

Role of Medial Prefrontal Cortex Serotonin 2A Receptors in the Control of Retrieval of Recognition Memory in Rats

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Often, retrieval cues are not uniquely related to one specific memory, which could lead to memory interference. Controlling interference is particularly important during episodic memory retrieval or when remembering specific events in a spatiotemporal context. Despite a clear involvement of prefrontal cortex (PFC) in episodic memory in human studies, information regarding the mechanisms and neurotransmitter systems in PFC involved in memory is scarce. Although the serotonergic system has been linked to PFC functionality and modulation, its role in memory processing is poorly understood. We hypothesized that the serotonergic system in PFC, in particular the 5-HT_{2A} receptor (5-HT_{2AR}) could have a role in the control of memory retrieval. In this work we used different versions of the object recognition task in rats to study the role of the serotonergic modulation in the medial PFC (mPFC) in memory retrieval. We found that blockade of 5-HT_{2AR} in mPFC affects retrieval of an object in context memory in a spontaneous novelty preference task, while sparing single-item recognition memory. We also determined that 5-HT_{2AR}s in mPFC are required for hippocampal–mPFC interaction during retrieval of this type of memory, suggesting that the mPFC controls the expression of memory traces stored in the hippocampus biasing retrieval to the most relevant one.

Introduction

Remembering is more than the activation of a single memory trace. So how are we able to retrieve specific memories? As retrieval cues are often not uniquely related to a particular memory, there should be a mechanism to allow selective memory retrieval by focusing on relevant (and ignoring irrelevant) information. This is important during episodic memory (Squire, 2004). Most of what we know about the mechanisms that control interference comes from human studies (Ferbinteanu et al., 2006). Although the anatomical basis of episodic memory has been extensively studied, the neurobiological mechanisms are largely unknown (Wheeler et al., 1995; Nyberg et al., 2000; Burgess et al., 2001; Hayes et al., 2004). Thus, the development in animals of behavioral tasks that model episodic memory is essential. Some aspects of episodic memory have been modeled using object recognition (Eacott and Norman, 2004; Ergorul and Eichenbaum, 2004; Warburton and Brown, 2010; Easton et al., 2012). Animal studies

using tasks that employ a subset of episodic memory features have provided relevant mechanistic information (Farovik et al., 2008; Langston and Wood, 2010; Warburton and Brown, 2010; Navawongse and Eichenbaum, 2013). Spontaneous recognition memory tasks are short, simple, and free from stress (for review, see Winters et al., 2010). They require judgment regarding the previous occurrence of stimuli, which can be made on the basis of the relative familiarity, recency, or by integrating information concerning objects and location (Brown et al., 1987; Gaffan, 1992; Fahy et al., 1993; Li et al., 1993; Meunier et al., 1993; Ennaceur et al., 1996; Murray and Bussey, 1999; Bussey et al., 2000; Brown and Aggleton, 2001; Kesner and Ragozzino, 2003; Hannesson et al., 2004a, b; Browning et al., 2005; Barker et al., 2007; Barker and Warburton, 2011; Navawongse and Eichenbaum, 2013). Rodents naturally tend to approach and explore novel objects, which are assumed to have no natural significance to the animal and which have never been paired with a reinforcing stimulus. They also show an innate preference for novel over familiar objects. They readily approach objects and investigate them physically by touching and sniffing them, rearing upon and trying to manipulate them with their forepaws (Aggleton et al., 1989; Ennaceur and Aggleton, 1994). This behavior can be easily quantified and used to study simple recognition memory as well as more complex spatial-, temporal-, and episodic-like memory in rodents.

The prefrontal cortex (PFC) is a key area involved in top-down cognitive control, like response selection, inhibitory control (Miller, 2000; Fuster, 2001; Miller and Cohen, 2001; Miller and Wang, 2006), suppression of unwanted memories, recall of remote memories (Anderson et al., 2004; Frankland and Bontemp, 2005), and episodic recollection (see above). The PFC

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receives a broad range of sensory and limbic inputs that can activate contextually appropriate representations of goals or task rules (Miller and Cohen, 2001). It directly projects to the perirhinal cortex and to the hippocampus via the parahippocampus, the subiculum, and the presubiculum (Goldman-Rakic et al., 1984; Price, 1999; Petrovich et al., 2001). Reciprocal connections project back to the PFC.

Although the serotonergic system has been linked to PFC function (Martín-Ruiz et al., 2001; Puig et al., 2004, 2005), its role in memory processing is poorly understood. The 5-HT_{2A} receptor (5-HT_{2AR}) is one of the main postsynaptic serotonergic receptor types and it is highly expressed in the PFC. (Boulougouris et al., 2007). The expression pattern of 5-HT_{2AR} and its role in PFC cellular physiology suggest that it is important for the modulation of PFC-dependent functions, including attentional control, cognitive flexibility, and impulsivity (Winstanley et al., 2003; Carli et al., 2006; Baker et al., 2011). However, the role of serotonin and, particularly, 5-HT_{2AR} in memory processing remain largely an important question. A handful of studies suggest a role for 5-HT_{2AR} in episodic memories in humans (de Quervain et al., 2003; Wagner et al., 2008). A few animal studies have addressed a potential role of 5-HT_{2AR} during memory consolidation (Meneses et al., 1997; Meneses, 2007; Wagner et al., 2008). However, we know very little regarding the role of the serotonergic system and particularly, 5-HT_{2AR} in episodic memory retrieval. Moreover, since 5-HT_{2AR} is highly expressed in PFC, we hypothesized that 5-HT_{2AR} signaling in PFC could have a role in the control of episodic memory expression.

In this study we chose different versions of the spontaneous novel object recognition task that model some features of episodic memory to analyze the role of the serotonergic modulation via the 5-HT_{2AR} in the medial PFC (mPFC) during memory retrieval. We hypothesize that top-down control from mPFC through 5-HT_{2AR} signaling would be required for accurate memory retrieval only in the cases in which the task cannot be solved using an item-only strategy and a combination of multiple elements is required.

Materials and Methods

Subjects

162 male adult Wistar rats (weight 180–250 g) were housed five per cage and kept with water and food *ad libitum* under a 12 h light/dark cycle (lights on at 7:00 A.M.) at a constant temperature of 23°C. Experiments took place during the light phase of the cycle (between 10:00 A.M. and 5:00 P.M.) in a quiet room with dim light. The experimental protocol for this study was approved by the National Animal Care and Use Committee of the University of Buenos Aires.

Surgery and drug infusions into the mPFC

Rats were deeply anesthetized with ketamine (60 mg/kg) and xylazine (8 mg/kg) and placed in a stereotaxic frame. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. After small burr holes were drilled, a set of 22 g guide cannulae were implanted bilaterally into the mPFC [anterior–posterior (AP) +3.20 mm/lateral (L) ± 0.75 mm/dorsoventral (DV) –3.50 mm] (see Fig. 1B). Cannulae were fixed to the skull with dental acrylic. A dummy cannula was inserted into each guide cannula to prevent clogging. At the end of surgery, animals were injected with a single dose of meloxicam (0.2 mg/kg) as analgesic. Behavioral procedures commenced 5–7 d after surgery.

On the experimental day, the dummy cannulae were removed and a 30 g injection cannula extending 1 mm below the guide cannula was inserted. The injection cannula was connected to a 10 μ l Hamilton syringe. Cannulated rats received bilateral 0.8–1 μ l infusions of the appropriate drug or vehicle into the mPFC 15 min before testing. MDL 11,939 (Tocris Bioscience), ergotamine (Sigma), and SB 242084 (Tocris Biosci-

ence) were dissolved in pure dimethylsulfoxide (DMSO) and diluted in saline to final concentration of 300 ng/ μ l (<10% DMSO) and 8-OH DPAT (Sigma) was dissolved in saline.

Surgery and drug infusions for the disconnection experiment

For the disconnection experiment (see Fig. 6), rats were anesthetized and put in the stereotaxic frame as described before. Two pairs of 22 g cannulae were used. One pair was bilaterally implanted in the mPFC and the other pair was implanted bilaterally in the hippocampus (AP –3.90 mm/LL ± 3.00 mm/DV –3.00 mm). On the experimental day, rats were infused with MDL 11,939 (300 ng/ μ l) in the mPFC and muscimol (0.1 μ g/ μ l; Sigma) in the hippocampus.

For this experiment rats were divided into four groups. The first group received a bilateral injection of MDL 11,939 in the mPFC and vehicle in the hippocampus. The second group received a bilateral injection of vehicle in the mPFC and muscimol in the hippocampus. The third group received a contralateral injection of MDL 11,939 in the mPFC and muscimol in the hippocampus. The fourth group received an ipsilateral injection of MDL 11,939 in the mPFC and muscimol in the hippocampus.

Behavioral experiments

Statistical analysis. Behavioral data were analyzed using unpaired Student's *t* test when two groups were compared and one-way ANOVA followed by Newman–Keuls multiple comparisons or Tukey's multiple comparisons as indicated in the figure legends.

Simple object recognition and temporal order recognition tasks. Simple object recognition (SOR) and temporal order recognition (temporal memory object recognition, TMOR) tasks were conducted in a Y-shaped apparatus, as described previously (Winters et al., 2004; Forwood et al., 2007). Briefly, the Y-apparatus had high, homogenous white walls constructed from Plexiglas to prevent the rat from looking out into the room, thereby maximizing attention to the stimuli. All walls were 40 cm high, and each arm was 27 cm in length and 10 cm wide. The start arm contained a guillotine door 18 cm from the rear of the arm. This provided a start box area within which the rat could be confined at the start of a given trial. Duplicate copies of objects made from plastic, ceramic, glass, and aluminum were used. The height of the objects ranged from 8 to 24 cm and they varied with respect to their visual and tactile qualities. All objects were affixed to the floor of the apparatus with an odorless reusable adhesive to prevent them from being displaced during each session. As far as we could determine the objects had no natural relevance for the rats and they have never been associated with a reinforcer. The objects, floor, and walls were cleaned with ethanol 50% between experiments.

All rats were habituated to the context the day before the beginning of the task. They were allowed to explore the empty Y-shaped apparatus for 10 min. For these habituation sessions, the rat was placed in the start box, and the guillotine door was opened to allow the rat to explore the main area of the apparatus. The guillotine door was lowered when the rat exited the start box to prevent re-entry into this area of the apparatus. Testing began 24 h later in each experiment.

SOR. To address whether simple object discrimination was affected by 5-HT_{2AR} blockade, we used the SOR task. Each trial consisted of two phases (see Fig. 3A). In the sample phase, two identical objects (A1 and A2) were placed in the Y-shaped apparatus, one at the end of each exploration arm. The rat was placed in the start box with the guillotine door lowered. The guillotine door was then raised to allow the rat into the exploration area of the maze and was allowed to explore the objects for 5 min. The time spent exploring the two objects was scored by an experimenter viewing the rat. Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose. Turning around or sitting on the object was not considered exploratory behavior.

At the end of the sample phase, the rat was removed from the Y-shaped apparatus and returned to its home cage for the duration of the retention period. After the delay, the rat was placed back in the start box of the Y-shaped apparatus and released into the exploration area for the choice phase. The Y-shaped apparatus now contained an identical copy of the sample (familiar) object (A3) in one arm and a new object (B) in the other. The exploration arms in which the choice objects were placed were

counterbalanced between rats. The rat was allowed to explore the objects for 3 min, at the end of which it was removed and returned to its home cage. We calculated a discrimination ratio defined as the proportion of total exploration time spent exploring the novel object (i.e., the difference in time spent exploring the novel and familiar objects divided by the total time spent exploring the objects).

TMOR. To address if recency memory retrieval is affected by 5-HT2AR blockade, we conducted a TMOR task. This task comprised two sample phases and one test trial (see Fig. 2A). It was conducted in the same apparatus as the SOR task. In each sample phase, the subjects were allowed to explore two copies of an identical object for 5 min. Different objects were used for sample phases 1 and 2, with a 1 h delay between the sample phases. The test trial (3 min duration) was given 3 h after sample phase 2. During the test trial, a copy of the objects from sample phase 1 and a copy of the objects from sample phase 2 were used. The positions of the objects in the test and the objects used in sample phase 1 and sample phase 2 were counterbalanced between the animals.

Simultaneous oddity discrimination task. The simultaneous oddity discrimination (SOD) task was conducted to evaluate if infusion of the 5-HT2AR antagonist affected attention and perception. The oddity apparatus incorporated the same considerations used to design the Y-apparatus. The exploration area was triangular in shape (Fig. 3E). The oddity apparatus had high, homogenous white walls constructed from Perspex to prevent the rat from looking out into the room. All walls were 30 cm high and the three sides of the triangular area were 75 cm long. The back wall was 84 cm wide and 59 cm tall, and the objects were placed 2.50 cm apart along the back wall. All rats were habituated to the empty apparatus for 10 min in which they were allowed to freely explore it. For the habituation session, the rat was placed in the triangular apparatus, facing the corner of the triangle away from the stimuli. Testing began 24 h after the habituation session. All object sets used in a given trial were placed in the apparatus before the rat was placed in the apparatus. The rat was placed in the corner of the triangular apparatus (away from the stimuli), and the trial did not begin until the rat turned around and entered the main exploration area. The time spent exploring the three objects during a testing phase was scored by an experimenter viewing the rat. Exploration of an object was defined as directing the nose to the object at a distance of <2 cm and/or touching it with the nose.

Object in context recognition task. To evaluate if retrieval of an object in a particular context was affected by infusion of MDL 11,939 in the mPFC, we used the object in context (OIC) recognition task. For OIC recognition, we used two arenas with different physical features. The first apparatus (context 1) was a 50 cm wide × 50 cm length × 39 cm height arena with black plywood walls and floor, divided into nine squares by white lines. The second apparatus (context 2) was a 60 cm wide × 40 cm length × 50 cm height acrylic box. The floor was white as well as two of its walls, which had different visual clues. The frontal wall was transparent and the back wall was hatched. All rats were habituated in a daily session in which they were allowed to explore each apparatus for 10 min. The test began 24 h later, using objects that had the same characteristics described in the previous experiments.

On sample phase 1, rats were placed in context 1 facing the wall opposite the objects and were allowed to explore two identical objects (A1 and A2) for 5 min (Fig. 1A). In sample phase 2, conducted 1 h later, rats were placed in context 2 together with two identical new objects (B1 and B2) and were allowed to explore the objects for 5 min. Memory was tested 3 h later. On the memory test, rats were reintroduced to context 1 or 2 (pseudorandomly assigned) and were allowed to explore *ad libitum* for 3 min one copy of object A and one copy of object B. The time spent exploring the two objects was scored during the testing phase.

OIC recognition task with three objects. The same contexts were used and animals were habituated as previously described. Animals were exposed to three identical objects (A1, A2, and A3) for 5 min and re-exposed to the same context and objects 15 min later (see Fig. 4C). One hour later the animals were placed in context 2 together with three identical new objects (B1, B2, and B3) and were allowed to explore them for 5 min. On the memory test rats were reintroduced to the first context and allowed to explore *ad libitum* for 3 min one copy of A, one copy of B, and

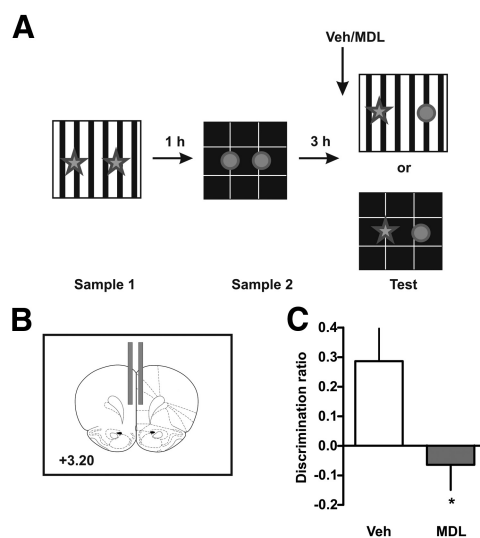


Figure 1. Blockade of 5-HT2AR in mPFC impairs OIC recognition memory retrieval. **A**, Training and testing scheme. Animals were exposed to a context containing two identical copies of an object for 5 min. An hour later they were exposed to a different context containing two identical copies of a different object. Three hours later they were re-exposed to context A or B containing one copy of each of the objects. **B**, Schematic representation of the sites of injection. Fifteen minutes before the test session rats were infused with 300 ng/ μ l MDL 11,939 or vehicle (Veh) into the mPFC. **C**, Discrimination index was calculated as the time spent exploring the incongruent object minus the time spent exploring the congruent object over the total exploration time during the test session; $n = 8–9$ per group, $*p < 0.05$, Student's *t* test.

Table 1. Total exploration scores during sample phase for the TMOR, OIC, NOR, object-location, and OIC with novelty tasks

	A	A'	<i>p</i>	B	B'	<i>p</i>	<i>N</i>
TMOR	18.37 ± 1.81	19.05 ± 1.9	0.8	17.39 ± 2.68	16.58 ± 2.48	0.82	16
OIC	15.32 ± 1.43	13.91 ± 1.43	0.49	13.67 ± 1.23	16.37 ± 1.76	0.22	18
SOR	24.33 ± 2.43	25.84 ± 2.43	0.66				15
Object location	16.15 ± 2.82	14.23 ± 2.4	0.62				8
OIC with novelty	14.71 ± 1.36	13.50 ± 1.42	0.55	13.00 ± 1.21	15.84 ± 1.67	0.178	24

Values are expressed in seconds as mean ± SEM. A, A' and B, B' indicate each one of the identical objects used. There were no significant differences in time exploring each one of the objects, Student's *t* test.

a new object (C). The order of the context exposure was pseudorandomly assigned.

Results

Blockade of mPFC 5-HT2AR affects the OIC recognition task

The OIC recognition task is a three-trial procedure (Wilson et al., 2013). In this case, the rats are exposed, during the sample phase, to two different pairs of identical objects presented in different contexts (Fig. 1A). These presentations are separated by an hour. During the retention trial, performed 3 h after the last presentation, a new copy of each of the objects used before is presented in one of the contexts (pseudorandomly assigned). Thus, one of the objects is presented in an “incongruent” context, while the other is being presented in a “congruent” one. In this task, novelty comes from a novel combination of an object and a context, and exploration will be driven by retrieval of a particular “what” and “which context” conjunctive representation. The animals showed no differences in the exploration levels in each context during the training session (see Table 1), neither did they show differences in the exploratory level during the test session (see Table 2). However, blockade of the mPFC 5-HT2AR with the 5-HT2AR antagonist MDL 11,939 15 min before the retention trial produced a significant difference in the level of exploration of both objects compared with vehicle-treated rats. As it has been

Table 2. Total exploration scores during test phase for the TMOR, OIC, NOR, SOD, object-location, and OIC with novelty tasks

	Veh	MDL	<i>p</i>	<i>N</i>
TMOR	28.46 ± 3.27	30.84 ± 4.77	0.69	8
OIC	10.69 ± 2.02	12.48 ± 1.13	0.45	9
SOR	22.48 ± 3.90	21.21 ± 2.08	0.77	8
SOD	24.33 ± 4.24	17.20 ± 2.66	0.16	7
Object location	14.54 ± 4.85	16.39 ± 2.11	0.73	4
OIC with novelty	15.63 ± 4.12	15.11 ± 1.78	0.9	10

Values are expressed in seconds as mean ± SEM. MDL-injected rats showed no differences in total exploration scores compared with Veh-injected rats, Student's *t* test.

shown before, vehicle-treated rats showed a preference for the incongruent object as reflected by their increased levels of exploration of this object compared with the congruent one (Fig. 1C; $t_{(15)} = 2.54$, $p = 0.0223$). In contrast, rats infused with MDL 11,939 showed no preference for any of the objects. There were no significant differences in the total levels of exploration across groups, suggesting that the blockade of 5-HT_{2A} affected the capability to discriminate in which context they had seen the objects and not their exploratory drive. This result strongly suggests that blockade of mPFC 5-HT_{2A} affects the capability of the animals to discriminate between two known objects that have been previously shown in different contexts.

Blockade of mPFC 5-HT_{2A} disrupts the temporal order object recognition task

The TMOR measures the ability of the animals to discriminate the relative recency with which they have seen two different objects. This task is a three-trial procedure, composed of two sample trials separated by 1 h and a retention trial 3 h later. It has been shown that rats display a preference for the “older” object during the retention trial and this has been taken as a measure of recency memory (Barker et al., 2007). Rats were trained in TMOR and their memory was tested. Total exploration times during testing showed no differences between groups (see Table 2). Injection of MDL 11,939 in the mPFC 15 min before the retention test affected the animals response in the TMOR compared with the animals injected with vehicle (Fig. 2B; $t_{(12)} = 2.85$, $p = 0.0146$). The discrimination ratio shows that rats infused with MDL 11,939 explored both objects to the same extent showing no recency discrimination. There were no significant differences in level of exploration during the training phase (see Table 1).

No effect of mPFC 5-HT_{2A} blockade in the SOR task or in the object-location task

The amnesia observed in the TMOR and OIC tasks could be simply explained by a deficit in object recognition, that is, the rats are forgetting both objects, independently of whether they were explored more or less recently or in different contexts. To investigate whether mPFC 5-HT_{2A} is also necessary for an SOR in which no requirement is imposed for associating objects and context or order, we tested rats in an SOR task, which implies one sample trial and a retention trial 3 h later. Infusion of MDL 11,939 into the mPFC 15 min before the retention test did not affect the ability of the rats to discriminate between a familiar and a novel object as it is shown by the discrimination ratio (Fig. 3B; $t_{(13)} = 1.207$, $p = 0.2490$) neither did it affect the total exploration time (see Table 2). These results suggest that the activity of 5-HT_{2A} in the mPFC is not necessary for retention in the SOR task and thus the deficit in the TMOR and OIC tasks cannot be explained by an impairment in object discrimination per se. Another possibility is that blockade of mPFC 5-HT_{2A} impairs retrieval of

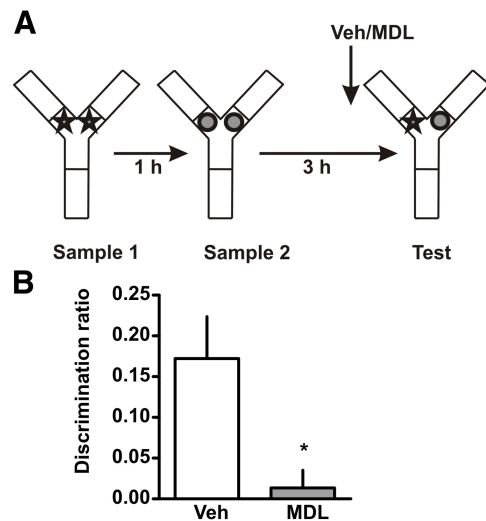


Figure 2. Blockade of 5-HT_{2A} in mPFC impairs recency discrimination during retrieval. **A**, Training and testing scheme. Animals were exposed to an arena containing two identical copies of an object and an hour later re-exposed to the same arena containing two identical copies of a different object. Three hours later rats were exposed to the same context containing one copy of each object. **B**, Fifteen minutes before the test session rats were infused with 300 ng/μl MDL 11,939 or vehicle (Veh) into the mPFC. Discrimination index was calculated as the time spent exploring the older object minus the time spent exploring the recent object over the total exploration time during the test session; $n = 7$ per group, * $p < 0.05$, Student's *t* test.

the context, a treatment that could cause the deficit in the OIC task. This can be ruled out since infusion of MDL 11,939 into the mPFC 15 min before the retention test did not affect performance in an object-location task (Fig. 3D). This task implies one sample phase and a retention trial phase (Fig. 3C). Novelty is determined by a change in the location of one of the objects, but not the object identity. If retrieval of the context had been affected, then rats should have explored both objects equally during the retention test (and show a lower discrimination ratio), but there were no differences between groups.

Blockade of mPFC 5-HT_{2A} does not affect attention and perception

The mPFC is highly involved in attention and perception processes as has been shown across different tasks (Robbins, 1996; Miller and Cohen, 2001). Since our results could be explained by differences in attention or perception we decided to directly test this hypothesis using the SOD task (Bartko et al., 2007). This task consists of a single trial in which animals are exposed simultaneously to three objects from which two of them are identical (Fig. 3E). It has been shown that animals tend to divide their exploratory time between the two identical copies resulting in an apparent preference for the single object. We found that independently of the infusion of MDL 11,939 into the mPFC, both groups explore more the odd object (Fig. 3F; $t_{(12)} = 0.3181$, $p = 0.7559$) with no significant differences in the total exploration time (see Table 2), suggesting that the differences observed in the OIC and TMOR tasks are not due to changes in attention or perception as a result of the infusion of the 5-HT_{2A} antagonist.

Performance is not affected by blockade of mPFC 5-HT_{2A} if a familiar object is replaced by a novel object after OIC training

The next set of experiments was focused in understanding the nature of the OIC task, as we wanted to assess the mechanisms involved in the what, where aspect of episodic memory. Blockade of mPFC

5-HT_{2A}R precluded the capacity of the rats to discriminate between congruent and incongruent objects in the OIC task. Thus, one hypothesis could be that the infusion of MDL 11,939 causes forgetting of both objects in this different setup (i.e., two contexts and two sets of familiar objects). To test this hypothesis we designed a similar experiment in which animals were trained in the same OIC task as described before but the incongruent object was swapped for a novel object during the testing phase (Fig. 4A). We found that both groups, independently of the infusion of MDL 11,939 or vehicle, spent more time exploring the novel object compared with the congruent one (Fig. 4B; $t_{(16)} = 0.6017$, $p = 0.5558$). Although this experiment suggests that animals infused with MDL 11,939 are able to recognize novelty, we modified the OIC task to assess the level of exploration of the congruent, incongruent, and novel object simultaneously. This design allowed us to evaluate if the blockade of mPFC 5-HT_{2A}R produced a deficit in the retrieval of the previously known objects (both objects are seen as novel) or if the blockade allowed interference between the traces of the two known objects (both objects are seen as congruent or incongruent). In animals infused with vehicle, we found a significant difference in the level of exploration across the three objects (Fig. 4D; one-way ANOVA, $F_{(3)} = 72.77$, $p < 0.0001$). *Post hoc* analysis (Newman–Keuls multiple comparisons) showed that they significantly explored more the novel object than the other two ($p < 0.05$) and explored more the incongruent than the congruent one ($p < 0.05$). However, the animals infused with MDL 11,939 showed a different pattern of exploration. An ANOVA analysis showed there were significant differences in the level of exploration ($F_{(3)} = 12.20$, $p < 0.0001$). They significantly explored the novel object more than the other two (Fig. 4D, right; $p < 0.05$) but showed no significant differences in the level of exploration between the congruent and the incongruent one ($p > 0.05$). Interestingly, the level of exploration of both objects is similar to the level of exploration of the incongruent one from the control group. Thus, SOR is unaffected by MDL 11,939, but the OIC component of the task is abolished by this treatment. This means that rats remember having seen the objects, but they do not remember in which context they have seen them.

Blockade of mPFC 5-HT_{2A}R but no mPFC 5-HT_{2C}R affects the OIC task

Although the 5-HT_{2A}R is one of the main excitatory serotonergic receptors in the cortex, there are other serotonergic receptors expressed in cortical cells, like the 5-HT_{2C}R. 5-HT_{2C}R are closely related to the 5-HT_{2A}R and MDL 11,939 has been shown to have some affinity for them (Pehek et al., 2006). For that reason, we wanted to evaluate the role of the 5-HT_{2C}R in the OIC task. We found that the infusion of SB 242058, a potent and very specific 5-HT_{2C}R antagonist (Kennett et al., 1997), did not affect

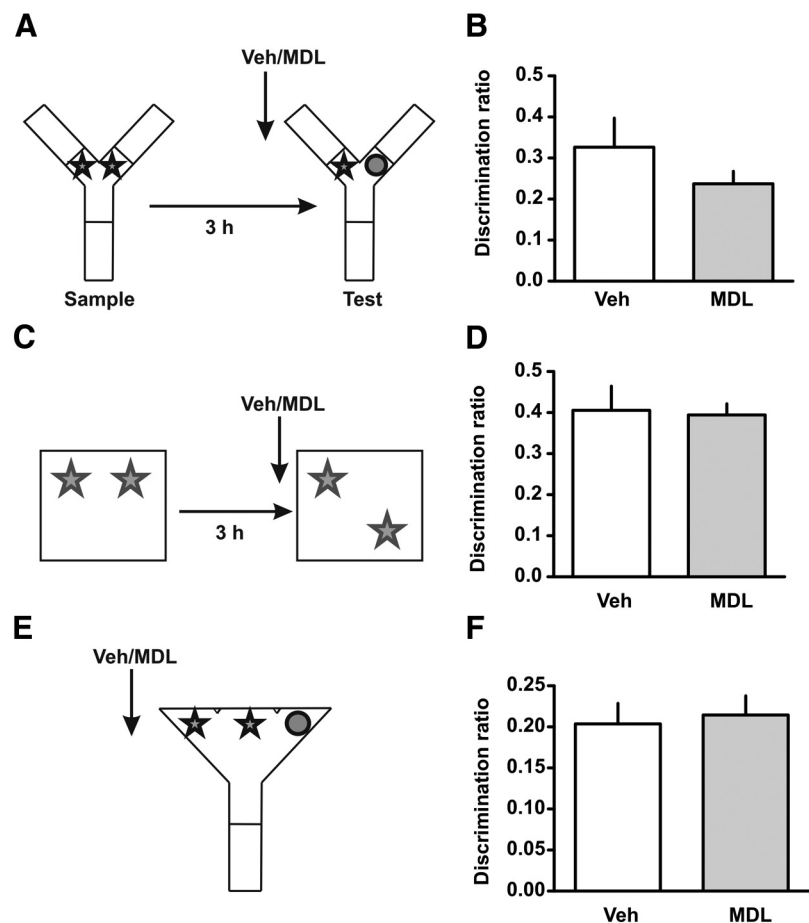


Figure 3. Spontaneous novel object recognition, objection location, and perception are not affected by the blockade of 5-HT_{2A}R in mPFC. **A**, Training and testing scheme for the SOR. **B**, Animals were trained in SOR and 15 min before the test session were infused with 300 ng/ μ l MDL 11,939 or vehicle (Veh) into the mPFC. Discrimination index was calculated as the time spent exploring the novel object minus the time spent exploring the familiar object over the total exploration time during the test session. **C**, Training and testing scheme for the object-location task. **D**, Fifteen minutes before testing rats were infused with MDL 11,939 or Veh into the mPFC. Discrimination index was calculated as the time exploring the object in the novel location minus the time exploring the object in the familiar location over the total exploration time during the test session. **E**, Scheme for the SOR paradigm. **F**, Fifteen minutes before the experiment rats were infused with 300 ng/ μ l MDL 11,939 or vehicle (Veh) into the mPFC. Discrimination index was calculated as time spent exploring the odd object minus half mean of the time exploring the repeated object divided by total exploratory time. $n = 4–8$ per group, $p > 0.2$, Student's *t* test.

the response of the animals in the OIC task (Fig. 5B; one-way ANOVA followed by a *post hoc* analysis; $F_{(4)} = 6.685$, $p > 0.005$), strongly suggesting that the effect described before is due to the blockade of 5-HT_{2A}R specifically.

Activation of 5-HT_{1A}R also affects the OIC task

5-HT_{1A}R are the main serotonergic receptors in the cortex and are coupled to inhibitory G_i proteins. It has been shown that a high proportion of cortical cells coexpress 5-HT_{1A}R and 5-HT_{2A}R (Amargós-Bosch et al., 2004; Andrade, 2011). When we infused a 5-HT_{1A}R agonist (8-OH DPAT) into the mPFC before the retention test, we found a deficit in the level of discrimination between the congruent and incongruent object, similar to the one described for the 5-HT_{2A}R antagonist in the OIC task (ANOVA, $F_{(4)} = 6.685$, $p < 0.05$; Fig. 5B). Activating 5-HT_{1A}R also affected the total exploratory time, something that we did not see for any of the other drugs used. These results indicate that serotonin signaling in mPFC controls retrieval through both the 5-HT_{2A}R and the 5-HT_{1A}R.

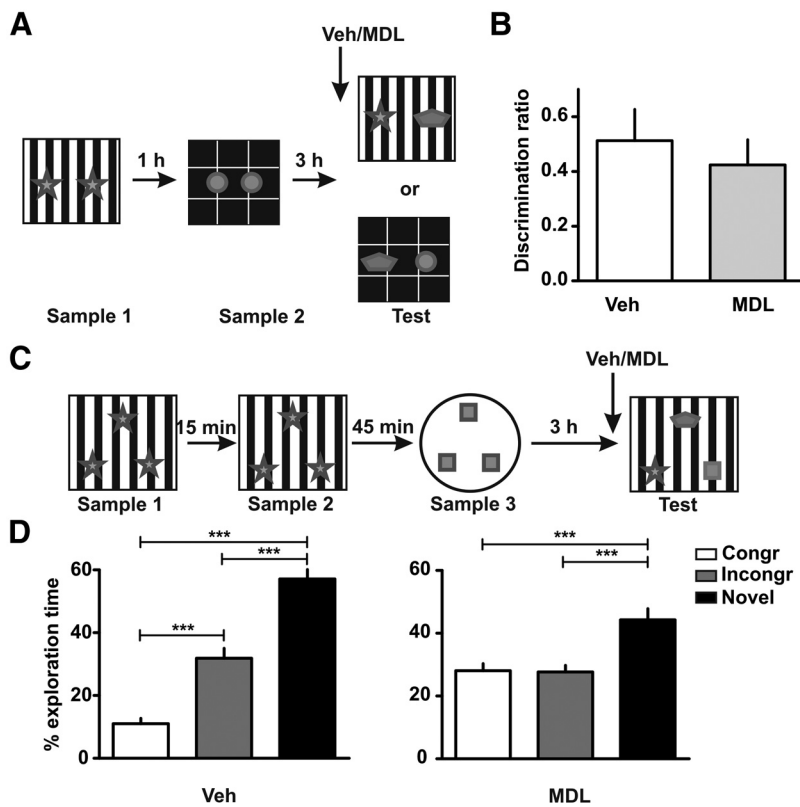


Figure 4. Blockade of 5-HT2AR in the mPFC before retrieval does not affect recognition of a novel object. **A**, Training and testing scheme. The training session was identical to the OIC task. For the testing session the incongruent object was replaced by a novel object. **B**, Fifteen minutes before testing rats were infused with MDL 11,939 or vehicle (Veh) into the mPFC. Discrimination index was calculated as the time spent exploring the novel object minus the time spent exploring the congruent object over the total exploratory time during the test session. **C**, Training and testing scheme for a three-object design. The training scheme is similar to the OIC except that the rats were exposed on three sample phases to three copies of each of the two objects (two sample phases for object A and one for object B). **D**, Fifteen minutes before the testing session rats were infused with MDL 11,939 or Veh. Rats were exposed to one of the two contexts where one of the objects was replaced by a novel one. Exploratory time is expressed as percentage of time exploring each object for Veh-infused animals (left) and MDL 11,939-infused animals (right). $n = 9–12$ per group, $***p < 0.0001$, one-way ANOVA followed by Newman–Keuls multiple comparisons.

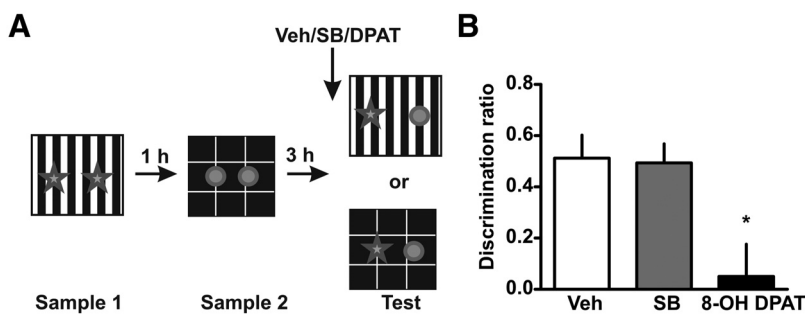


Figure 5. Activation of 5-HT1AR but not blockade of 5-HT2CR in the mPFC before retrieval affect OIC recognition. **A**, Training and testing scheme. **B**, 5-HT1ARs, but not 5-HT2CRs, are involved in the serotonergic modulation of mPFC activity during retrieval of recognition memory. 8-OH DPAT or SB 242084 were infused in the mPFC 15 min before retrieval; $n = 4–5$ per group, $*p < 0.05$, Student's *t* test.

Connectivity between the mPFC and hippocampus is required for the OIC task

We next wanted to analyze whether mPFC was involved in the retrieval of hippocampal memory traces. For this experiment, rats were implanted with dwelling cannulae in both the mPFC and the dorsal hippocampus. We had four different groups that were simultaneously tested: (1) rats injected bilaterally with MDL 11,939 in the mPFC and vehicle in the hippocampus (MDL/Veh), (2) rats injected bilaterally with vehicle in the mPFC and muscimol in the hippocampus (Veh/Musc), (3) rats injected with MDL 11,939 in mPFC on one hemisphere and muscimol in the hippocampus in the contralateral one (MDL/Musc contra), and (4) rats injected with MDL 11,939 in mPFC in one hemisphere and muscimol in the hippocampus of the same hemisphere (MDL/Musc ipsi). An ANOVA analysis showed there were significant differences between the groups ($F_{(4)} = 5.529, p < 0.005$). We first confirmed our previous experiment showing that rats infused bilaterally with MDL 11,939 in the mPFC and vehicle in the hippocampus (MDL/Veh group) showed a deficit in the OIC task as described previously (Fig. 6C). Then, the effect of bilateral inactivation of the hippocampus was tested in the Veh/Musc group. Bilateral infusion of muscimol into the hippocampus produced a profound deficit in the OIC task ($p < 0.05$), similar to the one observed by blockade of the 5-HT2AR in the cortex (Fig. 6C). The bilateral inactivation groups showed that both the 5-HT2AR in the mPFC and activity in the hippocampus are necessary for the correct resolution during the OIC task. To further investigate whether the interaction between the two structures is also necessary in the OIC task, we tested the MDL/Musc contralateral group. We found a deficit similar to the one observed for the bilateral inactivation of the hippocampus or bilateral infusion of MDL into the mPFC (Fig. 6C; $p < 0.05$, for multiple comparisons). Other disconnection studies suggest that, as described for the hippocampus and perirhinal cortex, the more dominant interactions between the mPFC and the hippocampus are ipsilateral (Churchwell et al., 2010; Churchwell and Kesner, 2011). To confirm that the main interaction between the mPFC and hippocampus is ipsilateral, we analyzed the effect of the ipsilateral infusion of MDL 11,939 in the mPFC and muscimol into the hippocampus (MDL/Musc ipsi group). This procedure should allow normal interactions between the mPFC and hippocampus in one hemisphere. We found normal levels of discrimination in the OIC in this group (Fig. 6C; $p > 0.05$, multiple comparisons). These results suggest that the interaction between mPFC and the hippocampus is required during retrieval in the OIC task.

Discussion

This study examined the role of mPFC 5-HT2R during retrieval of different versions of the novel object preference task that involve different types of to-be-remembered information. Our main findings are that (1) 5HT2AR in mPFC is required for retrieval of object recognition memory only in the cases in which the task cannot be solved by a single item strategy and (2) connectivity between mPFC and the hippocampus is necessary for

retrieval of an OIC memory. The SOR can be solved by using an item-only strategy, but TMOR and OIC tasks require that the animal remembers an association of the objects and their relative position in time or to the objects and the context in which they have seen them. Retrieval of both TMOR and OIC memories, but not SOR memory, requires 5-HT_{2A} function in mPFC. This might indicate that there is something qualitatively different between these behavioral paradigms in terms of mPFC requirement during retrieval.

Other studies have previously dissociated mPFC requirements in object preference tasks. For example, Barker et al. (2007) found that mPFC excitotoxic lesions affected performance in TMOR and in an object-in-place task in which the animals have to remember which object has been seen and where it was. mPFC lesions did not have any effect in an SOR or in an object-location task in which one of the two objects seen during the study phase is displaced to a novel location during test. However, ours is the first study, to our knowledge, to establish a role for mPFC serotonin receptors in the process of remembering this type of information. Moreover, our results establish that serotonergic signaling in mPFC is coupled to hippocampal activity during retrieval of the OIC task.

Our results are consistent with other animal studies like the ones mentioned, and also with human findings indicating that damage to dorsolateral PFC moderately affects standard item recognition (Wheeler and Stuss, 2003), but profoundly affects performance in tasks that require a recollection type of strategy (Janowsky et al., 1989; Gershberg and Shimamura, 1995; Alexander et al., 2003).

What is the mechanism by which mPFC participates in TMOR and OIC performance during retrieval? One possibility is that after blockade of 5-HT_{2A} in mPFC, rats are not able to appropriately select between competing memory traces, like during extinction (Quirk et al., 2006). During the test session of the OIC task, both objects are familiar, so the rat must be able to retrieve the object-context association rather than the item-only memory trace to select the relevant behavior. Thus, blockade of 5-HT_{2A} in mPFC might result in failure to identify a specific combination of an object and a context. It is unlikely that rats injected with the 5-HT_{2A} antagonist are impaired at remembering the context, since this treatment does not affect retrieval in an object-location task (Barker et al., 2007). In this sense, Navawongse and Eichenbaum (2013) have shown that mPFC inactivation in the rat decreases selectivity of hippocampal place cells for particular object-location combinations in a context-guided object association task. This is consistent with the view that mPFC does not participate in the spatial representation, but biases retrieval and selection of the appropriate contextual memory. Another possibility might be that the animals do identify the correct memory

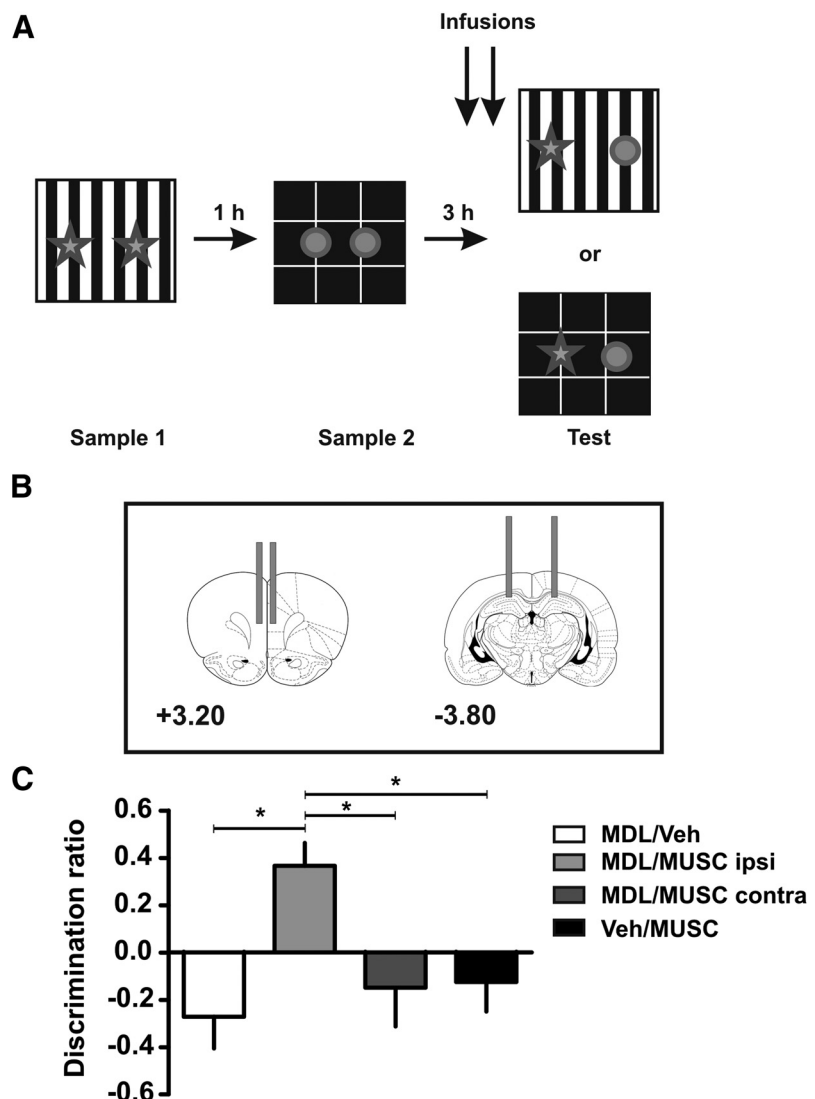


Figure 6. Blockade of 5-HT_{2A} in mPFC and activity in the hippocampus contralaterally affects the retrieval in the OIC task. **A**, Training and testing scheme. **B**, Illustration of the locations of the injection cannula tips in the mPFC and hippocampus. The numbers show distances from bregma. **C**, Effects of either contralateral (MDL/MUSC contra) or ipsilateral (MDL/MUSC ipsi) disconnection between the mPFC and hippocampus on performance during the OIC task. MDL 11,939 or vehicle (Veh) was infused in mPFC while muscimol (MUSC) or Veh was infused into the dorsal hippocampus. Deficits in the ability to discriminate between congruent and incongruent objects were found with the MDL/MUSC contra inactivation, as well as in the control groups (MDL/Veh and Veh/MUSC) but not in the MDL/MUSC ipsi condition. MDL 11,939 and muscimol were infused 15 min before the test session; $n = 5-9$ per group, $*p < 0.05$ ANOVA followed by Tukey's multiple comparisons.

traces, but are unable to select the appropriate motor response. Our experiments do not allow distinguishing between these two alternatives.

An interesting explanation could be that the animals fail at source monitoring, the ability to identify the source of remembered information (Johnson et al., 1993), for example, identifying when or where certain information was acquired. This process is suggested to be an essential part of episodic memory retrieval. Failure in source monitoring might be accompanied by misidentification of items or false memories (Johnson et al., 1993; Farovik et al., 2008). The experiment depicted in Figure 5 indicates that normal animals show an increasing degree of preference for congruent, incongruent, and novel objects. However, infusion of MDL 11,939 in mPFC equalizes exploration levels for congruent and incongruent objects, sparing novel object preference. Our results suggest that the animals do remember having

seen the objects, they just do not remember in which context they have seen them. Alternatively, the animals might falsely recall having seen the incongruent object in the testing context. Absolute levels of exploration suggest that the results could be a mixture of both impairments.

In agreement with our results, older human adults carrying a short allele of the serotonin transporter show an impairment in a source memory recognition task associated with less neural activity in PFC, compared with control carrying the long allele (Pacheco et al., 2012). This suggests that 5-HT signaling in PFC, possibly through activation of 5-HT_{2A}, is important for episodic memory in humans.

The PFC is highly enriched in 5-HT_{1A} and 5-HT_{2A}. In particular, 5-HT_{2A} is the main excitatory receptor type in the PFC. There is clear evidence that activation of 5-HT_{2A} modulates the response of PFC cells (Andrade, 2011). Interestingly, 5-HT_{1A} (a G_i-coupled receptor) and 5-HT_{2A} appear to be coexpressed in a large fraction of pyramidal cortical cells. This suggests that 5-HT_{1A} and 5-HT_{2A} receptors may cooperate to regulate how pyramidal neurons encode excitatory inputs (Araneda and Andrade, 1991; Béique et al., 2004). Interestingly, our results indicate that 5-HT_{1A} and 5-HT_{2A} receptors in mPFC might have opposite functions during retrieval of the OIC memory. This suggests that serotonin might exert a fine regulation over memory retrieval. Slice electrophysiological experiments indicate that one possible mechanism by which they could be doing this is by modulating the gain of mPFC neurons. In addition, *in vivo* blockade of 5-HT_{2A} in the mPFC affects gamma oscillations, suggesting that 5-HT_{2A} regulates the function of this structure at a system level (Puig and Gullledge, 2011). Although our experiments do not address how blocking 5-HT_{2A} might be affecting interference resolution, we could hypothesize that blocking 5-HT_{2A} during retrieval might alter the gain modulation of the cells and also the gamma oscillation activity of the system, altering the output to target structures such as the hippocampus.

It has recently been shown that the mPFC is connected with the hippocampus indirectly through the nucleus reuniens of the thalamus (Xu and Südhof, 2013). These authors very elegantly show that blocking the mPFC projections to the nucleus reuniens affects the activity of the hippocampus and increases the levels of generalization of a fear memory, suggesting that the three structures form a circuit involved in memory generalization. Our own experiments suggest that there is a functional connection between the mPFC and the hippocampus that is important for the correct performance in the OIC task. Then, we can hypothesize that blocking 5-HT_{2A} in the mPFC might result in changes to the nucleus reuniens and consequently its connection with the hippocampus. In summary, we have shown that serotonin signaling through the 5-HT_{2A} is required for the correct capability of the mPFC to guide retrieval of the more relevant memory trace. Retrieval of an episodic memory can be triggered by particular cues belonging to the original memory but that are usually common to other memory traces. We believe that the role of the mPFC could be to suppress memory interference allowing the selection of the correct memory trace during retrieval. Our results point at serotonin as a modulator of this process by means of the activation of 5-HT_{2A}. This mechanism could be seen as part of a broader set of cognitive functions. Accumulating evidence suggests that 5-HT_{2A}s are involved in behavioral flexibility. Interestingly, a recent paper has shown that systemic injection of 5-HT_{2A} antagonist enhances strategy switching by inhibiting responses on a previously relevant strategy (Baker et al., 2011).

One can argue that this process might require the inhibition of memory traces related to the previous strategy used to allow a change in the behavioral response. In this way, blockade of 5-HT_{2A} might impair retrieval of the previous strategy making it easier to learn the new one. Behavioral flexibility, as a part of cognitive flexibility processes is affected in different psychiatric conditions such as obsessive compulsive disorder (El Mansari and Blier, 2006), autism spectrum disorders (Brune et al., 2006), and schizophrenia (Clarke et al., 2004; Uçok et al., 2007). Selection of the relevant memory traces and the control of retrieval might be crucial for behavioral flexibility. In this scenario, specific pharmacological manipulations of the 5-HT_{2A} could ameliorate cognitive deficits associated with these disorders.

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