Signalling pathways associated with 5-HT₆ receptors: relevance for cognitive effects

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Abstract
A growing body of evidence supports the use of serotonin 5-HT₆ receptor antagonists as a promising mechanism for treating cognitive dysfunction. We evaluated 5-HT₆ receptor expression and associated biochemical mechanisms in the hippocampus of rats that had been trained in the Morris water maze (MWM), a spatial learning task. Training in the MWM induces a down-regulation of 5-HT₆ receptor protein and mRNA receptor expression. The learning procedure or the administration of the selective 5-HT₆ receptor antagonist SB-271046 induced an increase in pCREB1 levels while CREB2 levels were significantly reduced. However, although SB-271046 was able to improve retention in the MWM, no further changes in pCREB1 or CREB2 levels were found to be associated with the presence of the 5-HT₆ receptor antagonist during the learning procedure. The MWM procedure significantly increased pERK1/2 levels and interestingly, further increases were seen when treating with SB-271046 during the MWM. These results suggest that, in the hippocampus, biochemical pathways associated with pERK1/2 expression, and not with the CREB family of transcription factors, seem to be related to the cognitive-enhancing properties of 5-HT₆ receptor antagonists.

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Introduction
Normal and impaired memory involves several neurotransmitters and signal systems. Among them, growing evidence indicates that the serotonin (5-hydroxytryptamine, 5-HT), system, acting via multiple receptors, modulates normal, pathophysiological and therapeutic aspects of learning and memory (Buhot, 1997; Cross et al. 1984; García-Alloza et al. 2004; Lai et al. 2002; Meneses, 1999). 5-HT₆ receptors are one of the most recently identified serotonin receptor subtypes (Monsma et al. 1993; Ruat et al. 1993). These receptors are coupled to a G stimulatory protein, inducing cAMP through adenylate cyclase stimulation (Monsma et al. 1993). In-situ hybridization studies have shown robust mRNA expression in rat and human striatum, nucleus accumbens, cortex and olfactory tubercle, with moderate expression in the hippocampus and thalamus (Gérard et al. 1997, Monsma et al. 1993; Ruat et al. 1993; Ward et al. 1995).

The 5-HT₆ receptor has become an increasingly promising target for improving cognition (Meneses et al. 2007; Mitchell & Neumaier, 2005; Mitchell et al. 2007; Woolley et al. 2004). The 5-HT₆ receptor antagonist Ro 04-6790 improved retention of the Morris water maze (MWM) task (Woolley et al. 2001) and reversed a scopolamine-induced deficit in an autoshaping task (Meneses, 2001) and in a rodent test of recognition memory (Lieben et al. 2005). Two other structurally different 5-HT₆ receptor antagonists, SB-399885 and SB-271046, increased novel object recognition in adult rats treated either acutely or subchronically (Hirst et al. 2003; King et al. 2004) and improved water maze retention (Rogers & Hagan, 2001) although they failed to alter acquisition of spatial learning. However, it appears that 5-HT₆ blockade is more consistently effective in alleviating memory deficits rather than increasing memory in normally functioning animals (Marcos et al. 2008a). In contrast to the works cited above, Russell & Dias (2002) and Lindner et al. (2003) failed to detect any effects of Ro 04-6790 or SB-271046 upon acquisition of an autoshaping task,
scopolamine-induced deficits in contextual fear-conditioning, or retention of a water maze task. Moreover, Fone (2006) reported that several selective 5-HT₆ receptors appear to restore memory impairments in the novel object discrimination paradigm.

The mechanism of the cognitive-enhancing properties of drugs acting on 5-HT₆ receptors is not completely understood. Changes in the density of 5-HT₆ receptor related to cognition have been described, as 5-HT₆ over-expression in the striatum impairs instrumental learning (Mitchell et al. 2007). Furthermore, 5-HT₆ receptor density is altered after memory consolidation in an instrumental autoshaping learning task (Meneses et al. 2007).

At the biochemical level, it is well known that 5-HT₆ receptor activity leads to activation of cyclic adenosine 3′,5′-monophosphate (cAMP) signalling pathways, which are involved in the pharmacological cascade that leads to the activation of a conserved regulator of gene expression, the cAMP response element (CRE) binding protein (CREB) (Silva et al. 1998). CREB is a constitutive transcription factor from a family of genes that share similar structural domains and contain both activators and repressors. For example, CREB referred to as CREB1 regulates gene transcription by binding to CRE, a cis-acting enhancer element in the regulatory region of various genes. The function of CREB1 is regulated largely by its phosphorylation at Ser133, which results in activation of gene transcription (Meyer & Habener, 1993; Montminy et al. 1990). Phosphorylation of CREB1 at Ser133 can be induced by a number of protein kinases, including cAMP-dependent protein kinase A (PKA) and Ca²⁺/calmodulin-dependent kinase II (CaMKII) (Sheng et al. 1991).

In contrast, CREB2 (also known as ATF4) has been shown to be a transcriptional repressor inhibiting CREB1-mediated transcription (see review by Kandel, 2001). It has been shown that CREB2 reduced expression improves memory storage in mice (Chen et al. 2003) and that hippocampal CREB1 but not CREB2 is decreased in aged rats with spatial memory impairments (Brightwell et al. 2004). On the other hand, it has also been described that the carboxyl-terminal region of 5-HT₆ receptors interacts with the Fyn-tyrosine kinase, and further that 5-HT₆ receptors activate the extracellular signal regulated kinase1/2 (ERK1/2) via Fyn-dependent pathways (Yun et al. 2007).

In the present work we have studied mechanisms seemingly involved in the effects on cognition of drugs acting on 5-HT₆ receptors. The effects of the MWM, a spatial learning task on 5-HT₆ receptor (both protein and mRNA) expression, and downstream signalling modulated by 5-HT₆ receptors have been examined.

Methods

Experimental design

Male Wistar rats (Charles River Laboratories, Spain), weighing 230–250 g were used. Rats were housed 3–4 per cage. Animals were kept at constant room temperature (21 ± 1 °C) and relative humidity (55 ± 5%) with a 12-h light/dark cycle (lights on 08:00 hours) with free access to food and water. All the experiments were performed in strict compliance with the recommendations of EU (DOCE L 358/1/18/2/1986) for the care and use of laboratory animals. Animals were randomly assigned to control and MWM groups. All animals were sacrificed on the last day of MWM test. Brains were removed and dissected on ice to obtain the hippocampus, including the ventral and dorsal parts (for Western blot), or frozen immediately at −40 °C in isopentane, and stored at −80 °C until sectioning (for 5-HT₆ receptor autoradiographic experiments and mRNA determinations). 5-HT₆ receptor autoradiography and in-situ studies were performed in coronal rat brain sections of 14 μm at the level of the hippocampus (−3.3 mm relative to bregma) according to the atlas of Paxinos and Watson.

MWM

Equipment

The water maze consisted of a black circular pool (145 cm diameter × 55 cm high) constructed from black polyethylene and filled with water at 22 °C. The pool was divided into four equal quadrants identified as northeast (NE), northwest (NW), southeast (SE) and southwest (SW). A black invisible platform, 10 cm diameter, was located in a constant position in the middle of the SW quadrant. The water level was 40 cm, 2 cm above the platform, which was completely concealed. The training room offered black geometric paintings, to serve as visual extra-maze cues for the animals. The information was analysed using a video-tracking system (Ethovision 3.0, Noldus Information Technology B.V., The Netherlands).

Water maze procedure

The protocol was adapted from previous reports (Diez-Ariza et al. 2003; Marcos et al. 2008a). On day 1 of the experimental procedure, animals performed a single trial of 60 s, without the platform present, to become accustomed to the pool. In the acquisition phase, rats performed six training trials/day (120 s each) for two consecutive days (days 2 and 3) with the escape platform in a fixed position. Rats were allowed to swim freely until they found the submerged
platform or until 120 s elapsed. If the rat found the platform, it was allowed to remain there for 15 s and then returned to its home cage. If it was unable to find the platform within 120 s, it was then placed on the platform for 15 s, and a maximum score of 120 s was assigned. Results were expressed as latency to find the hidden platform, and swim speed (cm/s).

On day 4 (retention phase) a single transfer test was performed, in which the platform was removed from the pool. Rats were allowed to swim for 60 s in search of the platform. Time spent by the animals in the quadrant (SW) where the platform had been located was recorded. If the rat demonstrated a persistent preference during the trial to navigate in the pool quadrant where the escape platform had previously been placed this was taken as an index of acquisition of the spatial task (Gage et al. 1984). Rats were treated with SB-271046 (10 mg/kg p.o., in 1% methylcellulose solution, 1 ml/100 g, provided by GlaxoSmithKline, UK) every day except on the habituation day, 4 h before starting the MWM procedure, as the maximal whole brain SB-271046 concentration is not reached until at least 3 h after oral administration (Routledge et al. 2000).

**[125]I**SB-258585 binding to 5-HT₆ receptors

[125]I**SB-258585 binding was assayed essentially as previously reported (Hirst et al. 2000). Tissue samples were thawed and homogenized in 15 vol (mg/ml) of ice-cold 50 mM Tris–HCl buffer (pH 7.7) using an Ultra-Turrax homogenizer. The homogenates were centrifuged at 35 000 g for 20 min and the resulting pellet was re-homogenized in Tris–HCl. Following two further centrifugations, membranes were finally resuspended (~80 mg tissue/ml) in 50 mM Tris–HCl, 5 mM MgCl₂, 0.5 mM EDTA and 10 μM pargyline and stored at −80 °C until required. All determinations were carried out in duplicate. [125]I**SB-258585 binding assays consisted of 200 μl membrane suspension (corresponding to ~20 mg/ml tissue), 25 μl [125]I**SB-258585 (provided by GlaxoSmithKline) at concentrations from 0.1 to 3 nM, and 25 μl buffer or methiothepin (10 μM) for non-specific binding. At the end of incubations, tubes were rapidly filtered under reduced pressure using a cell harvester on GF/B filters (Whatman, UK) that had been pre-soaked in 0.3 % v/v polyethyleneimine (Sigma-Aldrich, UK) in ice-cold buffer. The amount of radioactivity bound to filters was measured in a Wallac liquid scintillation counter. Data were subjected to Scatchard analysis to determine the number of binding sites (Bₘₐₓ: fmol/mg protein) and the dissociation constant (Kᵤ: nM). Protein content was measured using the assay described by Bradford (1976), using bovine serum albumin as standard.

**5-HT₆ receptor autoradiography**

5-HT₆ receptor quantitative autoradiography studies were performed as described by East et al. (2002). The 5-HT₆ receptor was labelled with 1 nM of the selective radioligand [125]I**SB-258585 as previously described (Hirst et al. 2000). Non-specific binding was determined in the presence of methiothepin (Sigma, USA), 10 μM, in adjacent sections. Slides were apposed to [125]I-Hyperfilm (Amersham, UK) for 4 d at 4 °C with [125]I standards. Autoradiograms were quantified using an image analysis system (Scion Image, Scion Corporation, USA) and densitometry measurements of CA1, CA3 and dentate gyrus subfields of the hippocampus were taken. The average value of the duplicate sections was taken and non-specific signal subtracted. Results were expressed as nCi of [125]I/mg wet tissue.

**In-situ hybridization for 5-HT₆ receptor mRNA**

Slides were fixed in 4% paraformaldehyde in phosphate-buffered saline (PBS) for 45 min and then washed in PBS (3 x 5 min), at room temperature. Sections were acetylated with 0.25% acetic anhydride in 0.1 M triethanolamine buffer (1 x 10 min) (pH 8). After dehydration through graded ethanol, sections were immersed in chloroform (1 x 10 min), rehydrated with ethanol and air dried. The oligonucleotide used was (Marcos et al. 2008b): 5’-TGC GTG GGC CAG GGT GTG CAG CTC ATC CTG GAC CCC CTC CAA GGA GCG-3’ (Sigma Genosis, UK). Probe was 3′-labelled with [α-35S]dATP, specific activity > 1000 Ci/mmol (Amersham Biosciences), using terminal deoxyribo nucleotide transferase (Roche Diagnostics, USA). Negative controls including sense oligonucleotides showed minimal background signals. Labelled probes were purified by chromatography on Sephadex G-25 columns (Nick column, Amersham Biosciences). Sections were incubated overnight in humid chambers (containing 50% formamide in 4 x SSC) at 47 °C with 200 μl hybridization buffer (50% deionized formamide, 4 x SSC, 10% dextran sulphate, 1 x Denhardt’s solution, 500 μg/ml salmon sperm DNA, 20 mM sodium phosphate, 100 mM DTT, 250 μg/ml yeast tRNA and 1% N-lauroylsarcosine) containing 1 x 10⁶ cpm of the [35S]ATP-labelled antisense. After incubation, slides were washed in 2 x SSC buffer at 55 °C for 3 x 15 min, followed by 2 x 60 min and 5 min in H₂O(d) at room temperature. Sections were then
reaction buffer (53 mM assay) were preincubated for 15 min on ice in 150 litres of membrane suspension (6–12
Membrane preparations and enzymatic activity assay
Adenylate cyclase assay
from three sections of each animal were averaged and appropriate conversion factor. Densitometric values from three sections of each animal were averaged and expressed as nCi/g tissue.

**Adenylate cyclase assay**

Membrane preparations and enzymatic activity assay was carried out as described by Valdizán et al. (2003) from frozen hippocampal tissue. Twenty-five micro-litres of membrane suspension (6–12 μg protein/assay) were preincubated for 15 min on ice in 150 μl reaction buffer (53 mM N-[2-hydroxyethyl]piperazine-N’-[2-ethane-sulphonic acid] (pH 7.4), 0.3 mmol/l EGTA, 5 mM MgCl₂, 0.1 mg/ml bovine albumin, 1 mM dithiothreitol, 0.5 mM 3-isobuthylmethylxanthine, and the nucleoside triphosphate regeneration system of 5 mmol/l creatine phosphate, 50 U/ml creatine phosphokinase), and 20 μl water (basal activity), forskolin (1 μmol/l) (non-specific activity), or the selective 5-HT₆ agonist E-6801 (1 μmol/l; Romero et al. 2006, provided by Laboratorios Dr Esteve S.A., Barcelona, Spain). The reaction was started by addition of 0.5 mmol/l Mg-ATP and incubated at 30 °C for 10 min. The reaction was stopped by boiling the tubes for 4 min. Samples were centrifugated at 13,000 g for 5 min at 4 °C. Aliquots (50 μl) of supernatant were assayed for cAMP content using a commercial protein-binding assay kit (cAMP [³H] assay kit TRK 432; GE Healthcare, UK) combining the high specificity and affinity for cAMP of a highly purified and stabilized binding protein with an improved charcoal separation step. Membrane protein concentrations were determined using the Bio-Rad Protein Assay kit (Bio-Rad, Germany).

**Western blotting**

Cytosolic extract preparations from the hippocampus were homogenized in a 50 mM Tris buffer (pH 7.2, 4 °C). Each sample was adjusted to final protein concentration of 4 mg/ml (DC protein assays; Bio-Rad, USA). Extracts were mixed with Laemmeli’s sample buffer boiled for 5 min. Samples (40 μg for CREB1, 20 μg for CREB2, 50 μg for pCREB1, ERK1/2 and pERK1/2) were loaded onto 10% bisacrylamide gels and separated by SDS-PAGE. Separated proteins were electrophoretically transferred from gels to PVDF membranes. The different proteins were detected with monoclonal antibodies (CREB1: sc-186, 1:1000 TBST, Santa Cruz Biotechnology Inc., USA; CREB2: sc-200, 1:2000 TBST, Santa Cruz Biotechnology Inc.; pCREB1: sc-7978, 1:200 TBST, Santa Cruz Biotechnology Inc.; ERK1/2: p44/42 kinase 1:1000 TBST, Cell Signaling Technology, and pERK1/2: phospho-p44/42 MAPK (Thr202/Tyr204)(E10) 1:2000 TBST, Cell Signalling Technology). Results were normalized against β-actin (mouse monoclonal 1:10000, Sigma, USA). Immunopositive bands were visualized by a chemiluminescent method (ECL; Amersham, USA). The optical density of reactive bands (CREB1 and pCREB1 ~43 kDa; CREB2 ~36 kDa and ERK1/2 and pERK1/2 ~44 and 42 kDa, respectively) visible on X-ray film was determined densitometrically.

**Statistical analysis**

Normality was checked by Shapiro–Wilk’s test (p > 0.05) before any other statistical analysis. In the acquisition phase of the MWM, differences between trials within the MWM group were analysed using a factorial ANOVA with replicates. In the retention phase, differences between times spent in quadrants were analysed using a one-way ANOVA followed by Tukey’s B test. Statistical differences in Bmax values of 5-HT₆ (radioligand binding assays) and densitometric measurements of 5-HT₆ protein and mRNA were determined by a Student’s t test. Adenylate cyclase assay and Western blotting results (CREB1, pCREB1, CREB2, ERK1/2 and pERK1/2) were analysed using a two-way ANOVA (learning task x treatment) followed when necessary by Student’s t test.

**Results**

**MWM**

In the acquisition phase of MWM task (Fig. 1), rats progressively learnt the platform position, as indicated by their decreasing escape latencies over trials (repeated-measures two-way ANOVA: F₁,₁₀ = 11.473, p < 0.001). Overall analysis (repeated-measures ANOVA: F₁,₁₀ = 8.150, p > 0.05) showed no significant effect of SB-271046 in latency to find the platform (n = 10 for all groups).

In the retention phase, SB-271046 produced a statistically significant improvement (one-way ANOVA: F₁,₁₀ = 3.808, p < 0.05) in the retention phase (Fig. 1b), measuring time spent in the SW quadrant where the platform had been located (n = 10 for both groups).

Swim speed was not affected by SB-271046 in either the acquisition phase or the retention phase. Values
were as follows (in cm/s): 25.63 ± 1.06 for controls and 22.91 ± 1.09 for the MWM group.

[125I]SB-258585 binding to 5-HT6 receptors in hippocampus

5-HT6 receptor density in the hippocampus was significantly (Student’s t test, p < 0.005) lower in the MWM group (n = 7) compared to controls (n = 7): \( B_{\text{max}} \) (in fmol/mg protein) was significantly higher (25.78 ± 5.35) in the control group than in the MWM group (11.41 ± 0.58). \( K_d \) values (in nM) were similar in the control (2.10 ± 0.27) and MWM (1.83 ± 0.38) groups.

5-HT6 autoradiography

5-HT6 receptor density in the hippocampus, measured by autoradiography binding with the selective radioligand [125I]SB-258585, was significantly decreased (Student’s t test, p < 0.001, n = 7 per group) in the MWM group (Fig. 2). Reductions observed reached 35% in the hippocampus.

In-situ hybridization for 5-HT6 receptor mRNA

As illustrated in Fig. 3, spatial learning (MWM procedure) induced a significant decrease in 5-HT6 receptor mRNA in the CA3 region of the hippocampus (Student’s t test, p < 0.001, n = 7 per group).

Adenylate cyclase activity

There was a significant interaction between learning group (control and MWM) and 5-HT6 receptor agonist treatment (basal and E-6801, 1 μmol/l) on adenylate cyclase activity (\( F_{1,30} = 7.955, p < 0.05 \), two-way ANOVA). Post-hoc analysis revealed that even though changes did not reach statistical significance, there was a strong tendency for E-6801 to increase adenylate cyclase activity (\( p = 0.07 \)), that was not observed in animals subjected to a combined MWM + E-6801 treatment. Absolute values were as follows (in pmol/mg protein): 418.17 ± 76.41 for controls, 781.20 ± 110.30 for the MWM group, 726.25 ± 122.48 for the E-6801 group, and 504.17 ± 91.95 for the MWM + E-6801 group (n = 6–8 per group).
Biochemical mechanisms associated with 5-HT₆ receptors: CREB and ERK1/2 signalling pathways

There was a significant interaction between learning group (control and MWM) and 5-HT₆ receptor antagonist treatment (basal and SB-271046) on CREB1 and pCREB1 levels in the hippocampus ($F_{1,22} = 4.104$, $p < 0.05$ and $F_{1,22} = 18.112$, $p < 0.001$, respectively, two-way ANOVA, $n = 6$ per group). Post-hoc analysis (Student’s $t$ test, $p < 0.05$ in each case) revealed that there were significant increases in CREB1 and pCREB1 levels in the MWM or SB-271046 groups. These increases tended to be lower in the combined MWM + SB-271046 group. Regarding the expression of the inhibitory transcription factor CREB2, there was a significant interaction between group (control and MWM) and 5-HT₆ receptor antagonist treatment (basal and SB-271046) ($F_{1,22} = 5.475$, $p < 0.05$, two-way ANOVA, $n = 6$ per group). Post-hoc analysis revealed significant decreases in CREB2 levels associated with MWM or SB-271046 treatments (Student’s $t$ test, $p < 0.05$). However, no changes in CREB2 were found in the combined MWM + SB-271046 group. All these results are depicted in Fig. 4.

As shown in Fig. 5, no interaction (learning group × 5-HT₆ receptor antagonist treatment) was found when analysing levels of total ERK1/2 ($F_{1,24} = 0.881$, $p > 0.05$, $n = 6$ per group). There was a main effect of MWM procedure ($F_{1,24} = 19.045$, $p < 0.001$), while SB-271046 treatment did not affect ERK1/2 levels ($F_{1,24} = 0.476$, $p > 0.05$). However, levels of pERK1/2 showed a significant interaction between learning group and pharmacological treatment ($F_{1,24} = 9.473$, $p < 0.01$). Post-hoc analysis revealed that there was a significant increase (around 300% over controls or SB-271046 alone, and 200% over MWM group) in pERK levels in the MWM group treated with SB-271046 (Student’s $t$ test, $p < 0.05$).

Discussion

The first aim of the present study was to determine if memory formation might modify 5-HT₆ receptor expression in a hippocampal-dependent behavioural task. The task chosen was spatial learning in the MWM, one of the most frequently used tests in the study of the neurobiology and neuropharmacology of spatial learning and memory (Morris, 1984). Experimental studies have consistently shown that the hippocampus is essential for spatial learning (see review by D’Hooge & De Deyn, 2001), and among the different hippocampal subfields, it has been demonstrated that the CA3 region is crucial for spatial acquisition and memory consolidation (Frederickson et al. 1990; Florian & Roulet, 2004; Lee & Kesner, 2002). In our
study, the learning procedure induced a down-regulation of hippocampal 5-HT₆ receptor expression. In the same sense, in a recent paper it has been demonstrated that increased 5-HT₆ expression in the striatum reduced acquisition or consolidation of instrumental learning (Mitchell et al. 2007). A note of caution should be made regarding the present results, as it has been described that that expression of 5-HT₆ receptors might be differentially regulated depending on levels of circulating adrenal corticoids (Marcos et al. 2008b), and the water maze could be a stressful event for a rat. However, in a situation of increased HPA axis responsiveness, such as supposedly the MWM, no differences were found in the expression of the 5-HT₆ gene in the hippocampus or frontal cortex (Marcos et al. 2008b) and further, non-significant increases in corticosterone levels were found to be associated with the learning procedure (A. Briones, personal communication, data not shown).

It might be possible that the 5-HT₆ receptor, as described for other molecules (Abel & Kandel, 1998; Chen et al. 2003), will exert inhibitory constraints during acquisition and retention of memory and will be down-regulated to restore equilibrium. It could be argued that reductions in 5-HT₆ receptor expression may represent an effort to enhance acetylcholine levels, as there is growing evidence that the supposed 5-HT₆ receptor’s influence on memory is mediated at least partially by increased acetylcholine neurotransmission (Bentley et al. 1999; Marcos et al. 2006; Riemer et al. 2003; Shirazi-Southall et al. 2002). Alternatively, it can also be suggested that 5-HT₆ receptor modulation of glutamate may also contribute to the effect of 5-HT₆ receptor antagonists on memory processing. In this regard, Dawson et al. (2000, 2001) showed an increase in glutamate release in the rat cortex and hippocampus following treatment with a 5-HT₆ receptor antagonist. Furthermore, Meneses and colleagues (2007) have recently shown that 5-HT₆ expression is increased in the CA3 region of hippocampus after treatment with scopolamine (cholinergic antagonist) or dizocilpine (NMDA receptor antagonist).

From the present results it might be expected that a 5-HT₆ receptor blockade may improve cognition, e.g. present results using the selective 5-HT₆ receptor antagonist SB-271046. However, contradictory results have been described (see Introduction), and moreover, 5-HT₆ receptor agonists also appear to restore memory impairments (Fone, 2006). Therefore, it would be of interest to study the effects of the learning procedure on the biochemical pathways activated by 5-HT₆ receptors. As a first step in this study, even though the
present results are not conclusive, there is a strong tendency ($p=0.07$) in the data that supports the view that down-regulation in the expression of receptors is accompanied by a decrease in adenylate cyclase activity.

cAMP signalling pathways are involved in the pharmacological cascade that leads to the activation of CREB (Silva et al. 1998). It has been demonstrated that the hippocampus is a region in which CREB-dependent gene expression is induced after spatial training (Guzowski & McGaugh, 1997; Taubenfeld et al. 1999) and is necessary for formation and consolidation of long-term memory (Florian et al. 2006; Kandel, 2001; Kogan et al. 1997). In our study, both the spatial learning task or the administration of the 5-HT$_6$ receptor antagonist had similar effects on levels of pCREB1 or the learning repressor CREB2. The seemingly surprising results obtained when SB-271046 was present during the MWM procedure may be explained in terms of the down-regulation of the receptor during the learning task. These results suggest the existence of an alternative intracellular signalling mechanism, not involving changes in adenylate cyclase activity and subsequent biochemical pathways, responsible for the effects of 5-HT$_6$ receptors in cognition. Yun et al. (2007) described that the carboxyl-terminal region of 5-HT$_6$ receptors interacts with the Fyn-tyrosine kinase, which in turn may lead to an activation of ERK1/2. The physiological importance of Fyn in the CNS has been suggested. Using Fyn-mutant mice, it was shown that a Fyn deficiency results in various neuronal defects, including defective long-term potentiation and impaired spatial memory (Grant et al. 1992). We have studied an apparent mediation of ERK1/2 in the cognitive-enhancing properties mediated by 5-HT$_6$ receptors. Based on the present results it seems that activation by phosphorylation of ERK1/2 is mediated in 5-HT$_6$ receptor effects. Even though the main target of cAMP is PKA, which has been proposed to play an essential role in the ERK1/2 cascade (Vossler et al. 1997), the differential effects of 5-HT$_6$ receptor antagonists on pCREB and pERK supports the work by Yun et al. (2007) that stimulation of 5-HT$_6$ receptor-activated ERK1/2 is via a pathway independent of activity on adenylate cyclase (which confers the definition as agonist/antagonist upon 5-HT$_6$ receptors). Therefore, these results would explain why both 5-HT$_6$ receptor agonists and antagonists may be effective in improving cognition.

In summary, the present results show that 5-HT$_6$ receptor expression is modulated by learning. It can be suggested that the existence of an intracellular signalling mechanism involves changes in ERK1/2 activity. Future studies on the molecular mechanisms responsible for the involvement of 5-HT$_6$ receptors on spatial learning are needed to give new insights into the complex mechanisms involved in learning and memory.

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Statement of Interest
None.

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