Serotonin signaling in the rat prefrontal cortex is required for Retrieval-Induced Forgetting

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Abstract

Forgetting is a ubiquitous phenomenon that is actively promoted in many species. The act of remembering some experiences can cause the forgetting of others in both humans and rats. We previously found that when rats need to retrieve a memory to guide exploration, it reduces later retention of other competing memories encoded in that environment. As with humans, retrieval-induced forgetting (RIF) relies on prefrontal control processes, is competition-dependent, and is cue-independent. RIF is thought to be driven by inhibitory control signals from the prefrontal cortex that target areas where memories are stored. Serotonin plays a crucial role in behaviors requiring high cognitive demand, including memory processes, partly through its modulation of Prefrontal Cortex activity. However, its potential involvement in regulating forgetting remains unexplored. Here, we exposed rats to the RIF tssk and employed a pharmacological approach to manipulate the activity and signaling of serotonin receptors 5-HT1A, 5-HT2A, and 5-HT2C in the medial prefrontal cortex (mPFC) of rats, as well as to inhibit downstream effectors. Our findings reveal a distinct role for prefrontal serotonin signaling in RIF. While 5-HT2C receptor manipulation had no effect, blocking 5-H21A or 5-HT2A receptors

in the mPFC abolished RIF, demonstrating their necessity in this process. Strikingly, further analysis identified the PI3K/AKT pathway as a key downstream effector of 5-HT2A receptor activation, suggesting a specific molecular mechanism through which serotonin modulates inhibitory control over memory. These results uncover a previously unrecognized serotonergic modulation of adaptive forgetting, linking prefrontal serotonin signaling to the regulation of memory competition.

Key words: forgetting, memory, serotonin, 5-HT2A, prefrontal cortex, retrieval.

Introduction

In the face of changing circumstances, allowing obsolete information to be forgotten may be an adaptive strategy (Shuai et al., 2010; Epp et al., 2016; Benna and Fusi, 2016; Sangha et al., 2005). Historically, forgetting was conceptualized as a passive mechanism triggered by memory interference caused by changes in contextual cues needed to retrieve older memories or by losing the engrams underlying a particular memory. However, evidence showing the existence of active forgetting mechanisms in flies and rodents began to accumulate (for a review, see Medina 2017), some of which could account for findings from earlier human studies. For example, when people and rats recall a past event, competing memories that interfere with retrieval are more likely to be forgotten (Anderson et al., 1994; Bekinschtein et al., 2018). In humans and rats, this retrieval-induced forgetting (RIF) requires prefrontal engagement during the selective retrieval practice phase (Wu et al., 2014; Bekinschtein et al., 2018). As for humans, this effect is competition-dependent and cue-independent, meaning the forgotten memory cannot be retrieved using other cues (Bekinschtein et al., 2018).

RIF is a type of incidental forgetting, and although several mechanisms have been proposed to explain this phenomenon, most can be grouped under inhibition-based forgetting hypotheses (Murayama et al. 2014). According to this perspective, when trying to recall a particular memory, related memories are also reactivated. However, these associated memories are inhibited because they are irrelevant to the current situation. Moreover, repeated exposure to one of these memories may help prepare the organism for similar future situations. Thus, during the selective practice of memories, inhibition may primarily reduce future interference, optimizing the animal's behavioral adaptation.

Most research on RIF attributes the source of inhibitory control to the prefrontal cortex (PFC) (Kuhl et al. 2007; Staudigl, Hanslmayr, and Bäuml 2010; Ferreira et al. 2014; Hanslmayr et al. 2010; Penolazzi et al. 2014). According to the Prefrontal Control Hypothesis (Anderson and Hulbert 2021), active forgetting (e.g., RIF) originates from top-down control signals mediated by the PFC. In humans, the lateral PFC, particularly on the right side, plays a key role in

reducing the accessibility of disruptive memories through inhibitory mechanisms that overlap with those used to manage the targeted memory. However, despite advances in understanding the behavioral manifestations of RIF, the neurobiological mechanisms underlying this phenomenon, including the specific neuronal circuits and neurotransmitters involved, remain largely unexplored.

Serotonergic fibers originating from the medial and dorsal raphe nuclei innervate various subregions of the PFC (Molliver 1987; Hensler 2006) and serotonin receptors, particularly 5-HT1A, 5-HT2A, and 5-HT2C, playing distinct roles in modulating executive control in the PFC (Švob Štrac, Pivac, and Mück-Šeler 2016; Puig and Gulledge 2011; Leiser et al. 2015). These receptors have been implicated in regulating various cognitive processes that modulate behavior, including attention (Passetti, Dalley, and Robbins 2003), working memory, response inhibition (Keeler and Robbins 2011; Macoveanu et al. 2013; Hadamitzky and Koch 2009; Wischhof, Hollensteiner, and Koch 2011), and cognitive flexibility (Roberts 2011; Kehagia, Murray, and Robbins 2010; Boulougouris, Dalley, and Robbins 2007; Baker et al. 2011) through the concert action of multiple serotonin receptors. We have also demonstrated that the 5-HT2A receptor in the rat mPFC is critical for retrieving an object-in-context memory but not the identity of an object (Bekinschtein et al. 2013). Thus, serotonin signaling via the 5-HT2A receptor in the mPFC might play a role in resolving interference during retrieval on a model of episodic memory. In the context of RIF, it may specifically assist in forgetting competing memories. Given its evolutionarily conserved modulatory role and ability to influence opposing aspects of cognition and behavior, serotonin is a strong candidate to play a key role in RIF (Bacqué-Cazenave et al. 2020).

In this study, we sought to determine whether serotonin-mediated control processes in the mPFC contribute to the adaptive forgetting of competing memories. Our findings reveal that blocking 5-HT1A or 5-HT2A receptors in the rat mPFC completely disrupts RIF, while activating 5-HT2A receptors promotes it. In contrast, blocking 5-HT2C receptors does not affect RIF. Moreover, we report that at least one of the pathways through which the 5-HT2A receptor modulates RIF involves PI3K kinases. Forgetting plays a crucial role in memory function, with excessive and insufficient forgetting being linked to significant disruptions in mental health (Ryan and Frankland, 2022). Investigating how natural forgetting mechanisms are regulated and the neurological circuits underlying them can provide insights into psychiatric conditions where these processes are disrupted.

Materials and Methods

Ethics statement

All experimental procedures were performed according to institutional regulations and following those of the National Animal Care and Use Committee of the University of Buenos Aires and Favaloro University (CICUAL Presentation DCT0204-16 / PICT 2015-0110).
All efforts were made to minimize the number of animals used and their suffering. In all possible cases, once the experiment was completed, animals were referred to other research activities (e.g., setting a surgical protocol) or teaching tasks within the institution that took place in the immediate or short term. No person related to this work declares any type of conflict of interest.

Subjects

We used 68 male and 120 female Wistar rats from our breeding colony, weighing 200-300 grams at the beginning of each experiment. The animals were kept in groups of 4 to 5 individuals with access to food and water ad libitum under a 12-hour light/dark cycle (7 a.m.–7 p.m.) and a temperature between 20 and 23 °C. The number of animals in each experiment is stated below. All experiments were performed during the light phase of the cycle. In all cases, the animals were handled for two consecutive days before the start of the experiment.

Apparatus

Animals were exposed to 3 of 4 available arenas with different visual cues (figures or patterns) on their walls made with self-adhesive paper of different colors. Arena 1 (50 cm width, 50 cm length, 40 cm height) is square-shaped and made of acrylic; the floor and wall backgrounds are white. Arena 2 (60 cm width; 40 cm length; 40 cm height) is rectangular and constructed from acrylic; the floor and three walls' backgrounds are white, while one wall is transparent, allowing unobstructed viewing from one side. Arena 3 is an equilateral triangle made of foamboard, with each side measuring 53 cm, and it stands 53 cm tall. The floor is made of white acrylic, while the walls are primarily white, with one wall contrasting with black. Arena 4 (57 cm diameter; 42 cm height) is circular and made of flexible plastic; the floor is white acrylic, while the walls are burgundy.
The assignment of the contexts was randomized; no context was associated with a particular condition or treatment. During the animals' exposure to the context, the lighting of the behavioral room was dim (40 lux) and constant within the contexts (no shadows were generated that could guide the animal's preference for a particular region of the arena).

Objects

For all experiments, we used objects that differed in shape, texture, size (between 8 and 24 cm), and color and that did not have systematically aversive or attractive characteristics to the animals. We have duplicates of all objects to be presented in pairs. Objects varied between trials of the same experiment for a particular subject and between experiments. The position of the novel object was balanced between treatments, such that the novelty was not associated with one specific context position. Objects were attached to the context floor with a reusable odorless adhesive (Patafix, UHU) to prevent them from shifting during each session. After each exposure, the contexts' objects, floor, and walls were cleaned with 50% ethanol. For clarity, we refer to the objects in the description and diagram of the behavioral task using letters (e.g., object A, object B, etc.). These letters do not indicate that the same objects were used across treatments or between trials. Besides, we counterbalanced the objects between conditions so that, for example, object A for animal 1 in the RP group differed from object A for animal 2 within the RP group.

Retrieval-Induced Forgetting task

Overview

We used the task described by Bekinschtein et al. (Bekinschtein et al. 2018) that modified the spontaneous object recognition procedure to include three phases equivalent to the ones present in human studies of RIF (Anderson et al., 1994; Ciranni and Shimamura, 1999; Maxcey and Woodman, 2014; Wimber et al., 2015): encoding, practice, and test. Also, it involves three conditions: Retrieval Practice (RP), Interference Control (IC), and Time Control (TC). Then, the task consists of three conditions, each composed of three phases (Fig. 1A). The RP condition is designed to reveal the forgetting of an object memory induced by the successive retrieval of a related object memory. The IC condition allows for assessing the effect of repeated presentation of objects on the recall of the studied objects. This condition helps rule out the possibility that the mere presentation of objects could cause the forgetting of elements from the previous phase. On the other hand, the TC condition evaluates the possible effect that the time elapsed from learning to evaluation could have on the retention of the memory of the objects.

For each experiment, different cohorts of animals were used. After the shaping phase, animals were randomly assigned to one of the three conditions (see below). The treatment assignment across the two weeks of the experiment was pseudorandomly assigned. All the experiments described followed this within-subject design for the condition.

The general retrieval practice paradigm

Shapping: It was done only once to habituate the animals to subsequent experimental practices of manipulation and exposure to contexts and objects. They were previously exposed to a context (10 minutes) and then to a pair of objects in that same context (3 minutes). Neither the context nor the objects were later used during the actual task, which began 24 to 48 hours later.

Day 1 - Context Habituation: The animals were allowed to freely explore the context where the relevant objects would be presented for 10 minutes.

Day 2:

Encoding: This phase consists of two consecutive 5-minute sessions separated by 25 minutes. Twenty-four hours after the habituation, animals were reintroduced to the now familiar context and exposed to two identical copies of an object (e.g., object A) and, in a second session, to two copies of another object (e.g., object B), which they could freely explore. These would eventually be defined as the practiced object (RP+) and the competitor object (RP-). The order of object presentation was balanced across treatments.

Practice: 60 minutes after the last acquisition session, the animals were exposed to 3 sessions of 3 minutes each (separated by 15 minutes) in which objects were presented depending on the animal's experimental condition:

RP: We pseudorandomly assigned one of the objects presented in the sample phase as the retrieval practiced object (RP+). Each session presented a novel object (different in each session, X/Y/Z). The location (right or left) where the studied object appeared during retrieval practice was randomly assigned for each trial. IC: A pair of identical, completely novel objects (differing between sessions as XX/YY/ZZ) was presented in each session.

TC: Animals remained in their usual cages the entire time.

Day 3 - Test: Twenty-four hours after the training and practice sessions, we assessed the memories of the two originally studied objects in the RP condition animals. First, we evaluated the memory for the competitor object by exposing the animals to a copy of the RP- object alongside a completely novel object (object C). Twenty minutes later, we reintroduced the animals to the context with the practiced object, introducing a copy of the RP+ object and another novel object (object D). We consistently tested memory for the RP- object before the RP+ object to minimize any interference effects that retrieving a stronger memory might have on the attempt to recover a weaker one. For the control conditions (IC and TC), the object whose memory was assessed was randomized across animals. In all cases, the location of the familiar object was counterbalanced among the animals.

The modified retrieval practice paradigm

To evidence the role of the 5-HT2A receptor during the practice phase, modulating its activity bidirectionally, we used a modified version of the protocol that does not allow the expression of RIF (Gallo et al. 2022). Briefly, we increased the number of sessions during the encoding phase, spaced the practice from the encoding phase for 24h, and retained all the other sessions as previously described (Fig. 2A).

Quantification of behavior

Behavior was recorded during the encoding, practice, and test phases using cameras placed over the arenas. Exploration times for each object were then quantified using digital stopwatches (Stopwatch, Center for Behavioral Neuroscience, Emory University, Atlanta, GA). An animal was considered to be exploring an object if it directed its nose toward the object at a distance of 1 cm or less and/or touched the object with its nose. Walking on the object, sitting on it, or using it for support was not considered part of exploration (Ennaceur and Meliani 1992). Quantification was performed blindly, and a subset of the videos from the test phase was randomly selected to be independently re-quantified in duplicate.

For each phase of the experiment, we measured the total exploratory time for each object and calculated the following indexes as a proxy of preference or discrimination as applicable. *Encoding:* Total exploring time (TT) of Object A and Object B copies; Preference Index: $TT_{Object\ A} - TT_{Object\ B} / (TT_{Object\ A} + TT_{Object\ B})$. *Practice:* TT of Object A and Objects X/Y/Z along the sessions; Discrimination Index (DI): $TT_{Object\ X/Y/Z} - TT_{Object\ A} / (TT_{Object\ X/Y/Z} + TT_{Object\ A})$. *Test:* TT of the familiar and novel object. DI: $TT_{Novel} - TT_{Familiar} / (TT_{Novel} + TT_{Familiar})$. Positive values of the Discrimination Index indicate a bias toward exploring the novel more than the familiar object. Levels close to zero indicate no preference between the novel and the familiar object.

Surgery

Guide cannulas (22G) were implanted in the medial Prefrontal Cortex (mPFC) of both hemispheres, following the coordinates measured relative to the bregma of the skull (anteroposterior +3.20 mm; lateral ±0.75 mm; dorsoventral −3.50 mm; Paxinos and Watson, 1998).

After being handled for at least two consecutive days, the rats were habituated to the operating room for at least one hour on the day of surgery. Pharmacological anesthesia was administered intraperitoneally (ketamine Dr. Gray SACI, 80 mg/kg, and xylazine Richmond, 8

mg/kg), and analgesic treatment was administered subcutaneously (Meloxicam, 0.2 mg/kg). The effect of anesthesia was confirmed by evaluating the paw reflex to a pinch. The animal was then placed in a stereotaxic frame. Within a time window of 35 to 45 minutes, the necessary craniotomies and cannula implantations were performed. The cannulas were secured to the skull with dental acrylic, and to prevent occlusion until the day of infusion, "flags" made from needles with a smaller diameter than the inside of the guide cannula were placed; they were removed on the day of drug infusion. At the end of the surgery, animals were injected with a single dose of antibiotic (gentamicin, 0.6 mg/kg) and given a nutritional supplement to prevent weight loss. Handling of the animals resumed at least five days after surgery, after evaluating the absence of signs of distress (e.g., lack of postural reflex or rapid weight loss).

Cannulae placement

Once the experiments were completed, post-mortem histological controls of the cannula placement were performed. A 1 µl solution of 4% methylene blue in saline was infused through each cannula, and 10-15 minutes later, the animals were sacrificed in CO2 chambers following the institutional protocols. The brains were sectioned, and the cannula locations were verified through examination.

Drug infusion

Handling was done gently to minimize stress. At the time of drug administration, the infusion cannula, with a 0.3 mm (30G) gauge, was inserted into the guide cannula. The infusion cannula is 1 mm longer than the guide cannula, so it protrudes when inserted. The entire procedure takes between 3 and 5 minutes. After each infusion, the infusion cannula was kept inside the guide cannula for one minute to allow for drug diffusion and prevent reflux.
1µL per hemisphere was infused 10 to 15 minutes before the practice phase of the following solutions or their respective vehicles, following the doses used in previous studies: 8-OH-DPAT (Sigma; 300 ng/µL in saline); MDL11,939 (Tocris Bioscience; 300 ng/µL DMSO 5%); SB242084 (Tocris Bioscience; 300 ng/µ in saline); LY294002 (Tocris Bioscience; NUEVA DOSIS in saline); PP2 (Tocris Bioscience; 33,3 ng/µL in DMSO 5%).

Experimental design and statistical analysis

Mixed experimental designs were used for all the experiments involving pharmacological manipulations. The animals were randomly assigned to one of the three experimental conditions (RP, IC, or TC) and, in different weeks, received the possible pharmacological treatments (e.g., drug and vehicle). The sample size used was based on results from previous

studies in the laboratory, assuming that if a treatment effect were found, it would be of a similar magnitude (Bekinschtein et al. 2018; Gallo et al. 2022).

Multiple linear regression models were proposed, detailed in each case, to determine the statistical significance of fixed effects given by the treatment, taking into account possible interactions between factors. Repeated measures were incorporated as a random factor in the models in all cases. Post-hoc contrasts (Bonferroni) were performed, adjusting the p-value for multiple comparisons.

To ensure that an initial preference for one of the objects during the encoding phase did not influence the Discrimination Index in the test phase, we analyzed the correlation between initial exploration preference and the test Discrimination Index across all experiments and control conditions. In no case was the correlation coefficient statistically significant for any group. We verified the assumptions of normality and homoscedasticity of the residuals in all the main experiments using the Shapiro-Wilk and Levene tests, respectively.

A significance level of 0.05 was established in all cases. Any difference was considered significant if its p-value was less than this threshold. All data processing and statistical analysis were conducted using the open-access software RStudio 2023.06.1.

Subjects exclusion criteria

Behavioral: Exclusion criteria were established based on the animals' exploratory behavior over the different phases of the protocol. Animals would be excluded if they do not meet the following requirements. Encoding: the total exploration time for each pair of objects must be greater than 10s/encoding session. Practice (RP): The total exploration time for the familiar and the novel objects across the practice sessions must be higher than 10s. Practice (IC): The exploration time must exceed 5 seconds in each practice session. Test: The total exploration time must be higher than 10 seconds.

Statistical: Extreme values (+/- 3 standard deviations from the mean) were identified for all calculated variables and excluded from the analysis to prevent these values from significantly impacting the statistical analysis.

Other: Subjects who showed signs of distress (e.g., due to the cannula implantation surgery) were excluded and sacrificed immediately. Animals were also excluded if they learned to escape from the context or had incorrect cannula placement.

In total, 21 subjects were excluded from the analyses, representing 10% of the initial subjects.

Results

Differential involvement of serotonin receptors in the mPFC: 5-HT1AR and 5-HT2AR but not 5-HT2CR activity are essential for RIF

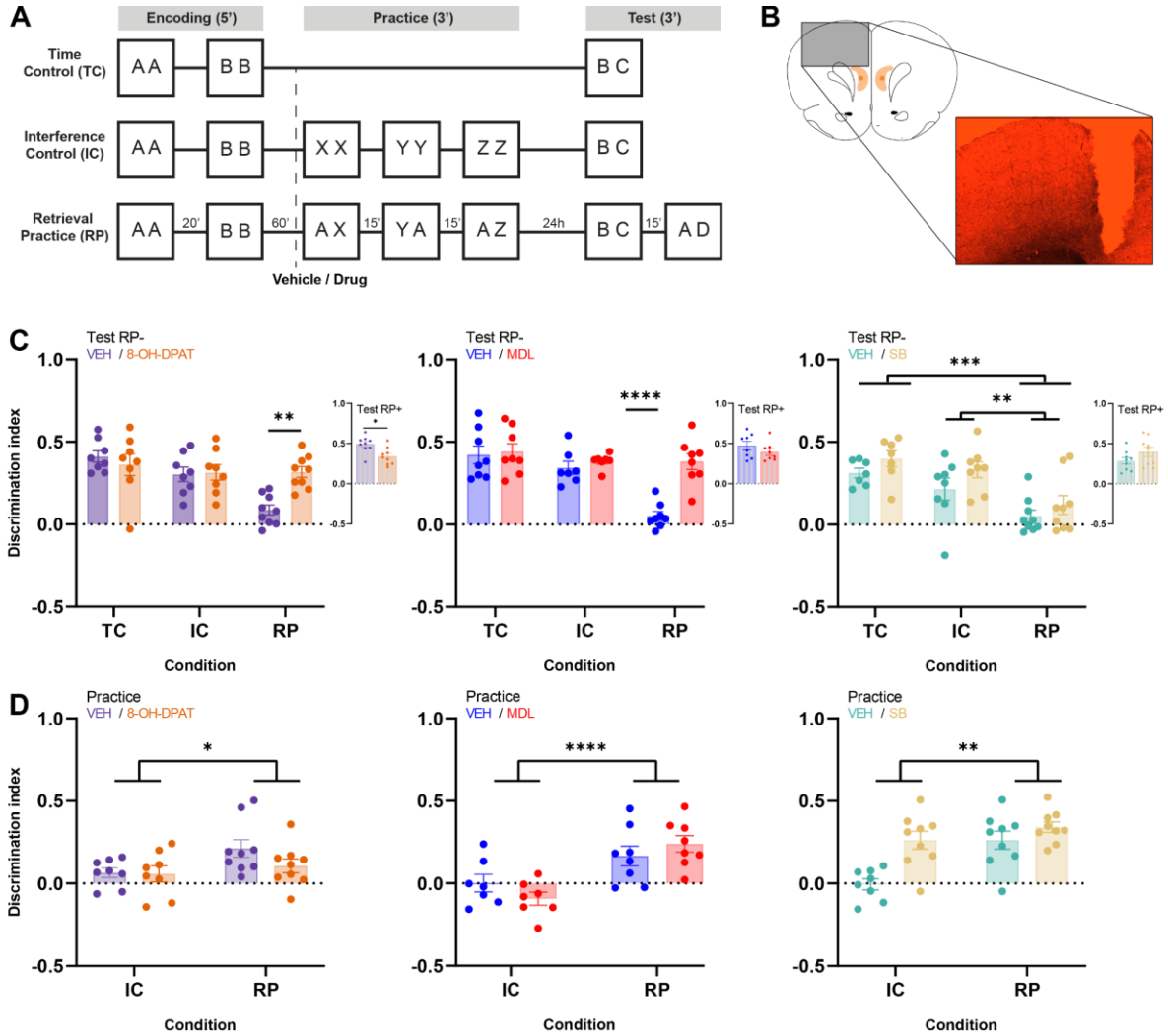

Figure 1. 5-HT1AR and 5-HT2AR but not 5-HT2CR activity are essential for RIF. A. *Schematic representation of the behavioral protocol. After the acquisition, animals were divided into RP, IC, and TC conditions. For all the experiments described, animals performed the task twice, once with a drug infusion and once with a vehicle infusion, pseudorandomly assigned. The experimental design is between subjects by condition and within subjects by pharmacological treatment. The dashed line indicates the infusion of the specific drug or its vehicle in the mPFC 15 min before the practice phase.* ***B.*** *Schematic representation of the infusion target area within the mPFC, alongside a histological section showing the cannula trace. Note that the actual infusion site lies deeper than the cannula tip due to the infusion needle extending 1 mm beyond the guide cannula.* ***C.*** *Discrimination index ± SEM for the testing phase of three different experiments. Left: 5-HT1AR agonist (8-OH-DPAT; n = 25); center: 5-HT2AR antagonist (MDL11,939; n = 23); right: 5-HT2CR antagonist (SB242084; n = 25). Asterisks show significant differences between treatments (Bonferroni post hoc comparisons). 8-OH-DPAT and MDL impaired, in the RP condition, the specific forgetting of the competitor object. The interaction between condition and drug on the Discrimination Index was insignificant in the*

SB experiment. Here, the asterisks show the main effect of the condition. Inset: Discrimination index ± SEM for the practiced *object.* ***D.*** *Discrimination index ± SEM during the Practice phase for the IC and RP groups across the three pharmacological experiments. Asterisks show the main effect of the condition on exploratory behavior.*p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.*

To investigate the specific role of serotonin modulation in the mPFC during retrieval-induced forgetting, we assessed the involvement of various serotonin receptors in rats performing the RIF task using selective antagonists. Serotonin 1A (5-HT1AR), serotonin 2A (5-HT2AR), and serotonin 2C (5-HT2CR) are among the most abundant serotonin receptors expressed in the rat mPFC (Pazos, Cortés, and Palacios 1985; Pazos and Palacios 1985; Pompeiano, Palacios, and Mengod 1992; Leiser et al. 2015; Pompeiano, Palacios, and Mengod 1994). These receptors play distinct roles in various cognitive tasks that involve mPFC function, underscoring the serotonergic system's complex regulation of this structure (Zhang and Stackman 2015; Bacqué-Cazenave et al. 2020).

Our initial analysis focused on the role of the Gi-coupled 5-HT1AR receptor in RIF. Following the encoding phase, rats were randomly assigned to the RP, IC, or TC conditions (Fig. 1A). Within each condition, animals were infused bilaterally in the mPFC with a selective 5-HT1AR agonist (8-OH-DPAT) or vehicle (VEH) before the practice phase or at a corresponding time in the TC group. As previously shown (Bekinschtein et al. 2018; Gallo et al. 2022), the selective practice of one object (A, RP+) prevented the expression of memory of a competing object (B, RP-) in the final test phase. In this case, we predicted that this process is inversely related to mPFC 5-HT1AR activity during the practice. We measured the exploration time in the test phase and calculated the DI, our dependent variable. We fitted a linear mixed model to analyze how the Condition and Drug influenced the Discrimination Index (DI ~ Condition x Drug). The Subject was treated as a random effect in the model. We found a statistically significant interaction between $\text{Condition}_{RP} \times \text{Drug}_{8\text{-OH-DPAT}}$ ($\beta = 0.28$, $_{95\%}$CI [0.10, 0.46], $t_{(22)} = 3.26$, $p < .01$). RIF was identified by a lower DI in the RP_{VEH} condition than in the IC_{VEH} and TC_{VEH} conditions ($[TC\text{-}RP]_{VEH}$: mean = 0,324, SE = 0.061, df = 22, t-ratio = 5.359, $p < .001$; $[IC\text{-}RP]_{VEH}$: mean = 0,215, SE = 0.061, df = 22, t-ratio = 3.571, $p < .05$). Notably, post hoc comparisons within each condition revealed no differences between the pharmacological treatments in the IC condition ($\text{mean}_{VEH\text{ - }8\text{-OH-DPAT}} = -0.012$, SE = 0.062, df = 22, t-ratio = -0,195, p = 1) and the TC condition ($\text{mean}_{VEH\text{ - }8\text{-OH-DPAT}} = 0.049$, SE = 0.062, df = 22, t-ratio = 0.791, p = 1), but showed significant differences in the RP condition ($\text{mean}_{VEH\text{ - }8\text{-OH-DPAT}} = -0.230$, SE = 0.059, df = 22, t-ratio = -3.921, $p < .01$) suggesting that activating mPFC 5-HT1AR can interfere with the ability of the cortex to induce forgetting of competing objects (Fig. 1B, *left*).

We also evaluated the memory of the RP+ object to delineate the specificity of the previously observed forgetting, which should be specific to the competitor object. Despite the negative effect of the pharmacological treatment on RP+ object memory expression ($\beta = -0.15$, $_{95\%}$CI [-0.26, -0.04], $t_{(8)} = -3.05$, $p = 0.016$), both conditions retained memory of the practiced objects (Fig. 1B, *left, inset*) as manifested by positive DIs (one-sample t-test; VEH: $t = 14.276$, $df = 8$, $p < .0001$; 8-OH-DPAT: $t = 9.59$, $df = 8$, $p < .0001$).

We evaluated the exploratory behavior during the practice phase to exclude potential pre-existing differences that might account for the observed effects in the test phase (Fig. 1D, *left*). We found a significant positive main effect of the Condition_{RP} ($\beta = 0.15$, $_{95\%}$CI [0.01, 0.28], $t_{(15)} = 2.32$, $p < .05$) on the DI across the three practice sessions, which was expected because of the protocol design, but no effect of the drug. Infusion of 8-OH-DPAT did not affect the total exploratory time during the practice phase, neither in the RP nor in the IC groups, indicating that it did not affect locomotor affective, motivational functions, or the ability to recognize familiar objects. Unless otherwise stated, assume that neither the condition, the pharmacological treatment, nor their interaction affected the total exploration time during the analyzed phase.

We then analyzed the serotonin receptor family 2, composed of three excitatory receptors. Notably, within this family, the 5-HT2A and 5-HT2C receptors are highly expressed in the cortex (Pazos, Cortés, and Palacios 1985; Pazos and Palacios 1985; Pompeiano, Palacios, and Mengod 1992, 1994; Leiser et al. 2015).

Subsequently, we asked if mPFC 5-HT2AR might participate in the mechanisms of RIF. In this case, animals were locally infused with VEH or MDL11939 (MDL), a selective 5-HT2AR antagonist, before the practice phase (Fig. 1A). Twenty-four hours after the encoding, we tested the memory of the competitor object (B, RP-). RP_{VEH} animals showed comparable exploration of the familiar and novel objects, a distinct pattern from that observed in the IC and TC groups ($[TC\text{-}RP]_{VEH}$: mean = 0.370, SE = 0.058, df = 20, t-ratio = 6.346, $p < .0001$; $[IC\text{-}RP]_{VEH}$: mean = 0.291, SE = 0.060, df = 20, t-ratio = 4.826, $p < .001$). As anticipated, RP_{VEH} animals exhibited clear evidence of RIF, consistent with the expected behavioral pattern. The 5-HT2AR antagonist treatment showed varying effects depending on the condition ($\text{Condition}_{RP} \times \text{Drug}_{MDL}$, $\beta = 0.31$, $_{95\%}$CI [0.21, 0.41], $t_{(20)} = 6.77$, $p < .001$). Only in the RP group, MDL-infused animals displayed a significantly higher DI than the VEH-treated animals ($RP_{VEH\text{-}MDL}$: mean = -0.329, SE = 0.032, df = 20, t-ratio = -10.165, $p < .0001$; $IC_{VEH\text{-}MDL}$: mean = -0.041, SE = 0.035, df = 20, t-ratio = -1.174, $p > .05$; $TC_{VEH\text{-}MDL}$: mean = -0.019, SE = 0.032, df = 20, t-ratio = -0.590, $p = 1$), suggesting that blockade of mPFC 5-HT2AR interferes with the mechanism underlying RIF (Fig. 1C, *center*). No differences were observed between treatments for the memory of the RP+ object (Fig. 1C, *center, inset*).

Regardless of the pharmacological intervention, in the practice phase, animals in the RP condition showed higher DI than the IC control group ($Condition_{RP}$, β = 0.16, $_{95\%}$CI [4.07e-03, 0.32], $t_{(13)}$ = 2.22, p = 0.045), indicating that they preferred the novel object compared with the practiced one (Fig. 1D, *center)*. In this phase, we also found a simple effect of the condition on the Total Exploring Time (β = 16.43, 95% CI [0.04, 32.82], t(13) = 2.17, p = 0.050), but it cannot fully account for the test effect, as it was independent of the infused drug.

In this case, to study the role of 5-HT2CR in RIF, animals were infused bilaterally with VEH or SB242084 (SB), a selective 5-HT2C antagonist, before the practice sessions (Fig. 1A).
In the test phase, we found that, independently of the pharmacological treatment, animals in the RP group showed lower DI than the IC and TC groups, highlighting RIF ($Condition_{RP}$: β = -0.26, $_{95\%}$CI [-0.41, -0.11], $t_{(21)}$ = -3.65, p = 0.001; $mean_{TC\text{-}RP}$ = 0,270, SE = 0.054, df = 21, t-ratio = 5.040, p < .001; $mean_{IC\text{-}RP}$ = 0,188, SE = 0.052, df = 21, t-ratio = 3.637, p < .01). No significant differences were observed when the memory of the RP+ object was tested between vehicle and drug-infused animals.
SB infusion did not affect the animals' ability to discriminate between a novel and a familiar object during the practice sessions, as presented for the RP condition ($Condition_{RP}$, β = 0.27, 95% CI [0.11, 0.42], t(15) = 3.73, p = 0.002), nor the exploration time of entirely novel objects, like the ones presented for the IC group.

Infusion of a selective 5-HT2A receptor agonist elicits forgetting of a competing memory

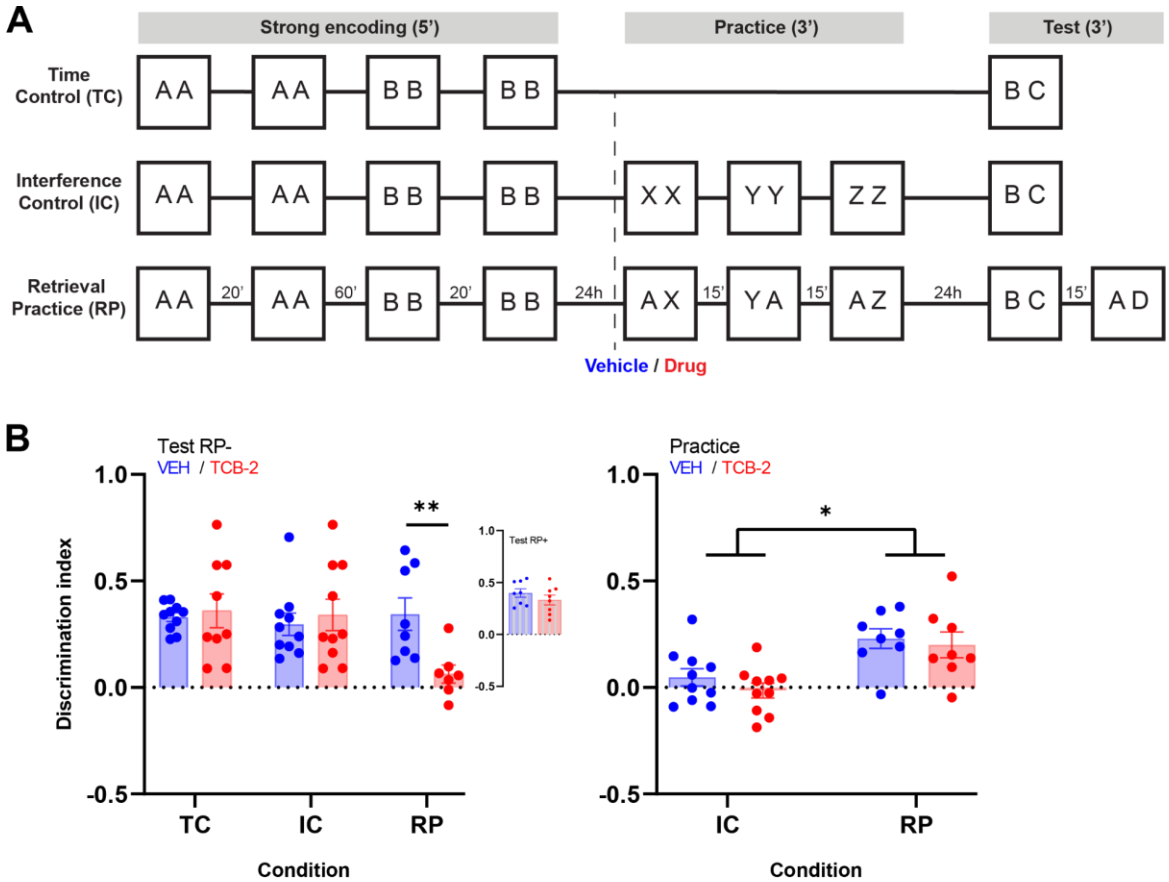

Figure 2. 5-HT2AR agonist in the mPFC infused before the practice phase promotes RIF. A. *Schematic representation of the behavioral protocol. In contrast to previous experiments, this protocol doubled the number of encoding sessions and separated this phase from the practice one for 24 hours. The dashed line indicates the infusion of the specific drug or its vehicle in the mPFC 15 min before the practice phase.* ***B.*** *Left: Discrimination index ± SEM for the testing phase. 5-HT2AR agonist (TCB-2; n = 28) promotes RIF in a behavioral protocol designed to resemble the main features of a general retrieval practice paradigm but without manifest forgetting; asterisks show significant differences between treatments (Bonferroni post hoc comparisons). Inset: Discrimination index ± SEM for the practiced object. Right: Discrimination index ± SEM during the Practice phase for the IC and RP groups; asterisk shows the main effect of condition on exploratory behavior.*

**p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.*

Previous studies have established the critical role of the 5-HT2AR in the mPFC in resolving memory interference (Bekinschtein et al. 2013). We now provide evidence for this role in RIF, where memory interference must also be managed through inhibitory control mechanisms. Thus, it is compelling and necessary to investigate the underlying signaling pathway. Moreover, we examine the effects of positively modulating its activity using an agonist to ensure bidirectional manipulations.

We focused the following experiments on the 5-HT2AR because of its important role in executive function and memory retrieval. Interestingly, we have previously found that blockade of mPF 5-HT2AR affects object retrieval when presented with potential interferent information, suggesting it might also be a key player in RIF.

Since blocking mPFC 5-HT2AR impairs the forgetting of the competitor object, we asked if activation of 5-HT2AR might be necessary to trigger RIF. To do this, we employed a version of the task that does not elicit RIF. An extra training session was added for each studied object, and the practice phase was separated from the encoding for 24h (Fig. 2A), preventing RP-objects from undergoing RIF (Gallo et al. 2022). Under these conditions, we assessed whether pharmacological activation of the 5-HT2AR with the selective agonist TCB-2 could facilitate RIF.

If signaling derived from 5-HT2AR activation promotes forgetting of a competing memory, we expect only $RP_{TCB\text{-}2}$ animals to explore the unpracticed object (B, RP-) similarly to an utterly novel object in the test phase. In contrast, the rest of the subjects would preferentially explore the novel object. As predicted, the $RP_{TCB\text{-}2}$ animals indistinguishably explored both objects presented in the test phase. They differed from what we observed for the $IC_{TCB\text{-}2}$ and $TC_{TCB\text{-}2}$ groups ($[TC\text{-}RP]_{TCB\text{-}2}$: mean = 0.291, SE = 0.079, df = 25, t-ratio = 3.683, $p < .01$; $[IC\text{-}RP]_{TCB\text{-}2}$: mean = 0.282, SE = 0.079, df = 25, t-ratio = 3.572, $p < .01$). Since the $Condition_{RP} \times Drug_{TCB\text{-}2}$ interaction was found significant ($\beta = -0.31$, $_{95\%}CI$ [-0.53, -0.08], $t_{(25)} = -2.80$, $p = .01$), we analyzed the differential effect of the infused agonist within each condition (Fig. 2B, *left*). Interestingly, only in the RP group, TCB-2-infused animals showed lower DI compared with the VEH-treated animals ($RP_{VEH\text{-}TCB\text{-}2}$: mean = 0.286, SE = 0.081, df = 25, t-ratio = 3.520, $p < .01$; $IC_{VEH\text{-}TCB\text{-}2}$: mean = -0.043, SE = 0.073, df = 25, t-ratio = -0.596, $p = 1$; $TC_{VEH\text{-}TCB\text{-}2}$: mean = -0.019, SE = 0.073, df = 25, t-ratio = -0.258, $p = 1$). No differences between treatments were observed in the performance of the practiced object (A, RP+) test phase (Fig. 2B, *left, inset*). As in the previous experiments, we did not observe that infusion of a selective 5-HT2A agonist before the retrieval practice phase modified the performance of the animals during this phase since we only detected a statistically significant effect of the Condition ($\beta = 0.18$, $_{95\%}CI$ [0.05, 0.32], $t_{(16)} = 2.83$, $p < .05$), indicating that infusion of TCB-2 does not affect attention, exploratory drive, or motivation (Fig. 2B, *right*).

PI3K mediates the 5-HT2AR effect on RIF in the CPFm during Retrieval Practice

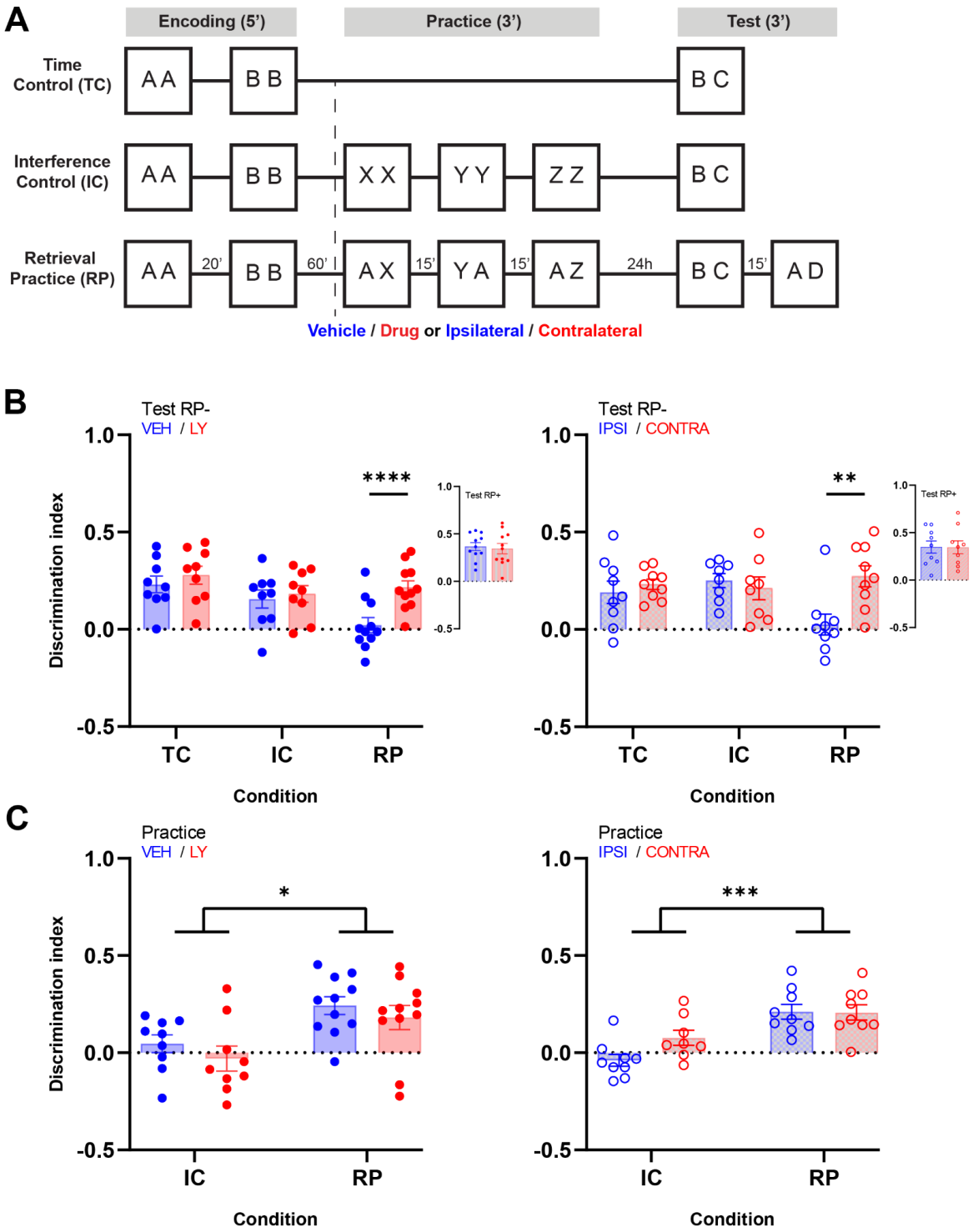

Figure 3. PI3K is involved in 5-HT2A signaling during Retrieval Practice. A. *Schematic representation of the behavioral protocol. In two separate experiments, we investigate the role of PI3K in RIF. First, we reproduce the approach used in previous experiments and compare the effect of a PI3K inhibitor between conditions. Then, we disrupt the functional connection between PI3K activity and 5-HT2AR, infusing the PI3K inhibitor with the 5-HT2AR antagonist simultaneously in the same (ipsilateral) or different (contralateral) hemispheres. The dashed line indicates the infusion in the mPFC 15 min before the practice phase.* ***B.*** *Discrimination index ± SEM for the testing phase. Left: PI3K inhibitor (LY 294002; n = 30); right: PI3K inhibitor + 5-HT2AR antagonist (LY 294002 + MDL11,939,*

n = 28). Asterisks show significant differences between treatments (Bonferroni post hoc comparisons). Bilateral infusion of LY alone into the mPFC or in a contralateral configuration with MDL blocked RIF. Inset: Discrimination index ± SEM for the *practiced object.* ***C.*** *Discrimination index ± SEM during the Practice phase for the IC and RP groups across the two pharmacological experiments. Asterisks show the main effect of the condition on exploratory behavior. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.*

The PI3K/AKT pathway, though not the primary signaling mechanism of 5-HT2AR, plays a pivotal role in neuronal homeostasis, growth, and plasticity. Its association with long-term memory processes (Horwood et al., 2006) highlights its potential relevance in RIF. Subsequently, we decided to analyze if the PI3K pathway is involved in the control of RIF. We trained a group of animals in the RIF task as previously described. Using the same experimental design as used for 5-HT2AR blockade, animals were randomly assigned to the RP, IC, or TC conditions, and in different weeks, the PI3K inhibitor LY 2940021 (LY) was infused into the mPFC before the practice phase (Fig. 3A). If PI3K is recruited during selective practice to facilitate the forgetting of competing memories, we predicted that RP_{LY} animals would show no signs of RIF. The results support our hypothesis (Fig. 3B, *left*): RP_{LY} animals showed a preference for the novel object compared with the RP- object ($Condition_{RP} \times Drug_{LY}$: $\beta = 0.13$, $_{95\%}CI$ [0.01, 0.25], $t_{(25)} = 2.18$, $p < .05$; $RP_{VEH\text{-}LY}$: mean = -0.193, SE = 0.039, df = 25, t-ratio = -4.907, $p < .001$. No effect of LY was observed in the memory performance of control conditions; $IC_{VEH\text{-}LY}$: mean = -0.026, SE = 0.044, df = 25, t-ratio = -0.602, p = 1; $TC_{VEH\text{-}LY}$: mean = -0.063, SE = 0.045, df = 25, t-ratio = -1.408, p >.5). This indicates that PI3K activity inhibition is detrimental for RIF specifically and does not cause a general long term memory deficit. We detected a main effect of the $Condition_{IC}$ over the total exploration time in this phase, an effect that did not appear in any other experiment ($\beta = -10{,}10$, $_{95\%}CI$ [-19.54, -0.66], $t_{(27)} = -2.19$, $p < .05$). No differences were observed between treatments for the memory of the RP+ object (Fig. 3B, *left, inset*). Similar to the previous experiments, the infusion of LY before the practice phase did not alter the animals' performance during this phase (Fig. 3C, *left*). We only observed a statistically significant effect of the Condition ($\beta = 0.20$, $_{95\%}CI$ [0.03, 0.36], $t_{(18)} = 2.50$, $p < .05$).

We then analyzed if the PI3K pathway mediated 5-HT2AR modulation of RIF through a molecular disconnection experiment. This approach is inspired by the classic brain disconnection experiments that assess functional interactions between brain structures (Jo and Lee 2010; Bekinschtein et al. 2013). This design has two main assumptions: that most functional communication between the structures occurs ipsilaterally and that one intact hemisphere is sufficient for proper behavior. This design relies on two primary assumptions:

first, that functional communication between the structures predominantly occurs ipsilaterally, and second, that a single intact hemisphere is sufficient to sustain normal behavior. Similarly, in the molecular disconnection paradigm, if both molecules interact within a brain structure in one hemisphere, inhibiting their activity should not impact behavior, as the molecules and pathways in the opposite hemisphere remain intact and functional (Miranda et al. 2017, 2018). Conversely, disrupting one molecule in one hemisphere and the other in the other hemisphere is expected to impair the behavioral response (e.g., RIF) if the interaction between the molecules is essential. Following this rationale, we trained cannulated rats in the RIF paradigm (Fig. 3A). Before the practice phase of the task, rats were infused in the same or different hemispheres (ipsi and contralateral infusions, respectively) in the mPFC with MDL and LY following a within-subject design by condition. When evaluating the competitor object's memory (B, RP-), animals that received contralateral infusion of MDL and LY could distinguish between a novel and the RP- object (Fig. 3B, *right*; Condition_{RP} x Drug_{CONTRA}, $\beta = 0.21$, $_{95\%}$CI [0.02, 0.40], $t_{(22)} = 2.25$, $p < .05$; $RP_{IPSI\text{-}CONTRA}$: mean = -0.247, SE = 0.065, df = 23, t-ratio = -3.799, $p < .01$; $IC_{IPSI\text{-}CONTRA}$: mean = -0.040, SE = 0.069, df = 23, t-ratio = 0.577, p = 1; $TC_{IPSI\text{-}CONTRA}$: mean = -0.040, SE = 0.065, df = 23, t-ratio = -0.621, p = 1). This experiment then supports the hypothesis that the activation of the PI3K pathway at least mediates 5-HT2AR modulation of RIF since RIF was effectively disrupted in the RP_{CONTRA} condition. No differences were observed between drug infusion schemes for the memory of the RP+ object (Fig. 3B, *right, inset*). We found that none of the infusion designs affects the response of the animals during the practice phase (Fig. 3C, *right,* Condition_{RP}, $\beta = 0.25$, $_{95\%}$CI [0.13, 0.36], $t_{(15)} = 4.54$, $p < .001$), supporting the idea that 5-HT2A and PI3K signaling do not interfere with the expression of the memory of the practiced object or with the ability to explore novel objects.

Src activity is required during Retrieval Practice for RIF but is not functionally linked to 5-HT2AR signaling

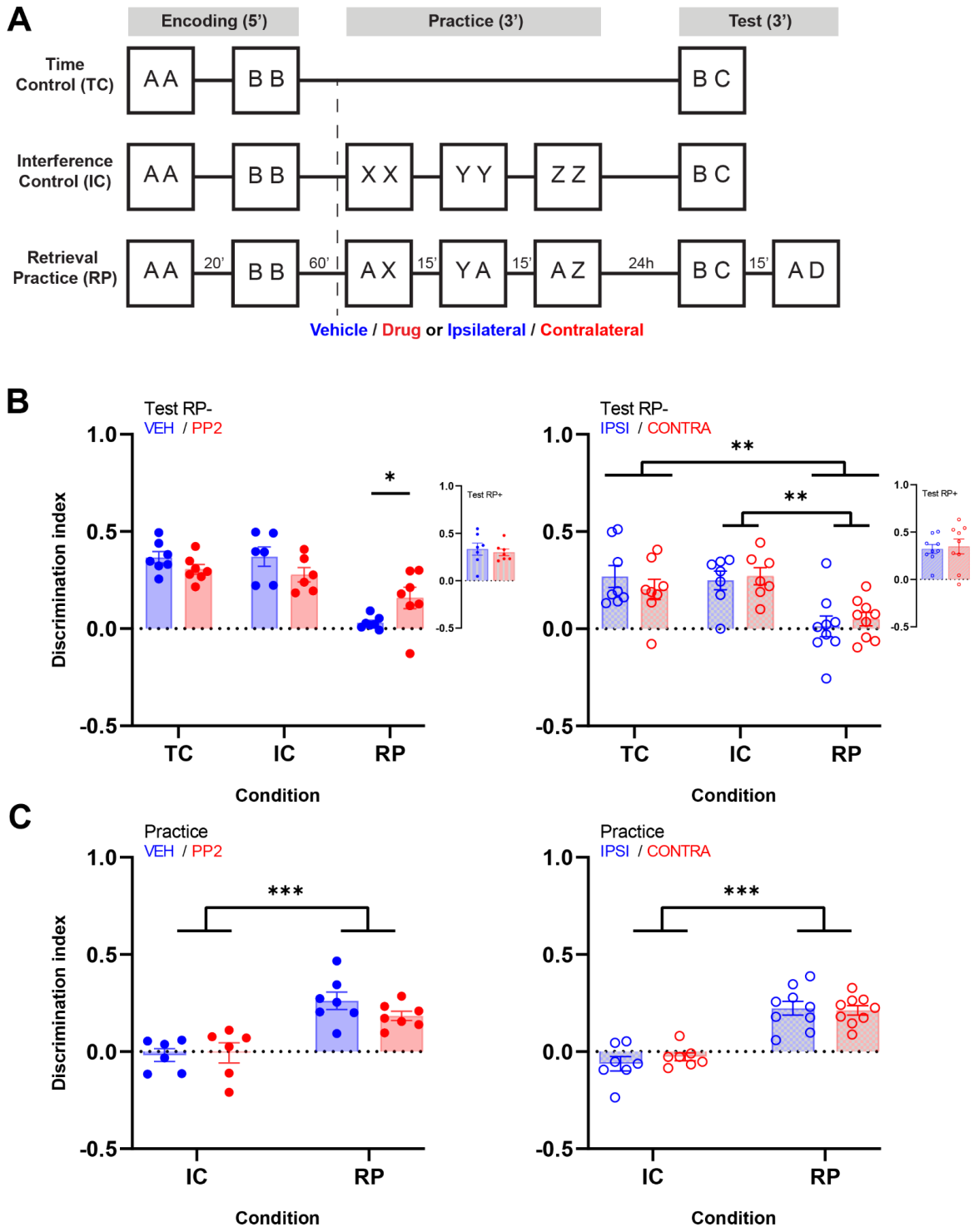

Figure 4. Src activity is necessary during Retrieval Practice for RIF but operates independently of 5-HT2A receptor signaling. A. *Schematic representation of the behavioral protocol. In two separate experiments, we investigate the role of Src in RIF. First, we reproduce the approach used in previous experiments and compare the effect of a Src inhibitor between conditions. Then, we disrupt the functional connection between Src activity and 5-HT2AR, infusing the Src inhibitor with the 5-HT2AR antagonist simultaneously in the same (ipsilateral) or different (contralateral) hemispheres. The dashed line indicates the infusion in the mPFC 15 min before the practice phase.* ***B.*** *Discrimination index ± SEM for the testing phase. Left: Src inhibitor (PP2; n = 20); right: Src inhibitor + 5-HT2AR antagonist (PP2 + MDL11,939, n = 24). Asterisks show significant differences between treatments or conditions (Bonferroni post*

hoc comparisons). Bilateral infusion of PP2 alone into the mPFC blocked RIF but not when it was combined in a contralateral configuration with MDL. Inset: Discrimination index ± SEM for the practiced object. ***C.*** *Discrimination index ± SEM during the Practice phase for the IC and RP groups across the two pharmacological experiments. Asterisks show the main effect of the condition on exploratory behavior. *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001.*

The Src kinase family (we will refer to Src as representing the entire SFK family) is a subfamily of non-receptor tyrosine kinase family, and many of its members (e.g., Src, Fyn, Yes, Lyn, and Lck) are expressed in the brain (Omri et al. 1996; Kalia, Gingrich, and Salter 2004; Bongiorno-Borbone et al. 2005) and participate in a wide variety of neuronal processes. In particular, Src and Fyn had been implicated in the modulation of synaptic transmission and plasticity (Kalia, Gingrich, and Salter 2004; Ohnishi et al. 2011; Schenone et al. 2011). Furthermore, Schmid and Bohn (Schmid and Bohn 2010) provided evidence that serotonin activates the formation of a 5-HTA2AR signaling complex involving β-arrestin2, Src, and Akt, and that blocking any individual component, including PI3K, prevents the full expression of the head-twitch response, highlighting the physiological significance of this complex. Given the role of Src signaling in different neurophysiological processes and the observed effects of PI3K inhibition in RIF, we hypothesized that inhibiting the Src would produce similar disruptions in RIF expression.

The results align with our hypothesis (Fig. 4B, *left*): RP_{PP2} animals exhibited a preference for the novel object over the RP- object in the test phase ($Condition_{RP} \times Drug_{PP2}$: $\beta = 0.19$, $_{95\%}CI$ [0.04, 0.34], $t_{(17)} = 2.60$, $p < .05$; $RP_{VEH\text{-}PP2}$: mean = -0.127, SE = 0.051, df = 17, t-ratio = -2.477, $p < .05$. No significant effect of PP2 was observed on memory performance in control conditions; $IC_{VEH\text{-}PP2}$: mean = 0.094, SE = 0.055, df = 17, t-ratio = 1.688, p >.5; $TC_{VEH\text{-}PP2}$: mean = 0.062, SE = 0.051, df = 17, t-ratio = 1.205, p >.5). These findings suggest that inhibiting Src activity specifically impairs RIF without affecting general long-term memory function. Additionally, no treatment-related differences were observed in the memory of the RP+ object (Fig. 4B, left, inset). Consistent with previous experiments, the infusion of PP2 prior to the practice phase did not alter the animals' performance during that phase (Fig. 4C, *left*). Only a significant effect of Condition was detected ($\beta = 0.28$, $_{95\%}CI$ [0.16, 0.40], $t_{(11)} = 5.03$, $p < .001$). Next, we aimed at testing the functional interaction between Src and the 5-HT2AR using a molecular disconnection approach similar to the one described above. In line with this approach, we trained cannulated rats in the RIF paradigm (Fig. 4A). Before the practice phase, rats received infusions of MDL and PP2 in the mPFC, either in the same hemisphere (ipsilateral infusion) or in opposite hemispheres (contralateral infusion), following a within-subject design based on the condition. In the competitor object's memory test (B, RP-), animals that received contralateral infusion of MDL and PP2 could distinguish between a novel and the RP- object: contrary to our expectations, we did not observe a significant interaction

between $Condition_{RP}$ x $Drug_{CONTRA}$. Instead, we found a main effect of the condition (Fig. 4B, *right*; $Condition_{RP}$, β = -0.26, $_{95\%}$CI [0.40, -0.12], $t_{(21)}$ = -3.84, p < .001; IC-RP: mean = 0.229, SE = 0.061, df = 21, t-ratio = 3.754, p < .01; TC-RP: mean = 0.205, SE = 0.059, df = 21, t-ratio = 3.490, p < .001). Thus, we did not find evidence supporting a functional interaction between Src signaling and the 5-HT2AR receptor during the retrieval practice to promote RIF. No differences between treatments were observed in the performance of the practiced object (A, RP+) test phase (Fig. 4B, *right, inset*). In line with prior experiments, the pharmacological treatment did not alter the animals' performance during the retrieval practice phase (Fig. 4C, *right*, β = 0.29, $_{95\%}$CI [0.19, 0.38], $t_{(14)}$ = 6.55, p < .001).

Discussion

Historically, neurobiological studies of memory have predominantly focused on understanding how memories are encoded, stored, and retrieved, while forgetting has often been regarded as memory failure (Davis and Zhong 2017; Medina 2018). However, emerging perspectives, such as those proposed by Richards and Frankland (Richards and Frankland 2017), suggest that the interaction between memory persistence and transience optimizes decision-making processes in dynamic environments. Among these active processes involved in forgetting is RIF. Cognitive models propose that RIF relies on inhibitory control processes, wherein attempts to retrieve target memories inadvertently activate competing, contextually irrelevant memories, which are subsequently suppressed to reduce interference and enhance retrieval efficiency. This mechanism would facilitate future retrieval of relevant information, preparing the organism for similar circumstances and optimizing behavioral responses. Therefore, forgetting can be conceptualized as an active mechanism that improves behavioral flexibility that mechanistically relies on cortical inhibitory processes.

Our findings align with a growing body of evidence indicating that forgetting involves active neurobiological processes rather than passive decay alone. Traditionally studied in the context of cognitive control and mood disorders, the serotonergic system has emerged as a key modulator of learning and memory mechanisms, particularly within the mPFC. Here, we extend the role of the serotonergic system to include its involvement in the adaptive forgetting of competing memories, even without explicit reward or external instructions. Specifically, we propose that serotonergic modulation within the mPFC facilitates inhibitory control processes during retrieval, suppressing competing memory traces and enabling more efficient access to relevant information.

We provide compelling evidence supporting the involvement of a serotonin-dependent inhibitory control mechanism in RIF. All the effects found were specific to the RP condition, as no changes were observed when the pharmacological treatment was administered before the encoding of interfering materials (interference control) or during rest in the home cage (time control). These results suggest that serotonergic modulation in the mPFC selectively influences processes engaged during retrieval practice rather than nonspecific factors such as novelty, salience, or mood that would otherwise impact performance across all conditions.

Our main result demonstrates that RIF depends on serotonin modulation in the mPFC during successive attempts to retrieve a specific memory. Infusing a 5-HT2AR antagonist into the mPFC during the practice phase blocked RIF without altering the practiced object memory (Fig. 1C, *center*), demonstrating the necessity of 5-HT2A receptor activity for this process. Importantly, our findings also reveal the involvement of the 5-HT1AR in this phenomenon. Administration of a 5-HT1AR agonist in the mPFC of rats during the practice phase impaired the forgetting of the competitor object memory (Fig. 1C, *left*). 5-HT2CRs are also expressed in the mPFC, and it has been demonstrated that MDL11,939 exhibits some affinity for them (Elizabeth A. Pehek et al. 2006). We found that the infusion of SB242058, a potent and highly specific 5-HT2CR antagonist (Kennett et al. 1997), did not affect the response of the animals in the RIF task, indicating that serotonergic modulation influences the mechanisms underlying RIF by 5-HT2A and 5-HT1A but not 5-HT2C receptors.

Though serotonin cells densely project to the mPFC, the role of this neuromodulator on the mPFC function is not fully understood. This is partially due to the widespread localization of serotonin receptors within the PFC that modulate neuronal activity in a complex way and to the fact that the serotonin system interacts heavily with other neuromodulatory systems, influencing various cognitive processes. 5-HT1A and 5-HT2A receptors are among the most densely expressed in this area, both in excitatory and inhibitory neurons. In vitro studies have shown that 5-HT1A receptor activation reduces neuronal firing, while 5-HT2A receptor activation enhances it, creating a balance that modulates prefrontal cortical activity (Araneda and Andrade 1991; Andrade 2011; Mengod, Palacios, and Cortés 2015; Amargós-Bosch et al. 2004) and in vivo modulates neural network oscillations (Puig et al. 2010). Though the role of 5-HT1A receptors in cognition appears to be limited, the role of 5-HT2A receptors is more clearly established. Behaviorally, cortical 5-HT2ARs are implicated in cognitive processes (Aznar and Hervig 2016) functions like working memory, cognitive flexibility, inhibition (Harman et al. 1966; Janiger and Dobkin de Rios 1989; Boulougouris and Tsaltas 2008; Frecska et al. 2012; Friedman and Robbins 2022) as well as memory retrieval (Bekinschtein et al. 2013; J. F. Morici et al. 2022).

Notably, 5-HT2ARs are implicated in cognitive functions that require the control of prepotent responses. If control of memory retrieval takes advantage of a mechanism sensitive to serotonin modulation, we hypothesized that serotonin might play a role in controlling RIF. Our results support this hypothesis, demonstrating that serotonergic modulation operates through the coordinated activation of specific receptor subtypes, such as 5-HT1A and 5-HT2A—but not 5-HT2C—. 5-HT2AR bidirectionally modulates RIF. Specifically, blocking mPFC 5-HT2AR prior to the retrieval practice phase impaired RIF, whereas activating these receptors in the mPFC immediately before retrieval practice induced retrieval-induced forgetting. What are the mechanisms by which serotonin modulates RIF? Active inhibition of downstream areas like the medial temporal lobe might be triggered by the mPFC in a serotonin-dependent manner, inhibiting the memory of the competitor object. Temporary interactions between low- and high-frequency brain waves are proposed to coordinate network activity across the cortical surface during cognitive tasks (Canolty et al., 2006). In the PFC, 5-HT2ARs and 5-HT1ARs jointly contribute to generating gamma oscillations (Puig and Gulledge, 2011). However, they exert opposing influences on gamma wave power: 5-HT1AR antagonism enhances cortical gamma activity, while 5-HT2AR antagonism disrupts gamma wave synchronization (Celada et al., 2013). Comparable effects on gamma and theta rhythms have been reported in mice, suggesting that blocking 5-HT2AR or activating 5-HT1AR could disrupt the balance essential for serotonergic regulation of cortical network dynamics during active inhibition. Active inhibition may depend on the synchronization between the mPFC and downstream structures. Human studies have demonstrated that reduced theta and gamma power are linked to the forgetting of unpracticed items (Spitzer et al. 2009). This suggests that serotonin may enhance the expression of theta and gamma rhythms, potentially serving as the mechanism underlying the inhibition of unpracticed objects.

Interestingly, we found that, in this paradigm, blockade of 5-HT2AR does not affect retrieval itself since the infusion of the antagonist has no effect during the retrieval practice session but impairs the retrieval of the competitor memory on a later test. Previously, we have shown that mPFC 5-HT2ARs are involved in the control of retrieval only when competitor objects are simultaneously presented. Still, it does not participate in the ability to distinguish between a familiar object from a new one (Juan Facundo Morici et al. 2018). Then, we argue that retrieval control and RIF mechanisms are intrinsically distinct, though both can be modulated by serotonin in a 5-HT2AR-dependent manner.

Carhart-Harris and Nutt (Carhart-Harris and Nutt 2017) propose, based on the roles of 5-HT1A and 5-HT2A receptors related to learning and cognition, that signaling via the 5-HT2AR mediates the role of serotonin in sensing environmental changes and consequent behavioral adaptation (Branchi 2011). The promotion of plasticity through 5-HT2AR signaling is central to their proposal that, along with enhancing stress tolerance, a key function of serotonin

transmission in the brain is to engage processes necessary for change when change is needed. We propose that the practice phase could be a context in which the conformation of the 5-HT2A receptor with high affinity for serotonin is favored under normal conditions, whose adaptive role is to decrease future access to memories that are no longer useful in an environment that guides the retrieval of another related memory.

5-HT2A is a GPCR with a complex system of signaling pathways. The classical pathway is $G_{q/11}$-mediated activation of PLC and Ca^{2+} release from the endoplasmic reticulum. However, studies in heterologous expression systems identified several other signaling transduction pathways coupled to the receptor. Different pathways appear to be selectively activated depending on the type of agonist used to test them (Porter et al. 1999; Kurrasch-Orbaugh et al. 2003; Berg et al. 1998). This phenomenon, which seems to be a feature of GPCRs, is known as biased agonism. In recent years, trying to identify the signaling pathway downstream of 5-HT2AR gained a lot of attention as a renewed interest in hallucinogenic drugs as therapeutic agents increased. However, the natural 5-HT2AR ligand, serotonin, can also activate different signaling pathways due to their release kinetics or cellular milieu; 5-HT2AR activation also induces ERK and tyrosine kinase pathway as well as β-arrestin2/PI3K/Src/Akt cascade (Schmid and Bohn, 2010). Since different pathways may contribute uniquely to various cognitive processes, we focused on the PI3K/Akt pathway as a potential mechanism involved in the modulation of RIF by 5-HT2AR. The PI3K/Aktpathway is a critical regulator of brain function, playing essential roles in neuronal homeostasis, growth, and plasticity. Although the PI3k/Akt pathway is not the canonical 5-HT2AR signaling route, its involvement is particularly intriguing due to the relationship between Akt and long-term memory processes (Horwood et al. 2006)

In this work, we found that the activation of the PI3K pathway in the mPFC is necessary for the expression of RIF. We also found a functional interaction between 5-HT2AR and PI3K, which is essential for 5-HT2AR activation during the retrieval practice phase. Independently of the complete pathway involved, our results strongly support a role for PI3K as a key element in the 5-HT2AR signaling during the retrieval practice phase of the task, underscoring its pivotal role as a molecular bridge between serotonergic signaling and active forgetting. PI3K is crucial for synaptic plasticity mechanisms across several brain regions, including the hippocampal CA1 (Sanna et al. 2002; Karpova, Sanna, and Behnisch 2006), dentate gyrus (Kelly and Lynch 2019), insular cortex (Slouzkey, Rosenblum, and Maroun 2013), and the amygdala (C. H. Lin et al. 2001). Furthermore, activation of the PI3K pathway in the hippocampus is necessary for both contextual memory recall and extinction (Barros et al. 2001; C. H. Lin et al. 2001; Chen, Hough, and Li 2003; C.-H. Lin et al. 2003; Kritman and Maroun 2013), while dysfunction in this pathway has been linked to various neurodegenerative

and psychiatric disorders (Browning et al. 2005; Nakano et al. 2017; Sánchez-Castillo et al. 2022) suggesting that plastic mechanisms within the mPFC might be involved in RIF. PI3K signaling appears to be an interesting pathway due to its downstream effectors. RAC1 is a molecule crucially involved in cell differentiation, migration, and the regulation of cytoskeletal dynamics, which are essential for spine morphology, dendritic spine formation, and synaptic plasticity (Heasman and Ridley 2008). Notably, recent studies have linked RAC1 to active forgetting (Davis and Zhong 2017; Medina 2018). In Drosophila, constitutively active Rac1 in mushroom body neurons accelerates memory decay in Pavlovian olfactory aversive conditioning, while constitutively inactive Rac1 inhibits intrinsic forgetting (Shuai et al. 2010). Similarly, spaced training in contextual fear conditioning inhibits hippocampal Rac1 activity in rats, resulting in heightened contextual fear (Jiang et al. 2016). Conversely, pharmacological inhibition of Rac1 enhances fear memory in massed training. In contrast, intrahippocampal injection of a Rac1 activator impairs contextual fear memory in spaced training paradigms. Though we have not analyzed the role of RAC1 in retrieval-induced forgetting, RAC1 might be a target of PI3K-Akt pathway and a key mediator of RIF by 5-HT2AR activation. Further studies are necessary to deepen our understanding of the molecular mechanisms underlying RIF. Advancing this knowledge will not only enhance our comprehension of this critical cognitive process for managing memory interference but also shed light on whether drugs tested for hallucinogenic or antidepressant effects might disrupt a mechanism essential for individuals to adapt to environmental changes.

The 5-HT2ARs have been reported to be coupled with the activation of Src in neuronal cells (González-Maeso et al. 2007; Schmid and Bohn 2010; Tang and Trussell 2015).
There is evidence that Src participates in memory and learning, possibly via phosphorylation of N-methyl-D-aspartate receptors, but its role is poorly understood. Overexpression of Src affects excitatory synaptic transmission in area CA3 of the mammalian hippocampus and impairs fear memory (Yan et al. 2017). After spatial maze learning, upregulation of Src mRNA was observed in area CA3 (Zhao et al. 2000). Increased autophosphorylation of Src enhances hippocampal long-term potentiation and spatial memory(Wang et al. 2018). If little is known about the role of Src in memory, even less is understood regarding its involvement in active forgetting, and even more so in RIF. While it was reasonable to hypothesize that Src is involved in the RIF process due to its connection to a shared pathway with the 5-HT2A receptor and PI3K, we tested this assumption using our paradigm. Our results show that inhibiting Src during the practice phase interferes with the expression of RIF, suggesting that Src activity is essential for RIF to occur, although it is not directly linked to 5-HT2AR signaling during the retrieval phase. This finding does not conflict with the rest of our results, as Src is a family of proteins activated by multiple signaling pathways and could potentially converge

on Akt without being directly activated by 5-HT2AR. Few studies have explored the direct interaction between GPCRs and Src or their activation mechanisms. Like arrestins, Src offers an alternative signaling pathway for GPCRs, contributing to their physiological functions. Further research is needed to clarify the specific pathways mediated by Src and their role in GPCR signaling (Berndt and Liebscher 2021).

Previously, we have shown that dopamine acting on D1Rs in the mPFC modulates the control processes involved in RIF in rats (Gallo et al. 2022). The serotonergic and dopaminergic systems often interact, influencing various functions including cognition and mood regulation (De Deurwaerdère, Drutel, and Di Giovanni 2018; Di Giovanni et al. 2008). Although dopamine and serotonin are closely related and have a similar degree of functional relevance, compared to the former, we have less specific understanding of the mechanisms by which serotonin affects behavior (De Deurwaerdère, Chagraoui, and Di Giovanni 2021) . Regarding the serotonin 5-HT2A receptor, it has been proposed that it may influence dopamine release. Systemic administration or local infusion of DOI, a selective 5-HT2A agonist, in the PFC results in an increase in local dopamine, and this effect can be blocked by selective 5-HT2A receptor antagonists (E. A. Pehek et al. 2001; Elizabeth A. Pehek et al. 2006; Bortolozzi et al. 2005). In addition, 5-HT2AR is present in approximately 55% of pyramidal neurons projecting to the ventral tegmental area (VTA) (Vázquez-Borsetti, Cortés, and Artigas 2009). Half of these latter neurons synapse in the DR, suggesting that mPFC 5-HT2A might be involved in the concerted modulation of the ascending serotonergic and dopaminergic activity (Vázquez-Borsetti et al. 2011). Dopamine and serotonin might work in concert during RIF, but further studies are necessary to test this hypothesis.

Individuals with Major Depressive Disorder (MDD) exhibit specific characteristics in episodic memory retrieval and the emotional valence of recalled events, which may stem from difficulties in inhibiting irrelevant information (Lemogne et al. 2006). Notably, both anxiety and depressive disorders are associated with reduced RIF (Groome and Sterkaj, n.d.; Law et al. 2012), suggesting that the inhibitory processes crucial for RIF are disrupted in these conditions. RIF is known to depend on PFC activity, and the functionality of this region is markedly altered in depression (Downar and Daskalakis 2013; Riva-Posse et al. 2018). Could the impaired inhibitory control observed in individuals with MDD be the mechanism responsible for their diminished RIF? (Giebl et al. 2016). Our findings further position serotonin 5-HT2A and 5-HT1A receptors, both implicated in depressive phenotypes (Celada et al. 2004; Savitz, Lucki, and Drevets 2009; Albert and François 2010), as critical contributors to the processes underlying RIF. Incorporating antidepressant use as a modulatory variable and

examining its correlation with RIF performance could provide valuable insights into the complex interactions between depressive states and memory processes.

This serotonergic influence on RIF may have significant implications for understanding the neurobiological underpinnings of active forgetting and its relationship with psychopathological conditions. Deficits in serotonergic modulation and inhibitory control have been implicated in disorders such as depression, anxiety, obsessive-compulsive disorder, and impulsivity-related conditions. By positioning the serotonergic system in the mPFC as a critical regulator of memory suppression, these findings contribute to a broader framework for understanding how active forgetting supports cognitive flexibility and adaptive behavior in dynamic environments.

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