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Research report

Memory consolidation and amnesia modify 5-HT$_6$ receptors expression in rat brain: An autoradiographic study

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Abstract

Traditionally, the search for memory circuits has been centered on examinations of amnesic and AD patients, cerebral lesions and, neuroimaging. A complementary alternative might be the use of autoradiography with radioligands. Indeed, *ex vivo* autoradiographic studies offer the advantage to detect functionally active receptors altered by pharmacological tools and memory formation. Hence, herein the 5-HT$_6$ receptor antagonist SB-399885 and the amnesic drugs scopolamine or dizocilpine were used to manipulate memory consolidation and 5-HT$_6$ receptors expression was determined by using [^{3}H]-SB-258585. Thus, memory consolidation was impaired in scopolamine and dizocilpine treated groups relative to control vehicle but improved it in SB-399885-treated animals. SB-399885 improved memory consolidation seems to be associated with decreased 5-HT$_6$ receptors expression in 15 out 17 brain areas. Scopolamine or dizocilpine decreased 5-HT$_6$ receptors expression in nine different brain areas and increased it in CA3 hippocampus or other eight areas, respectively. In brain areas thought to be in charge of procedural memory such basal ganglia (*i.e.*, nucleus accumbens, caudate putamen, and fundus striate) data showed that relative to control animals amnesic groups showed diminished (scopolamine) or augmented (dizocilpine) 5-HT$_6$ receptor expression. SB-399885 showing improved memory displayed an intermediate expression in these same brain regions. A similar intermediate expression occurs with regard to amygdala, septum, and some cortical areas in charge of explicit memory storage. However, relative to control group amnesic and SB-399885 rats in the hippocampus, region where explicit memory is formed, showed a complex 5-HT$_6$ receptors expression. In conclusion, these results indicate neural circuits underlying the effects of 5-HT$_6$ receptor antagonists in autoshaping task and offer some general clues about cognitive processes in general.

Keywords: Autoshaping; 5-HT; [^{3}H] SB-258585; Memory; Learning; Receptors; Rat

1. Introduction

Normal and impaired memory involves several brain areas, neurotransmitters and signal systems and growing evidence indicates that the serotonin (5-hydroxytryptamine, 5-HT) system modulates normal, pathophysiological and therapeutic aspects of learning and memory [13–15]. Indeed, the etiology of Alzheimer's disease (AD) includes dysfunctions of cholinergic and 5-HT systems. Serotonin modulates the cortical cholinergic tone through multiple 5-HT receptors [1]. The identification [7] of seven families of 5-HT (5-HT$_1$ to 5-HT$_7$) receptors have allowed the study of their participation involved in learning and memory [13]. *In vitro* studies have established that with the exception of the 5-HT$_3$ receptors which are a ligand-gated ion channel, all of the serotonin receptors belong to the G-protein coupled receptor (GPCR) superfamily. At least, six 5-HT receptors families couple to G protein, including 5-HT$_6$ which couple to G$_s$ protein [7].

AD and aging apparently are associated with changes in diverse 5-HT markers, including 5-HT$_6$ receptors [2,21]. In recent years, 5-HT$_6$ receptors have been extensively implicated in normal memory and recovery for amnesia [3]. However, there are no previous studies about how memory and pharmacological treatments alter 5-HT$_6$ receptors expression and what might be their memory circuit. This is important since traditionally, the search for brain areas involve in learning and memory has been centered on examinations of amnesic and AD patients, cerebral lesions, and neuroimaging. In this regard, a complementary

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alternative consists of using radioligands. Several techniques have been used to explore the distribution of 5-HT_6 receptors (see [3,4] for review), including northern blot, *in situ hybridization*, RT-PCR, immunohistochemistry, and autoradiography. Showing that 5-HT_6 receptors are almost exclusively localized in the CNS, to be most dense in striatum, olfactory tubercles, and nucleus accumbens; with moderate levels in the cerebral cortex, hippocampus (CA1–CA3, dentate gyrus), hypothalamus, amygdala [3,4], important brain areas for memory formation [13]. 5-HT_6 receptors seem control cholinergic [4] or glutamatergic central function [3,21].

Expression of several 5-HT receptors including 5-HT_6 seems to be necessary to memory formation. In this regard, in a recent work [5] reported that reverse transcriptase-polymerase chain reaction (RT-PCR) analysis revealed that there was a higher level of expression of several 5-HT receptors mRNAs in autoshaping-trained relative to untrained groups. Actually, pharmacological naive untrained and autoshaping-trained rats showed significant differences, the latter groups expressing, in decreasing order, 5-HT_{1A} < 5-HT_6 < 5-HT_4 < or = 5-HT_7 receptors mRNA in prefrontal cortex and hippocampus. These findings provide further support to the notion that 5-HT_6 receptors participate in memory tasks such as water maze, passive avoidance, autoshaping, novel object discrimination [3], resulting their blockade in a facilitation of memory formation or reversing amnesia. Interestingly, Fone [6] reported that several selective 5-HT_6 receptor agonists have become available and these also appear to restore memory impairments in the novel object discrimination paradigm. It is unclear why and how 5-HT_6 receptor agonists and antagonists might improve memory or reverse amnesia (see [12]). Notably, animals overexpressing 5-HT_6 receptors in the striatum developed amnesia in a simple operant learning task, which was reversed by the selective 5-HT_6 receptor antagonist SB-258585 [7]. Hence, it seems to be a heuristic approach testing groups treated with well-know amnesic drugs scopolamine (cholinergic antagonist) and dizocilpine (non-competitive glutamatergic antagonist). Importantly, these amnesic drugs affect different neurotransmission system relevant for memory [1]. Since we had a limited quantity of the radioligand [^{3}H] SB-258585, (4-iodo-*N*-[4-methoxy-3-(4-methylpiperazin-1-yl) phenyl]benzene-sulfonamide) (see Fig. 1) unfortunately we were unable to include groups treated with amnesic drugs and SB-399885 (*N*-[3,5-dichloro-2-(methoxy)phenyl]-4-(methoxy)-3-(1-piperazinyl)benzenesulfonamide) (Fig. 1). In this work the aim was to determine 5-HT_6 receptors expression in animals submitted to a Pavlovian/instrumental autoshaping learning task determining 5-HT_6 receptors expression by using the radioligand [^{3}H] SB-258585 in animals treated with vehicle or the selective 5-HT_6 receptor antagonists SB-399885, which previously was reported to improve consolidation and reverse amnesia induced by cholinergic and glutamatergic antagonists [8,9]. In addition, an attempt was made to model memory deficits by using the well-known amnesic drugs scopolamine (a cholinergic and muscarinic antagonist) and dizocilpine (a glutamatergic non-competitive antagonist). Herein will be used a complex learning task such as Pavlovian/instrumental autoshaping learning task had been use to study 5-HT receptors protein

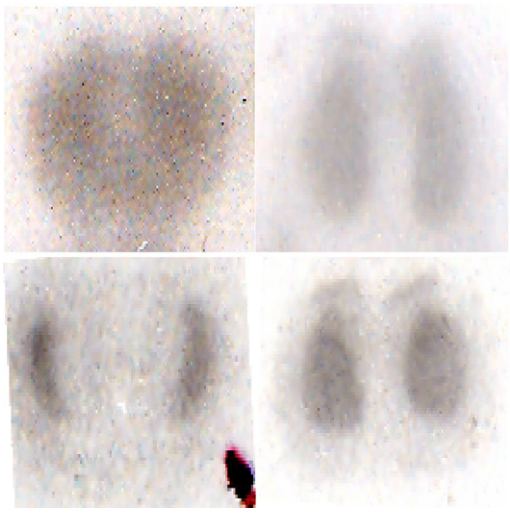
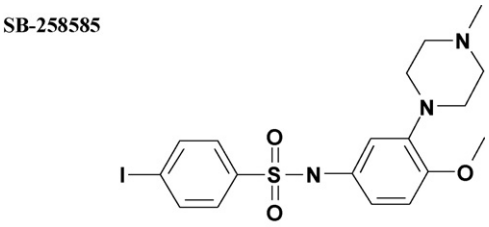
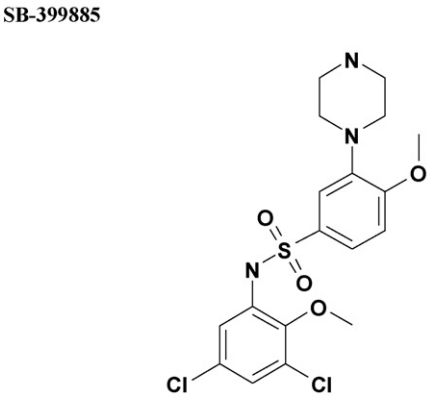


Fig. 1. Top: Autoradiograms showing cortical, hippocampal, septal and amygdalar areas of groups treated with saline (left) or SB-399885 (right). Bottom: amygdala of scopolamine (left) and basal ganglia areas of dizocilpine-treated animals (right).

[5,10–12], as measured with [^{3}H]-5-HT [11] or RNAm expression [5] in areas such as prefrontal cortex, striatum, hippocampus (for review see [12]). Thus, autoshaping task allows to combine declarative/explicit (*i.e.*, hippocampus dependent) and non-declarative/implicit (*i.e.*, striatum dependent) memory.





2. Materials and methods

2.1. Animals

Three-month-old male Wistar rats were used. Animals were collectively (10 per cage) housed in a temperature- and light-controlled room under a 12 h