepped down onto the grid with all four paws. The second day rats were placed again in the platform and the latency to step down was measured in seconds (no foot-shock was given during the test). Intra-mPFC infusions were made 30 minutes before the test.

2.4.4. Data Analysis

Data are presented as mean ± SEM or aligned dots (before-after graphs). Schapiro-Wilk's test was used to verify data normal distribution. Data were analyzed by Student's t-test or two-way repeated measures of ANOVA (RM-ANOVA: time x mPFC treatment, with time as the repeated factor) with the GraphPad Prism software. When a significant interaction was found, a simple effect test with Holm-Sidak correction for multiple comparisons was used. In case of no interaction between factors, the main effect of ANOVA was analyzed. Significant differences were set at $p<0.05$. A total of 9 rats were eliminated from the analysis because of cannula misplacement (n=5) or following the GraphPad Outliers Grubbs' test with alpha 0.05 (n=4).

3. Results

3.1. mPFC α7 nAChRs are not involved in the expression of aversive associative memories

We have previously shown that mPFC α7 nAChRs are involved in the expression of cocaine-associated memory (Pastor et al., 2021; see also Figure 2b below). Here, we asked whether mPFC α7 nAChRs control of memory retrieval is specific for the appetitive value of the cue or if those receptors are also involved in the expression of aversive associative memories. We reasoned that if the MLA effect was independent on the value, it should block aversive associative memory retrieval. First, we conducted CPA experiments where animals were trained to avoid the chamber previously associated with the aversive effect of an injection of LiCl (Figure 1a). Following conditioning, time spent in the black (LiCl-associated) chamber decreased in comparison with time spent in that chamber during pretest in vehicle-infused rats (Figure 1b), consistent with the expected conditioned place aversion. The bilateral infusion of MLA before the test did not influence CPA memory test performance as those animals also showed a decrease of the time spent in the black chamber (Two-way RM ANOVA: $F_{(1,23)}$=10,92; p=0,0031 for time; $F_{(1,23)}$=1,625; p=0,2151 for mPFC treatment; $F_{(1,23)}$=0,737; p=0,3993 for the interaction). Accordingly, we found no differences in the CPA score between MLA-infused and vehicle control rats (Figure 1c; Student's t- test: $t_{(23)}$=0.859; p=0.399). The number of entries to both CPA chambers was assessed to control locomotor activity during the test and no differences were found between groups (Figure 1d). Thus, α7 nAChRs antagonism in the mPFC did not affect LiCl CPA retrieval.

To test the role of mPFC α7 nAChRs in memory retrieval with another aversive task, we performed a CFC where the animals were infused with MLA 30 minutes before the test (Figure 1e). We assessed the percentage of time during which the animals were freezing during the test (Figure 1f). We found no differences between MLA-infused and vehicle control rats (Student´s t-test: $t_{(15)}$=0,3716; p=0.7154). Thus, mPFC α7 nAChRs blockade did not influence CFC memory retrieval. In addition, we performed IA experiments with a mild shock, where we found no differences in memory expression between MLA-infused and vehicle control rats (latency to step-down: 60.5 $\pm$ 16.7 sec for vehicle-infused rats; 115.9 $\pm$ 40.4 sec for MLA-infused rats; Student´s t-test: $t_{(13)}$=1,330; p=0,2064; n=7-8). Altogether, these results suggest that, differently from what we

reported for cocaine-associated memory expression, mPFC α7 nAChRs are not involved in aversive associative memory retrieval.

3.2. MLA in the mPFC blocked cocaine-associated memory retrieval in a dopamine-sensitive manner.

As we commented above, we previously showed that α7 nAChRs antagonism by MLA in the mPFC blocked one-trial cocaine CPP retrieval 24 and 29h following conditioning (Pastor et al., 2021). Considering the clinical relevance of behavioral and pharmacological strategies that can weaken drug-context memories in a long-term manner, we extended our previous work by assessing if MLA-induced blockade of cocaine CPP persisted 48h following conditioning (Figure 2). Moreover, given that α7 nAChRs are involved in prefrontal cortical DA release (Livingstone et al., 2009), we asked if cholinergic signaling through α7 nAChRs was needed in the mPFC to allow cue-induced cocaine memory retrieval in a dopamine-sensitive manner. D1Rs are one of the main DA receptors in the mPFC (Santana & Artigas, 2017) and are needed for cocaine CPP expression (Shinohara et al., 2017). Thus, we assessed whether the blockade of cocaine memory retrieval induced by the infusion of MLA in the mPFC could be prevented by SKF, a D1Rs agonist. Different groups of rats received an infusion of vehicle, MLA, SKF or a co-infusion of MLA and SKF before the 24h CPP test, as shown in the experimental scheme (Figure 2a).

To assess cocaine memory retrieval, we compared the time spent in the white (cocaine-associated) CPP chamber during the pretest and test sessions, i.e., before and after conditioning (Two-way RM-ANOVA. $F_{(2,76)}$=12,75; p<0,0001 for time; $F_{(3,38)}$=1,456; p=0,2417 for mPFC treatment; $F_{(6,76)}$=2,532, p=0,0274 for the interaction between factors; Figure 2b). As expected, cue-induced retrieval took place 24h following conditioning in control animals infused with vehicle. This was evidenced as an increase of the time spent in the white chamber in the 24h test compared with the pretest (***p=0.0003). Moreover, cocaine-associated memory was evidenced 48h following conditioning (*p=0.0327). In contrast, MLA infused rats did not show any changes on the time spent in the white chamber at 24h following conditioning. Importantly, MLA blocking effect was still present 48h after conditioning with no additional infusion. Similar to control rats, animals infused only with SKF showed a significant increase on the time spent in the white chamber 24h and 48h following conditioning (**p=0.0033 and **p=0.0069, respectively),

indicating that SKF has not an effect per se. Critically, MLA-induced blockade of cocaine place conditioning was prevented if the animals were co-infused with SKF before the 24h test (**p=0.0013). In this group, cocaine CPP was also evidenced 48h following conditioning (*p=0.0436). In Figure 2c, we quantified the difference between the time spent in the white CPP chamber during test and pretest (CPP score) to compare the behavior between groups. Vehicle, SKF alone and MLA+SKF groups showed significantly higher 24h and 48h CPP scores than rats infused with MLA alone (Two-way RM-ANOVA: $F_{(3,38)}$=5,044; p=0,0049 for mPFC treatment; $F_{(1,38)}$=0,7354; p=0,3965 for time; $F_{(3,38)}$=1,565; p=0,2137 for the interaction between factors). The number of entries to both CPP chambers was assessed to control locomotor activity during the 24h and 48h tests and no differences were found between groups (Figure 2d, e).

These results showed that MLA blocked cue-induced cocaine memory retrieval in a long-term manner and suggest that MLA may interfere with D1R-mediated neurotransmission occurring in the mPFC during the exposure to drug cues.

3.3. MLA alone did not induce any preference or aversion for the conditioning chambers.

To test if MLA infusion in the mPFC had any rewarding or aversive effect per se, we made control experiments by using the same protocols than in CPA and CPP experiments, except for the fact that rats were conditioned with (i.p.) saline solution in both CPP/CPA chambers (Figure 3). When using the CPA protocol, we found that MLA infusion before the 24h test did not induce any change on the time spent in the black chamber in the test compared to pretest (Two-way RM-ANOVA: $F_{(1,10)}$=0,2457, p=0,6308 for time; $F_{(1,10)}$=0,1285, p=0,7274 for mPFC treatment; $F_{(1,10)}$=0,1577, p=0,6997 for the interaction between factors; Figure 3a). Accordingly, no differences were found in the CPA score (Student´s t-test: $t_{(10)}$=0,3971, p=0,6997; Figure 3b). The same was found when using the CPP protocol: MLA did not induce any change on the time spent in the white chamber during the test compared to pretest (Two-way RM-ANOVA: $F_{(1,10)}$=0.7308, p=0,4126 for time; $F_{(1,10)}$=0,0024, p=0,9618 for mPFC treatment; $F_{(1,10)}$=0,0031, p=0,9565 for the interaction between factors; Figure 3c) and no differences were found between groups on the CPP score (Student´s t-test: $t_{(10)}$=0,05590; p=0.9565; Figure 3d). Thus, the infusion of MLA in the mPFC did not induce per se any preference or aversion for the conditioning chambers.

4. Discussion

In this study, we found 1) a differential role of prefrontal α7 nAChRs on aversive and rewarding memories, and 2) that α7 nAChRs and D1Rs interact in the mPFC to modulate cocaine-associated memory retrieval. mPFC is responsible for regulating both rewarding and aversive memory processing, which is essential for approaching potential rewards and avoiding potential threats. These behaviors are crucial for survival, but impairments in their control have been linked to neuropsychiatric conditions such as anxiety, post-traumatic stress disorders, and substance use disorders. Understanding the mechanisms behind the role of the mPFC in these processes is important for developing effective treatments for these and other conditions that involve abnormal memory processing.

Previous research has shown that different projection neurons are activated in the mPFC in response to rewarding or aversive stimuli (Ye et al., 2016). These neurons may be re-activated during the exposure to associated cues for allowing memory retrieval and guiding behavior accordingly (Frankland, Josselyn, & Köhler, 2019; Josselyn & Tonegawa, 2020). Neural activity in the mPFC is known to be crucial for memory expression of different rewarding and aversive learning tasks, including those used in the present study (Corcoran & Quirk, 2007; Einarsson & Nader, 2012; María C Gonzalez et al., 2014; Maria Carolina Gonzalez, Villar, Igaz, Viola, & Medina, 2015; Zhang et al., 2020). However, which are the neuronal systems that control the retrieval of rewarding and aversive memories is a question which remains unanswered. Neuromodulatory systems projecting to the mPFC are likely to be involved (Gritton et al., 2016; Pastor & Medina, 2021; Vander Weele, Siciliano, & Tye, 2019). They have a crucial role in healthy individuals (Bloem, Poorthuis, & Mansvelder, 2014; Del-Arco & Mora, 2008; Hu, 2016; Picciotto, Higley, & Mineur, 2012), and their impairment leads to many neuropsychiatric diseases where memory processing is impaired (Koukouli & Changeux, 2020; Sara & Bouret, 2012). ACh phasic release in the mPFC is involved in the detection of sensory cues that are related to both reward and aversion (Gritton et al., 2016; Parikh, Kozak, Martinez, & Sarter, 2007). Moreover, basal forebrain cholinergic neurons -the main ACh source of the mPFC- were suggested to encode reward-predictive value of sensory cues (Hangya, Ranade, Lorenc, & Kepecs, 2015; Leonor Teles-Grilo Ruivo et al., 2017) and they respond to both water reward and air puff punishment (Hegedü, Sviatkó, Martínez-Bellver, & Zs Hangya, 2023). Thus, the cholinergic system is well positioned to regulate both rewarding and aversive memory processing in the mPFC. Moreover, mPFC cholinergic

interneurons are involved in sustained attention which is essential to guide behavior. (Obermayer et al., 2019) Thus, despite the need of more research on this topic, this local source of ACh could also be important for rewarding and aversive memory processing.

Place conditioning involves the association of sensory cues and the emotional value of an unconditioned stimulus. It can be used to assess the effectiveness of potential treatments for conditions that involve aberrant learning and memory processes, such as substance use disorder (Thomas M. Tzschentke, 2007) or post-traumatic stress disorder (Mineka & Oehlberg, 2008). One of the benefits of using place conditioning is that it allows for the evaluation of both rewarding and aversive types of memories in a single behavioral task. In this study, we performed CPA experiments where we found that rats infused with MLA before the test, still showed a decrease on the preference for the black (LiCl-associated) compartment, similar to control rats infused with vehicle. Thus, in contrast to the importance of mPFC α7 nAChRs for the expression of cocaine CPP (Pastor et al., 2021; and Figure 2b), the antagonism of mPFC α7 nAChRs before the test did not impair aversive memory retrieval. This is the first evidence showing that, differently from what is observed with cocaine CPP, mPFC α7 nAChRs are not involved in the retrieval of LiCl-associated CPA. Moreover, we used inhibitory avoidance and contextual fear conditioning tasks to confirm that mPFC α7 nAChRs are not involved in aversive memory retrieval. Our results are in line with a recent study showing that the antagonism of α7 nAChRs before the test did not affect the retrieval of a trace fear conditioning in rats (Miguelez Fernández, Molla, Thomases, & Tseng, 2021), although it was previously reported that α7 nAChRs were necessary for cued- and context-trace fear conditioning retrieval in a mice model (Raybuck & Gould, 2010). These discrepancies could be attributed to the difference in the rodent model, the behavioral protocol and MLA concentration used.

Altogether, these results indicate that the retrieval of rewarding and aversive associative memories is differently modulated in the mPFC. Different mechanisms could be responsible for this differential effect. One possibility is that mPFC pyramidal neurons projecting to subcortical areas involved in behaviors promoting approach (e.g., the nucleus accumbens, NAc) *vs* avoidance (e.g., paraventricular nucleus of the thalamus, PVT) (Otis et al., 2017; Siemsen et al., 2022; Ye et al., 2016) may be differentially modulated by α7 nAChRs (Figure 4a). Although in our experiments the infusion of MLA diffused to all mPFC layers, it is important to consider that nAChRs modulation of mPFC activity is layer specific (Bloem et al., 2014). α7 nAChRs have not been implicated in the modulation of layer VI (Poorthuis, Bloem, Verhoog, & Mansvelder, 2013), where pyramidal neurons projecting to the PVT reside (Li & Kirouac, 2012; Vertes, 2002). This circuit is known to be involved in fear memory retrieval (Do-Monte, Quinõnes-Laracuente, & Quirk, 2015). Another possible mechanism which is not exclusive is that GABAergic interneurons in the mPFC may be modulating aversive behaviors more strongly than rewarding ones. This could occur through disinhibition processes (Letzkus, Wolff, & Lüthi, 2015; Poorthuis, Enke, & Letzkus, 2014), and α7 nAChRs may not be

involved. In that sense, it has been recently shown that different populations of somatostatin GABAergic neurons in the mPFC are differently engaged in aversive vs rewarding memory expression (Cummings, Bayshtok, Dong, Kenny, & Clem, 2022). How these interneurons are modulated by the cholinergic system during memory retrieval is still unknown.

Regarding rewarding memories, we confirmed and extended our previous evidence by showing that the antagonism of α7 nAChRs in the mPFC blocks cocaine-associated memory retrieval in a long-term manner. This was evidenced by the absence of cocaine CPP not only 24h but also 48h following conditioning in MLA-treated rats. Even though MLA is a reversible antagonist, we found that MLA has a lasting blocking effect of cocaine memory retrieval. Thus, MLA may be interfering with lasting synaptic changes induced by plasticity processes, which are essential for cocaine memory retrieval (Otis et al., 2018; Otis & Mueller, 2017). These mechanisms may be triggered by α7 nAChRs activation during the exposure to cocaine-associated cues, considering their role in mPFC synaptic plasticity (Pastor & Medina, 2023; Udakis, Wright, Wonnacott, & Bailey, 2016). For assessing this possibility, it would be necessary to use multi-trial experiments given that one-trial cocaine CPP does not last more than 48h (Kramar, Barbano, & Medina, 2014).

The mPFC is influenced by different neuromodulators, including ACh and DA (Pastor & Medina, 2021; Vander Weele et al., 2019). However, the precise mechanisms by which these neuromodulators interact in the mPFC for regulating behavior are still not fully understood. Cholinergic α7 modulation of DA release in the mPFC has been previously evidenced by *in vitro* and *in vivo* experiments (Jaffé & Hernández, 1989; Livingstone et al., 2009). Moreover, it was shown that cocaine associated cues increase mPFC DA release (Milella et al., 2013) and that mPFC D1Rs are necessary for cue-induced cocaine memory retrieval (Shinohara et al., 2017). Considering that mPFC α7 nAChRs are involved in cocaine memory processing (Pastor et al., 2021), we asked if α7 nAChRs are needed in the mPFC to allow cue-induced cocaine memory retrieval by interacting with D1Rs activation. We found that MLA blocking effect was prevented by the concomitant infusion of a D1Rs agonist in the mPFC before the first CPP test. These results indicate that, in the absence of α7 nAChRs activation, D1R exogenous activation in the mPFC is needed for cue-induced retrieval of cocaine-associated memory. How are α7 nAChRs and D1Rs interacting in the mPFC to drive cue-induced cocaine memory retrieval? α7 nAChRs are highly expressed in presynaptic terminals, modulating other neurotransmitter release, such as DA or glutamate (Cheng & Yakel, 2015). For example, glutamatergic inputs from hippocampus and amygdala are modulated by α7 nAChRs signaling (Miguelez Fernández et al., 2021). Then, it would be possible that α7 nAChRs in the mPFC facilitate DA neurotransmission by acting on DA terminals or indirectly by acting on glutamatergic terminals which may facilitate DA release (Lima et al., 2013; Figure 4b). We cannot totally rule out the possibility that D1Rs-mediated neurotransmission and α7 nAChRs systems act as complementary but independent mechanisms in the control of cocaine-associated memory expression. However, mPFC infusion of SKF alone did not

change cue-induced cocaine memory retrieval in comparison with control rats which received an infusion of vehicle. Thus, the modulatory role of D1Rs was evident only in the presence of α7 nAChRs antagonism supporting the interaction between α7 nAChRs and D1Rs neurotransmission during cue-induced cocaine memory retrieval. Additionally, other neurotransmission systems such as the noradrenergic one may be contributing to nAChRs modulation of cocaine memory retrieval, especially under stress conditions, considering the role of noradrenaline in mPFC neuronal activity and cocaine memory retrieval (Shinohara, Arakaki, Amano, Minami, & Kaneda, 2020; Wada et al., 2020). Further research is needed to address this possibility.

The α7 nAChRs subtype is being extensively studied as a new pharmacological target in clinical trials of neurological disorders in which memory processing is impaired such as schizophrenia and Alzheimer's disease (Bertrand & Terry, 2018; Greenfield et al., 2022). Knowing the role of α7 nAChRs in different types of memories is of translational importance to provide a deeper understanding of the underlying mechanisms involved in memory processing and ultimately lead to the development of more targeted and effective treatments for those neurological disorders. Here, we reported that MLA infusion in the mPFC selectively disrupt cocaine-associated memory retrieval without affecting associative memories of aversive events and without causing any rewarding or aversive effects on its own. The results of the present study suggest that the blockade of α7 nAChRs in the mPFC could be a viable method for disrupting the retrieval of rewarding memories which could have significant clinical benefits in the future.

Conflict of Interest: The authors declare no conflict of interest. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Data Availability Statement: The data that support the findings of this study are available from the corresponding author upon reasonable request.

Acknowledgements

This work was supported by FONCyT (PICT 1116-2018 to JHM) and the International Society for Neurochemistry (CAEN 2021 to VP). We greatly appreciate the technical assistance of Marina F. Berisso with CPA experiments.

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Legends to Figures

Figure 1: The antagonism of α7 nAChRs in the mPFC before the test did not affect the expression of aversive memories. a) Experimental scheme of LiCl-induced CPA. Bars represent b) the time spent in the black (LiCl-associated) chamber during pretest and test sessions; c) the CPA score, for rats conditioned with

LiCl and infused with MLA or vehicle before the CPA test; and d) the number of entries to CPA chambers during the test. Two-way RM-ANOVA: **p<0.01 (n=12-13). e) Experimental scheme of CFC; f) Percentage of time spent freezing during the CFC test for rats infused with MLA or vehicle. No differences were found between groups (n=8-10). g) Representative coronal rat brain photograph (left half) and scheme (right half) of the aimed cannula placement site, showing the methylene blue diffusion. CFC: contextual fear conditioning; CPA: conditioning place aversion; MLA: methyllycaconitine; mPFC: medial prefrontal cortex; VEH: vehicle.

Figure 2: Dopaminergic D1/D5Rs activation reversed MLA-induced blockade of cocaine CPP retrieval. a) Experimental scheme. Bars represent b) the time spent in the white (cocaine-associated) chamber during pretest, 24h and 48h test sessions or c) the CPP score, for rats conditioned with cocaine and infused with vehicle, MLA, SKF or MLA+SKF before the 24h CPP test. Two-way RM-ANOVA: *p<0.05; **p<0.01; ***p<0001 (n=9-11). MLA: methyllycaconitine; mPFC: medial prefrontal cortex; ns: non-significant; SKF: SKF-38393; VEH: vehicle.

Figure 3: The infusion of MLA in the mPFC did not induce any preference or aversion for the conditioning chambers. Bars represent a) the time spent in the black chamber during pretest and test sessions; b) the CPA score; c) the time spent in the white chamber during pretest and test sessions; or d) the CPP score, for rats conditioned with saline in both chambers and infused with vehicle or MLA before the test. See the text for details. No significant differences were found in any case (n= 6). MLA: methyllycaconitine; mPFC: medial prefrontal cortex; Sal: saline; VEH: vehicle.

Figure 4: Schematic representation of possible neuronal and molecular mechanisms underlying α7 nAChRs role in memory processing. a) One possible scenario for explaining the differential effect of the antagonism of alpha7 nAChRs in rewarding vs aversive memory retrieval is that these receptors may be differently modulating mPFC pyramidal neurons projecting to subcortical areas involved in behaviors promoting approach (e.g., the nucleus accumbens, NAc) *vs* avoidance (e.g., paraventricular nucleus of the thalamus, PVT). b) A possible molecular mechanism for explaining our results would be that α7 nAChRs presynaptically control DA release directly by acting on DA terminals or indirectly by acting on glutamatergic terminals promoting DA release. Ach: acetylcholine; DA: dopamine; Glu: glutamate; mPFC: medial prefrontal cortex; NAc: nucleus accumbens; nAChRs: nicotinic acetylcholine receptors; PVT: paraventricular nucleus of the thalamus.

HIGHLIGHTS

- mPFC α7 nicotinic receptors differentially affect appetitive and aversive memories
- MLA in the mPFC blocks cocaine-associated memory retrieval in a long-term manner
- MLA blocking effect is prevented by the activation of dopamine D1 receptors
- α7 nicotinic receptors in the mPFC are not involved in aversive memory retrieval

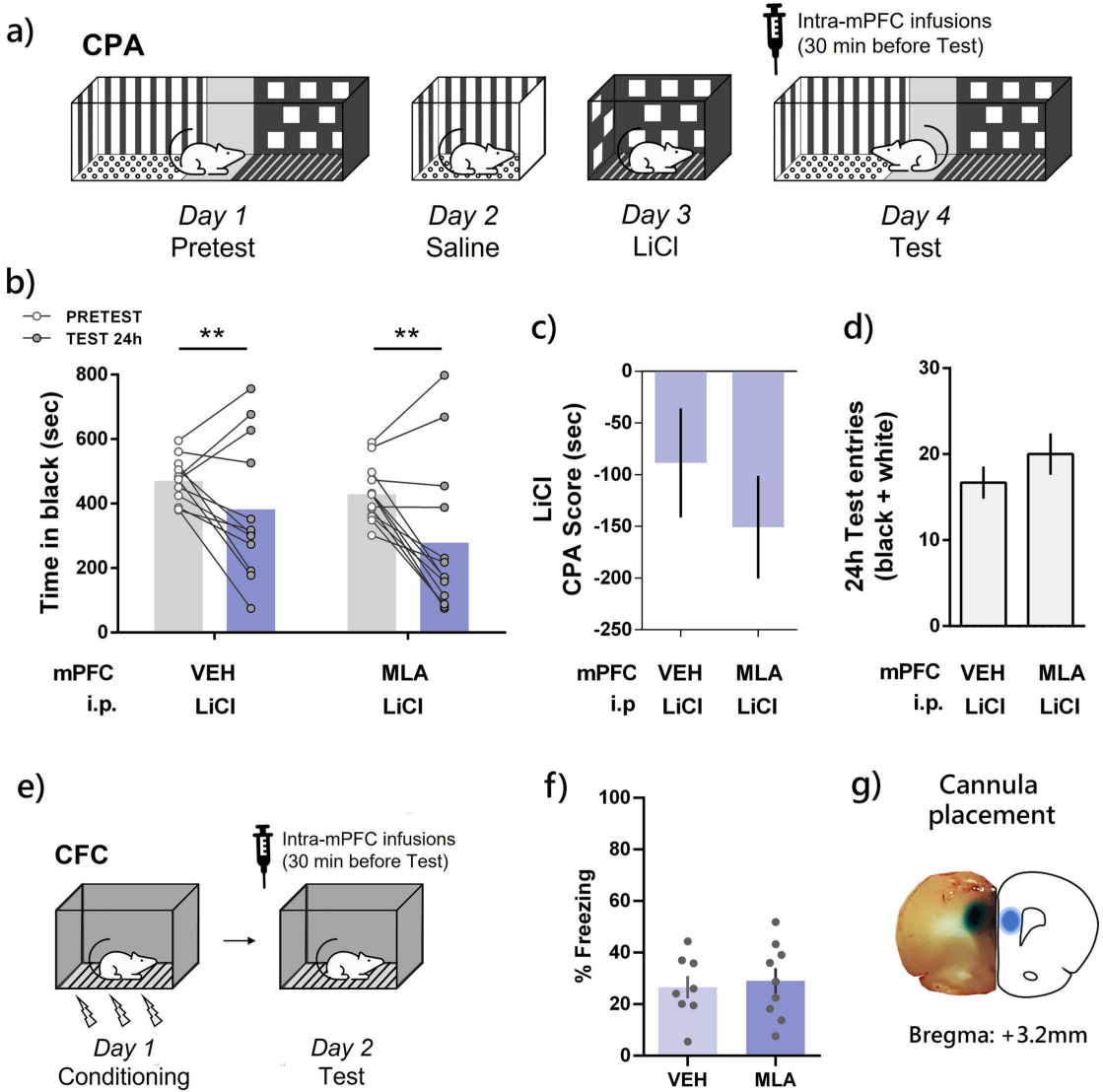
a)
CPA
Intra-mPFC infusions
(30 min before Test)
Day 1
Pretest
Day 2
Saline
Day 3
LiCl
Day 4
Test
b)
PRETEST
TEST 24h
**
**
Time in black (sec)
800
600
400
200
0
mPFC
i.p.
VEH
LiCl
MLA
LiCl
c)
LiCl
CPA Score (sec)
0
-50
-100
-150
-200
-250
mPFC
i.p
VEH
LiCl
MLA
LiCl
d)
24h Test entries
(black + white)
30
20
10
0
mPFC
i.p.
VEH
LiCl
MLA
LiCl
e)
CFC
Intra-mPFC infusions
(30 min before Test)
Day 1
Conditioning
Day 2
Test
f)
% Freezing
100
80
60
40
20
0
VEH
MLA
g)
Cannula
placement
Bregma: +3.2mm

Figure 1

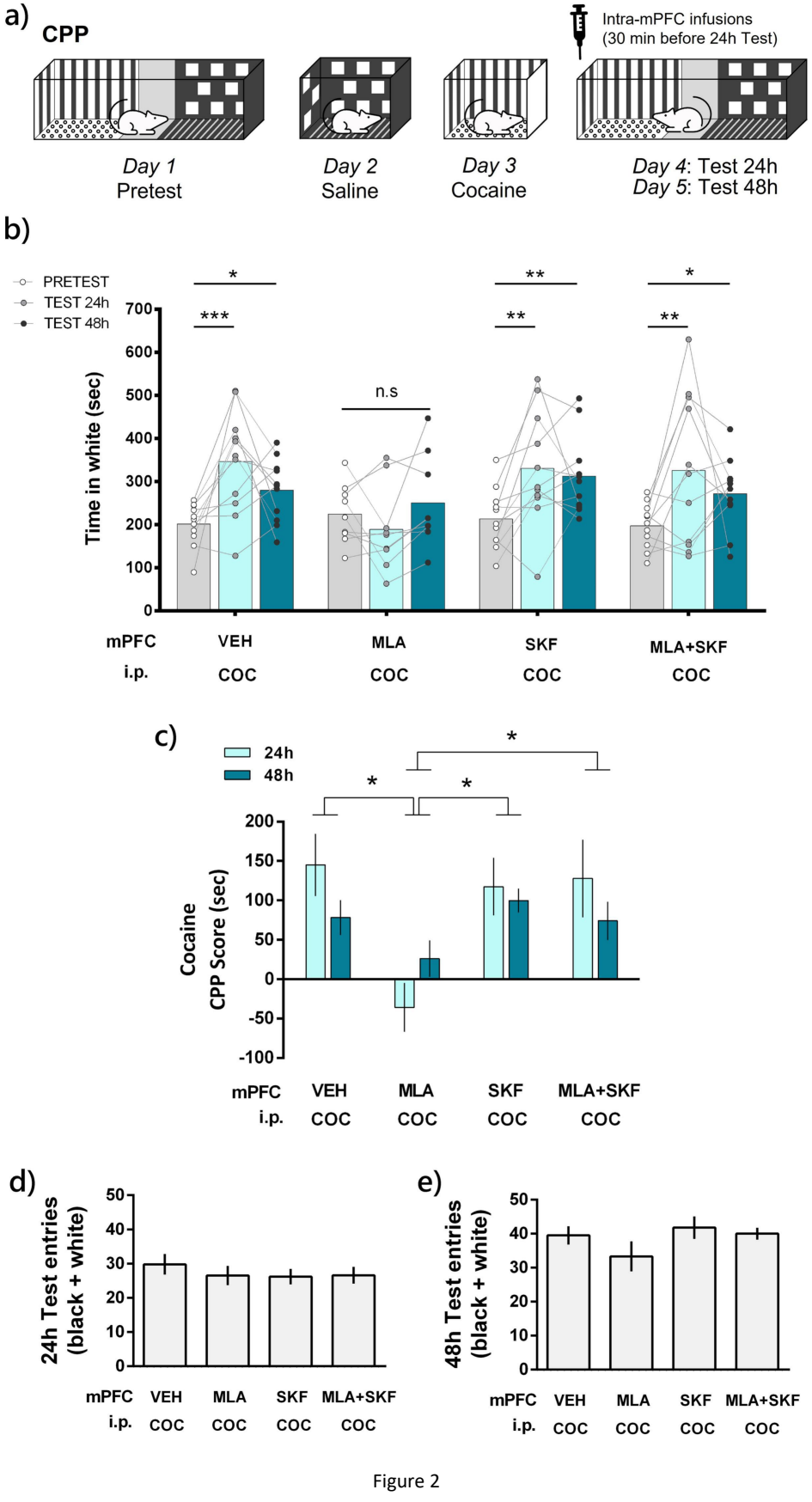
a)
CPP
Intra-mPFC infusions
(30 min before 24h Test)
Day 1
Pretest
Day 2
Saline
Day 3
Cocaine
Day 4: Test 24h
Day 5: Test 48h
b)
PRETEST
TEST 24h
TEST 48h
Time in white (sec)
n.s
mPFC
i.p.
VEH
MLA
SKF
MLA+SKF
COC
c)
24h
48h
Cocaine
CPP Score (sec)
d)
24h Test entries
(black + white)
e)
48h Test entries
(black + white)

Figure 2

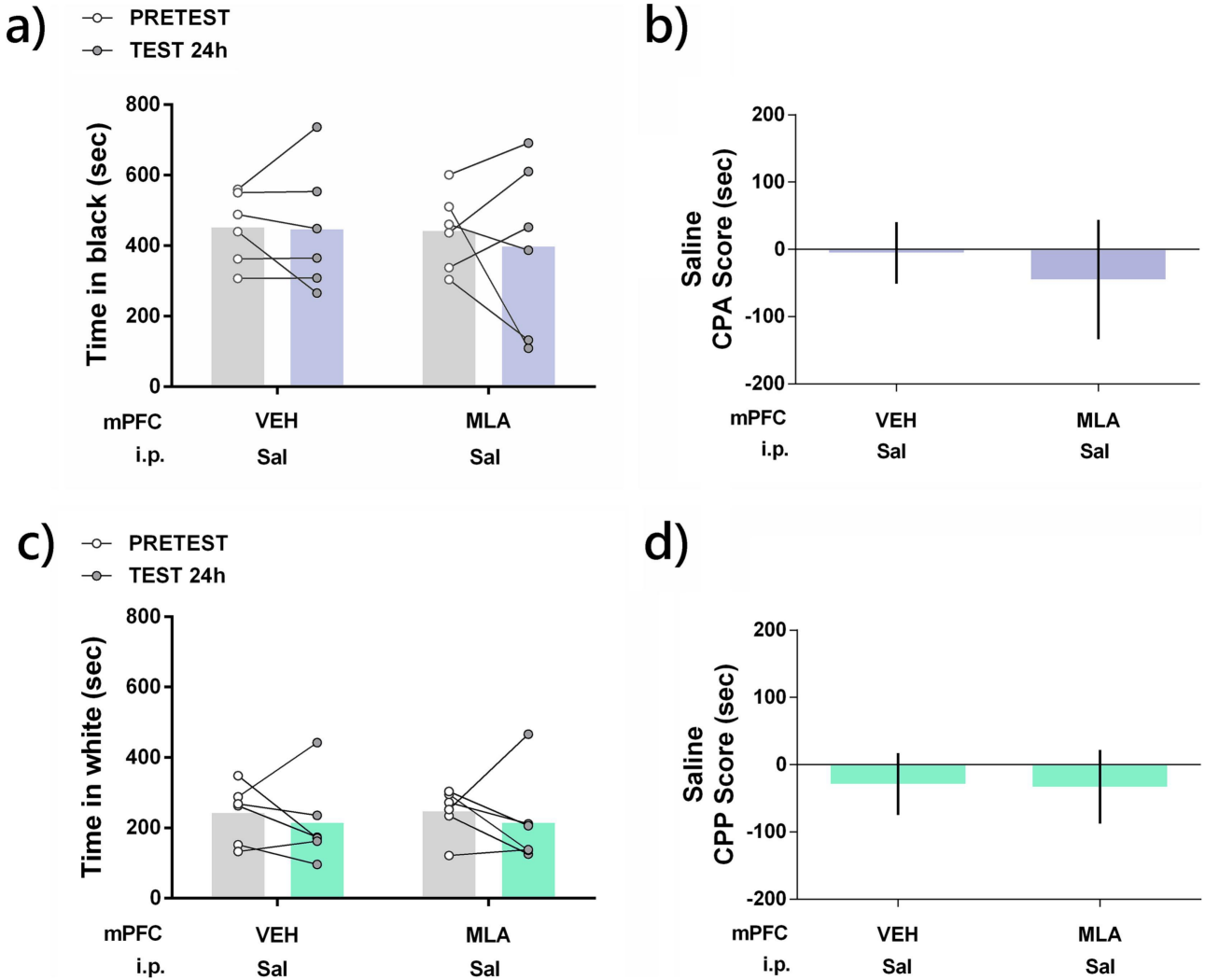
a)
PRETEST
TEST 24h
Time in black (sec)
800
600
400
200
0
mPFC
i.p.
VEH
Sal
MLA
Sal
b)
Saline
CPA Score (sec)
200
100
0
-100
-200
mPFC
i.p.
VEH
Sal
MLA
Sal
c)
PRETEST
TEST 24h
Time in white (sec)
800
600
400
200
0
mPFC
i.p.
VEH
Sal
MLA
Sal
d)
Saline
CPP Score (sec)
200
100
0
-100
-200
mPFC
i.p.
VEH
Sal
MLA
Sal

Figure 3

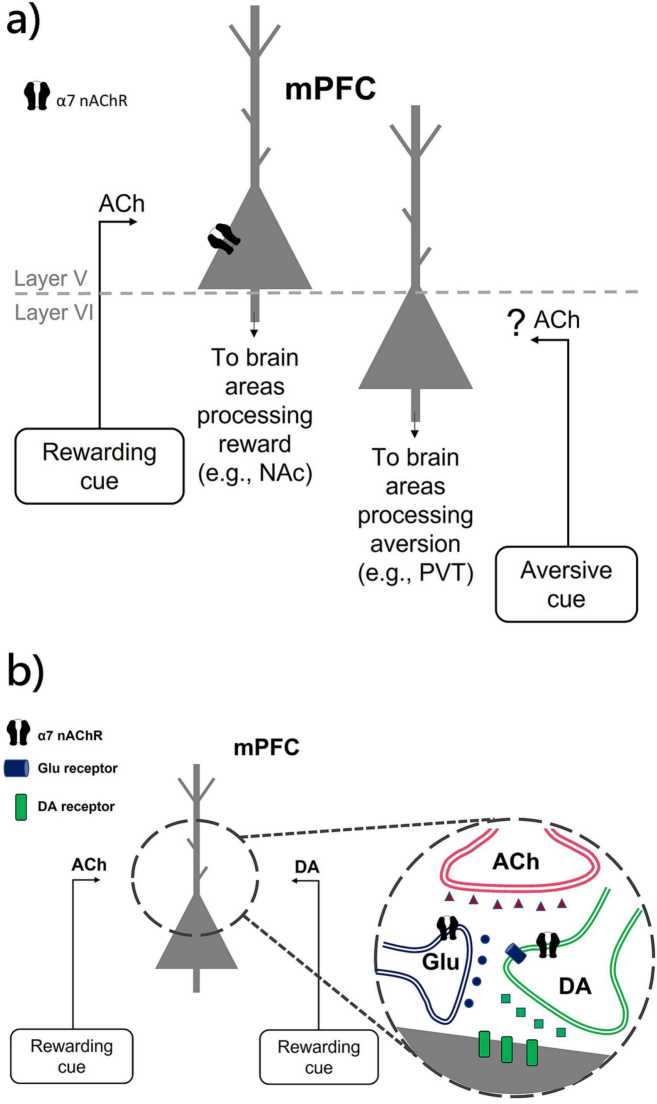
a)
α7 nAChR
mPFC
ACh
Layer V
Layer VI
? ACh
To brain areas processing reward (e.g., NAc)
To brain areas processing aversion (e.g., PVT)
Rewarding cue
Aversive cue
b)
α7 nAChR
Glu receptor
DA receptor
mPFC
ACh
DA
ACh
Glu
DA
Rewarding cue
Rewarding cue

Figure 4