



Hippocampal and prefrontal dopamine D_{1/5} receptor involvement in the memory-enhancing effect of reboxetine

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Abstract

Dopamine modulates cognitive functions through regulation of synaptic transmission and plasticity in the hippocampus and prefrontal cortex (PFC). Thus, dopamine dysfunction in depression may be particularly relevant for the cognitive symptoms. The norepinephrine transporter inhibitor reboxetine facilitates memory processing in both healthy volunteers and in depressed patients and increases dopamine release in both the hippocampus and PFC. We investigated the potential involvement of the hippocampal and PFC dopamine D_{1/5} receptors in the cognitive effects of reboxetine using the object recognition test in rats. Infusion of the D_{1/5} antagonist SCH23390 into the dorsal hippocampus or medial PFC prior to the exploration of the objects impaired memory. Conversely, infusion of the D_{1/5} agonist SKF81297 into the dorsal hippocampus or medial PFC facilitated memory. Reboxetine similarly facilitated recognition memory in healthy rats and the D_{1/5} antagonist SCH23390 reversed this effect when infused into the dorsal PFC, but not when infused into the hippocampus. Moreover, systemic reboxetine increased the levels of the NMDA subunit GluN2A in the PFC but not in the hippocampus. Finally, we demonstrate that a single dose of reboxetine does not affect immobility in the forced swim test but improves recognition memory in the Flinders sensitive line (FSL) rat model for depression. The present data in rats are in line with effects of reboxetine on memory formation in healthy volunteers and depressed patients and indicate the involvement of PFC dopamine D_{1/5} receptors.

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Introduction

Dopamine modulates a range of higher cognitive functions including attention, reward, working memory as well as episodic memory. Of particular interest for this work, dopamine is a mediator of long-term memory formation for novel information and motivationally salient events (Shohamy and Adcock, 2010). These functions are attributed to regulation of synaptic transmission and plasticity by the dopaminergic projections from the ventral tegmental area (VTA) to the hippocampus and prefrontal cortex (PFC; Lisman and

Grace, 2005; Robbins and Arnsten, 2009). It has been shown that novelty or unexpected reward enhance activity of dopamine neurons in the VTA (Ljungberg et al., 1992; Nakahara et al., 2004; Cohen et al., 2012) and increase dopamine release in the hippocampus and PFC (Ihalainen et al., 1999; Bassareo et al., 2002). At the cellular level, stimulation of dopamine D_{1/5} receptors in either brain area enhances synaptic transmission and plasticity (Gurden et al., 2000; Seamans et al., 2001; Lemon and Manahan-Vaughan, 2006; Ortiz et al., 2010; Xu and Yao, 2010), a well-known mechanism of memory formation (Neves et al., 2008).

There is evidence for dopamine dysfunction in depression (Nestler and Carlezon, 2006) and dopamine can be an important antidepressant target in addition to serotonin and norepinephrine systems (Willner, 1997; Chaudhury et al., 2013). Dopamine dysfunction may be particularly relevant for the cognitive

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symptoms of the disease. Indeed, depression is characterized by both emotional and cognitive symptoms and interestingly, the cognitive symptoms include deficits in attention, working memory and episodic memory (Disner et al., 2011; Millan et al., 2012). These symptoms have been linked to anatomical and functional abnormalities of the hippocampus and PFC (Pittenger et al., 2008; Clark et al., 2009). Cognitive symptoms of depression often persist after remission of other psychopathological symptoms and reduced memory processing for positive information has been proposed as a fundamental factor for negative thinking and low mood in cognitive psychology models of depression. As such, these cognitive symptoms are an important risk factor for relapse of emotional symptoms (Disner et al., 2011; Millan et al., 2012) and the modulatory effects of dopamine on memory and plasticity in the hippocampus and PFC makes the dopamine system a potential target for antidepressant drugs.

It has been suggested that antidepressants which inhibit the norepinephrine transporter (NET) are more effective in improving cognitive function compared to antidepressants that selectively block the serotonin transporter (Ferguson et al., 2003; Herrera-Guzman et al., 2009). The antidepressant drug reboxetine selectively blocks NET and improves memory for information with positive valence in both healthy volunteers and depressed patients (Harmer et al., 2003, 2009). There is evidence for memory-enhancing effects of norepinephrine, but the mechanisms of action are not fully understood (Swanson-Park et al., 1999; Nirogi, et al., 2012; Warner and Drugan, 2012). Interestingly, reboxetine increases dopamine levels in both the hippocampus and PFC (Linner et al., 2001; Borgkvist et al., 2011). We therefore proposed activation of dopamine receptors in these brain regions as a potential mechanism for reboxetine's memory-enhancing effects. We used the novel object recognition test to investigate the hypothesis. This test is widely used as a model for episodic-like memory in rodents (Ennaceur and Delacour, 1988) and can be considered as a rodent model for positive memory formation (Bevins and Besheer, 2005). Consistent with this view we previously demonstrated deficits in exploratory motivation and recognition memory in the Flinders sensitive line (FSL) rat model for depression (Gomez-Galan et al., 2012). We investigated the modulatory role of dorsal hippocampal and medial prefrontal dopamine D_{1/5} receptors in object recognition memory and the facilitatory effect of reboxetine in this test. The effect of reboxetine on recognition memory was further validated in FSL rats.

Method

Animals and housing

All experiments were carried out on male Sprague-Dawley (SD) rats (Charles-River Laboratories, France or The Netherlands) or FSL rats that were bred in-house. The rats were aged 2–3 months at the time of testing. Animals were housed under standard laboratory conditions (20–22 °C and 50–60% humidity). Animals that underwent surgery were single-housed. General procedures are summarized in Fig. 1a. The experiments were approved by the Stockholm North Committee on Ethics of Animal Experimentation.

Surgery

Rats were anaesthetized with a mixture of Hypnorm (0.315 mg/ml fentanyl citrate and 10 mg/ml fluanisone; Janssen-Cilag, UK) and Dormicum [5 mg/ml midazolam; Roche AB, Sweden (5 ml/kg i.p.)]. Guide cannulae (Plastics One, USA) were implanted above the CA1 fields of the left and right dorsal hippocampus (mediolateral: ±3.0 mm, anteroposterior: –4.2 mm, dorsoventral: –1.3 mm relative to bregma and dura surface, with 0° angle from the vertical axis in the coronal plane) or above the prelimbic fields of the left and right medial PFC (mediolateral: ±2.5 mm, anteroposterior: +2.0 mm, dorsoventral: –3.0 mm relative to bregma and dura surface, with 20° angle from the vertical axis in the coronal plane) according to the stereotaxic atlas of Paxinos and Watson (1998). The implantation site was histologically verified post-mortem.

Object recognition testing

The object recognition testing was performed in a Plexiglas box (length, 80 cm; width, 35 cm; height, 35 cm) with patterned sides, as previously described (Gomez-Galan et al., 2012). The objects were constructed out of plastic toys. Rats were habituated to the test box for 20 min on the day prior to the training session. During the training session (S1) rats were allowed to freely explore the objects for 2 or 15 min, after which they were returned to their home cages. A 5 min test session (S2) was performed following a 24 h retention interval. One of the objects of the training session was replaced by a replicate object and the other by a novel object. In the experiments with FSL rats, the active object exploration time during the 15 min S1 was measured and the SD controls were allowed the same active object exploration time and were then taken out of the box, to correct for lower active object exploration in FSL rats. The behaviour

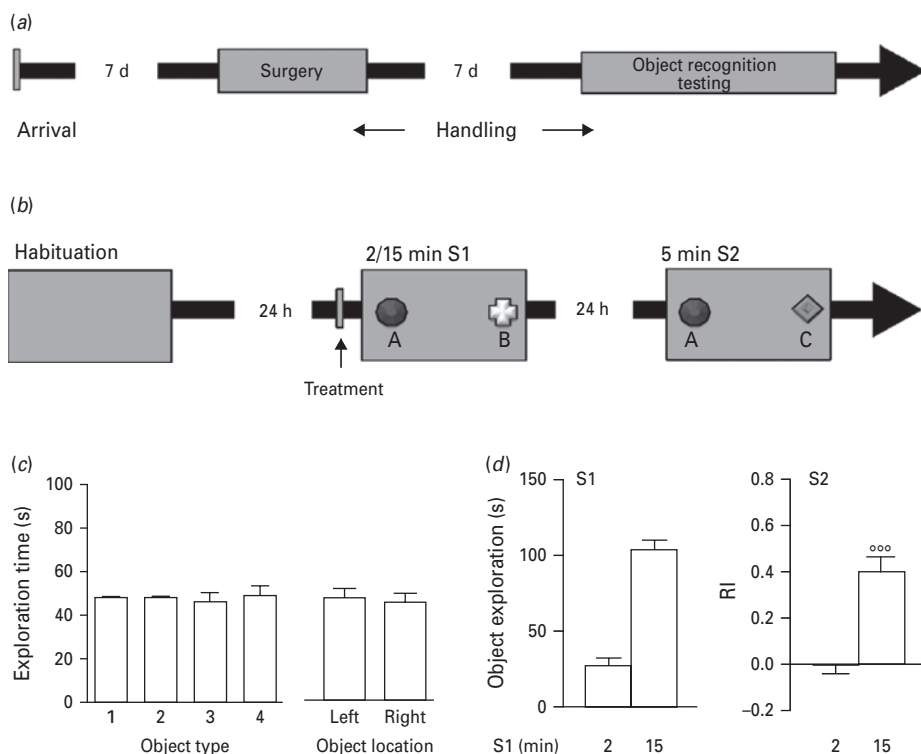


Fig. 1. Experimental design. (a) Following an acclimatization period, rats underwent stereotaxic surgery with bilateral cannulation for experiments with drug administration in the dorsal hippocampus or medial prefrontal cortex. Object recognition testing was carried out after a recovery period during which the animals were handled and habituated to drug administration procedures. (b) Object recognition testing was carried out on three consecutive days. On the first day, animals were habituated to the test box. On the following day, animals received drug treatments and were then allowed to explore a first set of objects (A, B) during the training session (S1). On the final day, the animals were allowed to explore a second set of objects (A, C) to evaluate long-term memory retention during the test session (S2). (c) The rats did not show preference for the objects ($n=6$ per object type) or object locations ($n=12$ per object location) during training (d) Rats spent significantly less time exploring the objects in a 2 min S1 compared to a 15 min S1. Free object exploration during a 2 min S1 did not result in significant preference for the novel object during S2 (right panel, $n=14$). Free object exploration during 15 min S1 (left panel) induced significant preference for the novel object during S2 (right panel, $n=12$). RI, Recognition index. *** $p < 0.001$ between groups; °°° $p < 0.001$ vs. chance level (0).

in each trial was filmed and the same observer blinded to the experimental conditions scored the exploration time for all experiments. Exploration was defined as sniffing, biting, licking or touching the object with the nose while facing it. A recognition index (RI) was calculated as $(C-A)/(C+A)$, with C being the time spent exploring the novel object and A being the time spent exploring the familiar object during S2. Animals were excluded from the study when one of the objects was not explored at all or when the total object exploration time was shorter than 5 s for either object during a 2 min S1, shorter than 20 s for either object during a 15 min S2, or shorter than 10 s for the total of both objects during S2. To assure the validity of the scoring, two people scored the control groups independently and the correlation between

the two results for total exploration time in S1 was calculated, giving a correlation of 0.82 (Pearson's test).

Forced swim test

We used a modified version of the Porsolt forced swim test in which the rats were tested for 5 min on only 1 d, as previously described (Gomez-Galan et al., 2012). The rats were immersed in a glass cylinder with water at 25 °C. Immobility was defined as no additional movements by the rat beyond those that were required to keep the head above the water.

Drugs and administration

SCH23390 [(R)-(+)-7-Chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine hydrochloride]

and SKF81297 [(RS)-6-Chloro-2,3,4,5-tetrahydro-1-phenyl-1H-3-benzazepine hydrobromide] were purchased from Tocris (UK). Reboxetine [2-(2-ethoxyphenoxy)-phenyl-morpholine] was provided by Pharmacia and Upjohn (USA). All drugs were dissolved in sterile saline (0.9% NaCl). Intracerebral administrations were performed using 33 G stainless steel infusion cannulae (PlasticsOne, USA) connected with flexible polyvinyl chloride tubing (PlasticsOne, USA) to microsyringes (Hamilton, Switzerland) in a microinfusion pump (CMA, Sweden). Solutions were infused at a rate of $1\ \mu\text{l}/\text{min}$ over 1 min after which the infusion cannula was left in place for 1 min. In experiments with a 15 min S1, drugs were infused 15 min prior to S1 and in experiments with a 2 min S1, drugs were infused immediately prior to systemic administration of reboxetine or saline. Reboxetine or saline were injected subcutaneously (s.c.) 30 min prior to S1.

Western blot

Animals that had undergone the novel object recognition test with systemic reboxetine or saline treatment were killed at the end of S2 and the hippocampus and PFC were dissected and immediately frozen on dry ice. Samples were homogenized by sonication in 1% SDS; protein concentration was measured using a BCA kit (Pierce, USA) and equal amount of proteins were loaded on a NuPAGE bis-tris gradient gel (Novex, USA). Proteins were transferred to a PVDF membrane (Millipore, Sweden) and incubated with antibodies against GluN1 (Tocris, USA) or GluN2A (Abcam, UK) as well as actin to ensure equal loading. Fluorescent secondary antibodies were used and the signal was detected with an Odyssey system (Li-cor, USA) allowing linear quantification over a large dynamic range. The amount of proteins was quantified using Image J software (NIH, USA).

PSD-95 fractions

Animals were injected with reboxetine 24 h before killing by decapitation. PFC and hippocampus were dissected on ice and homogenized in Hepes-buffered sucrose. The homogenate was centrifuged at 1000 g for 15 min and the supernatant was removed and centrifuged for 15 min at 10000 g. The resulting pellet was washed by resuspending the pellet in Hepes-buffered sucrose and centrifuged at 10000 g for 15 min. The pellet was agitated in a Hepes solution for 30 min and then centrifuged for 30 min at 16000 g. The yielding pellet was suspended in Hepes-buffered sucrose and put on top of a sucrose concentration gradient with

concentrations of 0.8, 1.0 and 1.2 M respectively and centrifuged in a swinging bucket rotor at 150000 g for 2 h. The layer between the 1.0 and 1.2 M sucrose solutions was extracted, diluted in 4 mM Hepes solution and centrifuged at 150000 g for 30 min. The resulting pellet was then resuspended in a solution containing 50 mM Hepes, 2 mM EDTA and 0.5% Triton X-100 and left to agitate for 15 min at 4 °C. The solution was then centrifuged at 16000 g for 40 min and the remaining pellet was suspended in 1% SDS and frozen until used for Western blot as described earlier. For PSD-95 fractions equal volume was loaded and the amount of GluN2A was normalized to the amount of PSD-95 (antibodies from Abcam, UK).

Data analysis

Results are presented as mean \pm S.E.M. Within-group comparisons to chance levels were performed using paired two-tailed *t* test and differences between groups were analysed using the unpaired two-tailed *t* tests or one-way analysis of variance (ANOVA) followed by Newman-Keuls *post hoc* test for comparison of multiple groups where applicable. Differences were considered significant for $p \leq 0.05$. Statistical analyses were performed using GraphPad Prism 5.0 (GraphPad Prism Software Inc., USA).

Results

Experimental set-up for testing long-term object recognition memory

We first established specific experimental conditions for the object recognition test to study pharmacological disruption or enhancement of long-term memory in rats. Following surgery and handling (Fig. 1a), rats were subjected to either a 2 or a 15 min training session (S1) and a 5 min test session (S2) on the following day (Fig. 1b). No bias was observed between the different objects (15 min exploration, $F_{3,20}=0.05$, overall $p > 0.05$, $n=6$ per object type, Fig. 1a) or object location in the test box (15 min exploration, $t_{23}=1.932$, $p > 0.05$, $n=12$ per object location, Fig. 1c). Control SD rats (receiving saline into the dorsal hippocampus or medial PFC) showed lower object interaction in a 2 min S1, compared to a 15 min S1 ($t_{25}=9.26$, $p < 0.001$, $n=12-14$, Fig. 1d). Rats that were allowed to explore the objects for 2 min during S1 did not discriminate between the familiar and the novel object during S2 (RI not significantly different from 0, $t_{13}=0.14$, $p > 0.05$, $n=14$, Fig. 1d), whereas rats that were allowed to explore the objects for 15 min during S1 showed a clear preference for the novel object during S2 (RI significantly > 0 ,

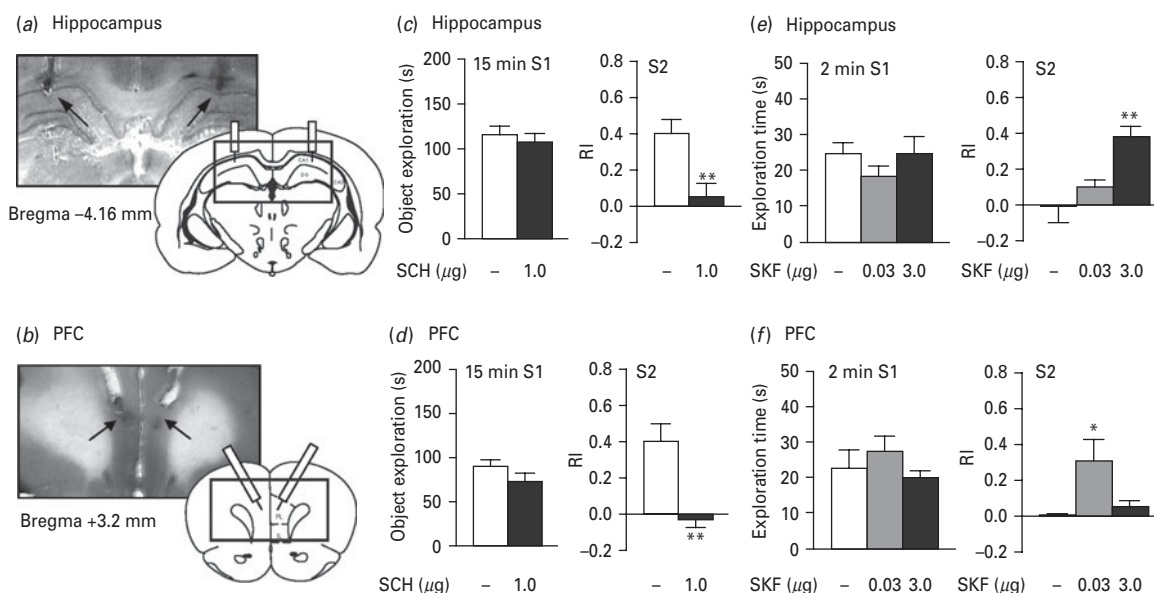


Fig. 2. Dopamine D_{1/5} receptor modulation of long-term object recognition memory. (a, b) Illustration of the infusion sites for the dorsal hippocampus (top panel) and medial prefrontal cortex (PFC; bottom panel). (c, d) Local infusion of saline or the D_{1/5} antagonist SCH23390 (SCH) into the dorsal hippocampus (top panels, $n=5-6$) or medial PFC (bottom panels, $n=6-7$) did not affect object exploration during the following training session (S1, left panels) but abolished novel object preference during the test session (S2, right panels) when using the protocol with 15 min S1. (e, f) Local infusion of the D_{1/5} agonist SKF81297 (SKF) into the dorsal hippocampus (top panels, $n=4-7$) or medial PFC (bottom panels, $n=4-7$) did not affect object exploration during the following training session (left panels) but enhanced novel object preference during the test session (right panels) at the dose of 3 μg per side in the hippocampus and 0.03 μg per side in the PFC when using the protocol with 2 min S1. RI, Recognition index. * $p < 0.05$ vs. saline; ** $p < 0.01$ vs. saline.

$t_{11}=6.1$, $p < 0.001$, $n=12$, Fig. 1d) indicating stable memory formation. Thus we have established two protocols where 15 min exploration leads to long-term memory whereas a 2 min exploration does not. The two protocols can be used to study memory impairing and facilitating effects respectively.

Modulation of object recognition memory by dopamine D_{1/5} receptors in the dorsal hippocampus and medial PFC

We next investigated the effect of local D_{1/5} antagonist or agonist infusion in the dorsal hippocampus (Fig. 2a) or medial PFC (Fig. 2b) on long-term memory formation in the object recognition task. Under conditions of persistent memory formation (15 min S1), the D_{1/5} receptor antagonist SCH23390 (1.0 μg per hemisphere) abolished novel object preference during S2 when infused 30 min prior to S1 either in the dorsal hippocampus (compared to saline controls, $t_9=3.7$, $p=0.0077$, $n=5-6$, Fig. 2c) or the medial PFC (compared to saline controls, $t_{11}=3.4$, $p=0.0078$, $n=6-7$, Fig. 2d). SCH23390 did not affect object exploration during S1 in either the dorsal hippocampus ($t_9=0.614$, $p > 0.05$,

$n=5-6$, Fig. 2c) or medial PFC ($t_{11}=1.448$, $p > 0.05$, $n=6-7$, Fig. 2d) groups. Conversely, under conditions where control rats did not remember the object 24 h later (2 min S1) we found that the D_{1/5} agonist SKF81297 dose-dependently increased exploration of the novel object during S2 when infused either in the dorsal hippocampus ($F_{2,14}=8.608$, $p=0.0036$, $n=4-7$, Fig. 2e) or medial PFC ($F_{2,15}=3.774$, $p=0.047$, $n=4-7$, Fig. 2f). Again, drug treatment did not affect object exploration during S1 in either the dorsal hippocampus ($F_{2,14}=0.812$, $n=4-7$, $p=0.46$, Fig. 2e) or medial PFC ($F_{2,15}=0.661$, $p=0.53$, $n=4-7$, Fig. 2f) groups. It should be noted that a dose of 0.03 μg SKF81297 was sufficient to improve object recognition memory when infused into the medial PFC (Newman-Keuls: $p < 0.05$ vs. saline) but had no effect in the dorsal hippocampus. Conversely, a high dose of 3 μg SKF81297 did not improve object recognition memory when infused into the PFC but induced a clear preference for the novel object during the test session when infused into the hippocampus (Newman-Keuls: $p < 0.01$ vs. saline). Our observations are consistent with other reports showing that high-affinity D_{1/5} receptor agonists improve cognitive function in low doses

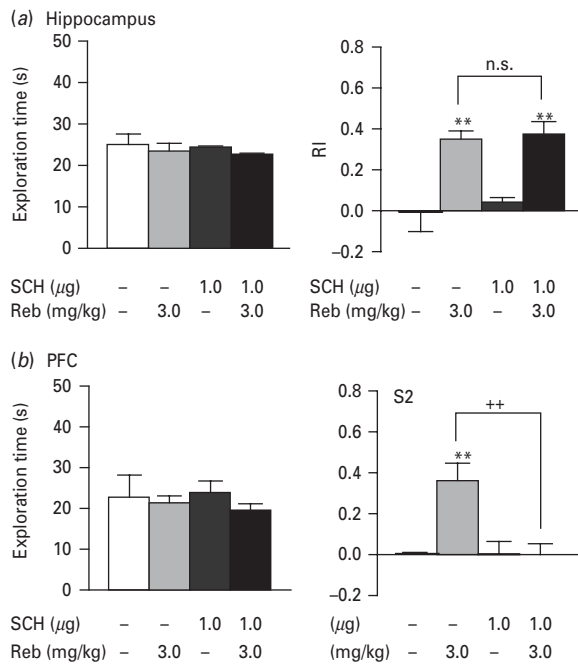


Fig. 3. Effects of reboxetine (Reb) on long-term object recognition memory. (a, b) Systemic Reb did not affect object exploration during the following training session (S1, left panels) when administered in combination with local infusions of saline or SCH23390 (SCH) into the dorsal hippocampus ($n=5-9$) or medial prefrontal cortex (PFC; $n=4-10$). Reb induced a clear preference for the novel object during the test session (S2, right panels) which was unaffected by local infusion of SCH into the dorsal hippocampus ($n=5-9$) but reversed by local infusion of SCH into the medial PFC ($n=4-10$). RI, Recognition index. ** $p < 0.01$ vs. saline/saline; ++ $p < 0.01$ Reb/saline vs. Reb/SCH.

(0.016–0.03 μ g) when infused into the medial PFC (Loiseau and Millan, 2009; Mizoguchi et al., 2009) whereas high doses (up to 10 μ g) improve memory when infused into the dorsal hippocampus (Rossato et al., 2009; da Silva et al., 2012). Taken together these results show that stimulation of the $D_{1/5}$ receptor in either the dorsal hippocampus or the medial PFC can facilitate long-term memory formation, whereas blocking these receptors during exploration blocks long-term memory formation.

Effects of reboxetine on long-term object recognition memory and NMDA subunit expression in the hippocampus and PFC

Systemic administration of the NET inhibitor reboxetine (3.0 mg/kg s.c.) induced a clear preference for the novel object during S2 when given 30 min before S1. This effect of reboxetine was not reversed by SCH23390 infused locally (1.0 μ g per hemisphere

immediately before systemic reboxetine) in the dorsal hippocampus ($F_{3,23}=9.76$, $p=0.0002$; Newman-Keuls: $p < 0.01$ for saline s.c. and saline locally vs. reboxetine s.c. and saline locally; $p < 0.01$ for reboxetine s.c. and saline locally vs. reboxetine s.c. and SCH23390 locally; $p > 0.05$ for other groups comparisons; $n=5-9$, Fig. 3a). However, the effect of reboxetine on object recognition memory could be completely abolished by a local infusion of the $D_{1/5}$ antagonist SCH23390 in the medial PFC ($F_{3,25}=6.96$, $p=0.0015$; Newman-Keuls: $p < 0.01$ for saline s.c. and saline locally vs. reboxetine s.c. and saline locally; $p < 0.01$ for reboxetine s.c. and saline locally vs. reboxetine s.c. and SCH23390 locally; $p > 0.05$ for other group comparisons; $n=4-10$, Fig. 3b). Systemic administration of reboxetine (3.0 mg/kg s.c.) did not affect object exploration during the S1 phase in combination with local infusions of saline or SCH23390 into the dorsal hippocampus ($F_{3,23}=0.78$, $p > 0.05$, $n=5-9$, Fig. 3a) or medial PFC ($F_{3,25}=0.77$, overall $p > 0.05$, $n=4-10$, Fig. 3b). Taken together we show that the NET inhibitor reboxetine can enhance memory formation and that this effect depends on stimulation of dopamine $D_{1/5}$ receptors in the PFC but not in the hippocampus.

Systemic reboxetine increases NMDA subunit GluN2A levels only in the PFC

Consistent with the idea that reboxetine can enhance memory formation through modulation of activity in the PFC but not in the hippocampus we found that reboxetine enhances the levels of the NMDA subunit GluN2A in the PFC but not in the hippocampus. We measured protein levels of GluN1, the ubiquitous NMDA subunit, and GluN2A, the dominant subunit in mature central nervous system, in homogenate of the hippocampus and PFC, 24 h after systemic administration of reboxetine in animals that undertook the object recognition procedure (Fig. 3c). GluN1 levels did not change significantly following reboxetine treatment in either the hippocampus ($85 \pm 6\%$ of control) or PFC ($98 \pm 8\%$ of control) thus GluN2A levels were normalized and expressed as GluN2A/GluN1. The normalized GluN2A levels were significantly higher in the PFC but did not change significantly in the hippocampus following reboxetine treatment (Newman-Keuls: $p < 0.05$ saline PFC vs. reboxetine PFC; $n=4-7$, Fig. 4a). To validate that the increase in total levels of GluN2A proteins corresponds to an increase of receptors at the synapse we purified PSD-95 complexes and measured GluN2A per PSD-95 unit. Again, we found that reboxetine treatment induces an increase of GluN2A in reboxetine-treated animals specifically

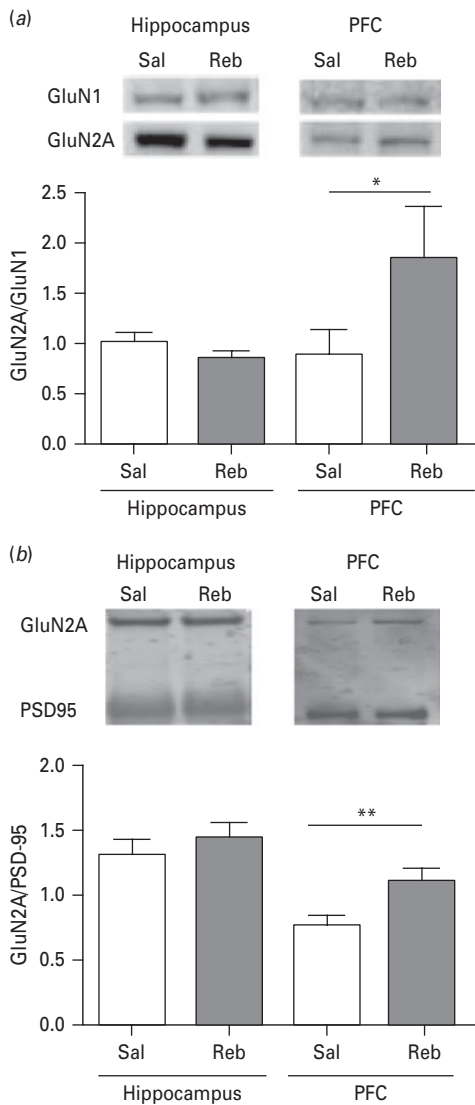


Fig. 4. Effect of reboxetine (Reb) on GluN2A and GluN1 NMDA subunits in the hippocampus and prefrontal cortex (PFC). (a) Rats received a systemic injection of saline (Sal) or Reb and went through the object recognition procedure. Representative immunoblots for GluN1 and GluN2A (top panel) and conversion to normalized GluN2A levels (bottom panel). Normalized GluN2A levels did not change significantly following Reb treatment in the hippocampus but were significantly higher in the PFC ($n=4-7$ per group). * $p<0.05$ vs. Sal. (b) GluN2A levels in the PSD-95 fraction is enhanced in the PFC but not in the hippocampus 24 h after a systemic injection of Reb. Representative immunoblots for GluN2A and PSD-95 (top panel) and amount of GluN2A normalized to PSD-95 (bottom panel); $n=6-9$ per group. ** $p<0.01$ vs. Sal.

in the PFC ($144\pm 8\%$ of control, Newman-Keuls: $p<0.01$, $n=6-9$) but not in the hippocampus (108 ± 9 of control, n.s., $n=9$).

Reboxetine modulation of object recognition memory and forced swim test immobility in FSL rats

We next confirmed the memory-facilitating effect of reboxetine in a genetic rat model for depression. FSL rats display emotional as well as cognitive symptoms of depression. We previously showed depressive-like behaviour in the forced swim test and memory dysfunction in the novel object recognition test in such rats (Gomez-Galan et al., 2012). FSL rats display low exploratory motivation compared to SD control rats in the object recognition task, leading to lower object exploration times. In the novel object recognition test we therefore matched the object exploration time of SD control rats to that of FSL rats during S1 by pairing up SD rats to FSL rats and stopping the S1 session when SD rats reached the object exploration time of the paired FSL rat during a 15 min S1. On average, SD rats required 206 ± 74 s to reach the same time of object exploration as FSL rats in a 15 min S1. Using this method, we found that a single administration of reboxetine (3.0 mg/kg i.p.) prior to the S1 reversed the recognition memory deficit in FSL rats ($F_{2,14}=8.22$, $p=0.0044$; Newman-Keuls: $p<0.05$ for SD saline vs. FSL saline; $p<0.01$ for FSL saline vs. FSL reboxetine; $n=5-6$ per group, Fig. 5a). Reboxetine did not affect object exploration during S1 ($F_{2,14}=0.06$, $p>0.05$; Newman-Keuls: $p>0.05$ for FSL saline vs. FSL reboxetine; $n=5-6$ per group, Fig. 5a), suggesting that exploratory motivation remained unaltered. The lack of effect on motivation was further confirmed when we showed that reboxetine has no acute effect in the forced swim test ($F_{2,19}=10.24$, $p<0.001$; Newman-Keuls: $p<0.01$ for SD saline vs. FSL saline; $p>0.05$ for FSL saline vs. FSL reboxetine; $n=7-8$ per group, Fig. 5b).

Discussion

Novelty has been shown to activate midbrain dopamine neurons (Ljungberg et al., 1992) and increase dopamine efflux in both the hippocampus and PFC (Ihalainen et al., 1999), providing a mechanism to enhance memory formation of new input (Lisman and Grace, 2005). Indeed, we found that sustained recognition memory, following object exploration in the presence of contextual cues, was blocked by administration of a D_{1/5} antagonist in either brain area, during the exploration phase (Fig. 2). If the exploration phase was too short however (2 min in our case), no stable memory was spontaneously formed, but D_{1/5} receptor stimulation in either the hippocampus or the PFC enabled sustained memory formation (Fig. 2).

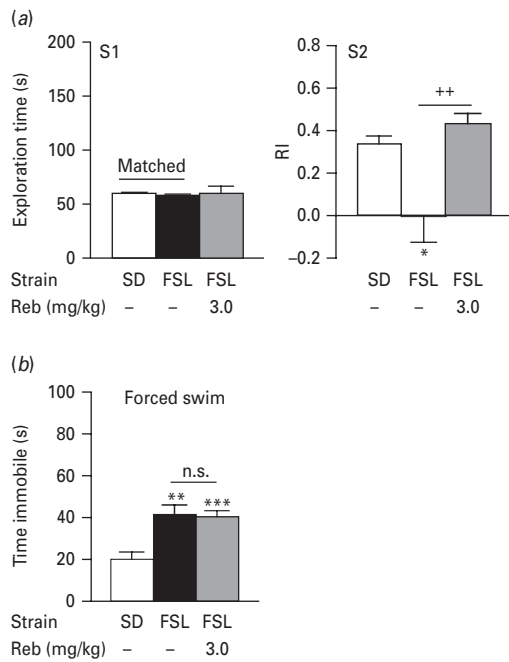


Fig. 5. Effects of reboxetine (Reb) on episodic-like memory and depression-like behavior in Flinders sensitive line (FSL) rats. (a) Reb did not affect object exploration in FSL rats during the training session of the object recognition task, but significantly increased preference for the novel object during the test session in FSL rats (right panel, $n=5-6$ per group). (a) FSL rats were more immobile compared to Sprague-Dawley (SD) rats in the forced swim test but a single dose of Reb did not affect immobility in FSL rats ($n=7-8$ per group). RI, Recognition index. * $p<0.05$ saline/FSL vs. saline/SD; ** $p<0.01$ saline/FSL vs. saline/SD; *** $p<0.001$ saline/FSL vs. saline/SD ++ $p<0.01$ saline/FSL vs. Reb/FSL.

This is well in line with previously shown memory facilitating or impairing effects of systemic administration of $D_{1/5}$ agonists and antagonists respectively (de Lima et al., 2011) and with previous studies showing that activation of $D_{1/5}$ receptors in the dorsal hippocampus and medial PFC is critically involved in the encoding of novel episodic-like memory (Bethus et al., 2005; Nagai et al., 2007). Moreover, the fact that we could abolish sustained memory formation by blocking $D_{1/5}$ receptors in either area suggests that efficient memory encoding in physiological conditions requires functional cooperation and $D_{1/5}$ activation in both the hippocampus and PFC. This finding is in agreement with lesion studies where both brain regions have been shown to interact with each other and with the perirhinal cortex to encode contextual associations in the object recognition task (Barker

et al., 2007; Barker and Warburton, 2011). Indeed, recognition memory is subdivided in distinct cognitive mechanisms: context-independent familiarity, in which the perirhinal cortex plays an essential role; context-dependent recollection, which is critically supported by the hippocampus and PFC (Eichenbaum et al., 2007; Farovik et al., 2008; Sauvage et al., 2008). Interestingly, when object exploration was restricted in time, local administration of a $D_{1/5}$ agonist in either the hippocampus or PFC was sufficient to facilitate sustained recognition memory. A level of functional redundancy may exist given that recognition memory can ultimately be sustained based on familiarity, independently of contextual associations, following lesions to both the hippocampus and PFC (Barker and Warburton, 2011). Therefore, it can be proposed that under conditions where no stable memory is formed, local pharmacological activation of $D_{1/5}$ receptors in the hippocampus or PFC may preferentially recruit either of these brain areas to facilitate memory formation, potentially by reinforcing the formation of contextual associations.

The antidepressant drug reboxetine is a NET blocker and has been shown to increase dopamine overflow in the rat PFC and hippocampus when administered in a dose yielding the equivalent of a therapeutic plasma concentration in humans (Linner et al., 2001; Borgkvist et al., 2011). We therefore hypothesized that activation of $D_{1/5}$ receptors in the dorsal hippocampus and/or medial PFC is an underlying mechanism for the memory-facilitating effect of reboxetine, as previously observed in humans (Harmer et al., 2003, 2009). In rats we similarly showed that administration of the same dose of reboxetine prior to a short object exploration session induced a clear preference for the novel object during the test session the following day. Despite the fact that blocking $D_{1/5}$ receptors in either the hippocampus or the PFC prevents long-term memory formation (Fig. 2), the memory enhancing effect of reboxetine was abolished when a $D_{1/5}$ receptor antagonist was given locally in the PFC, but not in the hippocampus. This differential effect can be explained by different sensitivity of the dopamine system in these areas. It is known for example that the PFC displays a two-fold higher dopamine $D_{1/5}$ receptor binding capacity compared to the hippocampus (Boyson et al., 1986). Moreover, in our experiments the effective dose of the $D_{1/5}$ agonist (Fig. 2), was 100-fold higher for the hippocampus compared to the PFC. Interestingly, short-term administration of reboxetine in healthy volunteers increased prefrontal functional magnetic resonance imaging activity during categorization of positive words, further indicating a role for the PFC

in the cognitive effects of reboxetine in humans (Norbury et al., 2008).

The privileged role of the PFC in the recognition memory-facilitating effect of reboxetine is further underscored by the observation that systemic reboxetine affects the NMDA receptor composition by enhancing the GluN2A levels only in the PFC and not in the hippocampus (Fig. 4). GluN2A-containing NMDA receptors are the predominant form of NMDA receptors in the mature brain and are important for the regulation of activity-induced synaptic plasticity. Mice with reduced levels of GluN2A display poor object recognition memory (Gao et al., 2011) and learning-induced increases in GluN2A have been proposed as a molecular mechanism for stabilization of learning-induced synaptic modifications (Quinlan et al., 1999, 2004). Thus, our observation of increased GluN2A levels in the PFC suggest that this may be a mechanism by which reboxetine improves recognition memory.

Memory deficits are common in depression and we previously showed impaired object recognition memory in a rat model of depression, the FSL rat, which was linked to abnormal glutamate transmission and suppressed long-term potentiation expression in the CA1 area of the hippocampus (Gomez-Galan et al., 2012). In the present study we show that a single injection of reboxetine prior to the exploration session (S1) is sufficient to overcome the memory deficit in this rat strain, confirming that reboxetine not only improves episodic-like memory in healthy rats, but also in an animal model of depression. These observations are consistent with the acute effects of reboxetine on episodic memory in healthy as well as depressed humans (Harmer et al., 2003, 2009). Interestingly, reboxetine did not have an acute effect on depressive-like behaviour (Fig. 5b) of FSL rats in the forced swim test. These data confirm that chronic treatment with antidepressants is typically required to improve depressive-like behaviour of FSL rats and supports the notion that memory facilitation alone is not sufficient to improve mood in depression (Femenia et al., 2012).

To the best of our knowledge, we provide the first experimental evidence that reboxetine improves memory in both healthy rats and in a rat model for depression. The results of the present study suggest a new mechanism whereby NET inhibitors enhance memory formation, through stimulation of the dopamine system in the PFC. Nevertheless, other brain regions may contribute to the effect of reboxetine on episodic-like memory. We propose that the object recognition task in rats can be used as an effective model to assess processing of and memory for positive

information in both healthy rats and in rat models for depression. The positive, rather than neutral, valence of objects in the object recognition task is further demonstrated by the observation that exposure to novel objects induces conditioned place preference in rats (Bevins and Besheer, 2005). Reduced memory processing for positive information has been proposed as a fundamental factor for negative thinking and low mood in cognitive psychology models of depression. As such, these cognitive symptoms are an important risk factor for relapse of emotional symptoms and constitute a prominent factor in disease burden (Disner et al., 2011; Millan et al., 2012). Cognitive therapies are effective in the treatment of depression and improve positive biases in emotional processing (Jakobsen et al., 2011). However, combined cognitive and pharmacological treatment of depression may reduce the effectiveness of either treatment alone (Browning et al., 2011). Identifying the effect of antidepressants on cognitive function may therefore be critical for future development of combination strategies. We propose that combination of cognitive therapy with antidepressants that specifically ameliorate cognitive processing through facilitation of prefrontal dopamine release may further improve the overall efficacy of antidepressant treatment.

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Statement of Interest

None.

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