SEROTONIN AND MEMORY

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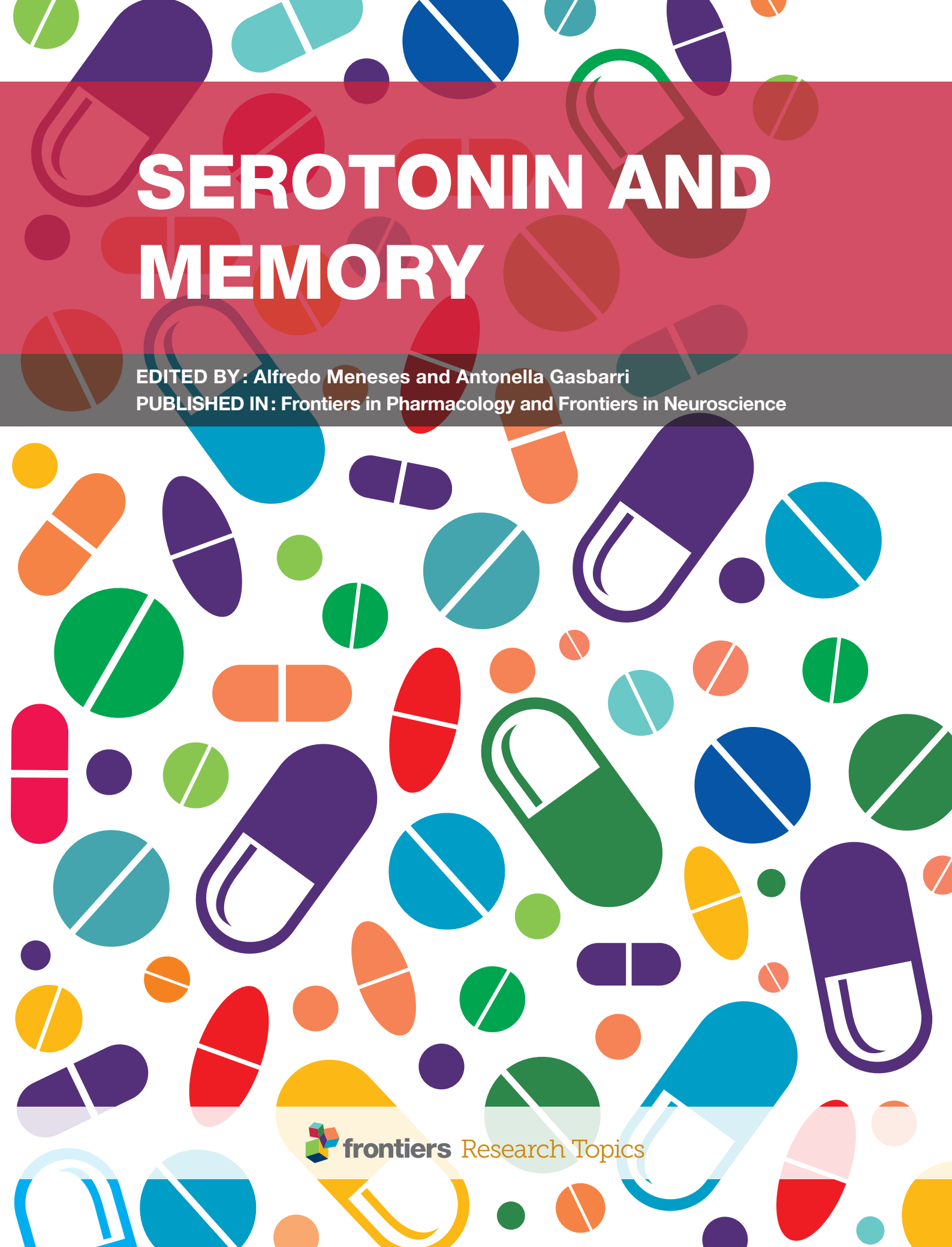

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SEROTONIN AND MEMORY

Topic Editors:
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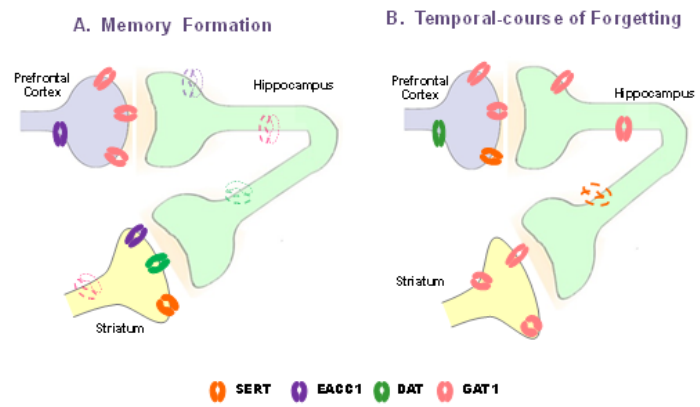

Schematic representation of changes with Western blot analysis of neural transporters in prefrontal cortex, hippocampus and striatum during memory formation and temporal-course of forgetting. Strong color refers to up-regulation, slight color refers to down-regulation. GAT1, GABA transporter 1; EAAC1, neuronal glutamate transporter excitatory amino acid carrier-1; DAT, dopamine transporter SERT, serotonin transporter. Image taken from Meneses A (2015) Serotonin, neural markers, and memory. Front. Pharmacol. 6:143. doi: 10.3389/fphar.2015.00143

The study of 5-hydroxytryptamine (5-HT) systems has benefited from the identification, classification and cloning of multiple 5-HT receptors (5-HT1 to 5-HT7). Increasing evidence suggests that 5-HT pathways, reuptake site/transporter complex and 5-HT receptors represent a strategic distribution for learning and memory. A key question still remaining is whether 5-HT markers (e.g., receptors) are directly or indirectly contributing to the physiological and pharmacological basis of memory and its pathogenesis or, rather, if they represent protective or adaptable mechanisms. Certainly, Alzheimer´s disease (AD) is a very complex neuropsychiatric disorder, where memory becomes progressively dysfunctional resulting in amnesia and dementia, whereas forgetting is a physiological phenomenon occurring all the time as adaptive mechanism. As dysfunctional memory occurs in several neuropsychiatric disorders, including schizophrenia, stroke, post-traumatic stress disorder. Hence, the aim of this call is collect recent and important findings related to information about serotonin and memory or 5-HT and learning or 5-HT and memory or serotonin and learning.

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EDITORIAL
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Editorial: Serotonin and Memory

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Keywords: serotonin, neural markers, therapeutic targets, memory, short-term, memory, long-term, memory disorders

The Editorial on the Research Topic

Serotonin and Memory

Several neurotransmission systems have been involved in function and dysfunctional memory (e.g., Myhrer, 2003; Decker and McGaugh, 2004; Reis et al., 2009; Cassel, 2010; Rodríguez et al., 2012; Komal and Nashmi, 2015), including serotonin (5-hydroxytryptamine, 5-HT), which accounts with multiple neural markers (receptors, transporter; e.g., Hannon and Hoyer, 2008; Saulin et al., 2012; Seyedabadi et al., 2014; McCorvy and Roth, 2015). Indeed, the 5-HT system can be manipulated in multiple ways with pharmacological tools and possesses well characterized downstream signaling in mammals' species (e.g., Marin et al., 2012; McCorvy and Roth, 2015). Emergent evidence indicates that this monoamine system might be a therapeutic target and neural marker regarding function and dysfunctional memory. This issue presents recent advances including the role of 5-HT_{2A} and 5-HT_{1A} receptors in the medial prefrontal cortex during recognition memory (Morici et al.). Hippocampal 5-HT_{1A} receptors and spatial and memory is revised by Glikmann-Johnston et al. Ochoa et al. report that post-training serotonergic depletions of the basolateral amygdala did not disrupt discrimination, retention or reversal learning; suggesting that this serotonergic activity is not required for formation and flexible adjustment of new stimulus-reward associations when the strategy to efficiently solve the task has already been learned. Hernández-Pérez et al. report that serotonin reduction in the supramammillary nucleus alters place learning and concomitant hippocampal, septal, and supramammillar theta activity in spatial memory. Zhang and Stackman review progress in the 5-HT_{2A} receptor distribution, signaling, polymerization, and allosteric modulation; as well as functions in learning and memory, hallucination and spatial cognition, and mental disorders. Pereira et al. show us that 5-HT_6 receptor agonism facilitates emotional learning and involves prefrontal cortex and hippocampal signaling. Serotonergic transporter function is reported by Sivamaruthi et al. demonstrating that cronobacter sakazakii infection alters serotonin transporter and improved fear memory retention. Stiedl et al. discuss the role of the serotonin receptor subtypes 5-HT_{1A} and 5-HT_7 and their interaction in emotional learning and memory; including the role of these receptors and their interplay at the molecular, neurochemical, and behavioral level. The potential involvement of serotonergic neural markers with respect to memory is reviewed by Meneses.

Special mention and thanks to the expert work of the referees, who made professional and careful reviews that improved the papers in this topic.

AUTHOR CONTRIBUTIONS

BG support as referee in several papers. AM was editor.

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Corrigendum: Editorial: Serotonin and Memory

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Keywords: serotonin, neural markers, therapeutic targets, memory, short-term, memory, long-term, memory disorders

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A corrigendum on

Editorial: Serotonin and Memory
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Due to an oversight, the name of Antonella Gasbarri in the Editorial article was reported as B. Gasbarri, which also rendered the citation of the Editorial article incorrect. This error does not change the scientific conclusions of the article in any way.

The original article was updated.

AUTHOR CONTRIBUTIONS

Both authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.

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REVIEW
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Hippocampal 5-HT$_{1A}$ Receptor and Spatial Learning and Memory

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Spatial cognition is fundamental for survival in the topographically complex environments inhabited by humans and other animals. The hippocampus, which has a central role in spatial cognition, is characterized by high concentration of serotonin (5-hydroxytryptamine; 5-HT) receptor binding sites, particularly of the 1A receptor (5-HT$_{1A}$) subtype. This review highlights converging evidence for the role of hippocampal 5-HT$_{1A}$ receptors in spatial learning and memory. We consider studies showing that activation or blockade of the 5-HT$_{1A}$ receptors using agonists or antagonists, respectively, lead to changes in spatial learning and memory. For example, pharmacological manipulation to induce 5-HT release, or to block 5-HT uptake, have indicated that increased extracellular 5-HT concentrations maintain or improve memory performance. In contrast, reduced levels of 5-HT have been shown to impair spatial memory. Furthermore, the lack of 5-HT$_{1A}$ receptor subtype in single gene knockout mice is specifically associated with spatial memory impairments. These findings, along with evidence from recent cognitive imaging studies using positron emission tomography (PET) with 5-HT$_{1A}$ receptor ligands, and studies of individual genetic variance in 5-HT$_{1A}$ receptor availability, strongly suggests that 5-HT, mediated by the 5-HT$_{1A}$ receptor subtype, plays a key role in spatial learning and memory.

Keywords: serotonin, 5-HT$_{1A}$ receptor, hippocampus, spatial cognition, memory

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INTRODUCTION

The idea that serotonin (5-hydroxytryptamine; 5-HT) is involved in learning and memory has gained traction in recent years, after having first been suggested in the 1980s (Altman and Normile, 1988). Early pharmacological studies mostly implicated spatial memory. More recent studies involving advanced methodologies such as neurotransmitter positron emission tomography (PET) and knockout mouse models have continued to link serotonin to spatial memory.

Spatial memory includes the ability to learn the topographical configuration of environments, to locate objects, to recall previously encountered locations, and to navigate within environments. Many day-to-day activities performed by animals and humans depend on spatial memory. Knowing where one is, where food and water resources are, and how to get to safety are examples of effective use of spatial memories that are essential for animal survival. Humans depend on their ability to remember the locations of objects in the environment on a daily basis, ranging from retrieving a mobile phone from a purse to making one's way to work and back home (McNamara, 2013).

At a clinical level, the study of spatial memory is of particular significance to several neurological disorders such as dementia of the Alzheimer's type where impairments in spatial cognition are a central feature. In addition, spatial memory, and particularly the ability to process and remember spatial descriptions of environments, has been linked to certain types of learning disabilities in children (Mammarella et al., 2014).

Functional neuroimaging studies show that spatial memory is largely mediated by mesial temporal areas (for example, Maguire et al., 1996b, 1997, 1998a,b; Burgess et al., 2001; Hartley et al., 2003), and within these areas, the hippocampus is a key structure for spatial memory. These regions are characterized by high concentration of the 5-HT$_{1A}$ receptor binding sites.

Involvement of the 5-HT$_{1A}$ receptor in cognition is undisputed. This receptor subtype has been suggested as a therapeutic target and neural marker of memory deficits (Meneses, 1999; Meneses and Perez-Garcia, 2007; Thomas, 2015). In this review, we argue that the 5-HT$_{1A}$ receptor plays a key role in spatial learning and memory, and we present evidence to support this proposition. We first consider the correspondence between the neuroanatomy of spatial memory and the 5-HT$_{1A}$ receptor distribution. We then review studies using various experimental methods that have illustrated the role of 5-HT$_{1A}$ receptors in spatial learning and memory.

NEUROANATOMY OF SPATIAL LEARNING AND MEMORY

Research on spatial memory has consistently implicated a hippocampal brain network consisting of the hippocampus proper, the parahippocampal cortices, fornix, parietal cortex, anterior thalamic nuclei, frontal cortex, and the striatum. The critical role of the hippocampal system in spatial learning and memory was first highlighted by Brenda Milner's early observations of "heightened" spatial memory deficits following temporal lobe excision for the relief of epileptic seizures (Milner, 1958, p. 251). Evidence for the importance of the hippocampus system has continued to accumulate, including very recent findings using single-neuron recording in human entorhinal cortex during virtual navigation (Miller et al., 2015). In terms of possible brain mechanisms underlying spatial learning and memory, findings have indicated that the rat hippocampus contains "place cells," and these cells exhibit location-specific activity (O'Keefe and Dostrovsky, 1971; O'Keefe and Speakman, 1987). This discovery led to the hypothesis that the hippocampus stores a cognitive map of the spatial layout of the environment (O'Keefe and Nadel, 1978). More than three decades later, in 2005, "grid cells" were found in the rat's entorhinal cortex, which is the chief gateway into the hippocampus (Hafting et al., 2005). Grid cells generate a coordinate system that allows exact positioning and pathfinding. Together with other cells in the entorhinal cortex that recognize the direction of the head of the animal and the border of the environment ("head-direction cells"; Taube, 1998), grid cells form networks with place cells in the hippocampus. Overall this circuitry constitutes a comprehensive positioning system, an inner global positioning system, or GPS, in the brain.

In addition to these cell recording studies, lesions and stimulation of the hippocampus in non-human primate (Parkinson et al., 1988; Angeli et al., 1993) and rodents (Morris et al., 1982; Buhot et al., 1991) were shown to impair spatial learning and memory. Similarly, in humans, medial temporal lesions, especially on the right side, have been shown to impair recall of spatial location of objects (Smith and Milner, 1981, 1989; Pigott and Milner, 1993; Bohbot et al., 1998; Smith et al., 2011), increase spatial memory errors (using the None-Box Maze, Abrahams et al., 1997, 1999), and impair performances on virtual reality topographical memory tasks (Spiers et al., 2001b).

More precise links between particular spatial memory functions and regions within the hippocampal network have been established in some studies. For example, early studies indicated lateralization of hippocampal involvement in memory, with the right medial temporal lobe predominantly associated with visuospatial recall (for example, Milner, 1965; Smith and Milner, 1981, 1989; Pigott and Milner, 1993; Abrahams et al., 1997; Maguire et al., 1997; Gleissner et al., 1998; Lv et al., 2014), and the left medial temporal lobe with verbal material recall (for example, Saling et al., 1993; Hermann et al., 1997; Martin et al., 2002; Lillywhite et al., 2007). In keeping with this idea, a patient with Pick's disease involving the left temporal lobe showed a complete dissociation between topographical memory and verbal memory (Maguire and Cipolotti, 1998), although more recent findings (for example, Maguire et al., 1996a,b; Grön et al., 2000; Spiers et al., 2001a; Astur et al., 2002; Glikmann-Johnston et al., 2008; Cánovas et al., 2011) support involvement of both the left and right medial temporal lobes in spatial learning and memory.

The cortices adjacent to the hippocampus, which provide the hippocampus with its main source of direct cortical input and output, have also been implicated in spatial learning and memory. For example, some studies indicated bilateral involvement of the parahippocampal gyri (Aguirre et al., 1996, 1998; Aguirre and D'Esposito, 1997; Epstein and Kanwisher, 1998; Mellet et al., 2000; Zeidman et al., 2012), whereas other studies indicate unilateral, predominantly right-sided involvement (Habib and Sirigu, 1987; Owen et al., 1996; Bohbot et al., 2000; Ploner et al., 2000). In terms of other regions of the hippocampal formation, in non-human primates, cells in the entorhinal cortex are active during the performance of a variation of the delayed matching to sample task (memory for objects) and the delayed matching to place task (memory for place) (Suzuki et al., 1997). Location-specific activity of neurons has also been recorded within the rat entorhinal cortex (Quirk et al., 1992). Furthermore, lesions to the entorhinal cortex in rats have been shown to result in deficits in acquisition and retention of the Eight-Arm Radial Maze and the Morris Water Maze (Cho and Jaffard, 1995; Nagahara et al., 1995; Davis et al., 2001; Devi et al., 2003). In humans, entorhinal stimulation applied during learning the locations of landmarks enhanced subsequent memory for these locations (Suthana et al., 2012). In a single-neuron recording study, entorhinal cortex neurons activated at multiple related areas of a virtual environment (Miller et al., 2015). Combined lesions of entorhinal and perirhinal cortices impaired rats' performance in spatial memory tasks (Otto et al., 1997; Kaut and Bunsey, 2001). In contrast, perirhinal lesions alone yielded inconsistent results, with some studies showing

impaired performance in certain tests of spatial memory (Wiig and Bilkey, 1994a,b; Liu and Bilkey, 1998a,b,c, 1999, 2001), while in others spatial memory was spared (Glenn and Mumby, 1998; Bussey et al., 1999, 2001; Machin et al., 2002; Ramos, 2002, 2013; Moran and Dalrymple-Alford, 2003). Thus, involvement of the perirhinal cortex in spatial learning and memory may be related to the specific memory paradigm employed.

In the following section, we provide an overview of 5-HT synthesis, electrophysiology, and receptor distribution to illustrate the concordance between 5-HT receptor distribution and brain areas involved in spatial memory, focusing on the hippocampus (see **Figure 1**). Subsequently, we review the evidence that 5-HT, mediated by the 5-HT$_{1A}$ receptor, is involved in the modulation of spatial learning and memory.

SEROTONIN (5-HYDROXYTRYPTAMINE; 5-HT) AND THE 5-HT$_{1A}$ RECEPTOR

Neurons that synthesize 5-HT are clustered in several nuclei along the midline of the brainstem, the most prominent of which are the raphe nuclei. Axons of these neurons innervate almost all regions of the central nervous system (CNS) and thus affect a great variety of behaviors, such as sleep/wake cycle, food intake, sexual behavior, emotional state, and cognitive processes, particularly learning and memory (Frazer and Hensler, 1994). 5-HT is synthesized from the amino acid tryptophan. The initial step in synthesis is the conversion of tryptophan to 5-hydroxytryptophan (5-HTP) by the enzyme tryptophan hydroxylase. Aromatic amino acid decarboxylase (AACD) then converts 5-HTP to 5-HT. 5-HT release occurs via exocytosis and is Ca^{2+}-dependent. After 5-HT release, the actions of 5-HT in the synapse are terminated by 5-HT transporters, located on the plasma membrane of serotonergic neurons, which reuptake 5-HT back into the serotonergic neurons. 5-HT catabolism occurs by monamine oxidase A (MAO-A) (Frazer and Hensler, 1994; Adell et al., 2002).

Seven types of 5-HT receptors have been identified, termed 5-HT$_{1\text{-}7}$, and among these are 14 distinct receptor subtypes. Each 5-HT receptor subtype has unique structural and pharmacological characteristics and a distinct distribution in the CNS. Of special interest is the 5-HT$_{1A}$ receptor, which is highly concentrated

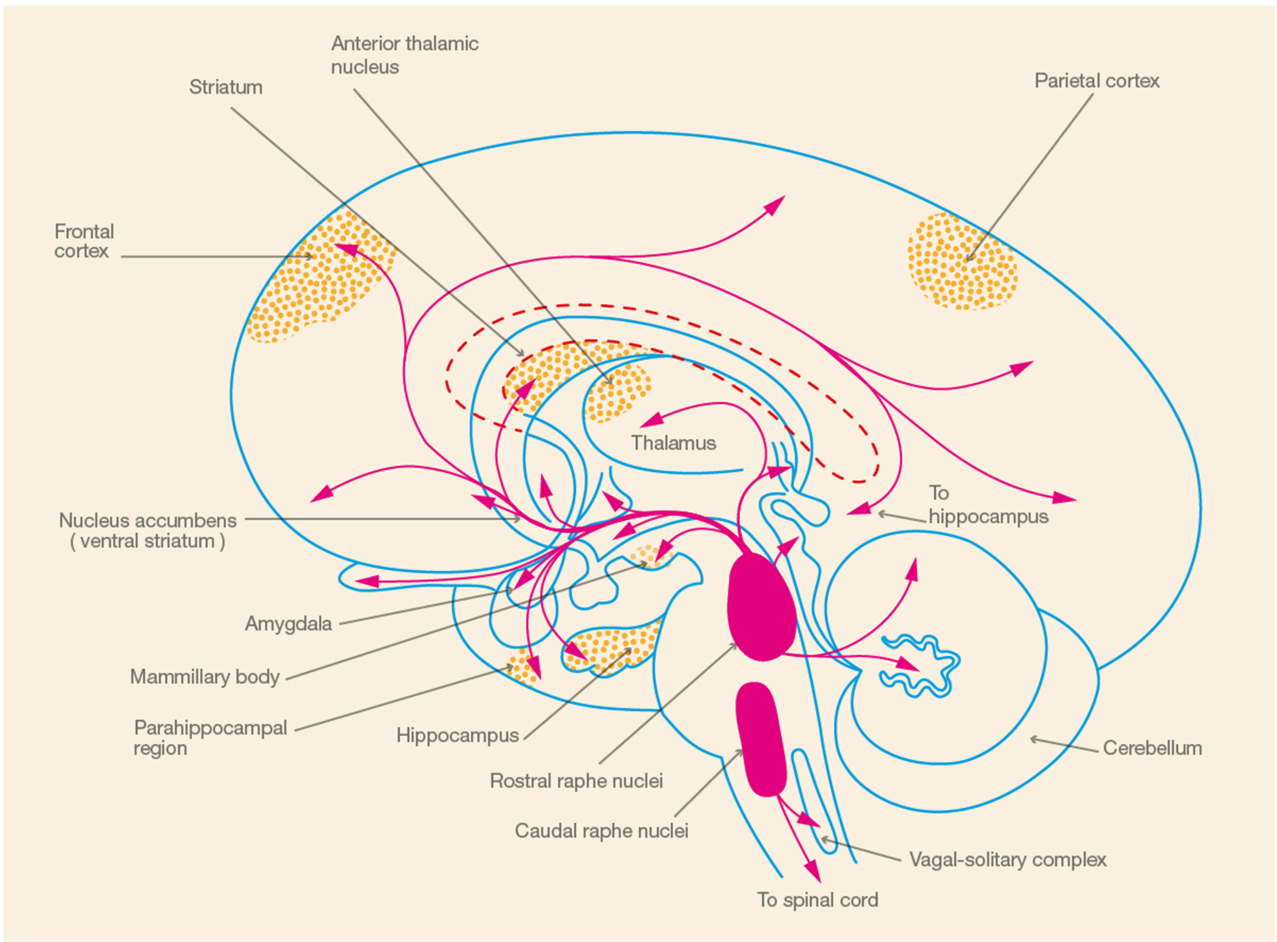

FIGURE 1 | Schematic illustration of brain areas involved in spatial memory ( ) and the corresponding serotonergic pathways ( ) (Adapted from Heimer, 1994, p. 227).

within the hippocampal system. 5-HT$_{1A}$ receptors are mainly concentrated in the limbic system, particularly the hippocampus (dentate gyrus and CA1), lateral septum, and amygdala, in cingulate and entorhinal cortices, and in the dorsal and median raphe nuclei, many of the regions implicated in spatial learning and memory. In contrast, only low concentrations are present in the striatum, substantia nigra, and the cerebellum (Barnes and Sharp, 1999; Lanfumey and Hamon, 2000). Autoradiography and immunohistochemical methods show that 5-HT$_{1A}$ receptors are located post-synaptically, as well as on the serotonergic neurons themselves in the raphe nuclei where they act as somatodendritic autoreceptors (Verge et al., 1985, 1986; Hoyer et al., 1986; Pazos et al., 1987; Zifa and Fillon, 1992; Hall et al., 1997; Lanfumey and Hamon, 2000). At the cellular level, 5-HT$_{1A}$ receptors reside on hippocampal pyramidal and granule cells (Lanfumey and Hamon, 2000). The highest density of these receptors are found in the granular layer (Hall et al., 1997).

In both hippocampus and dorsal raphe regions, 5-HT$_{1A}$ receptor activation results in neuronal hyperpolarization through the interaction with G-protein and the opening of K^+ channels (Hamon et al., 1990; Frazer and Hensler, 1994; Lanfumey and Hamon, 2000). Since 5-HT$_{1A}$ receptors are located pre- and post-synaptically, endogenous 5-HT and/or 5-HT$_{1A}$ receptor agonists have different effects. 5-HT$_{1A}$ somatodentritic autoreceptors modulate synaptic transmission. When activated via endogenous 5-HT and/or 5-HT$_{1A}$ receptor agonists, they inhibit the serotonergic neuron on which they reside, and reduce 5-HT release. In contrast, at post-synaptic receptors such as occur in the hippocampus, 5-HT$_{1A}$ agonists facilitate 5-HT neurotransmission (Lanfumey and Hamon, 2000). Brain areas that are critical for spatial learning and memory, such as those that are part of the hippocampal formation, harbor the post-synaptic 5-HT$_{1A}$ receptors.

5-HT$_{1A}$ AND SPATIAL LEARNING AND MEMORY

Evidence to support a role for the 5-HT$_{1A}$ receptor in spatial learning and memory comes from a variety of experimental methods, including mouse "knockout" models, direct receptor activation and blockade, neurotransmitter PET imaging, genetic studies, and manipulation of 5-HT concentrations. We organize this review according to the primary experimental method used. Studies cited here are summarized in **Table 1**.

Knockout Mouse Models

Studies using genetically modified animals, particularly those of single gene deletions in knockout mice, provide the strongest evidence for the role of the 5-HT$_{1A}$ receptor in learning and memory (see Bert et al., 2008 for a review of learning and memory in 5-HT1A-receptor mutant mice). Sarnyai et al. (2000) assessed 5-HT$_{1A}$-deficient mice on hippocampal-related spatial learning and memory tasks, the Morris Water Maze and the "Y" shape Maze. Their results showed that lack of 5-HT$_{1A}$ receptors is specifically associated with spatial learning and memory impairments. Wolff et al. (2004) demonstrated similar impairments in learning and retention of the Morris Water Maze in young-adult 5-HT$_{1A}$ knockout mice, but not in aged 5-HT$_{1A}$ knockout mice. The authors suggested that the reduced effect of the mutation in aged animals possibly reflects the lower efficacy of autoreceptors due to aging and/or a prevalence of hippocampal heteroreceptors.

5-HT$_{1A}$ Receptor Stimulation

5-HT$_{1A}$ agonists and antagonists modulate 5-HT neurotransmission and have been shown to directly alter spatial learning performance. Typically, antagonists have been found to impair spatial memory, whereas agonists are found to ameliorate the antagonist-induced spatial deficits, or allowed normal performance. For example, in a study by Micheau and Van Marrewijk (1999), intra-peritoneal administration of the 5-HT$_{1A}$ receptor agonist 8-hydroxy-2-(di-*n*-propylamino) tetraline (8-OH-DPAT) *improved* acquisition of a spatial discrimination task in an 8-arm radial maze. An intra-septal infusion of 8-OH-DPAT produced the same pattern of findings, although the improvement was less pronounced. Bertrand et al. (2000) showed contradictory findings, however, reporting that intra-septal infusion of 8-OH-DPAT *impaired* spatial learning. Administration of 8-OH-DPAT into the rat *dorsal raphe* had no effect on Delayed Non-Matching to Position (spatial working memory) task performance at any dose. In comparison, administration of the same compound into the *median raphe* improved performance accuracy. When 8-OH-DPAT was administered into the dorsal hippocampus, however, it produced a small impairment in performance (Warburton et al., 1997). 8-OH-DPAT also impaired performance on a water maze task (Carli et al., 1995) and on the eight-arm radial maze (Egashira et al., 2006). These findings demonstrate different effect for pre- and post-synaptic 5-HT$_{1A}$ receptor stimulation on spatial learning and memory tasks.

Additional evidence for the role of 5-HT$_{1A}$ receptor agonists in spatial memory comes from animal models of traumatic brain injury (TBI). In this model, animals are subjected to controlled cortical lesion to mimic TBI, and then memory is examined at different time points following injury and after administration of 5-HT$_{1A}$ agonists. These studies showed that TBI-induced spatial memory deficits are attenuated by treatment with the 5-HT$_{1A}$ receptor agonist buspirone (Olsen et al., 2012) and 8-OH-DPAT (Cheng et al., 2008). Furthermore, a combined therapeutic regimen of buspirone and environmental enrichment was found to be more effective than either alone in enhancing spatial learning in brain injured pediatric rats (Monaco et al., 2014).

Imaging Serotonergic Neurotransmission

Because the 5-HT$_{1A}$ receptor plays an important role in a range of physiological processes and in the pathophysiology of a variety of psychiatric and neurodegenerative disorders, synthesis of 5-HT$_{1A}$ receptor agents has been carried out primarily for their therapeutic potential. In recent years, more than 20 compounds have been labeled with carbon-11, fluorine-18, or iodine-123 for imaging and quantification of the 5-HT$_{1A}$ receptor with PET and SPECT (for review see Passchier and Van Waarde, 2001). The most successful radioligands thus far are [*carbonyl*-^{11}C] WAY-100635 (WAY), [*carbonyl*-^{11}C]*desmethyl*-

TABLE 1 | Summary of the studies cited according to the experimental method used.

Method	Citation	Findings
Knockout mouse models	Sarnyai et al., 2000	5-HT_{1A}-deficient mice were impaired on the Morris water maze and the "Y" shape maze.
	Wolff et al., 2004	Young-adults, but not aged, 5-HT_{1A} knockouts exhibited an impairment of learning and retention of the Morris water maze.
5-HT_{1A} receptor stimulation	Micheau and Van Marrewijk, 1999	Intra-peritoneal administration of 8-OH-DPAT[a] improved acquisition of a spatial discrimination task in an 8-arm radial maze. Intra-septal administration produced the same pattern of findings, but the improvement was less pronounced.
	Bertrand et al., 2000	Intra-septal injection of 8-OH-DPAT significantly impaired spatial learning in a water maze task.
	Warburton et al., 1997	Effects of 8-OH-DPAT on the rat performance of the Delayed Non-Matching to Position task varied according to the site of administration. In the dorsal raphe, the compound had no effect at any dose. Administration into the median raphe improved performance accuracy. In the dorsal hippocampus, 8-OH-DPAT produced a small impairment in performance.
	Carli et al., 1995	Rats treated subcutaneously with 8-OH-DPAT were impaired in choice accuracy on a two-platform spatial discrimination task. Spiroxatrine and (+)WAY100135[b] prevented the impairment of accuracy caused by 8-OH-DPAT.
	Egashira et al., 2006	Bilateral microinjections of 8-OH-DPAT into rats' dorsal hippocampus impaired spatial memory on the eight-arm radial maze. WAY-100135 and NAN-190[c] reversed the spatial memory impairment produced by 8-OH-DPAT.
	Olsen et al., 2012	Chronic treatment with buspirone[d] in rats attenuated traumatic brain injury-induced spatial learning and memory impairments on the Morris water maze.
	Cheng et al., 2008	Delayed and chronic treatment regimen with 8-OH-DPAT after cortical impact injury in rats facilitated motor recovery and acquisition of spatial learning in a water maze task.
	Monaco et al., 2014	A combined therapeutic regimen of buspirone and environmental enrichment was more effective than either alone in enhancing spatial learning in brain injured pediatric rats.
Imaging serotonergic neurotransmission	Glikmann-Johnston et al., 2015	Hippocampal asymmetry in PET [^{18}F]MPPF binding was associated with performance on a virtual object-location task. A lower binding potential in the right vs. the left hippocampus was related to better memory performance.
	Theodore et al., 2012	Using the PET ligand 18FCWAY, reduced left hippocampal 5-HT_{1A} receptor binding in temporal lobe epilepsy patients was related to delayed auditory verbal memory impairment, independent of the side of the epileptic focus.
Genetic variance in 5-HT_{1A} receptor availability	Roiser et al., 2006	MDMA[e] users and controls who are carriers of the *S* allele at the 5-HT transporter gene-linked polymorphic region (5-HTTLPR)[f] outperformed the *L* allele carriers on a visuo-spatial planning task, independent of drug use.
	Roiser et al., 2007	Carriers of the *S* allele at the 5-HTTLPR were more accurate than carries of the *L* allele on the CANTAB Pattern Recognition Memory.
	Jedema et al., 2010	Rhesus monkeys who are *S* allele carriers of the 5-HTTLPR were more accurate than carriers of the *L* allele on the delayed match to sample task.
Manipulations of 5-HT levels	du Jardin et al., 2014	PCPA[g] induced 5-HT depletion in rats and caused memory deficits on object recognition and Y-maze spontaneous alternation tests. Flesinoxan[h] significantly occupied 5-HT_{1A} receptors and restored PCPA-induced spatial memory deficits.
	Fox et al., 2000	Heavy MDMA users were impaired on several spatial memory components as tested by the CANTAB, including pattern recognition and spatial working memory.
	Skelton et al., 2006	MDMA-treated rats showed long lasting spatial learning deficits on the Morris water maze. Their performance on the Cincinnati water maze, a test of path integration learning, was initially impaired, but recovered over time.
	Vorhees et al., 2007	MDMA dose distribution had a long-term differential effect on different types of spatial learning. Path integration was mostly impaired following administration of a single dose. Spatial learning and reference memory was significantly impaired following administration of four divided doses.
	Fisk et al., 2011	Current and previous MDMA use was associated with visuospatial working memory impairment.
	Morford et al., 2002	Neonatal rats treated with D-fenfluramine[i] on the 11th–20th post-natal days exhibited infantile and adult spatial learning and memory deficits in the Morris water maze, and sequential learning impairments in a Cincinnati water maze.
	Vorhees et al., 1994	Rats exposed to methamphetamine[j] in early or late post-natal development exhibited impaired performance on a complex T-maze and on the Morris water maze.

(Continued)

TABLE 1 | Continued

Method	Citation	Findings
	Vorhees et al., 2000	Neonatal methamphetamine treatment in rats produced selective spatial learning and memory deficits on the Morris water maze during adulthood.
	Vorhees et al., 2008	Methamphetamine treatment in rats impaired path integration learning irrespective of dose. Only high doses of the drug impaired rats' spatial learning and memory performance on the Morris water maze. Enrichment of rearing conditions significantly improved acquisition of the task.
	Schröder et al., 2003	A neurotoxic regimen of methamphetamine rats induced damage to 5-HT terminals, as indicated by decreased [^{125}I]RTI-55 binding in the hippocampus, and impaired performance on an object recognition task, but not performance in the Morris water maze.

[a] *8-OH-DPAT is a 5-HT$_{1A}$ receptor agonist.*
[b] *Spiroxatrine and (+)WAY100135 are 5-HT$_{1A}$ receptor antagonists.*
[c] *NAN-190 is a 5-HT$_{1A}$ receptor antagonist.*
[d] *Buspirone is a 5-HT$_{1A}$ receptor agonist.*
[e] *Acute effects of MDMA include a rapid and significant increase in 5-HT, released from presynaptic vesicular stores. Repeated and high doses of MDMA cause decreased concentrations of 5-HT and its metabolite 5-HIAA.*
[f] *The S allele at the 5-HTTLPR is associated with reduced serotonergic neurotransmission relative to the L allele.*
[g] *Parachlorophenylalanine (PCPA) inhibits tryptophan hydroxylase, and thus reduces 5-HT synthesis.*
[h] *Flesinoxan is a selective 5-HT$_{1A}$ receptor agonist.*
[i] *D-fenfluramine is a substituted amphetamine that induces 5-HT release and inhibits its reuptake. Initially, D-fenfluramine increases 5-HT extracellular concentrations, but later causes a significant depletion.*
[j] *Methamphetamine induces long-lasting reductions of dopamine and 5-HT, inhibits presynaptic neurotransmitter reuptake, and reduces tyrosine and tryptophan hydroxylase activities.*

WAY 100635 (DWAY), 2′-methoxyphenyl-(*N*-2′-pyridinyl)-*p*-[^{18}F]fluoro-benzamidoethylpiperazine ([^{18}F]MPPF), and [^{11}C]robalzotan (NAD-299) (Passchier and Van Waarde, 2001). To the best of our knowledge, the only study that examined 5-HT$_{1A}$ receptor density and spatial learning and memory (i.e., object-location, navigation, and floor plan drawing) in humans using the PET ligand [^{18}F]MPPF was recently published by our group (Glikmann-Johnston et al., 2015). In this study, healthy participants performed spatial virtual environment tasks during PET scanning. We found an association between hippocampal asymmetry in [^{18}F]MPPF binding and performance on the object-location task. A lower binding potential in the right vs. the left hippocampus was related to better memory performance. This finding indicates that reduced right vs. left hippocampal 5-HT$_{1A}$ receptor availability enhances object-place associative memory. Although not within the scope of this review, it is important to note that Theodore et al. (2012) used similar experimental methodology in verbal memory using the 18FCWAY PET ligand. In their study, reduced left hippocampal 5-HT$_{1A}$ receptor binding in temporal lobe epilepsy (TLE) patients was related to delayed auditory verbal memory impairment, independent of the side of the epileptic focus. More cognitive serotonergic imaging studies are needed to build up the evidence for the role of 5-HT$_{1A}$ receptor in fundamental components of human spatial memory.

Genetic Variance in 5-HT$_{1A}$ Receptor Availability

Congenital differences in 5-HT$_{1A}$ receptor availability were found to be related to spatial memory, specifically length variations in the serotonin-transporter-gene-linked polymorphic region (5-HTTLPR). 5-HTTLPR is a 44-base pair insertion/deletion functional polymorphism in the promotor region of the serotonin transporter (5-HTT) gene (Lesch et al., 1996). This polymorphism produces two common alleles designated long (L) and short (S), and was found to affect 5-HT$_{1A}$ receptor availability (David et al., 2005). Human (Roiser et al., 2006, 2007) and primate (Jedema et al., 2010) carriers of S allele demonstrated superior performance compared to L carriers on a variety of cognitive tasks, including hippocampal-dependent visual memory tasks (a computerized version of the Block Design subtest of the Wechsler Adult Intelligence Test and the CANTAB Pattern Recognition Memory and Delayed Match to Sample).

Manipulations of 5-HT Levels

Pharmacological alterations of 5-HT concentrations, by altering either 5-HT release or reuptake, have been shown to influence spatial memory. Overall, increased extracellular 5-HT concentrations maintain or improve memory performance, and reduced levels of the neurotransmitter impair spatial memory. Changes in 5-HT release are thought to indirectly stimulate post-synaptic 5-HT$_{1A}$ receptors, which reside on areas important to spatial learning and memory, thereby affecting memory function (Lesch et al., 1996; Kuypers and Ramaekers, 2005). Support for this hypothesis is found in a study by du Jardin et al. (2014) with the use of parachlorophenylalanine (PCPA). This compound inhibits tryptophan hydroxylase, and thus reduces 5-HT synthesis. In their study, PCPA induced 5-HT depletion in rats and caused memory deficits on object recognition and Y-maze spontaneous alternation tests. The selective 5-HT$_{1A}$ receptor agonist flesinoxan significantly occupied 5-HT$_{1A}$ receptors and restored PCPA-induced memory deficits in both tests. Although other agents had similar effects on spatial memory function (e.g., ***3,4-methylenedioxymethamphetamine/MDMA***: Fox et al., 2000; Skelton et al., 2006; Vorhees et al., 2007; Fisk et al., 2011; ***D-fenfluramine***: Morford et al., 2002; ***methamphetamine***: Vorhees et al., 1994, 2000, 2008; Schröder et al., 2003), studies to date did not involve the 5-HT$_{1A}$ receptor directly. Even though

the 5-HT$_{1A}$ receptor is the most abundant in the hippocampus, it is not possible to exclude other receptor subtypes that 5-HT stimulate in this area (5-HT$_{2A}$, 5-HT$_6$, and 5-HT$_7$), and that may have an effect on spatial memory.

CONCLUSION

The findings reviewed here provide converging evidence in support of the hypothesis that 5-HT, mediated by the 5-HT$_{1A}$ receptor, plays a key role in hippocampal-dependent spatial memory in animals and humans. Strong evidence comes from knockout mouse models. These studies have shown that 5-HT$_{1A}$ receptor knockouts are specifically associated with deficits in performance on spatial memory tasks. A variety of agonists and antagonists active at the 5-HT$_{1A}$ receptor modulate 5-HT neurotransmission and induce a change in spatial learning. Blockade of the 5-HT$_{1A}$ receptor impairs spatial memory, while receptor activation ameliorates antagonist-induced spatial memory deficits. Another line of evidence emerges from studies that vary neurotransmitter levels pharmacologically. Typically, increased 5-HT extracellular concentrations maintain or improve memory performance, and reduction in neurotransmitter levels impairs spatial memory.

Recent advances in human neurotransmitter research methods allow for more direct quantification of 5-HT$_{1A}$ receptor availability during spatial learning and memory. Initial results from neuroimaging studies with the use of neurotransmitter PET indicate the contribution of endogenous serotonin release or 5-HT$_{1A}$ receptor density to spatial memory, particularly to the ability to recall the location of objects in the environment (Glikmann-Johnston et al., 2015). The mapping of the human genome provides further evidence at the individual person level for the association between 5-HT$_{1A}$ receptor density and spatial memory.

Theories of hippocampal involvement in spatial memory include: (a) the cognitive map theory of O'Keefe and Nadel (1978); (b) the theory proposed by Olton and colleagues (Olton et al., 1979; Olton and Paras, 1979), in which the hippocampus is crucial for working memory; and, (c) the theory that attributes a binding mechanism to the hippocampus to form spatial memories such as object location (for example, Chalfonte et al., 1996; Eichenbaum et al., 1996). The evidence reviewed in this paper involving 5-HT, particularly the 1A receptor subtype, and spatial memory is further supported by the well-established notion of the involvement of the hippocampus in spatial memory function.

A substantial number of studies have examined the role of 5-HT in spatial learning and memory and have demonstrated, particularly in animals, a strong relation between 5-HT and spatial memory. Yet several significant questions remain. We suggest that additional research is needed to clarify the relationship between 5-HT$_{1A}$ receptor modulation and specific aspects of spatial memory, including object location and spatial frames of reference, allocentric vs. egocentric representations, and navigation and episodic memory within a topographical framework (Burgess et al., 2002; Burgess, 2008). Also, research is needed into how the serotonergic system interacts with other major neurotransmitter systems, including the acetylcholineric system, to modulate spatial memory.

For patients with damage to the temporal lobes due to progressive pathology such as Alzheimer's disease, impairments of spatial memory are often the first symptoms reported. The idea that hippocampal 5-HT$_{1A}$ receptor plays a key role in spatial learning and memory may be informative for early intervention strategies, and for improving patient outcomes in diseases affecting the temporal lobes.

AUTHOR CONTRIBUTIONS

YG-J, MS, DR, and JS wrote the article, reviewed the article, and approved the final version for publication.

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Serotonin 2a Receptor and Serotonin 1a Receptor Interact Within the Medial Prefrontal Cortex During Recognition Memory in Mice

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Episodic memory, can be defined as the memory for unique events. The serotonergic system one of the main neuromodulatory systems in the brain appears to play a role in it. The serotonin 2a receptor (5-HT2aR) one of the principal post-synaptic receptors for 5-HT in the brain, is involved in neuropsychiatric and neurological disorders associated with memory deficits. Recognition memory can be defined as the ability to recognize if a particular event or item was previously encountered and is thus considered, under certain conditions, a form of episodic memory. As human data suggest that a constitutively decrease of 5-HT2A signaling might affect episodic memory performance we decided to compare the performance of mice with disrupted 5-HT2aR signaling (*htr2a*$^{-/-}$) with wild type (*htr2a*$^{+/+}$) littermates in different recognition memory and working memory tasks that differed in the level of proactive interference. We found that ablation of 5-HT2aR signaling throughout development produces a deficit in tasks that cannot be solved by single item strategy suggesting that 5-HT2aR signaling is involved in interference resolution. We also found that in the absence of 5-HT2aR signaling serotonin has a deleterious effect on recognition memory retrieval through the activation of 5-HT1aR in the medial prefrontal cortex.

Keywords: serotonin, 5-HT2A receptor, 5-HT1A receptor, recognition memory, interference control

INTRODUCTION

Serotonin (5-HT) is synthesized in neurons of the raphe nuclei localized in the brain stem. These cells project their heavily ramified axons throughout the brain (Jacobs and Azmitia, 1992). 5-HT exerts its multiple functions through 7 distinct families of receptors (Humphrey et al., 1993; Hoyer et al., 1994; Hoyer and Martin, 1996). Each family is composed by several members that differ in localization and downstream signaling (Hoyer et al., 2002; Seyedabadi et al., 2014).The serotonin 2a receptor (5-HT2aR), one of the principal post-synaptic receptors for 5-HT, is localized

in the cortex, ventral striatum, hippocampus, and amygdala (Pompeiano et al., 1994; Cornea-Hébert et al., 1999; López-Giménez et al., 2001), brain structures involved in memory processes. As many other 5-HT receptors, the 5-HT2aR is a G-coupled protein receptor. It has a complex signaling mechanisms including activation of Gq pathway, and scaffolding proteins, including Beta-arrestin 2 (Berg et al., 1998; Schmid et al., 2008; Schmid and Bohn, 2010). 5-HT2aR is expressed in excitatory and inhibitory cells. It has a very characteristic laminar distribution in all cortical sub regions with a dorsal ventral gradient (Jakab and Goldman-Rakic, 1998). The distribution of 5-HT2aR –highly expressed in the apical dendrites of pyramidal neurons in layer 5 of the cortex- suggests that cortical 5-HT2aR modulate cortical function via distinctive mechanisms (Jakab and Goldman-Rakic, 1998) and thus play a key role in the modulation of different cortical functions. Interestingly, the serotonin 1a receptor (5-HT1aR), a Gi coupled receptor, and 5-HT2aRs appear to be co-expressed in a large fraction of pyramidal cells (Araneda and Andrade, 1991; Amargos-Bosch et al., 2004; Béïque et al., 2004) in the medial Prefrontal Cortex (mPFC). Therefore, they may regulate in a cooperative manner the way pyramidal neurons encode excitatory inputs into action potential firing. However, how this interaction affects behavior is still unclear.

Episodic memory can be defined as the memory for unique events that have as a characteristic, particular temporal and spatial features that allows an experience to be considered as a sole event. This type of memory is fundamental for an individual to construct his/her own autobiographical memory (Tulving, 1984; Schacter et al., 2011). From human and animal studies we have gained information about the brain structures, mechanisms underlying this type of memory (Schott et al., 2006a,b; Seyedabadi et al., 2014) and it has been shown that the serotoninergic system plays a particular role on it (Meneses, 1999, 2015; de Quervain et al., 2003; Meneses et al., 2004, 2011; Meneses and Liy-Salmeron, 2012; Seyedabadi et al., 2014). In healthy individuals, 5-HT2aR might be involved in memory performance (de Quervain et al., 2003; Sigmund et al., 2008) and a common polymorphism at position 452 (His to Tyr) was associated with decrease episodic memory (de Quervain et al., 2003; Sigmund et al., 2008; Avgan et al., 2014). Also 5-HT2aR have been has been implicated in different neuropsychiatric and neurological disorders including schizophrenia, attention deficit hyperactive disorder, and Alzheimer's disease (Meltzer et al., 2003; Norton and Owen, 2005; Mestre et al., 2013; Selvaraj et al., 2014). All of them are associated with memory deficits.

Recognition memory can be defined as the ability to recognize if a particular event or item was previously encountered and is thus considered, under certain conditions, a form of episodic memory (Morici et al., 2015). In animal models, recognition memory can be evaluated using a spontaneous novel object recognition task (SNOR). This task and all its variants exploit the natural tendency of rodents to explore novel stimuli over familiar stimuli. A major advantage of these tasks is the fact that they are based on the natural preference of an animal to explore novel objects and are simple, and less stressful or time consuming than other traditional memory tasks. Using these tasks, we have previously showed that the blockade of 5-HT2aR in the mPFC before a test session affects the performance of rats in recognition tasks that cannot be solved by a single item strategy (Bekinschtein et al., 2013).

Memories are not isolated in the brain. Different experiences are often associated to the same cues which could diminish correct access to a given memory during retrieval. In this way, the memories for different experiences can compete during retrieval causing interference. Experiments in humans have suggested that the PFC participates in retrieval control and selection of the relevant memory traces (Squire et al., 2004; Ferbinteanu et al., 2006). Our results in animal studies allowed us to propose that 5-HT2aR signaling in the mPFC is involved in the ability of this structure to control memory interference during retrieval when retrieval cues are not unambiguously linked to a specific memory trace. Interestingly the same result was observed when 5-HT1AR are activated suggesting that the serotoninergic modulation of the mPFC during the retrieval of recognition memory task involves opposite effects through these two different receptors (Bekinschtein et al., 2013).

Because human data suggest that a constitutively decrease of 5-HT2A signaling might affect episodic memory performance (de Quervain et al., 2003; Sigmund et al., 2008), we decided to study recognition memory in a model that constitutively lacks 5-HT2aR activity. We compared the performance of mice with disrupted 5-HT2aR signaling (*htr2a*$^{-/-}$) with wild type (*htr2a*$^{+/+}$) littermates in recognition memory tasks. We also compared the performance of these mice in two working memory tasks that differed in the level of proactive interference. In order to understand the interaction within the serotoninergic system during the modulation of episodic memory, we also analyzed the role of 5HT1aR.

MATERIALS AND METHODS

Experimental Animals

Generation of genetically modified *htr2a*$^{-/-}$ mice and their control (*htr2a*$^{+/+}$) littermates was described elsewhere (Weisstaub et al., 2006). Animals were housed at 12 h light/dark cycle at 23°C with food and water *ad libitum*. Experiments took place during the light phase of the cycle (between 10 a.m. and 5 p.m., see exception below) in 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 (CICUAL). All experiments were performed on adult (8–16 weeks old) male mice. Eight to ten animals per genotype were used for each experiment.

Apparatus and Behavioral Experiments

Spontaneous novel object recognition and temporal memory object recognition tasks were conducted in a rectangular shaped apparatus. Briefly, the rectangular arena had homogenous gray walls constructed from opaque Plexiglas. The apparatus was 40 × 25 cm length × 30 cm high. For object in context task, an additional apparatus was used. It was a triangular arena made of homogenous walls constructed from opaque gray Plexiglas. It was 40 × 25 cm length × 30 cm high. Both contexts had the same surface area in order to avoid differences due to the size of the

arena. Duplicate copies of objects made from plastic, glass and aluminum were used. The height of the objects ranged from 8 to 12 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 for being displaced during each session. The objects were always located along the central line of the maze, away from the walls and equidistant from each other. As far as we could determine the objects had no natural relevance for the mice as they were never associated to any reinforcement. The objects, floor and walls were cleaned with ethanol 10% between experiments.

The Y-maze spontaneous alternation test was conducted in a maze with three identical arms of transparent Plexiglas ($40 \times 4.5 \times 12$ cm). Visual cues were located in the periphery of the room to allow spatio-visual orientation.

The radial arm maze (RAM) test was conducted in a radial 8-arm maze described elsewhere (Saxe et al., 2007). The apparatus consisted in an octagonal central platform connected to eight arms. From this platform, doors made of Plexiglas could be automatically lowered by the experimenter in order to allow the entry of the animals into the arms of the maze.

Spontaneous Novel Object Recognition Task (SNOR)

To address whether simple object recognition memory was affected by the constitutive lack of 5-HT2aR, we used a SNOR task. Each trial consisted of three phases (see **Figure 2A**). During habituation sessions, animals were introduced into the arena for 10 min during the first session. In the subsequent habituation sessions the mice were exposed for 5 min each time. During the sample phase, two identical objects (A1 and A2) were placed into the arena. The mice were re-introduced into the arena facing the wall (and not the objects). They were then allowed to explore the objects during 10 min. The time spent exploring the two objects were scored by an experimenter observing the mouse from a distance. 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. Mice that explored less than 5 s were excluded from the experiments.

At the end of the sample phase, the mouse was removed from the apparatus and returned to its home cage for the duration of the retention period of 24 or 3 h. After this delay, the mouse was placed back into the apparatus for the test session. In this case, the arena now contained an identical copy of the sample (familiar) object (A3) and a new object (B). The position (left or right) in which the objects were placed was counterbalanced between animals. The mouse was allowed to explore the objects for a period of 5 min, at the end of which it was removed and returned to its home cage. We calculated a discrimination index (DI) 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).

Object in Context Recognition Task (OIC)

In order to evaluate if the absence of 5-HT2a signaling was involved in other recognition tasks, we used the OIC task. The habituation phase was similar to the one used in SNOR, but in this case, the mice were habituated to two different contexts, 10 min in each context. On sample phase 1, subjects were placed in context 1 facing the wall opposite to the objects and were allowed to explore two identical objects (A1 and A2) for 10 min (see **Figure 1A**). In sample phase 2, conducted 1 h later, mice were placed in context 2 together with two identical new objects (B1 and B2) and were allowed to explore the objects for 10 min. The objects used had the same characteristics described in the previous experiments. Memory was tested 24 h later. On the test phase, mice were reintroduced to context 1 or 2 (pseudo randomly assigned) and were allowed to explore freely for 5 min one copy of object A and one copy of object B. The time spent exploring the two objects were scored during the testing phase. We calculated a discrimination index defined as the proportion of total exploration time spent exploring the object not previously associated to a given context (i.e., the difference in time spent exploring the object not previously associated to a given context ant the familiar object divided by the total time spent exploring both objects).

Temporal Order Recognition Task (TMOR)

To address if recency memory was affected by the constitutive blockade of 5-HT2aR expression, we conducted a TMOR task. This task comprised one 10 min habituation session, two sample phases and one test trial (see **Figure 3A**). It was conducted in the same arena used for the SNOR or in any of the arenas used for the Object in Context Task (OIC; see next paragraph). The habituation phase was similar to the one used in the SNOR task described above. During two sample phases, the subjects were allowed to explore two identical copies of an object for 10 min. Different objects were used for sample phases 1 and 2, with a delay between the sample phases of 1 h. The test trial (5 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 phase and the objects used in sample phase 1 and sample phase 2 were counterbalanced between the animals. We calculated a discrimination index defined as the proportion of total exploration time spent exploring the less recently presented object (i.e., the difference in time spent exploring the less recently presented object and the more recently presented object divided by the total time spent exploring both objects).

Radial 8-arm Maze Test

Food-deprived mice (85% of *ad-libitum* weight) were habituated for 10 days to retrieved food pellets at the end of the eight arms. The mice used distal visual cues located in the walls surrounding the maze for spatial orientation. After habituation sessions, mice were placed on the central orthogonal platform. In order to reduce inter-trial interference, subjects performed one trial per day, consisting of a sample phase and a test phase. During the sample phase, animals were allowed to explore only four (pseudo-randomly pre-dertermined) arms. After exploring these four arms, the experimenter closed the doors of these arms. During this sample phase, re-entering the previously visited arm was considered as an error. The test phase was then conducted 5 s

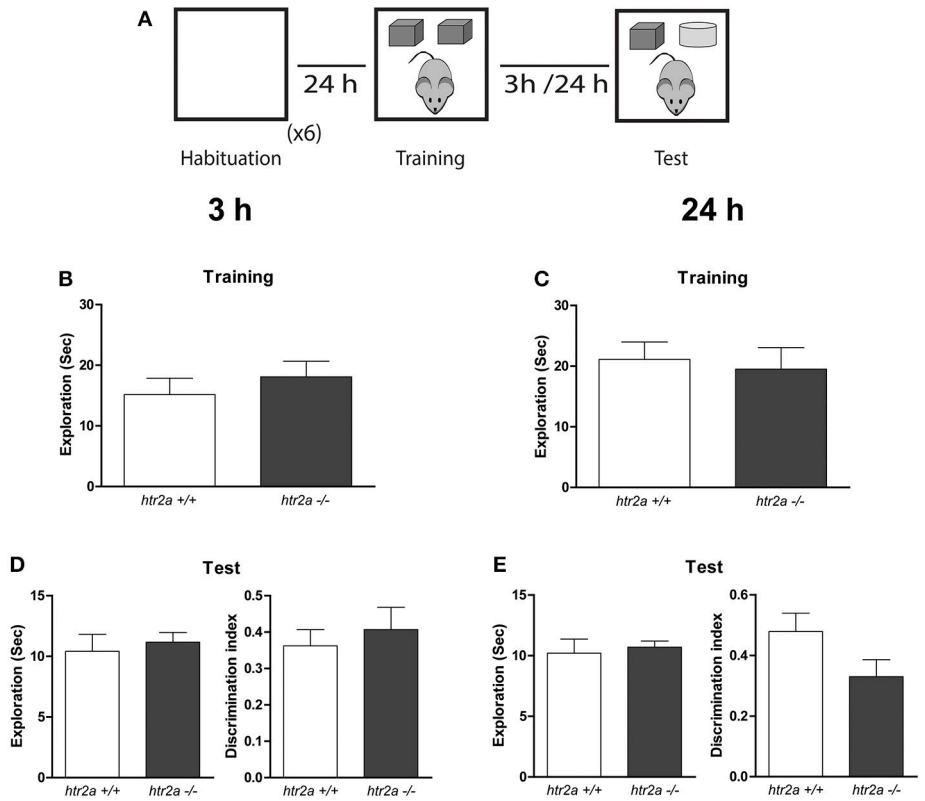

FIGURE 1 | 5-HT2aR is not required for the single-item object recognition. (A) Training and Testing scheme. The mice were exposed to a context containing two identical copies of an object for 10 min. Three or twenty-four hours later they were re-exposed for 5 min to the same context of the training phases containing one copy of the objects previously presented and a copy of a new object. **(B,C)** Exploration time measured in seconds made by the mice during the training. **(D,E)** Total exploration measured in seconds (left) and Discrimination index (right) obtained from the test phase delayed 3 or 24 h from the training phase. DI was calculated as the time spent exploring the novel object minus the time spent exploring the familiar object over the total exploration time. $n = 9$–11 per group, $p > 0.05$, Student's *t*-test.

after the sample phase had ended. During this test phase, all arms were opened but only the previously locked (and therefore not yet visited) arms contained food. The exploration of a previously visited arm (during sample phase), was considered as an error. Animals were exposed to one trial per day during 10 days.

Y-maze Spontaneous Alternation Test

The Y-shaped maze consisted of three identical arms of transparent Plexiglas (43 × 4 × 12.5 cm) placed at 120° angles to each other (Belforte et al., 2010; Braz et al., 2015). Mice were placed at the end of one arm facing the center and allowed to explore the maze freely for 8 min without training, reward, or punishment. All sessions were video recorded through a camera mounted above the maze allowing to analyze behavior of the mice by scoring the videos offline. Entries into each arm were scored and alternation behavior was defined as a complete cycle of consecutive entrances into each of the 3 arms without repetition. The percentage of spontaneous alternation was calculated as the number of alternations divided by the possible alternations [(# alternations)/(total arm entries − 2)]. Total entries were scored as an index of ambulatory activity in the Y maze and mice with scores below 7 were excluded as they showed a very low level of exploration. All experiments were conducted during the initial dark phase (6:00 p.m. to 9:00 p.m.) to maximize exploratory behavior to consistently obtained high number of entries (Belforte et al., 2010).

Surgery and Drug Infusions

The mice were deeply anesthetized with ketamine (150 mg/kg) and xilacine (6.60 mg/kg) and placed in a stereotaxic frame. The skull was exposed and adjusted to place bregma and lambda on the same horizontal plane. Small burr holes were then drilled and a set of 23 G guide cannulae of 0.5 cm were implanted bilaterally into the mPFC [anterior-posterior (AP) +1.5 mm; lateral(L) ±0.5 mm; dorsoventral (DV) −0.80 mm]. Cannulae were fixed to the skull with dental acrylic. At the end of surgery, animals were injected with a single dose of meloxicam (0.33 mg/kg) as analgesic and gentamicine (5 mg/kg) as antibiotic. Behavioral procedures commenced 5–7 days after surgery. We used a within subject design, each animal was evaluated twice, once with vehicle and once with the drug. Half of the animals were injected first with vehicle and half fist with the drug. Mice from each genotype received infusion of VEH and WAY-100135 separated by 7 days. The order of infusions was randomly assigned. On the test day, infusions were made using a 30 G injection cannula connected to a 10 μl Hamilton syringe. Cannulated mice received bilateral

0.5 μl infusions of WAY-100135 (5-HT1aR antagonist) or DMSO 13% into the mPFC 15 min before the test session. WAY-100135 was diluted in DMSO 13% into final concentration of 2μg/μl (Carli et al., 1995).

Statistical Analysis

Data were expressed as mean ± SEM and analyzed with Student's *t*-test, One-way analyses of variance (ANOVAs); Two-way ANOVA with and without repeated measures were also used when required. Factors were: Genotype for the One-way ANOVA and Genotype and Treatment for the Two-way ANOVA analyses were followed by *post-hoc* tests. Statistical analyses were performed using Graph Pad Prism 5. $P < 0.05$ was considered significant.

RESULTS

Htr2a$^{-/-}$ Response is Normal in the SNOR Task

To study whether 5-HT2aR deficiency caused a deficit in recognition memory, we exposed *htr2a*$^{+/+}$ and *htr2a*$^{-/-}$ mice to a SNOR task. This task can be solved by a single item strategy. Mice only require to recognize if the objects presented are familiar or novel. We found that the constitutive blockade of 5-HT2aR signaling has not affect on how mice distributed their exploratory time between the copies of the objects during the training phase [*htr2a*$^{+/+}$: $t_{(8)3h} = 1.03$, $p = 0.329$; $t_{(10)24h} = 1.41$, $p = 0.186$. *htr2a*$^{-/-}$: $t_{(7)3h} = 0.92$, $p = 0.386$; $t_{(8)24h} = 0.677$, $p = 0.517$] or the total exploratory levels [see **Figures 1B,C**; $t_{(15)3h} = 0.7832$, $p = 0.7745$; $t_{(18)24h} = 0.3515$, $p = 0.7396$]. Neither in the ability of the animals to discriminate between a familiar and a novel object as shown by a non-different discrimination index or total exploratory times when animals were tested 3 h. [see **Figure 1D**; $t_{(15)} = 0.5949$, $p = 0.4863$] or 24 h [see **Figure 1E**; $t_{(18)} = 1.777$, $p = 0.6230$] after training (sample phase). This result indicates that blockade of 5-HT2a signaling is not necessary for object recognition *per se* and that the *htr2a*$^{-/-}$ mice have a normal ability to acquire and consolidate recognition memory.

Htr2a$^{-/-}$ Mice Showed Deficits in the OIC Task

The OIC is a task that specifically evaluates the ability of the animals to recognize the "what and where" features of memory and, unlike the SNOR task, it has been shown to be dependent on the integrity of the PFC (Spanswick and Dyck, 2012; Bekinschtein et al., 2013). The OIC task is a three trial procedure divided in two sample phases and one test phase (see **Figure 2A**). During the sample phase, two different pairs of identical objects are presented in different contexts. During the test phase, a copy

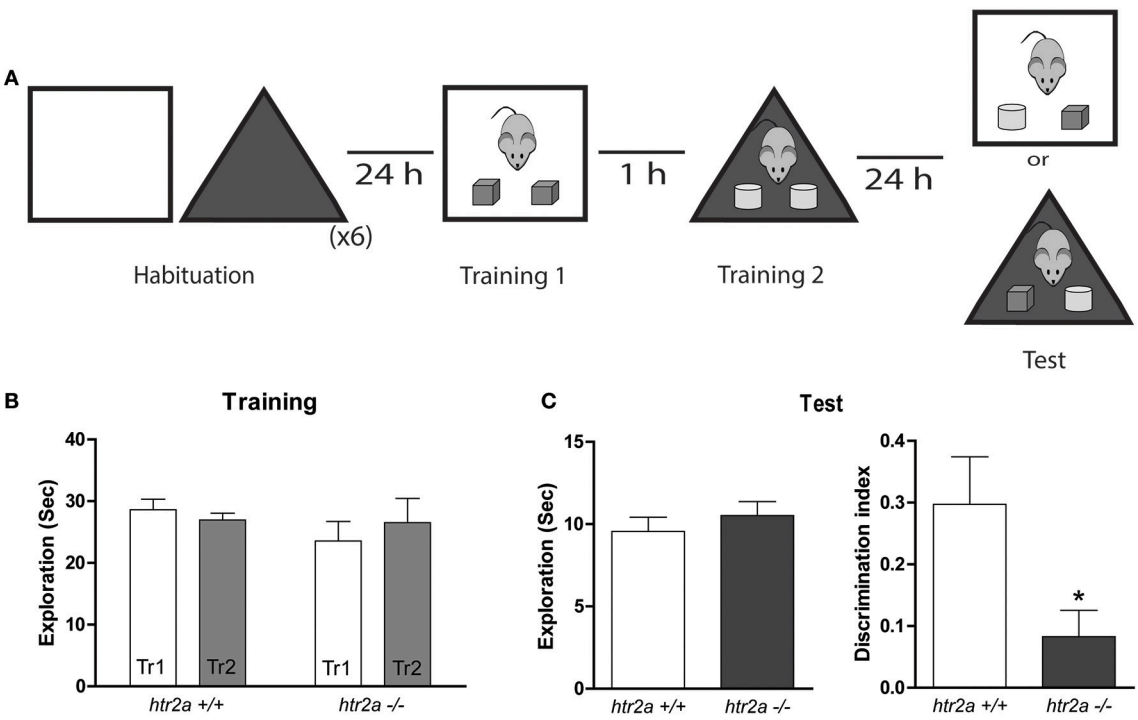

FIGURE 2 | 5-HT2aR is required for the object-in-context task. (A) Training and Testing scheme. Mice were exposed to a context containing two identical copies of an object. An hour later they were exposed to a different context containing two identical copies of a different object. Twenty-four hours later they were re-exposed to one of the context containing one copy of each of the objects. **(B)** Exploration time measured in seconds made by the mice during the first and the second Training phases (Tr 1 and Tr2). **(C)** Total exploration measured in seconds (left) and Discrimination Index (right). DI 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. $n = 10$–11 per group, $^*p < 0.05$, Student's *t*-test.

of each of the objects is presented in one of the previously experienced contexts. Thus, while one of the objects is presented in the same context experienced during the training session (congruent), the other object has not been experienced in this particular context, generating a discrepancy between the object and the context (incongruent). In this task, the novelty comes from the novel combination of an object and a context, and this will drive exploration. Recognition of this novel combination will be related to the ability of the animal to remember in which context an object presented during training. This task presents a higher load of interference than the SNOR, because during test the animals experience two familiar objects and these two memory traces can compete for retrieval.

We found that *htr2a*$^{-/-}$ mice showed a deficit in the level of discrimination of the congruent and incongruent objects as indicated by their null discrimination index during the test phase and compared with *htr2a*$^{+/+}$ [see **Figure 2C**; $t_{(19)} = 2.4998$, $p = 0.0218$]. This deficit was not due to differences in the total exploratory time during the test phase [see **Figure 2C**; $t_{(19)} = 0.789$, $p = 0.4397$] or during the sample phases [see **Figure 2B**; $F_{\text{genotype}(1, 20)} = 0.4908$ Two-way ANOVA] suggesting that the deficit might arise from the inability of *htr2a*$^{-/-}$ mice to recognize a novel combination of an object and a context. Although our model does not allow us to show which memory phase is affected by the mutation. The results obtained in the SNOR task suggest that the deficits observed in the OIC task are not due to a general deficit in acquisition, or consolidation but rather from something particular in the comparisons the animal has to make during retrieval.

Htr2a$^{-/-}$ Mice Showed Deficits in the TMOR Task

The TMOR measures the ability of the animals to assess the temporal order of two different object presentation events. The task is composed of two sample phase separated by 1 h and a retention phase performed 3 h later (see **Figure 3A**). In this paradigm, animals usually display a greater exploration time of the less recently presented "older" object. *Htr2a*$^{+/+}$ and *htr2a*$^{-/-}$ mice were trained and tested in this paradigm. There were no significant differences between genotypes in the total exploration time during the sample [see **Figure 3B**; $F_{\text{genotype}(1, 18)} = 0.5307$]; or test [see **Figure 3C**; $t_{(18)} = 0.7843$, $p = 0.1964$] phase. However, the distribution of the time exploring the objects differed between *htr2a*$^{+/+}$ and *htr2a*$^{-/-}$ mice. The discrimination index shows that *htr2a*$^{-/-}$ explored both objects to the same extent showing no recency discrimination while the *htr2a*$^{+/+}$ explored more the "older" object compared with the most "recent" one [see **Figure 3C**; $t_{(18)} = 3.153$, $p = 0.0055$] suggesting that 5-HT2aR signaling is necessary to be able to identify the order in which two objects were previously encountered.

Htr2a$^{-/-}$ Mice Showed Deficits in the Y-maze Task but Not in the Radial Arm Maze

In order to evaluate if the deficit observed was due to a general effect of-HT2a signaling in mPFC function we tested *htr2a*$^{+/+}$ and *htr2a*$^{-/-}$ mice in two working memory tasks. The first

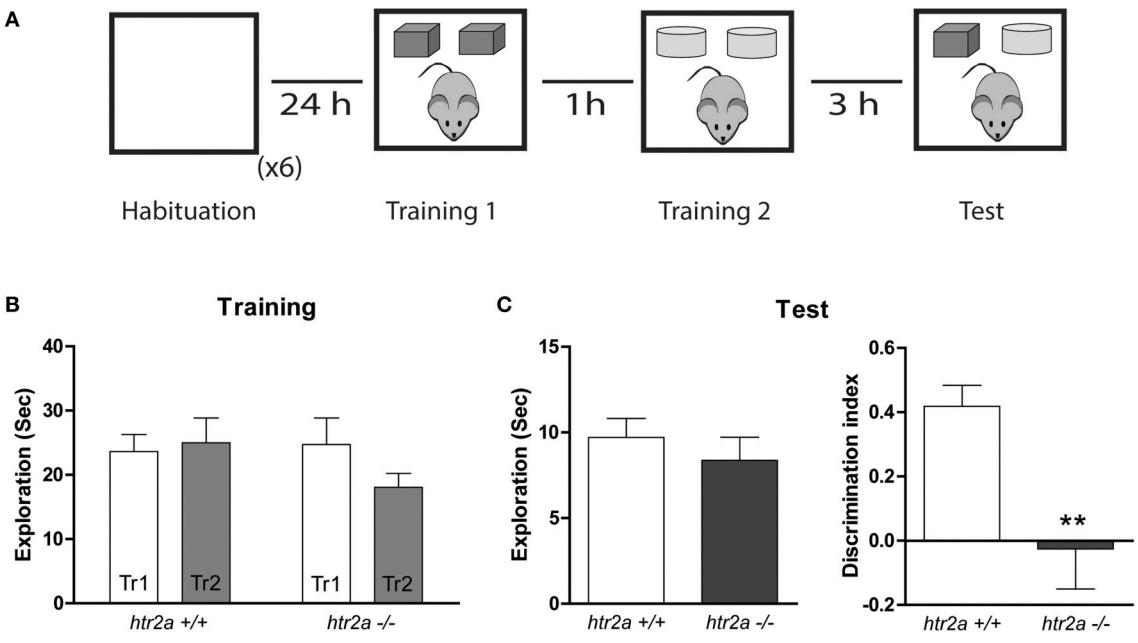

FIGURE 3 | 5-HT2aR is required for the temporal order recognition task. (A) Training and Testing scheme. The mice were exposed to an arena containing two identical copies of an object and an hour later they were re-exposed to the same arena containing two identical copies of a different object. Three hours later animals were exposed to the same context containing one copy of each object ("older" and "newer"). **(B)** Exploration time measured in seconds during the first and the second Training phases (Tr 1 and Tr2). **(C)** Total exploration measured in seconds (left) and Discrimination Index (right). DI was calculated as the time spent exploring the older object minus the time spent exploring the newer object over the total exploration time during the test session. $n = 10$ per group, $^{**}p < 0.01$, Student's *t*-test.

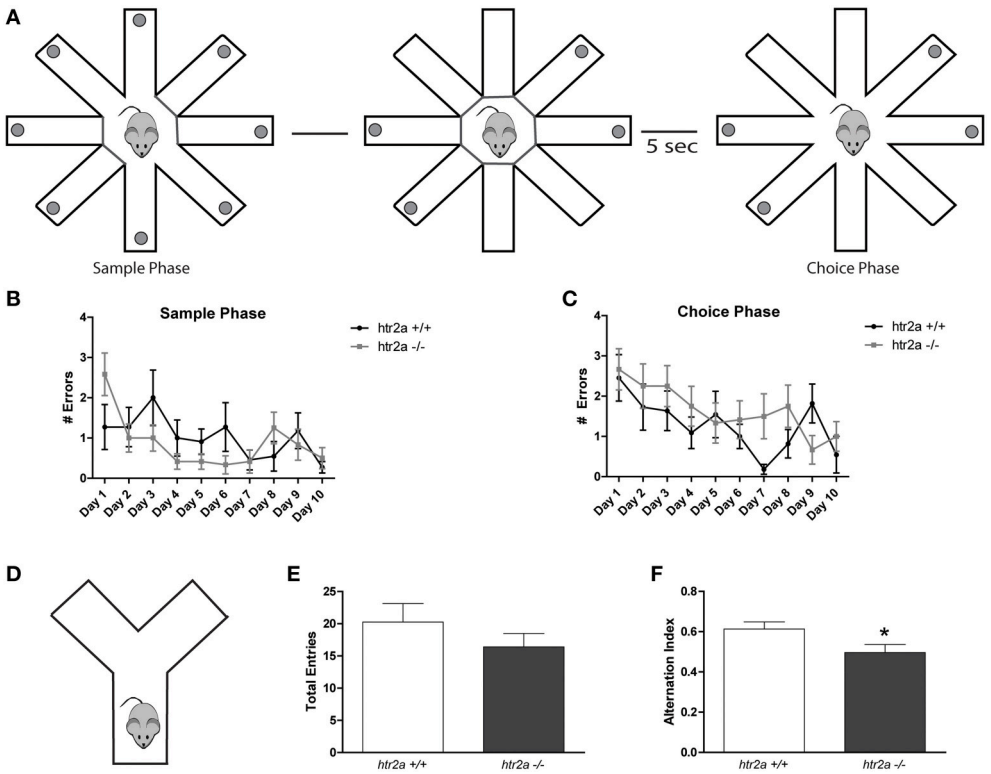

FIGURE 4 | Serotoninergic modulation via 5-HT2AR is required for the resolution of working memory tasks with high level of interference. (A) Training and test scheme for the Radial Arm Maze test. Food-deprived mice were exposed to a single trial per day during 10 days. The delay between sample and choice phase was 5 s. **(B)** Number of errors made during sample phase. **(C)** Number of errors made during choice phase. $n = 10$ per group, $p > 0.2$, Two-way ANOVA. **(D)** Scheme of the Y-maze employed. Animals were located at the end of one of the tree arms and were allowed to explore the maze for 8 min. **(E)** Total number of entries to the Y-maze arms. **(F)** Alternation index made by the mice during the Y-maze spontaneous alternation test. $n = 12$ per group, $^*p < 0.05$, Student's *t*-test.

one was the RAM maze (see **Figure 4A**). We used one trial per day and a fixed delay of 5 s between sample and test phase. In the sample phase animals were allowed to retrieve 4 food pellets from 4 of the 8 arms. In the choice phase all 8 arms were opened and visits to any of the arms opened during the sample phase were scored as working memory errors. We found that there were no significant differences between $htr2a^{+/+}$ and $htr2a^{-/-}$ mice in any of the phases of the experiments [see **Figures 4B,C**; $F_{\text{genotype sample phase}(1,\ 20)} = 0.3910$, $p = 0.5385$; $F_{\text{genotype choice phase}(1,\ 21)} = 1.148$, $p = 0.296$; $F_{\text{errors ph1}(9,\ 21)} = 3.171$, $p = 0.0014$; $F_{\text{errors ph2}(9,\ 21)} = 3.341$, $p = 0.0008$]. The second task was the spontaneous alternation Y-maze task (see **Figure 4D**). In this case, we found a deficit in alternation in $htr2a^{-/-}$ compared with $htr2a^{+/+}$ mice although they were no differences in the total number of entries performed during the task [**Figure 4E**; $t_{(22)} = 1.076$, $p = 0.2936$ and **Figure 4F**; $t_{(22)} = 2.184$, $p = 0.0399$]. During the RAM task, only one trial per day was used. The level of interference was thus very low between successive trials (separated by a 24 h delay). In contrast, the spontaneous alternation is a task but has a high level of interference since the animals were allowed to explore the maze as much as they wanted for 8 min without interruption. Our results thus suggest that the deficit observed in $htr2a^{-/-}$ mice might not be due to a working memory problem *per se* but to a deficit in interference control.

5-HT1aR Blockade Rescues the Deficit Observed in the OIC Task in *htr2a*$^{-/-}$

The mPFC is highly enriched with 5-HT1aR and 5-HT2aR. Thus, it was interesting to explore whether both receptors played a role in the serotoninergic modulation of mPFC function during the resolution of the OIC task. In order to test this possibility we infused a 5-HT1a selective antagonist, WAY-100135, in the mPFC 15 min before the test session (see **Figure 5A**). As was described before, there was no differences between genotypes during the training phase (see **Figure 5B**). We found an effect of the drug on total exploratory time [see **Figure 5C**; $F_{(1,\ 12)} = 0.1718$, $p = 0.047$] for both genotypes consistent with the previously reported result that WAY-100135 affects locomotion in a dose dependent manner (Wedzony et al., 2000). Concerning the discrimination levels between the congruent and incongruent object we found an interaction Genotype x Treatment [$F_{(1,\ 29)} = 14.44$, $p = 0.0011$]. The results of *post-hoc* analyses showed that WAY-100135 had no effect in the discrimination between the congruent and incongruent objects in $htr2a^{+/+}$ *mice* (see **Figure 5C**), but restores the ability to discriminate between the

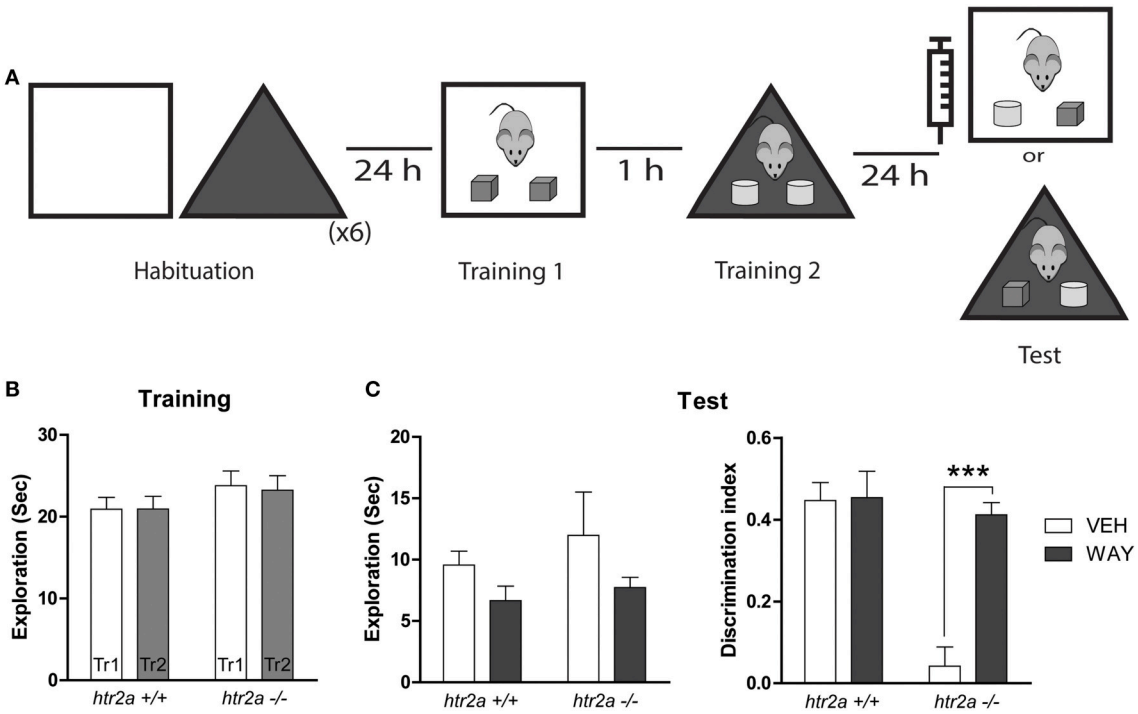

FIGURE 5 | Blockade of 5-HT1AR rescues the phenotype observed in the OIC task in *htr2a*$^{-/-}$ (A) Training and test scheme for the object-in-context (OIC) task. Fifteen minutes before the test session mice were infused with 1 μg/side of WAY 100135 or vehicle (VEH) into the mPFC. **(B)** Exploration time measured in seconds made by the mice during the first and the second Training phases (Tr 1 and Tr2). **(C)** Total exploration measured in seconds (left) and Discrimination Index (right). Discrimination index was calculated as the time spent exploring the incongruent object minus the time spent exploring the congruent one over the total exploration time during the test session. $n = 6$ per group, $^{***}p < 0.0001$, Two-way repeated measured ANOVA followed by Bonferroni's *post-hoc* test.

congruent and incongruent objects in the *htr2a*$^{-/-}$ mice (see **Figure 5C**).

CONCLUSIONS

In the current study the constitutive loss of 5-HT2aR produce deficits in particular class of recognition memory. The deficits were reserved to the OIC and TMOR task while the performance of *htr2a*$^{-/-}$ mice were normal in the SNOR. The deficit observed in the OIC task was rescued by antagonizing the 5-HT1aR in the mPFC before the test session. While the SNOR task can be solve only by taking into account the characteristics of the objects, the OIC and TMOR tasks require the animals to remember an association between the objects and the context in which they have seen them or the objects and their relative position in time. This suggested that 5-HT2aR signaling might be necessary to control the expression of the relevant memory traces when complex representations must be used for successful retrieval. Results from the two working memory tasks suggest that 5-HT2aR signaling is helpful to performance when the interference load is high, like when two familiar objects from different experiences are presented, but does not play a role when this interference load is low. These results then support a role of 5-HT2aR in interference control, probably acting at the mPFC level.

Previously, we have shown that blockade of 5-HT2aR with MDL 11939 in mPFC of rats during the test phase of an OIC task impaired the resolution of this task (Bekinschtein et al., 2013). Here we show that *htr2a*$^{-/-}$ mice recapitulate this phenotype suggesting that the constitutive absence of the receptor signaling does not generate compensatory mechanisms and that it affects a specific type of recognition memory.

Recognition memory involved the interaction of different structures including the hippocampus, perirhinal and prefrontal cortices. Our model does not allow us to identify directly which subpopulation is responsible for the deficits observed. However, some inference can be made based in the results obtained. The deficits were observed in tasks that cannot be solved by a single item strategy suggesting that 5-HT2a signaling is necessary for the ability to reduce memory interference. The RAM results together with results obtained using a Morris water maze (data not shown) indicate that *htr2a*$^{-/-}$ mice have no deficits in spatial navigation indicating normal hippocampal function in *htr2a*$^{-/-}$ mice. Neither the deficits could be explained by differences in locomotor activity since we had previously shown that *htr2a*$^{+/+}$ and *htr2a*$^{-/-}$ mice showed no significant differences in this measure in many different locomotors dependent tasks (Weisstaub et al., 2006). In addition, unimpaired performance of *htr2a*$^{-/-}$ mice in the SNOR suggests that the functional integrity of the perirhinal cortex—a structure that is essential for item recognition—(Barker et al., 2007; Bartko et al., 2007) is also

normal in our mice. Our studies also indicate that *htr2a*$^{-/-}$ mice have no deficits in the acquisition phase of these tasks and that they are able to distinguish a novel and familiar objects. Even more, our results observed in the different tasks evaluating recognition memory support the hypothesis that the deficit observed in *htr2a*$^{-/-}$ are due to the key role that the 5-HT2aR play in mPFC function.

The two tasks in which we did find deficits in *htr2a*$^{-/-}$ mice were the TMOR and OIC tasks. To solve them, mice have to integrate and compare information obtained during the training sessions. In one case (TMOR), the important information is of a temporal nature since the animals have to recognize the relative recency of the object experience (Barker et al., 2007; Bekinschtein et al., 2013). In the OIC task, the relevant information comes from the association of the context with the objects. In this case, both objects are familiar as well as the context in which they are presented during the test phase. The difficulty arises from the fact that one of the objects is presented in a different context during the sample phase. During the test phase there is an "inconsistency" between one of the objects and the context in which it is presented. Behaviorally, the animals explore more the "incongruent" than the "congruent" object. Although we do not know how the system solves this problem, we hypothesized that during the test phase the mPFC controls the retrieval of the memory traces, selecting the more relevant one. It has been shown that mPFC is important for the resolution of this type of tasks. Barker et al. found that mPFC excitotoxic lesions affected performance in TMOR and in an object-in-place task during which the animals have to remember which object has been seen and where it was (Barker et al., 2007; Barker and Warburton, 2011; Chao et al., 2015; de Souza Silva et al., 2015). Then, it is possible that the deficit observed in *htr2a*$^{-/-}$ mice results from a lack of 5-HT2aR signaling in the mPFC in a similar way to what was observed in our previous work with rats and in this way affects the ability of the mPFC to interact with other structures to solve the task.

Other experiments support this hypothesis. *htr2a*$^{-/-}$ mice showed deficits in the Y-maze spontaneous alternation task, without showing deficits in the RAM task. Both tasks assess working memory, a function highly dependent on mPFC integrity (Baeg et al., 2003; Benchenane et al., 2010; Wei et al., 2015). An important difference between both tasks resides in the designed used to test working memory. Our RAM task has a high memory load, since the animals need to keep in memory a certain number of arms (four) that already visited during sample phase in order to get the maximum amount of reward possible during a subsequent test phase. However, it has a low interference load as only one trial per day is presented to the animal. In the case of the Y-maze, the animals are allowed to explore the arms as much as they like and in the order they want and is based on the natural tendency of the mice to alternate the visits. Since this task has no reward associated with any of the visits, and the animals are left in the maze for a considerable lapse of time, the task is prone to produce high levels of interference between the successive visits of the arms. Then, we have two working memory tasks that differed in the memory load and level of interference and in which *htr2a*$^{-/-}$ mice respond differently. These differences indicate that *htr2a*$^{-/-}$ mice do not have a mPFC deficit in general or a working memory deficit *per se*. Instead they show a deficit in cases in which the interference level is high, suggesting that serotonin signaling through 5-HT2aR is involved in interference resolution necessary in specific type of working memory tasks. This interference control might act through a top-down executive control over other areas involved in the resolution of the tasks (Goldman-Rakic, 1995; Petrides, 1995; Kesner and Churchwell, 2011; Griffin, 2015). The deficits observed in the Y-maze task in absence of 5-HT2aR signaling could be explained by an imbalance in the top-down control made by the mPFC in the same way as what we saw in the TMOR and OIC tasks.

Although it is clear that serotonin plays an important modulatory role on mPFC function, how and through which receptors serotonin exerts these effects is far from clear. One of the main problems is the specific and sophisticated pattern of expression that show each 5-HT receptors subtype. Two of the main serotonergic receptors in the mPFC are the 5-HT2aR and 5-HT1aR. These two receptors exert, in the mPFC, opposite effects on neuronal activity. Since the interplay between these two receptor types is a key factor in serotonin modulation of cortical function, we decided to evaluate if they 5-HT1aR was also involved in the regulation for OIC task. We hypothesized that if both receptors played antagonistic roles in mPFC function, then we might be able to restore the deficit observed in *htr2a*$^{-/-}$ mice by manipulating 5-HT1aR activity. To do this, we infused our genetically modified mice with a selective 5-HT1aR antagonist directly into the mPFC. By combining genetic, pharmacologic and stereotaxic strategies we were able to show that mPFC 5-HT1aR are also involved in the resolution of the OIC task, and that during retrieval in the absence of 5-HT2aR signaling the main effect is through the activation of 5-HT1aR. 5-HT2aR is densely expressed in layer V of the cortex, both in excitatory and inhibitory neurons. Interestingly 60% of 5-HT2aR pyramidal cells also co-expressed 5-HT1aR. These cells showed a clear compartmentalization regarding the expression pattern of these two serotonin receptor subtypes. While 5-HT2aR are expressed predominantly in the basal part of the apical dendrite, 5-HT1aRs are expressed in the axon initial segment from where they exert an inhibitory role over the generation of action potentials (Puig and Gulledge, 2011; Celada et al., 2013a). This segregation has been postulated to be key in regulating neuronal excitability at a local level but will also have long range effects, since many of the pyramidal cells that express these receptors project to different structures, including the raphe nucleus (Celada et al., 2001, 2013b). The activation of 5-HT1aR hyperpolarizes pyramidal neurons whereas activation of 5-HT2aR results in neuronal depolarization, reduction of the afterhyperpolzarization and increase of excitatory postsynaptic currents and discharge rate (Celada et al., 2013b), then the response of the cortex to 5-HT stimulation can be inhibition, excitation or biphasic, *in vitro* as well as *in vivo* (Celada et al., 2001, 2013b; Avesar and Gulledge, 2012). The responses observed in the raphe are not only due to the differences in the modulation of projection cells from the mPFC but also to the activation of different receptors and cell types in the raphe itself (Celada et al., 2001,

2002, 2013a,b). Then the absence of 5-HT2aR probably affects not only the firing pattern of pyramidal cells in the mPFC (Weisstaub et al., 2006) but might also affect the response of the raphe nucleus to a particular stimulus. It is possible that the absence of 5-HT2aR, switch the balance to increase inhibition of projection cells, decreasing the stimulation received by the raphe and then affecting the release of 5-HT onto the cortex. If serotonin signaling in the cortex is important for retrieval control, then these changes could be, in part, responsible for the deficit observed in the *htr2a*$^{-/-}$ mice. Although our model does not allow us to identify if the effects observed behaviorally are due to the activation of 5-HT1aR that are co-expressed with 5-HT2aR or the ones expressed in other cortical cells, our results indicate that both receptor types are involved. More specific manipulations might allow in the future determining which subpopulations of 5-HT1aRs as well as 5-HT2aRs are responsible for the effects observed.

We have shown that the ablation of 5-HT2aR signaling throughout development produces a deficit in recognition memory. This deficit appears to be selective to tasks that cannot be solved by single item strategy suggesting that 5-HT2aR signaling is involved in interference resolution. The normal performance of *htr2a*$^{-/-}$ mice in the SNOR and RAM tasks support this hypothesis. In addition the phenotype observed in *htr2a*$^{-/-}$ mice is consistent with the phenotype we observed in rats (Bekinschtein et al., 2013) suggesting that the constitutive absence of the receptor signaling does not generate compensatory mechanisms. The congruence between the results in both species implies that the main effect of 5-HT2aR signaling in the mPFC is during the retrieval phase of the memory process. In the absence of 5-HT2aR signaling, the behavioral effect observed appears to be due to the activation of 5-HT1aR receptors in the mPFC suggesting that serotonin modulation of mPFC function is a key element for recognition memory in rodents.

Frequently serotonin and its receptors are associated with psychiatric disorders. However, deficits in serotonin system appear to be involved in processes that is seen as the main characteristics of these disorders and that span across many of them. These deficits are more specific and selective, even in the complete absence of 5-HT2aR expression; there are no global memory deficits but rather particular features of the memory process that are affected. Then, there is a potential for members of the serotoninergic system to be use as a biological marker of cognitive processes in the normal brain.

In summary, this work support emerging evidence that serotonergic system in the mPFC is involved in memory retrieval. Since episodic memory is affected in pathologies such as schizophrenia, Alzheimer, frontotemporal dementia and depression. Our results point out to the 5-HT1a and 5-HT2a receptors as novel target for drug development to improve episodic memory retrieval in these psychiatric and neurological disorders.

AUTHOR CONTRIBUTIONS

JM: Carried out the recognition memory experiments, analyzed the data and helped to wrote the manuscript. LC: Performed spontaneous alternation task. GM: Trained NW and supervised her for the RAM experiment, discussed the results and helped with the manuscript. JG: Provided the mice, discussed the results and helped with the manuscript. PB and NW conceived the project. PB discussed the results and helped with the manuscript. NW: Wrote the paper and supervised all aspects of the project.

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The role of serotonin 5-HT$_{2A}$ receptors in memory and cognition

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Serotonin 5-HT$_{2A}$ receptors (5-HT$_{2A}$Rs) are widely distributed in the central nervous system, especially in brain region essential for learning and cognition. In addition to endogenous 5-HT, several hallucinogens, antipsychotics, and antidepressants function by targeting 5-HT$_{2A}$Rs. Preclinical studies show that 5-HT$_{2A}$R antagonists have antipsychotic and antidepressant properties, whereas agonist ligands possess cognition-enhancing and hallucinogenic properties. Abnormal 5-HT$_{2A}$R activity is associated with a number of psychiatric disorders and conditions, including depression, schizophrenia, and drug addiction. In addition to its traditional activity as a G protein-coupled receptor (GPCR), recent studies have defined novel operations of 5-HT$_{2A}$Rs. Here we review progress in the (1) receptor anatomy and biology: distribution, signaling, polymerization and allosteric modulation; and (2) receptor functions: learning and memory, hallucination and spatial cognition, and mental disorders. Based on the recent progress in basic research on the 5-HT$_{2A}$R, it appears that post-training 5-HT$_{2A}$R activation enhances non-spatial memory consolidation, while pre-training 5-HT$_{2A}$R activation facilitates fear extinction. Further, the potential influence that 5-HT$_{2A}$R-elicited visual hallucinations may have on visual cue (i.e., landmark) guided spatial cognition is discussed. We conclude that the development of selective 5-HT$_{2A}$R modulators to target distinct signaling pathways and neural circuits represents a new possibility for treating emotional, neuropsychiatric, and neurodegenerative disorders.

Keywords: serotonin, 5-HT$_{2A}$ receptor, learning, memory, cognition

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Introduction

The serotonin (5-HT) 5-HT$_{2A}$ receptor (5-HT$_{2A}$R) is a GPCR of the type A family. It was defined as the classical D receptor initially by Gaddum and Picarelli (1957), and later referred as the 5-HT$_2$ receptor by Peroutka and Snyder (1979). The 5-HT$_{2A}$R gene is located on human chromosome 13q14-q21. *HTR2A* gene codes for a 471-amino acid sequence in rat, mouse, and human

Abbreviations: 5-HT, 5-hydroxytryptamine/serotonin; AD, Alzheimer disease; AMPAR, alpha-amino-3-hydroxy-5-methyl-4-isoxazole propionate receptor; BLA, basolateral amygdala; CS, conditioned stimulus; DOI, 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane; ERK, extracellular signal-regulated kinases; GFAP, glial fibrillary acidic protein; GPCR, G protein-coupled receptor; IP; inositol phosphate; LSD; lysergic acid diethylamide; mGluR2, metabotropic glutamate receptor; MUPP1, multiple PDZ protein-1; MWM, Morris water maze; NAc, nucleus accumbens; NMDAR, *N*-methyl-D-aspartate receptor; NOR, novel object recognition; OCD, obsessive–compulsive disorder; PDZ, postsynaptic density zone; PFC, prefrontal cortex; PKC, protein kinase C; PLC, phospholipase C; PSD, postsynaptic density; RSK2, ribosomal S6 kinase 2; sIPSC, spontaneous inhibitory postsynaptic current; sIPSP, spontaneous inhibitory postsynaptic potential; US, unconditioned stimulus.

(Sparkes et al., 1991). The rat 5-HT_{2A}R was cloned in 1988 (Pritchett et al., 1988) and the human 5-HT_{2A}R was reported by Julius et al. (1990). Central 5-HT_{2A}Rs exert diverse behavioral, physiological, and psychological influences (Hoyer et al., 2002; Hannon and Hoyer, 2008; Homberg, 2012). Abnormality in the structure and function of the 5-HT_{2A}R is associated with a number of disorders, including schizophrenia, depression/anxiety, and drug addiction. Furthermore, many hallucinogenic drugs exert their psychoactive effects by acting as agonists for 5-HT_{2A}Rs. Preclinical studies show that 5-HT_{2A}R blockade has antipsychotic (Meltzer, 1999), antidepressant (Kroeze and Roth, 1998; Roth et al., 1998) and anxiolytic properties (Cohen, 2005). Pharmacological studies indicate that high-affinity antagonists of 5-HT_{2A}Rs are effective atypical antipsychotics, due to their demonstrated efficacy to reduce both positive and negative symptoms of schizophrenia. Results from recent molecular biological and neuropharmacological studies suggest some exciting potential new avenues by which 5-HT_{2A}Rs influence CNS function. Here we review progress in understanding the contribution of 5-HT_{2A}Rs to modulation of learning and memory through an analysis of their (1) anatomy and biology: distribution, signaling, polymerization, and allosteric modulation; and (2) functions: learning and memory, hallucination and spatial cognition, and mental disorders. Based on the recent progress in 5-HT_{2A}R research, we suggest that selective 5-HT_{2A}R modulators targeting distinct signaling pathways may hold significant efficacy as new therapeutic approaches for several neurological disorders that present with cognitive impairment.

5-HT_{2A}R Anatomy and Biology in CNS

Cellular and Subcellular Distribution

Serotonin 5-HT_{2A}Rs are widely distributed in the CNS. In the rat brain, immunohistochemical studies show that 5-HT_{2A}Rs are broadly expressed in the cerebral cortex – especially in layers I and IV–V, the piriform and entorhinal cortex, the claustrum, endopiriform nucleus, and olfactory bulb/anterior olfactory nucleus, brainstem, as well as the limbic system and the basal ganglia; especially in the NAc and caudate nucleus (Xu and Pandey, 2000; Hannon and Hoyer, 2008). Interestingly, 5-HT_{2A}R binding appears to be absent from cerebellum (Xu and Pandey, 2000).

In human brain, autoradiographic analysis using [^{3}H] ketanserin indicates a high density of 5-HT_{2A}R binding in laminae III and V of the frontal, parietal, temporal, occipital, anterogenual cortexes, and entorhinal area. 5-HT_{2A}Rs are also visualized in the mammillary bodies of the hypothalamus, claustrum, and the lateral nucleus of the amygdala. The hippocampus, caudate, putamen, and accumbens nuclei present an intermediate density of binding. Areas such as the thalamus, brain stem, cerebellum and spinal cord contained only low to very low densities of binding (Pazos et al., 1987). *In situ* hybridization studies reveal that 5-HT_{2A}R mRNA is present in all neocortical areas, especially in layer 5 pyramidal neurons, and in putative interneurons. 5-HT_{2A}R mRNA was observed at minimal levels in the hippocampus and not in the raphe, cerebellum, substantia nigra or striatum (Burnet et al., 1995).

Morphological and double immunofluorescence analyses confirmed the presence of 5-HT_{2A}Rs on pyramidal neurons, interneurons, and glial cells in neocortex, amygdala and hippocampus (Willins et al., 1997; Bombardi, 2012, 2014). Thus, predicting the functional influence of activated cortical 5-HT_{2A}Rs is not straightforward, since these receptors would be capable of direct excitation and modulating feed-forward inhibition. In addition, 5-HT_{2A}Rs are located on cholinergic (Quirion et al., 1985) and glutamatergic neurons (Hasuo et al., 2002). 5-HT_{2A}R immunolabeling was also observed on glial cells in many forebrain regions: astrocytes were identified by double immunolabeling as cells in which 5-HT_{2A}R and GFAP was colocalized (Xu and Pandey, 2000); and on microglia (Glebov et al., 2015). These findings demonstrate that consideration of the serotonin-mediated signaling at 5-HT_{2A}Rs must include pathways that involve neurons and glial cells alike. It will be of interest to determine the degree to which functional influences expressed by the activation of 5-HT_{2A}Rs are dependent upon neurons, astrocytes, and microglial cells, and to determine whether clinically relevant features of 5-HT_{2A}Rs are related to changes in neurons or astrocytes.

At the subcellular level, 5-HT_{2A}R immunolabeling is found on cell bodies and processes of neurons (Cornea-Hebert et al., 1999; Xu and Pandey, 2000); in particular, at both pre- and post-synaptic compartments (Miner et al., 2003). However, the majority of evidence suggests a predominant expression at postsynaptic dendritic spines and shafts of non-5-HT neurons. Our own immuno-electron microscopy data revealed that 5-HT_{2A}R is also distributed in the dendritic spines, shafts, and presynaptic terminals of CA1 neurons in the mouse dorsal hippocampus (Zhang et al., 2015). Consideration should also be given to evidence suggesting that 5-HT_{2A}R subunits are extensively and dynamically trafficked between the cytoplasm and the neuronal membrane, as much 5-HT_{2A}R label has been identified at cytoplasmic rather than membrane bound compartments in adult rat neocortex (Cornea-Hebert et al., 1999). It will be of interest to determine the corresponding function of 5-HT_{2A}R subunit trafficking between the respective neuronal sub-compartments, and the intracellular signaling that promotes trafficking.

Interacting Proteins

Multiple interacting proteins regulate the function of 5-HT_{2A}Rs in the membrane. 5-HT_{2A}Rs interact with multiple PDZ protein-1 (MUPP1) and PSD-95 PDZ proteins (Jones et al., 2009). The 5-HT_{2A}R colocalizes with PSD-95 and with MUPP1 in a subset of dendritic spines of rat cortical pyramidal neurons. PDZ proteins are vital for docking 5-HT_{2A}R to the dendrites in cortical neurons and preventing the internalization of 5-HT_{2A}Rs (Xia et al., 2003). MUPP1 is enriched in dendritic spine PSD domains of pyramidal neurons and enhances the localization of 5-HT_{2A}R to the cell surface. Within cortical pyramidal neurons, PSD-95 regulates the functional activity of 5-HT_{2A}R by promoting apical dendritic targeting and stabilizing receptor turnover. The complex of 5-HT_{2A}R and PSD-95 plays a key

role in 5-HT_{2A}R-mediated head-twitch behavior in mice (Abbas et al., 2009). Binding of calmodulin to the 5-HT_{2A}R C-terminus impedes PKC-mediated phosphorylation of the 5-HT_{2A}R, thus, preventing its desensitization (Turner and Raymond, 2005). Conversely, association of p90-RSK2 with 5-HT_{2A}R (intracellular 3 loop) silences the GPCR's signaling (Sheffler et al., 2006). Caveolin-1 interacts with 5-HT_{2A}R and profoundly modulates its signaling by facilitating the interaction of 5-HT_{2A}R with Gαq (Bhatnagar et al., 2004). 5-HT_{2A}R and the light chain 2 domain of the microtubule-associated protein MAP1A are co-localized in the intracellular compartment of pyramidal neuronal dendrites of adult rats and may participate in intraneuronal signaling processes involving cytoskeletal elements (Cornea-Hebert et al., 2002). In consideration of these properties, we suggest that altering 5-HT_{2A}R-coupled proteins and pathways may enable an alternative method to selectively promote distinct modulatory functions of 5-HT_{2A}Rs.

Signaling

Activation of neuronal 5-HT_{2A}Rs can induce pleiotropic effects via G protein-dependent, ligand-dependent, and ligand-independent signaling pathways, including phospholipase signaling, ERK pathway, and tyrosine kinase pathway in neurons (Millan et al., 2008; Masson et al., 2012). In most circumstances, activation of 5-HT_{2A}Rs increases intracellular Ca^{2+} levels via $G_{\alpha q}$-PLC-IP3 signaling (Hagberg et al., 1998). In PFC, activation of 5-HT_{2A}Rs suppresses membrane $Ca_v1.2$ L-type Ca^{2+} currents via a $G_{\alpha q}$-mediated PLCβ/IP_3/calcineurin signaling pathway (Day et al., 2002). 5-HT_{2A}R activation also stimulates the $G_{\alpha 12/13}$-phospholipase A2 signal transduction pathway, which promotes arachidonic acid release (Kurrasch-Orbaugh et al., 2003a,b).

Besides PLC-mediated Ca^{2+} signaling, 5-HT_{2A}R activation also induces ERK phosphorylation via diverse intracellular signaling mechanisms (Gooz et al., 2006). Src and calmodulin promote 5-HT2AR-mediated phosphorylation of ERK. In the PC12 cell model system, ERK phosphorylation by 5-HT_{2A}R may not depend on PLC/PKC signaling, and instead requires an increase in intracellular Ca^{2+}, and the activation of CaM and Src (Quinn et al., 2002). The ERK target RSK2 directly acts on the third intracellular (i3) loop of 5-HT_{2A}R protein (Sheffler et al., 2006), leading to direct phosphorylation of the i3 loop at the conserved residue Ser-314 to suppress 5-HT_{2A}R signaling. In addition, RSK2 is required for tyrosine kinases, such as the epidermal growth factor receptor and the platelet-derived growth factor receptor, both of which have been demonstrated to attenuate 5-HT_{2A}R functioning in primary cortical neurons (Strachan et al., 2009, 2010).

Besides the G protein, 5-HT_{2A}Rs are also coupled to β-arrestin2. 5-HT binds 5-HT_{2A}R to stimulate Akt phosphorylation via the β-arrestin2/phosphoinositide 3-kinase/Src/Akt cascade (Schmid and Bohn, 2010). Application of the 5-HT_{2A}R agonist DOI to cultured cortical neurons induced phosphorylation of p21-activated kinase (PAK) via Rac guanine nucleotide exchange factor (RacGEF) kalirin-7 (Jones et al., 2009). The 5-HT_{2A}R also regulates the tyrosine kinase pathway activity (Quinn et al., 2002). Excitation of neuronal 5-HT_{2A}Rs activates transglutaminase which leads to transamidation of Rac1, a small G protein, resulting in constitutive activation of Rac1 (Dai et al., 2008). Chronic treatment with olanzapine, an atypical antipsychotic drug, causes the desensitization of 5-HT_{2A}R signaling. In rat frontal cortex, stimulation of the JAK-STAT pathway desensitizes the 5-HT_{2A}R-mediated PLC activation induced by olanzapine (Singh et al., 2010). Furthermore, constitutive activation of 5-HT_{2A}Rs induces $G_{q/11}$ phosphorylation and desensitization (uncoupling) (Shi et al., 2007).

As indicated above, 5-HT_{2A}Rs are also expressed in microglia and mediate 5-HT-induced exosome release (Glebov et al., 2015). Activation of 5-HT_{2A}R increases intracellular Ca^{2+} via PLC signaling in astrocytes (Hagberg et al., 1998) and Glu efflux from C6 glioma cells (Meller et al., 2002). Considering the diversity of signaling cascades that can be triggered by 5-HT_{2A}R activation, it is perhaps not surprising that serotonergic activation of 5-HT_{2A}Rs can have diverse influences on neuronal responses and CNS functions.

Oligomerization

The GPCRs can form homomers and heteromers, and thereby present distinct signaling and functional activities (Rios et al., 2001). Consistent with this, 5-HT_{2A}Rs have been shown to form oligomers (Lukasiewicz et al., 2010). Fluorescence resonance energy transfer and immunoprecipitation studies revealed that the human 5-HT_{2A}R homodimerizes in cultured cells (Brea et al., 2009). For 5-HT_{2A}R oligomers, the 5-HT_{2A}R agonist DOI caused an increase in energy transfer efficiency to the level of 12%, and ketanserin caused a decrease of 4.4%. Heterodimers of 5-HT_{2A}R and dopamine D_2 receptors respond to DOI and quinpirole, a DA D_2R agonist, with a decrease in FRET efficiency, while ketanserin and butaclamol increase the transfer efficiency value (Lukasiewicz et al., 2010). Heterodimers of 5-HT_{2A}R and mGluR2 receptor form via the linking domain in transmembrane-4 and -5 segments, and are present in the human brain. Post-mortem studies indicate a reduced density of these functional complexes in brains of schizophrenics (Gonzalez-Maeso et al., 2008). Delta-9-tetrahydrocannabinol (THC), the main psychoactive compound of marijuana, induces memory impairments, anxiety, dependence, and analgesia. Vinals et al. (2015) recently reported that amnesic and anxiolytic effects, but not analgesia, induced by THC were suppressed in 5-HT_{2A}R knockout mice. Molecular studies revealed that cannabinoid CB1 receptors (CB1R) and the 5-HT_{2A}R physically interact with each other to form heteromers, which are distributed extensively in hippocampus, cortex, and dorsal striatum, but not in the NAc. *In vivo* experiments have revealed that stimulation of CB1R and 5-HT_{2A}R reduces cell signaling, and the binding of an antagonist to one receptor blocks signaling of the interacting receptor. Heteromer formation leads to a switch in 5-HT_{2A}R-mediated G-protein coupling from $G_{\alpha q}$ to G_i. Synthetic peptides with the sequence of transmembrane helices 5 and 6 of CB1R disrupt CB1R and 5-HT_{2A}R heteromerization *in vivo*, leading to a selective abrogation of memory impairments, but not the antinociceptive properties caused by THC exposure (Vinals et al., 2015). The anatomy, biology and function of 5-HT_{2A}R

homomers and heteromers, including the dynamic formation and dissociation, distribution, signaling and function, remain elusive. Elucidation of 5-HT_{2A}R oligomers will be interesting for both basic science research and potential clinical applications.

Allosteric Modulation

Recent years have witnessed a tremendous advance in the research and development of novel compounds for GPCRs that bind allosteric sites to regulate receptor structure and function. These ligands provide high specificity, novel modes of efficacy and may open up a novel avenue for therapeutic agents against multiple mental and neurological disorders. Allosteric modulators bind to a site distinct from that of the orthosteric ligand-binding site. Usually the allosteric modulator induces a structure change within the GPCR to enhance or suppress the orthosteric ligand's functional activity (Conn et al., 2009; Melancon et al., 2012). Application of the amidated lipid, oleamide significantly potentiated 5-HT-induced hydrolysis of phosphoinositide in pituitary P11 cells expressing endogenously 5-HT_{2A}Rs (Thomas et al., 1997). Taken together, these results indicate that there are several binding sites present on 5-HT_{2A}Rs, and we suggest that it will be of interest to further characterize the functional significance of the distinct ligand-driven actions at the 5-HT_{2A}R.

Constitutive Activity

As mentioned above, 5-HT_{2A}Rs can also be constitutively active (i.e., via activating the receptor in an agonist-independent activity) *in vivo* (Berg et al., 2008). The inverse 5-HT_{2A}R agonists (e.g., risperidone and ketanserin) produce a great suppression of basal IP production, leading to a reduction of basal activity in the C322K mutant 5-HT_{2A}R (Egan et al., 1998). The "constitutively active" arrestin mutant (Arr2-R169E) induces agonist-independent 5-HT_{2A}R internalization, and a constitutive translocation of the Arr2-R169E mutant to the plasma membrane (Gray et al., 2003). The constitutive activity of 5-HT_{2A}Rs may represent another mechanism of regulating cellular function. The specific relationships of these constitutively active 5-HT_{2A}R-mediated properties to distinct behaviors have not been determined.

Electrophysiological Characteristics

Electrophysiological studies reveal complex effects of 5-HT_{2A}R activation on cortical neurons; however, mainly these receptors appear to mediate depolarizing effects on excitatory and inhibitory neurons. Slice recordings from prefrontal cortical neurons indicate depolarizing effects following 5-HT_{2A}R activation (Aghajanian and Marek, 1999; Zhou and Hablitz, 1999; Avesar and Gulledge, 2012). Local application of DOI, a 5-$HT_{2A/2C}$ receptor agonist, increases the firing rates of cortical neurons (Stein et al., 2000) and facilitates synaptic plasticity through an NMDAR-dependent mechanism in presumptive pyramidal neurons of the rat BLA (Chen et al., 2003). Meanwhile, α-methyl-5-hydroxytryptamine (a 5-HT_2R agonist) and DOI induce activation of GABAergic interneurons of the rat BLA (Stein et al., 2000). Double immunofluorescence labeling demonstrated that the 5-HT_{2A}R is primarily localized to parvalbumin-containing BLA interneurons. Accordingly, 5-HT primarily acts on 5-HT_{2A}Rs to potentiate GABAergic inhibition. 5-HT_{2A}R activation increases the frequency and amplitude of sIPSCs recorded from the pyramidal neurons in BLA of the juvenile rat (Jiang et al., 2009). DOI potentiates NMDAR-mediated changes in membrane potentials and calcium influx without affecting the neuronal resting membrane potential or input resistance. However, DOI does not affect AMPA/kainate receptor-mediated excitatory synaptic responses (Chen et al., 2003). The relationship of 5-HT_{2A}Rs to NMDARs is consistent with the view that 5-HT_{2A}Rs may be an effective target for modulating experience-dependent synaptic plasticity in the CNS. Globally, 5-HT_{2A}Rs have been shown to influence low-frequency field potential oscillations in rat frontal cortex (Celada et al., 2008). Taken together, these findings demonstrate that the 5-HT_{2A}R mediates 5-HT-induced excitation of cortical neurons. However, much remains to be determined as to the neurophysiological consequences of 5-HT_{2A}R activation, in particular as they relate to the regulation of specific behaviors.

Recent molecular and pharmacological research has made significant advances in the understanding of the functional selectivity of 5-HT_{2A}R. The multiple signaling pathways suggests bias agonism and bias signaling of 5-HT_{2A}Rs, which posit that an agonist can produce a mix of signaling, which is potentially determined by cell type and functional status.

5-HT_{2A}R Functions in CNS

Long-term declarative or episodic memory is supported by a network of brain structures in the medial temporal lobe of the mammalian brain. The medial temporal lobe memory system, which includes the hippocampus, dentate gyrus, and surrounding extrahippocampal cortical regions, influence decision-making processes guided by the PFC, and posterior parietal cortex (Squire et al., 2004, 2007; Preston and Eichenbaum, 2013). Serotonergic fibers originating from the raphe nuclei innervate many of the critical nodes within the medial temporal lobe memory system, including the hippocampus and amygdala, and on to the PFC (Vertes, 1991; Vertes et al., 1999). The modulatory influence of 5-HT on simple and more complex forms of learning and memory has been extensively examined in both invertebrate and vertebrate model systems (Kandel and Squire, 2000). The relevance of 5-HT to memory seems to generalize across mammals:, dietary tryptophan increases brain 5-HT levels and improves memory in rodents (Khaliq et al., 2006), the elderly, AD patients, and schizophrenics (Levkovitz et al., 2003; Porter et al., 2003). Further, reductions in brain 5-HT concentrations after acute or chronic tryptophan depletion has been demonstrated to impair contextual fear memory in mice (Uchida et al., 2007), object memory in rats (Jenkins et al., 2009), and declarative memory in humans (Schmitt et al., 2006). Below, we describe some evidence suggesting that the 5-HT_{2A}R may hold special significance as one of the substrates by which 5-HT regulates learning and memory (Meneses, 2007).

Learning and Memory

Polymorphisms in the human *HTR2A* gene are associated with altered memory processes. For example, a *HTR2A* gene polymorphism inducing the substitution of the His452 on the receptor subunit to a Tyr residue is associated with a significant impairment in memory recall amongst adults (de Quervain et al., 2003; Sigmund et al., 2008; Zhu et al., 2013). Carriers of the His452Tyr (rs6314) exhibited poor verbal delayed recall and recognition, but performed equivalent to controls on tests of immediate recall, attentional, and executive function (Wagner et al., 2008). Compared to His homozygotes, Tyr carriers exhibited a diminished hippocampal response to novel stimuli and a higher tendency to judge novel stimuli as familiar during delayed recognition (Schott et al., 2011). Amongst schizophrenics and healthy controls, those carriers of homozygous CC (T102C) and GG (A-1438G), or carriers of the so-called *T*-allele (rs6314), of the *HTR2A* gene polymorphisms exhibited significantly impaired short-term verbal memory (Alfimova et al., 2009), and spatial working memory (Blasi et al., 2013). Another polymorphism in the *HTR2A* gene, referred to as rs4941573 was found to be predictive of increased error rate in a spatial working memory task in an adult Chinese subject population (Gong et al., 2011). These results provide just a brief and incomplete view of a broad literature indicating the impressive degree to which alterations in the *HTR2A* gene relate to disordered cognitive functions in normal and abnormal human subjects.

The regional distribution of 5-HT_{2A}Rs can be predictive of the memory capacities that are sensitive to serotonin manipulation. The 5-HT_{2A}Rs are widely expressed in the neocortex and hippocampus of rats (Xu and Pandey, 2000; Hannon and Hoyer, 2008), rabbits (Aloyo and Harvey, 2000), primates (Jakab and Goldman-Rakic, 1998; Lopez-Gimenez et al., 1998), and humans (Hoyer et al., 1986; Lopez-Gimenez et al., 1998). **Table 1** summarizes the major findings of studies in which the learning and memory effects were examined after 5-HT_{2A}R pharmacological manipulations across distinct tasks and different species. The inconsistency of experimental results may be attributed to the species, selectivity and dose of drug, behavioral task and other effectors.

Object Memory

The spontaneous NOR task, which relies on rodents' inherent preference for exploring novel over familiar stimuli, has become a popular method for examining the neuropharmacological and neurophysiological mechanisms of object memory (Ennaceur, 2010; Cohen and Stackman, 2015). In the task, rodents are exposed to one or two novel objects in a familiar enclosure during a sample session (i.e., training). The rodent is removed from the enclosure after it has sufficiently explored the objects. After a delay of some length, the rodent is returned to the enclosure for a memory test session, during which the enclosure contains one familiar object and a novel object. If the rodent has successfully encoded and consolidated the memory of the original object from the sample session, then it is expected that the rodent will preferentially explore the novel object during the test session. The NOR task offers advantages for testing rodent memory in that the distinct memory processes of encoding, consolidation and retrieval are operationally defined as events occurring during the sample session, after the sample session, or during the test session, respectively. Another advantage is that the behavioral responses are spontaneous rather than requiring overt motivation such as electrical shock or food restriction. Our recent studies implicate the hippocampus as a key region in the rodent brain for object memory processes (Cohen et al., 2013; Cohen and Stackman, 2015). In light of the fact that 5-HT_{2A}Rs are densely expressed in the hippocampus (Luttgen et al., 2004), we examined the contribution of hippocampal 5-HT_{2A}Rs in object memory processes in male mice using an NOR task (see **Figure 1**). Systemic activation of 5-HT_{2A}Rs with the selective agonist, TCB-2 after the sample session significantly enhanced the time mice spent exploring the new object presented during the test session 24 h later (Zhang et al., 2013). The memory-enhancing effect of TCB-2, was blocked by pretreatment with the 5-HT_{2A}R antagonist, MDL 11,939, which suggests that 5-HT_{2A}R activation enhances the consolidation of object memory. Interestingly, when TCB-2 was administrated before the sample session, or before the test session, the 5-HT_{2A}R agonist failed to increase novel object preference relative to the respective control group. Together, these data suggest that 5-HT_{2A}R activation selectively potentiates memory consolidation. Furthermore, the selective local microinfusion of TCB-2 into the CA1 region of dorsal hippocampus recapitulated the memory enhancing effect observed after systemic treatment (Zhang et al., 2015). The relevance of the 5-HT_{2A}R for object memory processes was also demonstrated by results of a study showing that the local infusion of the 5-HT_{2A}R antagonist MDL 11,939 into the mPFC impaired retrieval of object-in-context memory in rats (Bekinschtein et al., 2013). Interestingly, the 5-HT_{2A}R agonist DOI was found to impair retrieval of memory for an operant response by adult rats in an autoshaping task (Meneses, 2007). Thus, it would appear that the influence of the 5-HT_{2A}R on memory is task- and memory system-dependent, and perhaps by the underlying neural circuitry that supports the respective memory process.

The encoding and consolidation of hippocampal-dependent memory appears, in part, to require fast glutamatergic neurotransmission, ensuing phases of synaptic plasticity, and dynamic replay of experience-dependent neurophysiological oscillatory activity within hippocampal cell populations (Eichenbaum, 1999; Karlsson and Frank, 2009). Our published (Zhang et al., 2013) data show that post-training activation of 5-HT_{2A}R s enhances object memory, likely by affecting consolidation. Prevailing views state that the hippocampus transfers recent to-be-remembered information to the neocortex during sharp wave ripples of the hippocampal local field potential (i.e., 100–200 Hz ripples; Chrobak and Buzsaki, 1996; Carr et al., 2011). During sleep, hippocampal neurons 'replay' patterns of spike trains present during a learning episode. As sharp wave ripples and replay may represent systems consolidation of memory, it would be of interest to examine the influence of 5-HT_{2A}R-sensitive drugs on sharp wave ripples and replay of spiking sequences during sleep episodes after a to-be-remembered experience. Postsynaptic 5-HT_{2A}Rs may modulate object memory consolidation by also influencing NMDAR-mediated synaptic plasticity. Consistent

TABLE 1 | Reported effects on learning and memory after pharmacological manipulation of 5-HT_{2A} receptors (5-HT_{2A}Rs).

Drug	Route; dose	Task	Species	Effect	Reference
M100907	0.01–0.1 mg/kg; i.p.	Probabilistic reversal learning	Mice	↑Acquisition	Amodeo et al., 2014; *BTBR T+tf/J mouse model of autism
M100907	0.01–0.1 mg/kg; i.p.	Serial spatial reversal learning task	Rats	↓ Retrieval	Boulougouris et al., 2008
M100907	0.02–2.0 nmol; olfactory bulb	Reversal-learning task	Rats	↓ Acquisition	Furr et al., 2012
MDL 11,939	0.067–6.7 μmol/kg; s.c.	Nictitating membrane conditioned responses	Rabbits	↓ Acquisition	Welsh et al., 1998
MDL 11,939	300 ng/μl; mPFC	NOR task	Rats	↓ Retrieval	Bekinschtein et al., 2013
Ritanserin,	2.5 mg/kg × 11 days; s.c.	Conditioned olfactory training	Rat pup	↑ Acquisition	McLean et al., 1996
Risperidone	1 mg/kg; i.p.	Reward-dependent operant conditioning task	Rats	↓ Acquisition and ↑ extinction	Frick et al., 2015
Risperidone	0.125 mg; i.p.	Probabilistic reversal learning	B6 mice	↓ Acquisition	Amodeo et al., 2014
Ketanserin Methysergide	1.0–3.0 mg/kg; s.c.; 3.0–15.0 mg/kg; i.p.	Delayed non-matching to position task (working memory)	Rats	↔ Retrieval	Ruotsalainen et al., 1997
Ketanserin	0.1 mg/kg × 14 days; i.p.	Passive avoidance paradigm and MWM	Rats	↓ Acquisition	Fedotova and Ordyan, 2010
DOI Ketanserin	0.01–0.1 mg/kg; i.p. 0.001–0.1 mg/kg, i.p.	Autoshaping learning task	Rats	↑ Consolidation	Meneses et al., 1997
M100907;α-methyl-5-HT	PFC	Oculomotor delayed-response tasks	Monkeys	↓ Acquisition ↑ Acquisition	Williams et al., 2002
TCB-2	1.0 mg/kg; i.p.	NOR task and Trace and delay fear conditioning	Mice	↑ Object memory acquisition; ↑fear memory extinction	Zhang et al., 2013
DOI	0.1–0.3 mg/kg; i.p.	Autoshaping learning task	Rats	↓ Consolidation	Meneses, 2007
LSD	0.43–12.9 μg/side; hippocampus	Trace eyeblink conditioning.	Rabbits	↑ Acquisition	Romano et al., 2010
LSD	1–300 nmol/kg; i.v.	Nictitating membrane response	Rabbit	↑ Acquisition	Gimpl et al., 1979
LSD	0.13 mg/kg/d × 11 days; s.c.	Bulbectomy-induced deficit in active avoidance learning	Rats	↑ Acquisition	Buchborn et al., 2014
Psilocybin	215 μg/kg; oral	Spatial working memory task	Humans	↔ Retrieval	Carter et al., 2005
Psilocybin	0.1–1.5 mg/kg, i.p.	Trace fear conditioning -	mice	↑ Extinction	Catlow et al., 2013
Psilocin	1.0 mg/kg, i.p. 4.0 mg/kg, i.p.	MWM; Carousel maze (CM)	Rats	↓Acquisition of CM; ↓Retrieval of MWM (4 mg/kg); ↔ Consolidation	Rambousek et al., 2014
Quipazine	1.25–10 mg/kg, s.c.	Conditioned avoidance response	Rats	↑ Acquisition	Alhaider et al., 1993

↑, *enhance;* ↓, *suppress;* ↔, *no effect.*

with this possibility, hippocampal 5-HT_{2A}Rs are predominantly expressed at dendritic sites on pyramidal neurons (Cornea-Hebert et al., 1999; Peddie et al., 2008). 5-HT_{2A}R-containing dendritic processes also were immunolabeled for the NMDAR subunit NR1 and GluR2 (Peddie et al., 2008). We have found that 5-HT_{2A}R activation increased the extracellular efflux of glutamate in the dorsal hippocampus, and increased the basal firing rates of CA1 pyramidal neurons in awake behaving mice (Zhang et al., 2015). These results suggest that the 5-HT_{2A}R activation induced facilitation of object memory consolidation, may result from the potentiation of hippocampal glutamate release, and pyramidal neuron temporal dynamics at a critical post-training time period. These data suggest that the 5HT_{2A}R may serve as a drug target for pharmacological interventions to treat memory impairments. It is conceivable that 5-HT_{2A}R activation promotes an increase in intracellular Ca^{2+}, combined with NMDAR-mediated Ca^{2+} influx, which together would facilitate the behavior-initiated synaptic plasticity. Aghajanian and Marek (1999) reported that activation of 5-HT_{2A}R produces an elevation in the frequency and amplitude of neuronal sEPSP/sEPSC. Consistently, 5-HT_{2A}R activation has been shown to facilitate NMDAR activity and synaptic plasticity in the cortex (Arvanov et al., 1999) and BLA (Chen et al., 2003). Furthermore, 5-HT_{2A}R directly interacts with PSD-95 to regulate receptor trafficking and signaling (Xia et al., 2003). 5-HT_{2A}R activation induces a transient increase in dendritic spinogenesis (Yoshida et al., 2011), phosphorylation of PAK, neuronal Rac guanine nucleotide exchange factor (Jones et al., 2009), BDNF expression (Vaidya et al., 1997), and Erk mitogen-activated protein kinase activity (Florian and Watts, 1998; Watts, 1998). Finally, the 5-HT_{2A}R inverse agonist pimavanserin was shown to reverse NMDAR antagonism-induced object memory impairments in

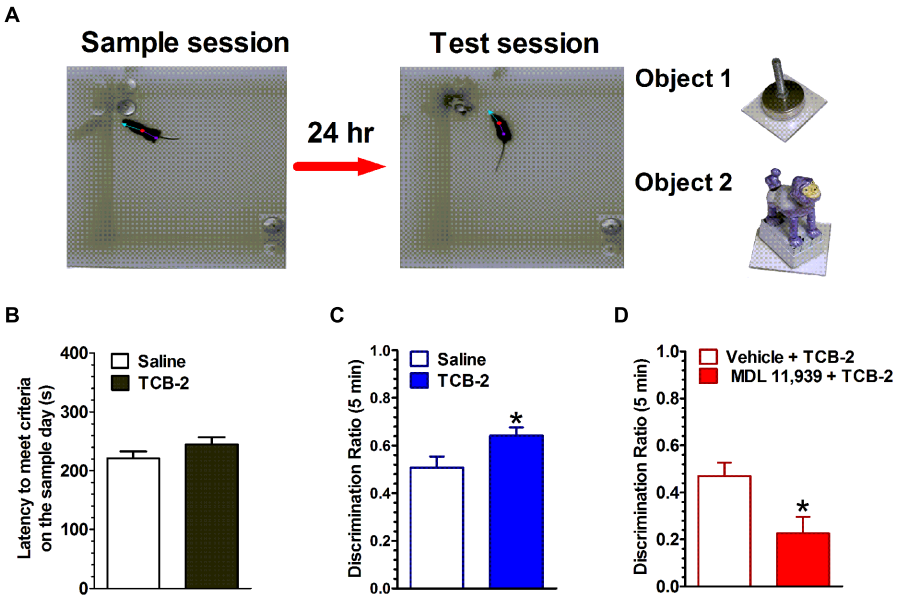

FIGURE 1 | Activation of 5-HT$_{2A}$Rs enhances the consolidation of object memory. (A) Experimental strategy. Left, during the sample session, mice were allowed to explore two identical novel objects each for at least 15 s, or either one for 18 s within a 10-min sample session. Middle, during the test session 24 h later, one of the objects (a cabinet leveling foot) was replaced with a novel object (a plastic toy monkey) and mice were individually reintroduced to the arena. Right, the objects used in this study. **(B)** There was no difference in the latency to achieve exploration criteria during the sample session between the saline- and TCB-2-treated mice. **(C)** Mice that received TCB-2 right after the sample session exhibited a significantly stronger preference for exploring the novel object during the test session: analysis revealed that TCB-2-treated mice had a higher mean discrimination ratio as compared to that of the saline group. **(D)** MDL 11,939, a selective 5-HT$_{2A}$R blocker suppressed TCB-2-induced enhancement in object memory (Zhang et al., 2013). *$p < 0.05$, vs. respective control condition.

combination with atypical antipsychotic drugs (Snigdha et al., 2010). These results support the view of a modulatory influence of 5-HT$_{2A}$R on NMDAR-dependent memory mechanisms. Considering the myriad potential influences of 5-HT$_{2A}$R on medial temporal lobe memory mechanisms, there would appear to be multiple downstream influences by which 5-HT$_{2A}$R activation could enhance memory.

Fear Memory

While there is a rich literature on the influence of serotonin on anxiety and an established contribution of serotonergic drugs to the remediation of anxiety disorders in humans, the present review will focus on the influence of 5-HT$_{2A}$Rs on fear memory encoded during Pavlovian conditioning sessions. Pavlovian fear conditioning has become a popular procedure for examining the neurobiological mechanisms of fear memory. As a Pavlovian conditioning procedure, fear conditioning lends itself well to defining processes of encoding, consolidation and retrieval of fear memory. Fear conditioning taxes a well-defined neural circuit within the amygdala, which in turn interacts with the hippocampus, anterior cingulate, or the PFC, depending on the elements of the conditioning session and the stage of memory processing (Zelikowsky et al., 2014). In addition, considerable attention has been given to investigations of the underlying biology of extinction of fear memory. During a delay fear conditioning session, an innocuous stimulus (e.g., a neutral tone or light) becomes a CS when it is repeatedly presented in such a way that it co-terminates with the presentation of a sufficiently aversive US (e.g., foot shock) (Zhang et al., 2013). The unconditioned response to the foot shock US is typically jumping and running, but the conditioned response to the CS is a defensive freezing response, or the cessation of all movement except for respiration. Thus, the freezing behavior provides a reliable post-conditioning measure of fear memory in rodents (Blanchard and Blanchard, 1969). During fear conditioning, the subject learns to associate the tone CS with the foot shock US, and under certain conditions, learns to associate the foot shock with the environment or context where the conditioning session was presented. Acquisition of both the tone-shock and the context-shock associations requires the amygdala; however, the context-shock associations are also dependent upon the hippocampus (Kim and Fanselow, 1992; Phillips and LeDoux, 1992). There has been considerable debate regarding the involvement of the hippocampus in contextual fear memory since there have been reports that hippocampal lesions impair contextual fear memory (Kim and Fanselow, 1992; Phillips and LeDoux, 1992; Anagnostaras et al., 1999; Stiedl et al., 2000), and others reporting that such lesions spare contextual fear memory (Cho et al., 1999; Wiltgen et al., 2006). Consensus seems to be building for the view that if the rodent is permitted sufficient time to acquire a hippocampal-dependent configural representation of the context (the chamber's geometry, olfactory, visual, tactile, and auditory cues) before the US is presented, then the hippocampus is engaged in associating the contextual memory with the foot shock (Rudy et al., 2002, 2004; Matus-Amat et al., 2004; Zelikowsky et al., 2014). In a trace fear conditioning

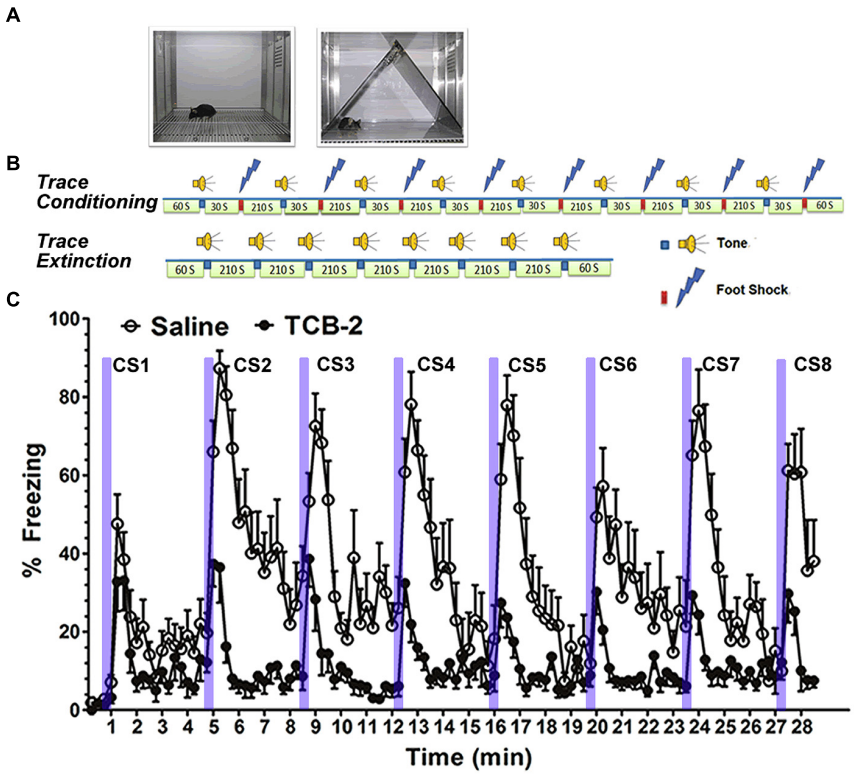

FIGURE 2 | Stimulation of 5-HT$_{2A}$Rs enhances the acquisition of extinction of trace fear memory. (A) Left, a chamber for fear conditioning (Context A) and contextual fear memory test; right, Context B, a modified chamber with different color, context, light density, and odor for cued fear memory test. **(B)** Trace fear conditioning training procedure. After a 60-s habitation to Context A, a tone was presented for 15 s followed by 30 s stimulus-free interval, and then a 0.5-s, 0.75 mA foot shock (US) was presented. The CS–US pairing was repeated eight times with a 210-s intertrial interval (ITI). Mice were removed from the conditioning chamber and returned to their home cages 60 s after the final CS–US pairing. Trace fear conditioning extinction procedure. Sixty seconds after placing the mouse into the modified chamber, eight unpaired 15-s tone CS were presented with a 120-s ITI. **(C)** Mice that received TCB-2 (1.0 mg/kg, i.p.) before a trace fear memory extinction test exhibited accelerated acquisition of extinction as indicated by significantly lower freezing scores earlier in the course of extinction as compared to those of vehicle-treated mice. TCB-2 significantly decreased percent freezing from the second to eighth CS presentation (Zhang et al., 2013).

procedure, a temporal gap is imposed between the offset of the tone CS and the onset of the foot shock US. The acquisition of an appropriately timed (i.e., anticipatory) conditioned freezing response occurs progressively over the course of the repeated CS–US pairings; this temporal fear memory is a form of declarative memory dependent on intact hippocampal function in rodents and humans (Clark and Squire, 1998; McEchron et al., 1998; Chowdhury et al., 2005). It should be clear that in deciphering an influence of 5-HT$_{2A}$R-sensitive drugs on the distinct processes of memory for contextual and/or cued fear, one must consider the specifics of the conditioning protocol used.

Finally, considerable attention has been directed toward defining the mechanisms of fear memory extinction, in part because of extinction's potential relationship to components of the human disorder post-traumatic stress disorder (Jovanovic and Ressler, 2010). Repeated presentations of the CS alone to the fear-conditioned rodent, promotes the acquisition of a new inhibitory association, which dampens or completely suppresses the expression of conditioned fear responses. Distinct subregions of the rodent PFC contribute differentially to fear extinction; that is, the prelimbic cortex appears to influence the expression of fear responses, while the infralimbic cortex influences the acquisition of extinction of fear memory (Quirk et al., 2010; Sierra-Mercado et al., 2011). Synaptic plasticity within mPFC-BLA neuronal circuits is induced during fear extinction training, resulting in increased inhibition of CS-elicited activity of BLA extinction neurons (Herry et al., 2008, 2010). Thus, converging evidence implicates the infralimbic and prelimbic cortices of the rodent brain and their differential projections to the amygdala sub-regions and to the hippocampus as contributing significantly to the synaptic plasticity that develops during the acquisition of fear extinction (see Tovote et al., 2015 for a recent review).

We found that systemic administration of the 5-HT$_{2A}$R agonist TCB-2 (see **Figure 2**) significantly enhanced the acquisition of fear extinction in mice that had undergone trace fear conditioning or delay fear conditioning (Zhang et al., 2013). Importantly, the 5-HT$_{2A}$R agonist did not affect locomotor responses or baseline freezing in the mice. Therefore, the effect of TCB-2 on fear extinction appeared to be specific to facilitating the acquisition of the new inhibitory memory that

suppressed fear expression. It is of interest to determine the site of action in the rodent brain at which TCB-2 works to facilitate fear extinction. In light of the plastic changes in neural circuitry that occur during the acquisition of fear extinction, it is possible that TCB-2 influences either the infralimbic cortical neurons or the "extinction neurons" of the BLA to facilitate fear extinction. Izumi and colleagues reported that an amygdala-selective reduction of 5-HT content via site-specific 5,7-DHT injection reduced the expression of conditioned fear responses in rats (Izumi et al., 2012). While this finding is difficult to reconcile with our report that 5-HT_{2A}R activation enhanced fear extinction, it is possible that the 5-HT denervation may have increased postsynaptic expression of 5-HT_{2A}Rs in the amygdala, which might in turn impair the expression of fear. It is clear that further studies are needed to clarify the neurophysiological influences of 5-HT, and the 5-HT_{2A}R in particular, on the neural circuitry supporting fear memory encoding, consolidation, retrieval, and extinction.

The influence of the 5-HT_{2A}R on the extinction and reconsolidation of fear memory may have significant impact on the development of therapeutic approaches for subjects with fear memory invasion, such as phobias and post trauma stress disorder (Quirk et al., 2010). For decades, the pharmacological manipulation of the 5-HT system has been a useful approach to treat emotional and mental disorders, such as depression and anxiety. Recent progress has suggested a promising therapeutic application of hallucinogenic 5-HT_2 agonists to treat depression and anxiety (Grob et al., 2011). These results suggest that despite the historical stigma associated with 5-HT_{2A}R activators as potential hallucinogens, such compounds may provide important medical potential for treating affective and cognitive symptoms associated with emotional and mental conditions.

Glutamatergic neurons in the amygdala, cortex and hippocampus are essential for memory extinction and reconsolidation. Local infusion of NMDAR antagonists into the BLA or CA1 region of hippocampus before extinction training suppresses fear memory extinction and reconsolidation (Baker and Azorlosa, 1996; Szapiro et al., 2003). The NMDAR partial agonist D-cycloserine facilitates the extinction of fear memory (Walker et al., 2002; Ledgerwood et al., 2003). Knockout of NMDAR in hippocampal CA1 pyramidal cells exclusively impairs the establishment of conditioning between the CS and the US during a trace fear conditioning task. These results suggest that the CS representation and conditioning are entrained within hippocampus cell ensembles, probably via NMDAR-dependent synaptic plasticity (McHugh et al., 1996; Huerta et al., 2000). Recall that 5-HT_{2A}Rs are expressed in the dendrites and dendritic spines of dentate gyrus neurons where NMDARs and AMPARs are assumed to be located (Peddie et al., 2008). 5-HT_{2A}R activation produces an elevation in the frequency and amplitude of cortical neuronal sEPSP/sEPSCs (Aghajanian and Marek, 1999), facilitates NMDAR activity and synaptic plasticity in the cortex (Arvanov et al., 1999) and BLA (Chen et al., 2003). It is worth while to examine the degree to which NMDARs expressed in the infralimbic and prelimbic cortices contribute to the 5HT_{2A}R-mediated enhancement in fear extinction.

Converging evidence demonstrates that activation of 5-HT_{2A}Rs via systemic injection, or by local microinfusion, appears to enhance two forms of hippocampal-dependent memory in mice: object memory and conditioned fear memory. Administration of a selective 5-HT_{2A}R antagonist alone was not found to significantly affect object memory or fear memory (Zhang et al., 2013), suggesting that memory consolidation does not require serotonergic activation of 5-HT_{2A}Rs and/or the antagonists do not affect the tonic effect the 5-HT_{2A}R. Activation of 5-HT_{2A}Rs with TCB-2 was also found to facilitate fear memory extinction in mice. These results offer promising support for the view that the 5-HT_{2A}R may be an important new target for consideration in the search for mechanisms by which long-term memory can be enhanced in humans.

Hallucination vs. Spatial Cognition

5-HT_{2A}R and Hallucination

Recent evidence suggests that activation of 5-HT_{2A}Rs may promote experiencing visual hallucinations by increasing neuronal excitability and altering visual-evoked cortical responses (Kometer et al., 2013). Hallucination is a type of misperception defined as the perception of an object without there being an object to perceive. Hallucinations are a significant characteristic found in a diversity of psychiatric and neurological states. Hallucinations can be triggered by at least three categories of drugs: psychedelics, (i.e., DOI, TCB-2, LSD, and psilocybin) via activation of 5-HT_{2A}Rs, psychostimulants (i.e., cocaine or amphetamine) via activation of dopamine D2 receptors and dissociative anesthetics (i.e., phencyclidine or ketamine) via blockade of glutamate NMDARs. The signaling and behavioral responses to each hallucinogen are distinct from each other. Activation of 5-HT_{2A}R is critical for the psilocybin (found in magic mushroom)-induced α oscillations, N170 visual-evoked potentials, and visual hallucinations (Kometer et al., 2013).

5-hydroxytryptamine/serotonin is an endogenous neurotransmitter and is not considered hallucinogenic. Interesting, *N*-methyltryptamines, a metabolite of 5-HT, also presents high affinity for 5-HT_{2A}R and can induce hallucinations in a manner independent of β-arrestin2/phosphoinositide 3-kinase/Src/Akt cascade (Schmid and Bohn, 2010). Signaling for hallucinogens is distinct. Lisuride (an antiparkinsonian agent) and LSD both bind cortical 5-HT_{2A}R, and thereby regulate PLC activity. LSD signaling involves pertussis toxin-sensitive heterotrimeric $G_{i/o}$ proteins and Src (Gonzalez-Maeso et al., 2007). Non-hallucinogenic agonists, for example lisuride, only stimulate cortical Gq in rats, whereas hallucinogens such as psilocybin (found in magic mushrooms), and LSD stimulate both $G_{q/11}$ and G_i (Gonzalez-Maeso et al., 2007). The β-arrestin pathway is involved in hallucinogen-mediated head shake responses in rodents (Schmid et al., 2008), and 5-HT induces a head shake response in mice via a β-arrestin-2-dependent signaling. However, the DOI invoked head shake behavior is not dependent upon β-arrestin-2 signaling. These findings suggest that the 5-HT_{2A}R-β-arrestin interaction may be exclusively for endogenous 5-HT action. Further examination of hallucinogen-mediated signaling may have major implications in drug development for treating emotional and mental disorders such

as depression and schizophrenia (Schmid et al., 2008). More research efforts will need to be focused on the hallucination-inducing aspects of 5-HT_{2A}R-sensitive drugs and, relevant to their potential therapeutic potential, it may be important to consider designing novel compounds that yield more of the beneficial effects, without activating those problematic sensory and perceptual effects.

5-HT_{2A}R-mediated Hallucination and Spatial Cognition

5-HT_{2A} receptors may affect spatial cognition. A human population-based study shows that 5-HT_{2A}R TT genotype of rs6313 is associated with better spatial cognitive performance (Gong et al., 2011). Kant et al. (1998) reported that the 5-HT_{2A}R agonist DOI (0.1 and 0.25 mg/kg, 30 min pretreatment) slowed rat performance as assessed by swim time on both a well-learned water maze as well as learning of a new maze, but DOI did not alter error rate on either task. Kant concluded that DOI impaired performance by suppressing motor activity on a water maze (Kant et al., 1998), which was in opposition to another report showing that manipulation of 5-HT_{2A}R did not impair the latency to a visible platform water maze test (Naghdi and Harooni, 2005). The serotonergic hallucinogens may impair the hippocampal-dependent spatial cognition by acting on 5-HT_{2A}Rs (Naghdi and Harooni, 2005). However, the direct evidence of 5-HT_{2A}R on visuospatial cognition and the central target has not been determined.

Serotonergic psychedelics may affect the integrity of visual functioning. Visual-directed spatial cognition and navigation are guided by exteroceptive (e.g., landmarks) and interoceptive (e.g., self-motion information) cues, and their integration. The hippocampus is a pivotal brain region receiving and integrating information for spatial memory and navigation in rodents (Broadbent et al., 2004; Eichenbaum, 2004). The MWM is a classic behavioral task for testing hippocampal-dependent visuospatial cognition, including place learning and memory, orientation and decision-making (Morris et al., 1982; Morris, 1984). Further, hippocampal place cells exhibit location-specific firing, and are considered to be fundamental components of network for spatial problem solving in the mammalian brain (for a review see Moser et al., 2008). The hippocampal neural circuit representing current location, directional heading and its integration is influenced by exteroceptive and interoceptive cues, and is considered to guide spatial cognition and navigation.

We recently found that pre-test activation of 5-HT_{2A}R with TCB-2 significantly delayed the initiation of an accurate search path by well-trained male mice in the hidden platform MWM (Zhang et al., 2015). Importantly, 5-HT_{2A}R activation did not affect swim performance or visual cue-triggered approach behavior in the visible platform water maze task. Taken together, our results suggest that the activation of 5-HT_{2A}R impairs the retrieval of hippocampal spatial memory, but not the accuracy of spatial information retrieval and decision-making. It is conceivable that the delayed initiation of accurate spatial search by TCB-2-treated mice might reflect the possible visual hallucinatory influences of the 5-HT_{2A}R agonist. For example, perhaps TCB-2-induced a brief aberration of visual input that slowed the perception of current position and local view of the mouse at the start of the water maze probe test. Once, reconciled or reoriented, the mouse was able to swim accurately to the remembered spatial location of the platform. It will be of interest to determine where in the brain TCB-2 is acting to alter spatial memory retrieval. The relatively weak influence of TCB-2-induced visual hallucination on spatial navigation may due to the difference in the visual information passing through the brain and central targets processing the information.

Taken together, the results we have reported here of memory effects after activation of the 5-HT_{2A}R represent a fairly complex picture. The post-training administration of TCB-2 enhanced consolidation of object memory in mice. Pre-test administration of TCB-2 did not affect retrieval of object memory, yet delayed retrieval of spatial memory. Pre-extinction training administration of TCB-2 facilitated the acquisition of extinction of both trace and delay fear memories. The facilitating effect of TCB-2 on fear extinction may have been the result of a combined effect of suppressing fear expression – possibly a consequence of impaired retrieval of fear memory, and enhancing the encoding and consolidation of fear extinction. To characterize the 5-HT_{2A}R agonist as a cognitive enhancer based solely on our object memory results, would be to ignore the other experimental findings. We are interested in conducting a more comprehensive analysis of the impact of TCB-2 on multiple forms of memory. For example, it will be interesting to examine whether post-conditioning TCB-2 might enhance the consolidation of fear memory, in a manner consistent with that observed in the NOR task. Likewise, it will be interesting to test whether post-extinction training TCB-2 facilitates the consolidation of fear extinction. Results of these experiments will help in better appreciating the modulatory influence of the 5-HT_{2A}R on long-term memory processes. This synthesis of recent findings of the influences of 5-HT_{2A}R activation should provide a credible argument that the 5-HT_{2A}R participates significantly to the well-documented contribution of 5-HT to memory (Meneses, 2013).

5-HT_{2A}R and Mental Disorders

A number of psychiatric and neurodegenerative disorders are associated with the variation of structure, expression, and function of 5-HT_{2A}Rs. Positron emission tomography (PET) molecular imaging has the sensitivity to quantify binding of 5-HT_{2A}Rs in CNS disorders. Medication-free depressed subjects presented greater 5-HT_{2A}R binding (Bhagwagar et al., 2006). There was a significant reduction in 5-HT_{2A}R binding in frontal polar, dorsolateral and medial frontal cortex, and parietal and temporal associative cortex of OCD patients and a significant correlation between 5-HT_{2A}R availability in orbitofrontal and dorsolateral frontal cortex and clinical severity (Perani et al., 2008). Schizophrenia patients present with very high 5HT_{2A}R occupancy in the frontal cortex (Talvik-Lotfi et al., 2000). These results suggest that the variation in the number, affinity and/or function of 5-HT_{2A}R participates in the etiology of mental disorders.

Alzheimer's Disease

It is interesting to note that neocortical 5-HT_{2A}R binding is significantly decreased in patients with early stage AD, and in those with mild cognitive impairment; especially in temporal lobe regions associated with long-term memory (Meltzer et al., 1998; Hasselbalch et al., 2008; Santhosh et al., 2009; Marner et al., 2011, 2012). Further, the severity of cognitive impairment in AD patients correlates with the decrease in 5-HT_{2A}R binding (Versijpt et al., 2003). Given the pattern of 5-HT_{2A}R distribution in neocortical regions and their expression on principal excitatory neurons, it is possible that the marked reduction in 5-HT_{2A}R in brains of AD is a direct product of neuron loss in key brain regions. Consistent with evidence from the human studies, the Alzheimer's-like neuropathology and associated memory deficits in rodents, which follow intra-hippocampal injection of ß-amyloid(1-42), are associated with a significant reduction in levels of hippocampal 5-HT_{2A}R expression (Christensen et al., 2008). Although we have focused this analysis on the influence of 5-HT_{2A}Rs on long-term, hippocampal-dependent memory, there is clear and compelling evidence to suggest that the 5-HT_{2A}R represents a potential new target by which human long-term memory may be modulated. We assert that it will be of interest in further examine the contribution of 5-HT_{2A}Rs to memory processes, and we are particularly interested in determining neurophysiological influences of 5-HT_{2A}R agonists which promote the enhancement of memory consolidation which we have reported in mice.

Drug Memory

Drug dependence, classified as an impulsive, compulsive, and relapsing psychiatric disorder, represents a devastating societal problem worldwide. The profound symptoms of drug abuse, in particular the cue-elicited relapse to drug use after even long periods of abstinence, are a consequence of robust experience-dependent synaptic plasticity within the brain's reward circuit. Like episodic, semantic, and habit memory, drug-associated memories are persistent and hold a strong influence on current and future behaviors. Of particular interest is the consideration of memory extinction as a psychological tool for remediating the problem of relapse in drug addicts. That is, if the problem of drug abuse is approached as a mental disorder of memory, then pharmacological manipulations that facilitate extinction may hold therapeutic utility for treating drug abuse. Drug exposure alters the expression and function of 5-HT_{2A}R, for example morphine decreases frontocortical 5-HT_{2A}R binding affinity in dogs (Adriaens et al., 2012). 5-HT_2Rs are up-regulated in amygdala, midbrain, pons, and medulla of morphine-tolerant and -dependent rats, but not in morphine-abstinent rats (Gulati and Bhargava, 1989). There is considerable evidence that 5-HT_{2A}Rs modulate the behavioral consequences of repeated exposure to addictive psychomotor stimulants. For example, M100907 suppresses hyperactivity elicited by cocaine (Fletcher et al., 2002), MK-801, amphetamine (O'Neill et al., 1999), and morphine (Auclair et al., 2004). DOM, a 5-HT_{2A}R agonist, attenuates locomotor-stimulating effects of morphine, which could be prevented by M100907 (Li et al., 2013). Furthermore, M100907 attenuated the ability of experimenter-administered cocaine to reinstate lever pressing (Fletcher et al., 2002) and attenuated the drug associated cue-induced reinstatement of cocaine-seeking behavior after extinction (Nic Dhonnchadha et al., 2009). M100907 also suppressed reinstatement induced by nicotine prime or nicotine-associated cue (Fletcher et al., 2012) and sensitization (Zaniewska et al., 2010). Intra-NAc infusions of M 100907 blocked the expression of cocaine-induced locomotor sensitization (Zayara et al., 2011). Intra-PFC M100907 decreased cue-elicited reinstatement of cocaine seeking-behavior (Pockros et al., 2011). Together, these results suggest that 5-HT_{2A}Rs modulate drug addiction-dependent behaviors such as craving and drug-seeking and pharmacological blockade of 5-HT_{2A}Rs may represent a therapeutic advance in suppression of cue-evoked craving and/or relapse in drug addicts.

Therapeutic Application of 5-HT_{2A}R

Preclinical and clinical studies have provided support for the use of pharmacological manipulation of 5-HT_{2A}R to treat the symptoms of mental disorders. Activation of 5-HT_{2A}R with TCB-2 in the medial septum-diagonal band of Broca complex enhances neuronal activity and working memory in hemiparkinsonian rats (Li et al., 2015). M100907 had no effect on attentional performance, but abolished the PCP-induced attentional performance deficits in rats (Poyurovsky et al., 2003). M100907 prevents impairment in attentional performance by NMDAR blockade in the rat PFC (Mirjana et al., 2004). There are a number of 5-HT_{2A}R drugs that have been evaluated or are being currently evaluated under clinical trials, for example quetiapine[1] for schizophrenia; M100907[2] for depression; ACP-103[3] for Parkinson's disease; pimavanserin for patients with AD psychosis[4] or with Parkinson's disease psychosis[5].

Conclusion

In this review, we have summarized recent progress in the signaling, polymerization and allosteric modulation of 5-HT_{2A}R; and have discussed the critical role of 5-HT_{2A}Rs in a number of cognitive processes. Based on the results of studies from our lab and others, it appears that activation of 5-HT_{2A}Rs may offer a novel approach to treat the impairment of learning and memory associated with several neurodegenerative disorders. Meanwhile, blockade of 5-HT_{2A}R may offer a feasible way to suppress drug craving and/or relapse. It will be very interesting to identify the corresponding signaling pathways by which 5-HT_{2A}Rs modulate these behavioral capacities. Of particular note, we reviewed evidence that 5-HT_{2A}Rs may dimerize with other receptors, and that certain pathways may promote constitutive activation of

[1]https://clinicaltrials.gov/ct2/show/NCT00207064?term=5-HT2A&rank=2
[2]https://clinicaltrials.gov/ct2/show/NCT00070694?term=5-HT2A&rank=5
[3]https://clinicaltrials.gov/ct2/show/NCT00086294?term=5-HT2A&rank=12
[4]https://clinicaltrials.gov/ct2/show/NCT02035553?term=5-HT2A&rank=41
[5]https://clinicaltrials.gov/ct2/show/NCT00477672?term=5-HT2A&rank=46

5-HT_{2A}Rs, which likely represent novel receptor signaling influences. Connecting such novel properties of 5-HT_{2A}Rs to distinct functional consequences of 5-HT-, or agonist-, specific activation of the 5-HT_{2A}Rs will be important for improving understanding the myriad influences of 5-HT_{2A}Rs in the CNS. The development of highly selective 5-HT_{2A}R ligands will be essential for further establishing the critical involvement of the 5-HT_{2A}R for a number of fundamental cognitive behaviors.

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5-HT6 receptor agonism facilitates emotional learning

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Serotonin (5-HT) and its receptors play crucial roles in various aspects of mood and cognitive functions. However, the role of specific 5-HT receptors in these processes remains to be better understood. Here, we examined the effects of the selective and potent 5-HT6 agonist (WAY208466) on mood, anxiety and emotional learning in mice. Male C57Bl/6J mice were therefore tested in the forced swim test (FST), elevated plus-maze (EPM), and passive avoidance tests (PA), respectively. In a dose-response experiment, mice were treated intraperitoneally with WAY208466 at 3, 9, or 27 mg/kg and examined in an open field arena open field test (OFT) followed by the FST. 9 mg/kg of WAY208466 reduced immobility in the FST, without impairing the locomotion. Thus, the dose of 9 mg/kg was subsequently used for tests of anxiety and emotional learning. There was no significant effect of WAY208466 in the EPM. In the PA, mice were trained 30 min before the treatment with saline or WAY208466. Two separate sets of animals were used for short term memory (tested 1 h post-training) or long term memory (tested 24 h post-training). WAY208466 improved both short and long term memories, evaluated by the latency to enter the dark compartment, in the PA. The WAY208466-treated animals also showed more grooming and rearing in the light compartment. To better understand the molecular mechanisms and brain regions involved in the facilitation of emotional learning by WAY208466, we studied its effects on signal transduction and immediate early gene expression. WAY208466 increased the levels of phospho-Ser^{845}-GluA1 and phospho-Ser$^{217/221}$-MEK in the caudate-putamen. Levels of phospho-Thr$^{202/204}$-Erk1/2 and the ratio mature BDNF/proBDNF were increased in the hippocampus. Moreover, WAY208466 increased c-fos in the hippocampus and Arc expression in both hippocampus and prefrontal cortex (PFC). The results indicate antidepressant efficacy and facilitation of emotional learning by 5-HT6 receptor agonism via mechanisms that promote neuronal plasticity in caudate putamen, hippocampus, and PFC.

Keywords: 5-HT6, antidepressant, memory, passive avoidance, forced swim test, c-fos, MAPK

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Introduction

Brain serotonin (5-HT) is implicated in a wide variety of physiological functions related to mood, cognition and movements. The mechanisms whereby 5-HT6 and its receptors exert its versatile functions are complex and often contradictory. For example, 5-HT6 agonists (Svenningsson et al., 2007; Carr et al., 2011; Kendall et al., 2011) and antagonists (Hirst et al., 2006; Wesolowska and Nikiforuk, 2007, 2008; Hirano et al., 2009) have procognitive and/or antidepressant-like effects in

animal models. Nonetheless, since many serotonergic compounds have entered, or are about to enter, the clinics, it is critically important to better delineate action of serotonergic compounds. This is particularly evident for 5-HT6 ligands as the combination of donepezil with a 5-HT6 antagonist, idalopirdine, improved the cognitive function of patients with Alzheimer's Disease (Wilkinson et al., 2014).

The exact role of 5-HT6 receptor activation for memory acquisition and consolidation is not yet completely understood. Virally mediated gene transfer to overexpress 5-HT6 receptors in the striatum had no effect on performance in the Morris water maze (hippocampus-dependent), but impaired the acquisition of a reward-based instrumental learning task (striatum-dependent), an effect rescued by treatment with the 5-HT6 antagonist, SB-258585 (Mitchell et al., 2007). However, treatment with the 5-HT6 agonist, WAY181187, facilitated extra-dimensional attentional set shifting [prefrontal cortex (PFC)-dependent] and increased c-fos expression in the PFC (Burnham et al., 2010). Administration of the 5-HT6 agonists, E-6801, or EMD-386088, reversed the cognitive deficits induced by scopolamine or MK-801 pretreatment in the conditioned emotion response, a cued and contextual fear memory (hippocampal, amygdala, and cortical-dependent; Woods et al., 2012). Paradoxically, both administration E-6801 and EMD-386088 as well as the 5-HT6 antagonists, SB-271046 and Ro 04–6790, improved the recognition memory (hippocampal-dependent; Kendall et al., 2011).

The PA test evaluates emotional memory (Burwell et al., 2004; Mitchell and Neumaier, 2005; Eriksson et al., 2008). PA is considered a complex memory test since it is comprised by both a Pavlovian component and also requires an instrumental response. In this test, animals are required to suppress the natural preference of a dark compartment to avoid a foot shock (e.g., Baamonde et al., 1992; Ogren et al., 2008). PA is hippocampal dependent and several studies have shown the importance of serotonin in this test (Misane and Ogren, 2000; Eriksson et al., 2008, 2013). The role of 5-HT6 agonists in PA is unknown, but other hippocampal dependent memories are modulated by 5-HT6 agonists (Kendall et al., 2011; Woods et al., 2012) and 5-HT6 antagonists (Lieben et al., 2005; Meneses et al., 2007; Kendall et al., 2011; Woods et al., 2012).

We have previously shown that the 5-HT6 agonist 2-ethyl-5-methoxy-N,*N*-dimethyltryptamine (EMDT), similarly to fluoxetine, induces antidepressant effect in the mouse tail suspension test and increases the phospho-Ser^{845}-GluA1 subunit of the AMPA receptor in the PFC and striatum (Svenningsson et al., 2007). Interestingly, the 5-HT6 antagonist, SB271046, blocked not only the effects of EMDT and but also counteracted effects of fluoxetine (Svenningsson et al., 2007).

The objective of this study was to further evaluate emotional processing along with antidepressant and anxiolytic actions by the highly selective and potent 5-HT6 agonist, WAY208466 (Schechter et al., 2008). Moreover, to understand molecular mechanisms of action and brain regions engaged by WAY208466, we also evaluated its effects on signal transduction and immediate early genes (IEGs) involved in neuronal plasticity. The roles of many IEGs are indeed related to neuroplasticity (Pei et al., 2004). Here we studied representative genes from two classes, a transcription factor (i.e., c-fos) and an effector (i.e., Arc – activity-regulated cytoskeletal associated gene; Clayton, 2000).

Materials and Methods

Animals

Adult male C57Bl/6J mice were obtained from Janvier labs (Scand-las Turku, Finland) and housed under controlled temperature and humidity with food and water *ad libitum* and in a 12 h light/dark controlled cycle. All experiments were carried out in agreement with the European Council Directive (86/609/EEC) and were approved by the local Animal Ethics Committee (N40/13; Stockholm Norra Djurförsöksetiska Nämnd). All efforts were made to reduce the number of animals used and to minimize their suffering.

Treatment

For all behavioral testing, animals were brought to the experimental room 30 min for habituation. Animals then received a single intraperitoneal injection of WAY208466 (3-[(-3-Fluorophenyl)sulfonyl]-*N*,*N*-dimethyl-1*H*-pyrrolo[2,3-*b*]pyridine-1-ethanamine dihydrochloride; Tocris Bioscience, Bristol, UK) or vehicle (saline) 30 min prior to behavioral tests. In an initial dose-response experiment, we examined three different doses (3, 9, and 27 mg/kg) of WAY208466 in the OFT and in the FST. Since the dose of 27 mg/kg impaired locomotor activity in the OFT and the dose of 3 mg/kg did not reduce the immobility in the FST, we used 9 mg/kg for subsequent tests (i.e., PA and EPM). In addition, and for comparison, naïve groups were treated with vehicle or 9 mg/kg of WAY208466 and euthanized.

Behavioral Tests

Forced Swim Test

The Porsolt forced swim test (FST) procedure was performed as described earlier (Cervo et al., 2005). Animals were individually placed in a vertical Plexiglas cylinder (height: 30 cm, diameter: 20 cm) filled with 15 cm depth water at 23–25°C. The water was changed between every animal. The animals were removed from the water after 6 min, and dried before they returned to their home cages. Behavior was analyzed in the last 4 min of the test (Guzzetti et al., 2008). The experiment was recorded and analyzed automatically using NOLDUS Ethovision XT9 software (Wageningen, The Netherlands).

Open Field Test

Mice were tested in the OFT for 5 min. The open field arena (46 cm × 46 cm) was illuminated by a reflected light of approximately 35 lux. Performance in the OFT was tracked and analyzed using an automated video tracking system (NOLDUS Ethovision XT9, Wageningen, The Netherlands).

Passive Avoidance Test

The step-through passive avoidance (PA) was performed as described earlier (Eriksson et al., 2013). Briefly, the PA apparatus

(25 cm × 50 cm × 25 cm) consisted of two equally sized compartments connected by a sliding door (7 cm × 7cm) (Ugo Basile, Comerio-Varese, Italy). The light intensities in the dark and the bright compartments were 2 and 250 lx, respectively. During PA training, each mouse was placed in the bright compartment and allowed to explore it for 60 s. The sliding door was then opened and the animal had a maximum of 300 s to step through to the dark compartment. Once the mouse had entered the dark compartment, the sliding door was automatically closed and, after 3 s, a weak electrical stimulus (0.3 mA, 2 s scrambled current) was delivered through the grid floor.

After 1 h short term memory (STM) or 24 h long term memory (LTM), the animal was again gently placed in the light compartment, and the latency to enter the dark compartment with all four paws was measured (retention latency) with a 9 min cutoff time for testing. No electrical stimulus was given during the second exposure. The parameters evaluated were retention latency, grooming, and rearing. All parameters were observed and registered manually during the experiment (latency to step through, grooming and rearing). The animals were euthanized 1 h after the test and their brains were later used for the *in situ* hybridization (described in Section "Immunoblotting").

Elevated Plus-Maze

The elevated plus-maze (EPM) was conducted as previously described (Kindlundh-Högberg et al., 2009). Mice were placed in the center facing an open arm and allowed to explore the apparatus for 5 min. Entries into the open (90 lux) and closed (20 lux) arms and time spent in each arm were measured by automated video tracking system (NOLDUS Ethovision XT9, Wageningen, The Netherlands). The animals were euthanized 1 h after the test and their brains were later used for the immunoblotting (described in Section "*In Situ* Hybridization").

Immunoblotting and Histological Measurements

Tissue Collection

Mice were sacrificed by decapitation; their brains were quickly dissected and dipped in isopentane, cooled in dry ice, for approximately 5 s. Samples were stored in −80°C freezer for further processing.

In Situ Hybridization

Fresh frozen coronal cryostat sections (14 μm) were prepared and hybridized with ^{35}S-radiolabeled antisense riboprobes against Arc and c-fos. The sections were exposed to Kodak MR film in room temperature for 7–21 days prior to development, according to a previously published protocol (Svenningsson et al., 1998, 2006). The areas selected for analysis were the PFC, the striatum/CPu, the nucleus accumbens (NAcc), the amygdala (basolateral nuclei of amygdala – LA/BLA), and the hippocampus (Hi – four different subareas: *Cornu Ammonis* – subareas CA1, CA2, CA3, and dentate gyrus – DG). Densitometric measurements were obtained from autoradiograms using the NIH ImageJ 1.40 software (National institute of Mental Health, Bethesda, MD, USA). All optical density values were normalized. For each target analyzed, the average of the control group (naïve group treated with saline) was normalized to 100% and results from each treatment group are presented as percentage of the control.

Immunoblotting

Tissues from PFC, hippocampus, and caudate-putamen (CPu) were sonicated and boiled in 1% sodium dodecyl sulfate (SDS) containing a protease and phosphatase inhibitor Cocktail (HaltTM, Pierce, Rockford, IL, USA). Protein concentration was determined in each sample using a bicinchoninic acid protein assay (BCA-kit, Pierce, Rockford, IL, USA). Equal amounts of protein (5–20 μg) were separated by SDS–polyacrylamide gel electrophoresis using 8% lower running gels. Proteins were transferred to Immobilon-P (Polyvinylidene Difluoride) membranes (Millipore, Bedford, MA, USA). Membranes were blocked by incubation in 5% (w/v) dry milk or bovine serum albumin (BSA) in TBS-Tween20 for 1 h at room temperature. Following overnight incubation with primary antibodies (**Table 1**), the membranes were washed three times with TBS-Tween 20 and incubated for 1 h with secondary horseradish peroxidase (HRP)-linked Anti-Rabbit IgG (H + L) (Dako, Glostrup, Denmark). Immunoreactive bands were detected by enhanced chemiluminescence (Bio-Rad, Bio-Rad, Hercules, CA, USA) and quantified by densitometry with ImageJ 1.40 software. All data are presented as values normalized to the levels of β-actin or calnexin. The level of the phosphorylated form of a protein was normalized to the total level of the same protein. For each target analyzed, the average of the saline group was normalized to 100% and results from each treatment group are presented as percentage of the saline group.

Statistical Analysis

Data were initially evaluated for outliers with the Grubb's test. Immunoblotting and behavior in the PA test and were analyzed with Student's *t*-test. Dose-response effects of WAY208466 on the OFT and FST were analyzed by one-way analysis of variance (ANOVA) with treatment as a factor. Arc and c-fos expression were analyzed with two-way ANOVA with training × treatment

TABLE 1 | List of the antibodies and dilutions used for the immunoblotting.

Antibody	Company	Catalog number	Dilution
Actin	Sigma	A5060	1:10000
Calnexin	Sigma	C4731	1:2000
Glu R1	Milipore	06–306	1:1000
P-Ser^{845} Glu R1	Milipore	04–823	1:1000
MEK	Cell signaling	9122	1:1000
P-Ser$^{217/221}$ MEK	Cell signaling	9121	1:1000
Erk1/2	Cell signaling	9107S	1:2000
P-Thr$^{202/204}$ Erk1/2	Cell signaling	9101S	1:1000
proBDNF	Alomone	ANT-006	1:200
mBDNF	Sigma	AV-41970	1 μg/ml

as factors. ANOVAs were followed by Fisher's least significance difference (LSD) *post hoc* test. All data are presented as mean ± SEM and significance was defined as $p < 0.05$.

Results

Behavioral Tests

Forced Swim Test

Analysis with one-way ANOVA showed a statistical difference between treatments ($F_{3,28} = 3.045$; $p < 0.05$). Fisher *post hoc* analysis showed that 9 mg/kg of WAY208466 decreased the immobility (**Figure 1A**).

Open Field Test

One-way ANOVA showed a statistical difference between treatments ($F_{3,28} = 6.70$, $p < 0.01$). WAY208466, at the highest dose (27 mg/kg), decreased locomotion (**Figure 1B**).

Passive Avoidance Test

No significant differences were observed between the saline group and WAY208466 (9 mg/kg) during the training session for either short term or long term memories in the latency to step through to the dark compartment ($t = -1.44$, $p < 0.17$; $t = 0.29$, $p < 0.2$, respectively). However, during the test section, student *t*-test showed a significant difference for both short ($t = -2.21$, $p < 0.05$) and long ($t = -2.64$, $p < 0.01$) term memories (**Figure 2**).

During the PA test, grooming and rearing were also examined. No significant differences were observed during training sessions for either grooming ($t = -1.55$, $p < 0.14$; $t = -0.17$, $p < 0.87$, respectively) or rearing ($t = 0.40$, $p < 0.69$; $t = -0.17$, $p < 0.87$, respectively). However, rearing was significant increased by WAY208466 (9 mg/kg) both in the test sessions for short and long term memories ($t = -5.22$, $p < 0.001$; $t = -3.55$, $p < 0.01$, respectively). Grooming was increased by WAY208466 in the STM paradigm ($t = -3.97$, $p < 0.01$), but not in LTM ($t = -0.36$, $p < 0.72$) (**Figure 3**).

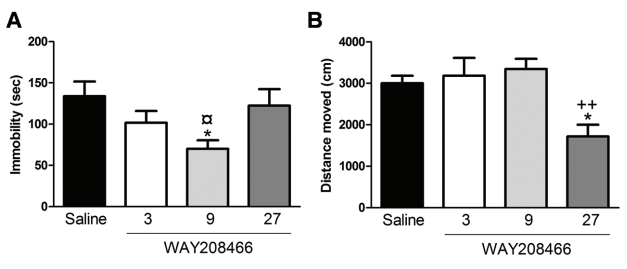

FIGURE 1 | Effects of WAY208466 treatment in the forced swim test (FST) and open field test (OFT). (A) Immobility duration in the FST showing antidepressant-like effect of WAY208466 after 9 mg/kg (ip). **(B)** Total distance moved during 5 min in the OFT. WAY208466 at 27 mg/kg (ip) caused motor impairment compared to the saline group. $^{*}p < 0.05$ compared to saline group, $^{¤}p < 0.05$ compared to 27 mg/kg. $^{++}p < 0.01$ compared to 9 mg/kg WAY208466 group. Mean ± SEM; $n = 8$ mice per group.

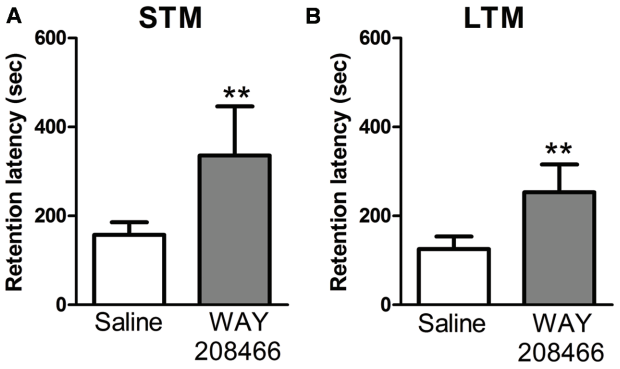

FIGURE 2 | Retention latency in the PA test. Animals were treated with saline or WAY208466 (9 mg/kg), 30 min prior test (ip). **(A)** Short term (1 h) memory (STM) comparison between treatments. **(B)** Long term (24 h) memory (LTM) comparison between treatments. $^{**}p < 0.01$ compared to saline group. Mean ± SEM, $n = 8$ mice per group.

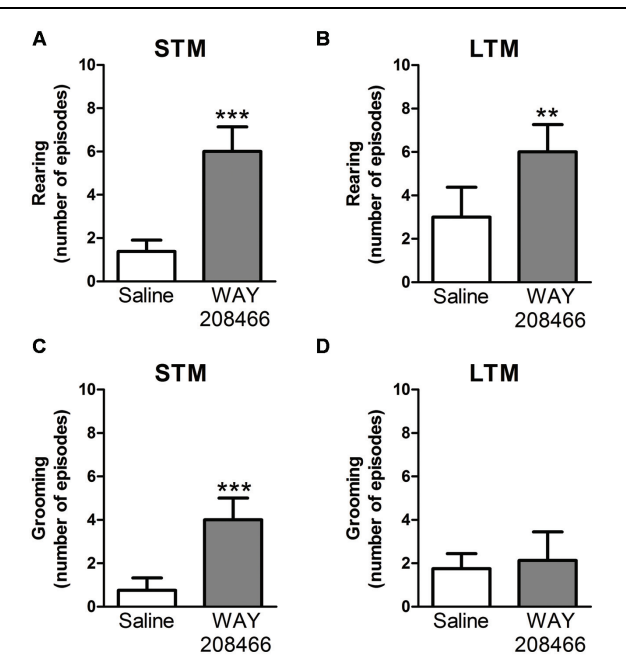

FIGURE 3 | Rearing (A,B) and grooming (C,D) during PA test. Differences in the number of rearings between treatments [saline or WAY208466 (9 mg/kg)] for STM **(A)** or LTM **(B)**. Differences in the frequency of groomings between treatments [saline or WAY208466 (9 mg/kg)] for STM **(C)** or LTM **(D)**. $^{**}p < 0.01$; $^{***}p < 0.001$ compared to saline group. STM, short term memory; LTM, long term memory. Mean ± SEM; $n = 8$ mice per group.

Elevated Plus-Maze

No significant differences were observed between the saline group and WAY208466 (9 mg/kg) in the EPM test. Student's *t*-test showed no differences in neither number of entries in the open arm of the maze ($t = 1.59$, $p < 0.13$), nor in time spent in the open arm ($t = 1.66$, $p < 0.11$) (**Table 2**).

TABLE 2 | Effects of saline and WAY208466 (9 mg/kg) treatment in the EPM test.

Treatment	Entries in the open arms	Time (sec) spent in the open arms
Saline	18.6 ± 0.92	117.5 ± 12.8
WAY208466	17.0 ± 1.10	99.11 ± 8.97

Data represented as mean ± SEM, n = 8 mice per group.

Immunoblotting and Histological Measurements

In Situ Hybridization

No significant changes in c-fos or Arc expression were observed in any of the analyzed areas (PFC, CPu, NAccs, amygdala, and hippocampus) in animals studied in the STM paradigm of PA.

However, in the LTM PA paradigm, two-way ANOVAs followed by Fisher's *post hoc* test showed increases of both hippocampal c-fos and Arc mRNAs in tested animals treated with WAY208466 in comparison with either saline/trained ($p < 0.05$ and $p < 0.01$, respectively) or treated/naïve ($p < 0.05$, $p < 0.01$, respectively) groups. (c-fos: treatment: $F_{1,21} = 1.88$, $p < 0.05$, training $F_{1,21} = 2.08$, $p > 0.05$, treatment × training interaction: $F_{1,21} = 5.26$, $p < 0.05$; Arc: treatment: $F_{1,21} = 5.67$, $p < 0.05$, training: $F_{1,21} = 11.10$, $p < 0.01$, treatment × training interaction: $F_{1,21} = 4.82$, $p < 0.05$) (**Figures 4E** and **5E**).

A two-way ANOVA also detected a treatment effect of WAY208466 in the Arc expression in the PFC in the LTM paradigm (treatment: $F_{1,21} = 4.75$, $p < 0.05$, training: $F_{1,21} = 2.56$, $p > 0.05$, treatment × training interaction: $F_{1,21} = 0.12$, $p > 0.05$). Specifically, WAY208466 treatment prevented the reduction in Arc expression observed in trained animals in comparison with the naïve groups ($p < 0.05$) (**Figure 5A**).

No significant changes were observed for the other analyzed areas in the long term memory paradigm (**Figures 4A–D** and **5B–D**).

Western Blot

Acute treatment with WAY208466 (9 mg/kg) increased the levels of phospho-Ser^{845}-GluA1 in the CPu ($t = 2.21$, $p < 0.05$), but not in the PFC or hippocampus ($t = 0.88$, $p > 0.2$; $t = 0.35$, $p > 0.2$, respectively) (**Figure 6C**). Similarly, phospho-Ser$^{217/221}$MEK was also increased in the CPu ($t = 2.31$, $p < 0.05$), but not in the PFC or hippocampus ($t = 1.03$, $p > 0.2$; $t = 0.68$, $p > 0.2$, respectively) (**Figure 6A**).

Levels of phospho-Thr$^{202/204}$Erk1/2 were increased in the hippocampus ($t = 2.71$, $p < 0.05$), but not in the PFC or CPu ($t = 0.33$, $p > 0.2$; $t = 0.10$, $p > 0.2$, respectively) (**Figure 6B**). Likewise, the ratio of mature BDNF/proBDNF was increased in the hippocampus ($t = 2.71$, $p < 0.05$), but not in the PFC or CPu ($t = 0.95$, $p > 0.2$, respectively) (**Figure 6D**).

Discussion

Our results show that the 5-HT6 agonist WAY208466 facilitates cognitive processing in the PA test and has an antidepressant-like effect in the FST. In agreement with a previous study performed in rats (Carr et al., 2011), we observed a U-shape dose-response curve in the antidepressant effect of WAY208466. The study of Carr et al. (2011) used rats as they have higher expression of 5-HT6 receptors when compared to mice (Hirst et al., 2003; Zhang et al., 2011). Nonetheless, our data demonstrates antidepressant properties of WAY208466 also in mice.

In addition of improving the performance in the PA test, WAY208466 also increased grooming and rearing during this test. Grooming/rearing in a new environment interacts with anxiety in a complex manner (Prut and Belzung, 2003). Furthermore, since Carr et al. (2011) found an anxiolytic-like

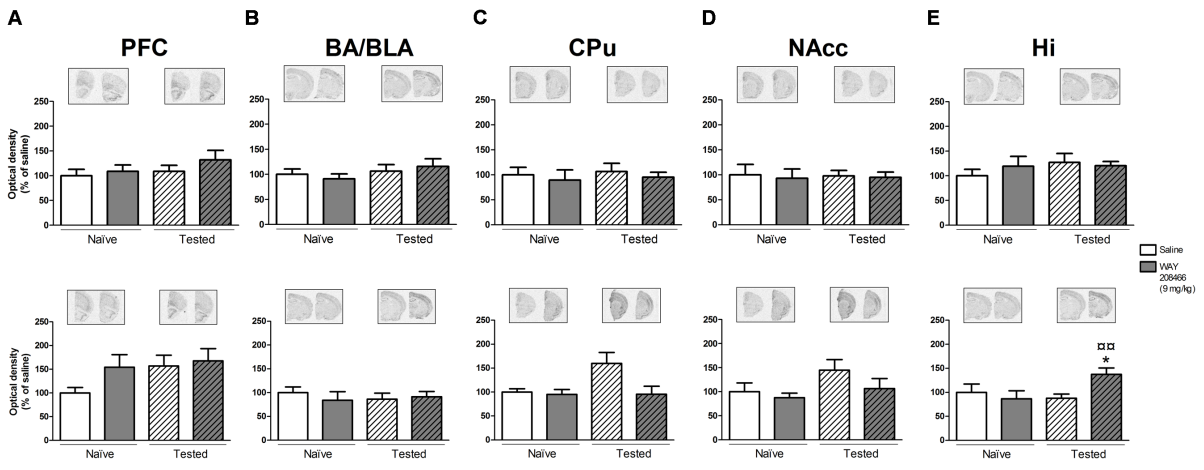

FIGURE 4 | Effects of saline and WAY208466 (9 mg/kg, ip) on the levels of c-fos mRNA after PA test for STM (upper) or LTM (lower) for (A) PFC, pre frontal cortex; (B) BA/BLA, lateral and basolateral amygdala; (C) CPu, caudate putamen; (D) NAcc, nucleus accumbens; (E) Hi, hippocampus.
$^{*}p < 0.05$ compared to saline/naïve group. $^{¤}p < 0.05$; $^{¤¤}p < 0.01$ compared to saline group. Mean ± SEM; $n = 6$–8 mice per group.

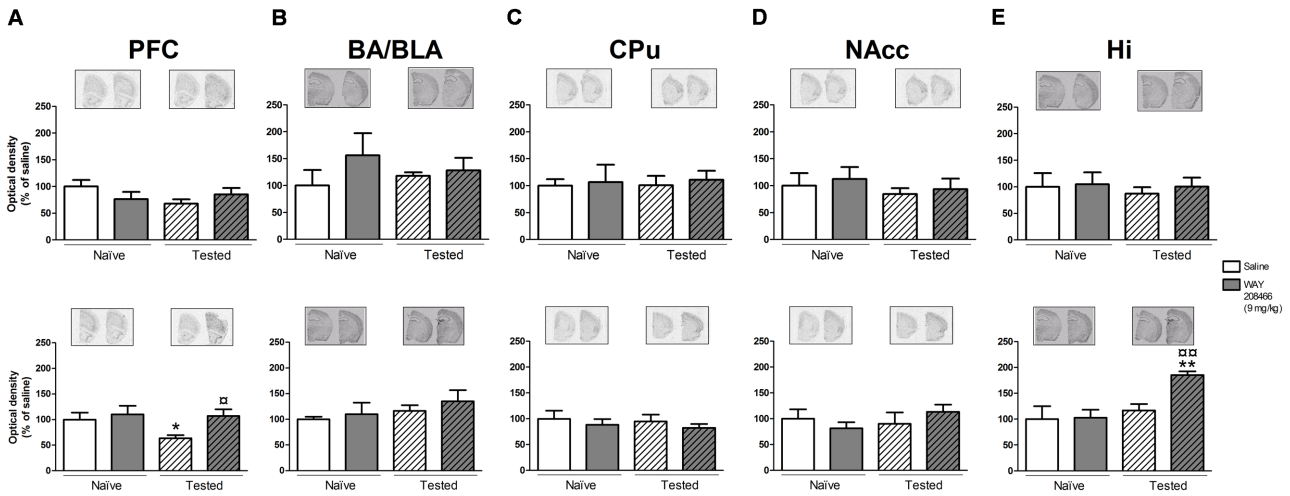

FIGURE 5 | Effects of saline and WAY208466 (9 mg/kg, ip) on the levels of Arc (activity-regulated cytoskeleton-associated protein) mRNA after PA test for STM (upper) or LTM (lower) for (A) PFC, pre frontal cortex; (B) BA/BLA, lateral and basolateral amygdala; (C) CPu, caudate putamen; (D) NAcc, nucleus accumbens; (E) Hi, hippocampus. $^{*}p < 0.05$; $^{**}p < 0.01$ compared to saline naïve group. $^{¤¤}p < 0.01$ compared to saline group. Mean ± SEM; $n = 6$–8 mice per group.

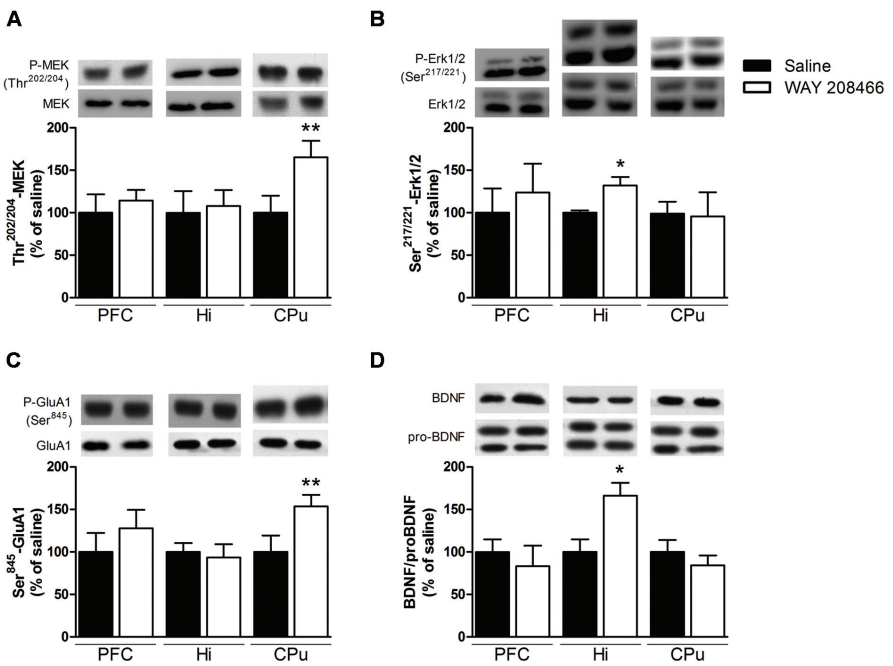

FIGURE 6 | Effects of saline or WAY208466 (9 mg/kg, ip) on the phosphorylation states of $Ser^{217/221}$-MEK (A), $Thr^{202/204}$-Erk1/2 (B), Ser^{845}-GluA1 (C) and the ratio matureBDNF/proBDNF (D) in the prefrontal cortex (PFC), hippocampus, and caudate-putamen (CPu). $^{*}p < 0.05$; $^{**}p < 0.01$ compared to saline group. Mean ± SEM; $n = 6$–8 mice per group.

effect of WAY208466, we performed experiments in EPM. Somewhat surprisingly, we did not find a significant effect of WAY208466 in the EPM. It is important to note that the absence of effect in our experiment might be related to the low sensitive of the EPM test to serotoninergic drugs.

The OFT was performed to evaluate for possible locomotor effects induced by WAY208466 that could bias the subsequent behavioral tests. We found that 27 mg/kg of WAY208466 reduced locomotion in the OFT. This result is inconsistent with a previous report (Carr et al., 2011), in which 30 mg/kg of WAY208466 caused no hypolocomotion in rats. The discrepancy between these results may, at least partly, be explained by different experimental designs. Carr et al. (2011) treated rats for 1 h before examining their locomotion for 30 min, whereas we

treated mice for 30 min before examining their locomotion for 5 min. A possible explanation for the different results is that our experiments are strongly influenced by a novelty response together with regular locomotor activity, whereas the results from Carr et al. (2011) were less influenced by novelty. Based on this result in the OFT we decided to not perform additional experiment with 27 mg/kg of WAY208466. 3 and 9 mg/kg of WAY208466 caused no hypolocomotion, but only 9 mg/kg decreased immobility in the FST. Based on these results, the subsequent EPM and PA experiments were only performed using 9 mg/kg of WAY208466.

To better understand the molecular mechanisms and brain regions involved in the facilitation of emotional learning by WAY208466, we correlated its behavioral effects with alterations on signal transduction and IEG expression. Studies have reported the importance of mitogen-activated protein kinase (MAPK) signaling pathway in the process of memory consolidation (Thomas and Huganir, 2004). We observed that WAY208466 increased the phosphorylation of MEK in the CPu and Erk1/2 in the hippocampus. Interestingly, treatment with the clinically used procognitive agent, memantine, has also been shown to both improve the PA performance and to increase hippocampal Erk1/2 phosphorylation (Liu et al., 2014).

Corroborating with our previous results using EMDT (Svenningsson et al., 2007), treatment with WAY208466 increased phosphorylation of the Ser^{845}-GluA1 receptors in the CPu. Phosphorylation of Ser^{845}-GluA1 in the ventral striatum has been reported to be important for spatial memory consolidation (Ferretti et al., 2014). The role of the CPu for learning the PA task is not as evident as that of hippocampus, although cholinergic blockade in the CPu impairs the memory formation in the PA test (Prado-Alcalá et al., 1985). Unlike our previous report with EMDT, (Svenningsson et al., 2007) WAY208466 treatment did not increase Ser^{845}-GluA1 phosphorylation in the PFC.

BDNF is critically important in multiple plastic changes regulating mood and cognition and was therefore also studied in the immunoblotting experiments. Treatment with WAY208466 did not change mature BDNF or proBDNF levels in hippocampus. However, the ratio of mature BDNF/proBDNF was increased, favoring neuronal plasticity.

To determine brain regions affected by the PA paradigm and 5-HT6 agonism, we evaluated the expression of the IEGs Arc and c-fos by *in situ* hybridization. Since the initial increase of c-fos and Arc after a neuronal stimuli can occur already after 15 min and last for many hours (Katche et al., 2010; McReynolds et al., 2010), we studied both short (1 h) and long (24 h) term PA paradigms. No changes in expression of these genes were found in response to the short term paradigm. However, the long term paradigm decreased Arc expression in the PFC and increase c-fos expression in the CPu. Interestingly, both these changes were counteracted by treatment with WAY208466. The long term paradigm of PA by itself had no effects on Arc and c-fos in hippocampus, but WAY208466 caused a significant increase of both these genes in this region. Our data is in agreement with previous data showing increased hippocampal and cortical Arc expression in animals treated with another 5-HT6 agonist, LY586713 (de Foubert et al., 2007). The 5-HT6 receptor is, indeed, coupled to Gαs proteins, which stimulate adenylate cyclase and downstream signaling mechanisms (Yun et al., 2007; Riccioni et al., 2011). It is therefore possible that some of the IEG activation seen here is a direct action of 5-HT6 agonism on hippocampal and cortical neurons. However, since these brain regions express relatively low levels of 5-HT6 receptors, it is also likely that these Arc and c-fos responses reflect indirect activation. There are dense projections from the midbrain to the PFC and hippocampus (Ongür and Price, 2000) and there are multisynaptic loops interconnecting ventral striatum, where 5-HT6 receptors are very high, with the PFC and hippocampus. Because both 5-HT6 agonists and antagonists are procognitive in several memory tasks, it would be interesting to compare their effects on IEG expression. To our knowledge, there are no publications describing IEG expression after treatment with a 5-HT6 antagonist.

Conclusion

WAY208466 facilitated emotional learning and induced antidepressant-like, but not anxiolytic, actions. Moreover, this 5-HT6 agonist stimulated molecular changes relevant for neuronal plasticity and memory formation in CPu, PFC and hippocampus. As noted above, the associations between behavioral responses and the molecular markers reported here are strictly correlational. To establish a causal relation between these events, experiments using gene knockouts would be necessary. Nonetheless, these data further emphasize an important role of 5-HT6 receptors in the regulation of neuronal signal transduction in relation to mood and cognition.

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Author Contributions

MP performed most of experiments, analysis, and writing. BM performed the immunoblottings, analyzed data and wrote the manuscript. RA contributed to the interpretation of the data and writing of the manuscript. PS designed the study and wrote the manuscript.

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REVIEW
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The role of the serotonin receptor subtypes 5-HT$_{1A}$ and 5-HT$_7$ and its interaction in emotional learning and memory

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Serotonin [5-hydroxytryptamine (5-HT)] is a multifunctional neurotransmitter innervating cortical and limbic areas involved in cognition and emotional regulation. Dysregulation of serotonergic transmission is associated with emotional and cognitive deficits in psychiatric patients and animal models. Drugs targeting the 5-HT system are widely used to treat mood disorders and anxiety-like behaviors. Among the fourteen 5-HT receptor (5-HTR) subtypes, the 5-HT$_{1A}$R and 5-HT$_7$R are associated with the development of anxiety, depression and cognitive function linked to mechanisms of emotional learning and memory. In rodents fear conditioning and passive avoidance (PA) are associative learning paradigms to study emotional memory. This review assesses the role of 5-HT$_{1A}$R and 5-HT$_7$R as well as their interplay at the molecular, neurochemical and behavioral level. Activation of postsynaptic 5-HT$_{1A}$Rs impairs emotional memory through attenuation of neuronal activity, whereas presynaptic 5-HT$_{1A}$R activation reduces 5-HT release and exerts pro-cognitive effects on PA retention. Antagonism of the 5-HT$_{1A}$R facilitates memory retention possibly via 5-HT$_7$R activation and evidence is provided that 5HT$_7$R can facilitate emotional memory upon reduced 5-HT$_{1A}$R transmission. These findings highlight the differential role of these 5-HTRs in cognitive/emotional domains of behavior. Moreover, the results indicate that tonic and phasic 5-HT release can exert different and potentially opposing effects on emotional memory, depending on the states of 5-HT$_{1A}$Rs and 5-HT$_7$Rs and their interaction. Consequently, individual differences due to genetic and/or epigenetic mechanisms play an essential role for the responsiveness to drug treatment, e.g., by SSRIs which increase intrasynaptic 5-HT levels thereby activating multiple pre- and postsynaptic 5-HTR subtypes.

Keywords: emotional learning, fear conditioning, fear memory, 5-HT$_{1A}$ receptor ligands, 5-HT$_7$ receptor ligands, passive avoidance, serotonin

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HTR, 5-HT receptor; cAMP, cyclic AMP; CNS, central nervous system; Epac, exchange proteins directly activated by cAMP; ERK, extracellular signal-related kinase; FC, fear conditioning; HR, heart rate; MAPK, mitogen-activated protein kinase; PA, passive avoidance; PKA, protein kinase A; SSRI, selective serotonin reuptake inhibitor.

Introduction

Serotonin (5-HT) is a biogenic amine acting as a neurotransmitter and neuromodulator. The distribution of serotonin-containing neurons in the CNS have been studied in different species and have been found to be localized exclusively in the brainstem (Hunt and Lovick, 1982; Takahashi et al., 1986; Ishimura et al., 1988). The majority of the serotonergic cell bodies reside in the dorsal and median raphe nuclei but send axons almost to the entire brain, including cortical, limbic, midbrain, and hindbrain regions (Charnay and Léger, 2010). As expected from the wide projection pattern of the 5-HT neurons, serotonin modulates variable physiological functions, such as sleep, arousal, feeding, temperature regulation, pain, emotions, and cognition (Bradley et al., 1986; Barnes and Sharp, 1999; Ögren et al., 2008; Berger et al., 2009; Artigas, 2015).

The pleiotropic behavioral effects of 5-HT are mediated by a family of at least 14 5-HTR subtypes (Hoyer et al., 1994). These 5-HTR subtypes are distributed in a brain- and cell-specific manner and regulate distinct physiological processes, through different and sometimes opposing signaling pathways (Hoyer and Martin, 1997; Hoyer et al., 2002).

The 5-HT$_{1A}$R is one of the best-studied 5-HTR subtypes due to its implication in anxiety-like behaviors (Heisler et al., 1998; Parks et al., 1998; Toth, 2003), in depression (Lucki, 1991) as well as in cognitive processes that are impaired in several psychiatric disorders (review by Ögren et al., 2008; Millan et al., 2012). Its potential role as a drug target has been also investigated (Tunnicliff, 1991; Den Boer et al., 2000; Blier and Ward, 2003). The most common antidepressants, the SSRIs, act by targeting the 5-HT$_{1A}$R (Hervas and Artigas, 1998; Artigas, 2015), supporting the key role of the 5-HT$_{1A}$R in the pathophysiology of mood disorders.

The 5-HT$_7$Rs are implicated in depression and anxiety, and evidence has been provided for their role in learning and memory (reviewed by Leopoldo et al., 2011). Interestingly, the 5-HT$_7$R and 5-HT$_{1A}$R exert opposing roles in the modulation of fear learning (Eriksson et al., 2008, 2012), pointing at the importance of both 5-HTR subtypes and their signaling interaction in the regulation of emotional learning.

After a brief introduction about the characteristics of 5-HT$_{1A}$ and 5-HT$_7$R (distribution, signaling, and ligands), this review will focus on the role of 5-HT$_{1A}$R, 5-HT$_7$R as well as its interplay in emotional learning processes. The interaction between the 5-HT$_{1A}$R and 5-HT$_7$R signaling will be discussed and results of studies using different available 5-HT$_{1A}$R and 5-HT$_7$R ligands on fear learning tasks are summarized. A considerable extent of this review will also be dedicated to describe the region-specific effects of 5-HT$_{1A}$R and 5-HT$_7$R, via local rather than systemic administration. Overall, the aim of this review is to draw general conclusions about the role of both 5-HT$_{1A}$R and 5-HT$_7$R in fear learning, which may contribute to our better understanding of the mechanisms underlying dysregulated learning and memory in affective disorders. The focus here is on fear learning because this one-trial learning task allows for exact timing of pharmacological manipulations to discriminate between different memory phases.

Characteristics of the 5-HT$_{1A}$ and 5-HT$_7$ Receptors

All the 5-HTR subtypes belong to the G protein-coupled receptor superfamily, with the exception of the 5-HT$_3$R as ionotropic receptor (Hoyer et al., 2002). The metabotropic 5-HTR subtypes consist of seven transmembrane domains and are classified into four groups based on the type of G proteins to which they are coupled. The 5-HT$_1$Rs (5-HT$_{1A}$R, 5-HT$_{1B}$R, 5-HT$_{1D}$R, 5-HT$_{1E}$R, 5-HT$_{1F}$R) couple to Gα_i/Gα_o proteins, whereas the 5-HT$_2$Rs (5-HT$_{2A}$R, 5-HT$_{2B}$R, 5-HT$_{2C}$) couple to Gα_q proteins, and the 5-HT$_4$R, 5-HT$_6$R, and 5-HT$_7$R couple to Gα_s proteins. For the 5-HT$_5$Rs (5-HT$_{5A}$R and 5-HT$_{5B}$R) G-protein coupling is not established yet (Bockaert et al., 2006).

5-HT$_{1A}$ Receptor Localization

5-HT$_{1A}$R was the first 5-HTR subtype to be cloned and is characterized by its high affinity for 5-HT (Nichols and Nichols, 2008). 5-HT$_{1A}$Rs are widely distributed throughout the CNS and are present in both pre- and postsynaptic sites. Presynaptically, 5-HT$_{1A}$Rs are exclusively located on the cell bodies and dendrites of 5-HT neurons in the dorsal and median raphe nuclei (Riad et al., 2000) and function as 5-HT$_{1A}$ autoreceptors which tightly regulate 5-HT neuronal activity.

Postsynaptically, the highest level of 5-HT$_{1A}$R is found in the limbic system based on receptor autoradiography and mRNA expression. Both techniques showed the distribution of the 5-HT$_{1A}$R in the lateral septum, cingulate and entorhinal cortices, with particularly high expression in the hippocampus (reviewed by Hannon and Hoyer, 2008). At the cellular level, the postsynaptic 5-HT$_{1A}$R is expressed in cortical pyramidal neurons as well as pyramidal, GABAergic and granular cells of the hippocampus (Hannon and Hoyer, 2008). At least in the hippocampal formation, the 5-HT$_{1A}$R is located on somata and dendrites of pyramidal and granular neurons, as well as on the dendritic spines of pyramidal neurons (Riad et al., 2000). Moreover, 5-HT$_{1A}$R immunoreactivity has been demonstrated in different subgroups of neurons in the septal complex with GABAergic septohippocampal parvalbumin-containing projection neurons, GABAergic calbindin D-28-containing neurons as well as cholinergic septohippocampal neurons (Lüttgen et al., 2005a). This indicates that systemic administration of 5-HT$_{1A}$R ligands can modify hippocampal function through effects on septohippocampal neurons that are responsible for the theta rhythm which plays an important role in memory functions (Elvander-Tottie et al., 2009).

5-HT$_{1A}$ Receptor Signaling

Activation of 5-HT$_{1A}$R leads to neuronal hyperpolarization, an effect mediated by pertussis-toxin-sensitive G$\alpha_{i/o}$ proteins. G$\alpha_{i/o}$ proteins are negatively coupled with the signaling pathway of adenylyl cyclase and thereby decrease the cAMP formation (De Vivo and Maayani, 1986; Weiss et al., 1986). Despite their high density in the dorsal raphe nucleus, 5-HT$_{1A}$ autoreceptors do not seem to inhibit AC, but mediate neuronal inhibition through different signaling pathways (Clarke et al., 1996). Both post- and presynaptic 5-HT$_{1A}$Rs inhibit neuronal firing via the activation

of G protein-coupled inwardly rectifying potassium channels as well as the inhibition of Ca^{2+} channels (Sodickson and Bean, 1998; Bockaert et al., 2006). A multitude of other signaling pathways and effectors has been also linked to the activation of the 5-HT$_{1A}$R (reviewed by Raymond et al., 2001; Bockaert et al., 2006).

5-HT$_7$R Localization

The 5-HT$_7$R was the last 5-HTR subtype to be cloned by using a targeted screening analysis of mammalian cDNA libraries and probes from already known receptors (Bard et al., 1993; Lovenberg et al., 1993; Ruat et al., 1993). Although 5-HT$_7$Rs demonstrate a high interspecies homology (>90%; To et al., 1995), they share a low homology with the other 5-HTR subtypes (<50%; Bard et al., 1993). Northern blot analysis and *in situ* hybridization studies demonstrate high expression of 5-HT$_7$R in the CNS and particularly in the hypothalamus (suprachiasmatic nucleus), thalamus, hippocampus, and cerebral cortex (Bard et al., 1993; Lovenberg et al., 1993; Ruat et al., 1993). Like 5-HT$_{1A}$R, the 5-HT$_7$R is also localized in the raphe nuclei in both rodent and human brain, which has raised questions about its role in the regulation of 5-HT levels (Martin-Cora and Pazos, 2004). At the neuronal level, 5-HT$_7$R is expressed in hippocampal CA pyramidal neurons with a higher density in CA3 than in CA1 (Bonaventure et al., 2004) and a differential expression, with selective localization on the cell bodies in CA1 pyramidal neurons (Bickmeyer et al., 2002). Little is known, however, about the expression patterns of 5-HT$_7$R in cortical neurons, where it is suggested that 5-HT$_7$R may have a role during the developing stages of cortical circuits (Béïque et al., 2007; Celada et al., 2013).

5-HT$_7$ Receptor Signaling

5-HT$_7$R activation activates adenylyl cyclase signaling and consequently the conversion of ATP to cAMP through coupling to Gα_s (Bard et al., 1993; Lovenberg et al., 1993; Ruat et al., 1993). Although cAMP activation is commonly mediated by the PKA, it has been demonstrated that Epac, a member of the cAMP-regulated guanine nucleotide exchange family, has a crucial role in PKA-independent signaling (Lin et al., 2003). For instance, 5-HT$_7$Rs activate the MAPK/ERK signaling pathway (Errico et al., 2001; Norum et al., 2003) via the stimulation of the Epac factor (Lin et al., 2003). Binding of cAMP to Epac leads to the activation of several other signaling pathways (reviewed by Holz et al., 2006).

Functional Roles of 5-HT$_{1A}$R and 5-HT$_7$ Receptors

The expression of 5-HT$_{1A}$R and 5-HT$_7$R in the limbic system (Hannon and Hoyer, 2008; Berumen et al., 2012) support a role in the modulation of functions like mood, memory processing as well as emotional association with memory. The 5-HT$_{1A}$R has been proposed to modulate anxiety based on studies with 5-HT$_{1A}$R knockout mice (Heisler et al., 1998; Parks et al., 1998; Toth, 2003) and the response to antidepressant drugs (Blier and Ward, 2003; Artigas, 2015). Several partial 5-HT$_{1A}$R agonists, e.g., buspirone, have been used to treat anxiety and depression (Tunnicliff, 1991; Den Boer et al., 2000), whereas co-administration of pindolol (β-adrenergic and 5-HT$_{1A}$R antagonist) with SSRIs enhances their therapeutic efficacy and shortens their onset of action (reviewed by Artigas et al., 2001). A considerable body of literature demonstrates the 5-HT$_{1A}$R involvement in various hippocampus-dependent learning and memory tasks (reviewed by Ögren et al., 2008).

In contrast, the available data on the function of 5-HT$_7$R is relatively limited, mainly due to the lack of selective agonists specific for this 5-HTR subtype (Misane and Ögren, 2000; Nichols and Nichols, 2008; Leopoldo et al., 2011). The physiological role of 5-HT$_7$R has been closely linked with the regulation of sleep, circadian rhythm, pain and also mood (reviewed by Leopoldo et al., 2011). Accumulating data implicates the 5-HT$_7$R in the action of antidepressant drugs, whereas the results from anxiety studies are contradictory (Leopoldo et al., 2011). Interestingly, studies using 5-HT$_7$R knockout mice revealed the crucial role of this receptor in hippocampus-dependent memory (Roberts et al., 2004; Sarkisyan and Hedlund, 2009).

5-HT$_{1A}$ and 5-HT$_7$ Receptor Ligands

General Receptor Ligand Principles

Agents that act as receptor ligands may be agonists or antagonists. Agonists initiate physiological changes by activating downstream signaling pathways, whereas antagonists bind to receptors without producing any effect (Rang et al., 2015). Ligands can be divided in three categories based on their function:

(1) Full agonists produce a maximal response equivalent to the endogenous agonist (here 5-HT). These agonists have high efficacy (i.e., the ability to initiate changes which leads to effects) for the binding receptor.
(2) Partial agonists are not capable of producing the maximal functional response even when they occupy the entire receptor population. These agonists present intermediate efficacy. Respectively, we could refer to partial antagonists that bind to the active site (competitive antagonism) but do not completely abolish the receptor-mediated effects.
(3) Mixed profile ligands that (appear to) act both as agonists and as antagonist in distinct receptor populations. More likely, they have different agonist profiles at different receptor sites (e.g., pre-versus postsynaptic 5-HT$_{1A}$R) and therefore appear to exert antagonist function in the presence of a full agonist, while acting as weak (partial) agonist thereby lowering the efficacy of the full agonist.

The function of any ligand used to study the role of 5-HT$_{1A}$R and 5-HT$_7$R is essential for the correct interpretation of the behavioral outcome. It is also important to mention that the intrinsic efficacy of a ligand is equally depended on the characteristics of response system; in our case the different brain populations of 5-HT$_{1A}$R and 5-HT$_7$R and their downstream signaling pathways. Agonists acting on the same receptor can produce different effects depending on their physicochemical

properties, brain distribution, full or partial agonism as well as the number of coupled receptors in a brain area. The specificity of the compounds used is another very important characteristic that should be always taken into consideration and is referred to the ligand's specific binding to the targeted receptor. Ligands with low specificity cannot be used to clarify the functional role of 5-HT$_{1A}$R and 5-HT$_7$R, since the produced effects can be also mediated via the binding to other proteins than the receptor of interest.

The physicochemical properties of compounds play an essential role for the drug uptake and diffusion with lipophilicity, solubility and molecular mass being among the most important properties (Waterhouse, 2003). The lipophilic nature of ligands is particularly important when they are administered locally. Increasing lipophilicity leads to enhanced blood–brain barrier diffusion, prevents the drug restriction in the area of interest and consequently produces wider effects, despite local application. This is evident from dorsohippocampal infusion of the blood–brain barrier penetrating drug 8-OH-DPAT, a full 5-HT$_{1A}$R agonist, which impairs tone-dependent memory (Stiedl et al., 2000a), whereas this does not occur when the NMDAR antagonist APV (Stiedl et al., 2000b) and the GABA$_A$R agonist muscimol are locally applied (Misane et al., 2013). The latter study is one of the few demonstrating the selective drug action in the dorsal hippocampus based on fluorescently labeled muscimol as bodipy conjugate. Besides the solubility of compounds and the applied dose, it is thus of high importance to consider other physico-chemical properties, such as half-life *in vivo*, to avoid misleading conclusions due to their wider spread (e.g., diffusion or potential active transport) in brain outside the target sites. The molecular weight of compounds can also provide valuable information about the diffusion capacity.

5-HT$_{1A}$ Receptor Agonists

The prototypic 5-HT$_{1A}$R agonist 8-OH-DPAT was the first full agonist developed (Arvidsson et al., 1981; Gozlan et al., 1983) and is still the most widely used to study the functional role of 5-HT$_{1A}$R in behavioral manipulations (Barnes and Sharp, 1999). Despite its high selectivity for the 5-HT$_{1A}$R, 8-OH-DPAT also acts as a 5-HT$_7$R agonist (Bickmeyer et al., 2002; Eriksson et al., 2008) and observed effects can be the result of an interplay between the two receptor subtypes (see below).

Additionally, several full and partial agonists have been synthesized (see **Table 1**), but only a few of them have been used in fear learning studies, such as the buspirone and tandospirone. Buspirone belongs to the arylpiperazine (partial) agonists (Hjorth

TABLE 1 | Selected overview on available 5-HT$_{1A}$ receptor agonists and ligands with mixed profile (reported function as presynaptic agonist and postsynaptic antagonist).

Function	Compound	Receptor Specificity	MW	Solvent	BBB penetr.	Behavior	Reference
Full/partial	Alnespirone (S-20499)	5-HT$_{1A}$ >> D$_2$ >> 5-HT$_{1B,2}$ >> α,β >> D$_1$ >> H$_1$ (pre-synaptic)	479	W	n.a.	A	Griebel et al. (1992)
Partial	Buspirone	5-HT$_{1A}$ = D$_2$ >> α$_1$,α$_2$	385.5	W	n.a.	A, L	Hjorth and Carlsson (1982), Quartermain et al. (1993)
Full	F-13640	5-HT$_{1A}$ >> n.a.	393.1	w	Yes	N	Deseure et al. (2002), Heusler et al. (2010); GtP
Partial	F-13714	5-HT $_{1A}$ >> 5-HT $_{1B-F,2-7}$	n.a.	w	n.a.	PPI	Assié et al. (2006)
Full	F-15599	5-HT$_{1A}$ (post-synaptic) >> 5-HT$_{1B-F,2-7}$	394.1	n.a.	Yes	FST	Maurel et al. (2007), Newman-Tancredi et al. (2009); GtP
Full	Flesinoxan	5-HT$_{1A}$ >> α$_1$ (antagonist) >> D$_2$	415.5	W	Yes	A	Ahlenius et al. (1991), Hadrava et al. (1995)
Partial	Ipsapirone (TVX Q 7821)	5-HT$_{1A}$ >> α$_1$ (antagonist)	401.5	w	Yes	A	Traber et al. (1984)
Partial/full	LY-228729	5-HT$_{1A}$ >> 5-HT$_{1B}$	n.a.	w	n.a.	L, FST	Swanson and Catlow (1992)
n.a.	NDO-008	5-HT$_{1A}$ >> n.a.	n.a.	w	n.a.	L	Misane et al. (1998)
Full	8-OH-DPAT	5-HT $_{1A}$ >> 5-HT$_7$ >> 5-HT$_4$ >> D$_2$	328.3	w	Yes	A, L	Arvidsson et al. (1981), Hadrava et al. (1995)
Full/Partial	Osemozotan (MKC-242)	5-HT$_{1A}$ >> α$_1$	379.8	w	n.a.	A	Matsuda et al. (1995), Sakaue et al. (2003)
Partial	PRX-00023	5-HT $_{1A}$ >> 5-HT $_{1B}$>α$_1$>α$_2$	n.a.	w	n.a.	A	Becker et al. (2006)
Full	Repinotan (BAY x 3702)	5-HT $_{1A}$ >> 5-HT$_7$ >> α$_1$>α$_2$>5-HT$_4$	400.5	HC1	Yes	L	De Vry et al. (1998), Schwarz et al. (2005)
Partial	Tandospirone (SM-3997)	5-HT$_{1A}$ >> D$_2$	383.5	w	n.a.	A, L	Shimizu et al. (1987)
Mixed profile	S-15535	n.a.	432.5	w	Yes	A, L	Millan et al. (1993), Carli et al. (1999)
Mixed profile	MDL-73005		n.a.	w	n.a.	L	Hajós-Korcsok et al. (1999), Bertrand et al. (2001)

A, anxiety; BBB, blood–brain barrier; D: FST; forced swim test; GtP, guide to pharmacology, see http://guidetopharmacology.org/; HCl, soluble in acidified aqueous solution; L, learning and memory tests; N, nociception; n.a., not available; penetr., penetrance; PPI, pre-pulse inhibition; W, soluble in water and/or saline.

and Carlsson, 1982) and acts also as antagonist with high specificity for the dopamine D_2 receptor (Witkin and Barrett, 1986). Tandospirone (SM-3997) is a 5-HT$_{1A}$R partial agonist and was initially studied for its anxiolytic properties in rats and mice (Shimizu et al., 1987). Similar to buspirone, tandospirone also exhibits dopamine antagonist action with a potency that is considerably lower than the one for the 5-HT$_{1A}$R (Shimizu et al., 1987). An overview of currently available 5-HT$_{1A}$R agonists is provided in **Table 1**.

5-HT$_{1A}$ Receptor Antagonists

WAY-100635 and NAD-299 are the most commonly used selective antagonists in the study of the 5-HT$_{1A}$R. Both ligands have high potencies and penetrate easily into the brain (Fletcher et al., 1996; Johansson et al., 1997; Stenfors et al., 1998). However, NAD-299 was found to have higher selectivity for the 5-HT$_{1A}$R than WAY-100635 (Fletcher et al., 1996; Johansson et al., 1997).

The last years novel compounds have been used to assess the role of 5-HT$_{1A}$R in emotional learning, such as the potent and selective 5-HT$_{1A}$R antagonists SRA-333 (lecozotan; Skirzewski et al., 2010), MC18 fumarate and VP08/34 fumarate (Siracusa et al., 2008; Pittalà et al., 2015).

The agents that were initially used as 5-HT$_{1A}$R antagonist were 2-methoxyphenylpiperazine derivatives with structural similarity to buspirone, such as BMY-7378 and NAN-190 (Greuel and Glaser, 1992). However, these ligands were characterized as partial 5-HT$_{1A}$R antagonist with antagonist properties only at the postsynaptic HT$_{1A}$R and lower affinity for the α-adrenergic receptors (Greuel and Glaser, 1992).

Finally, S-15535 is reported to act as a postsynaptic 5-HT$_{1A}$R antagonist while also behaving as an agonist on presynaptic 5-HT$_{1A}$ autoreceptors, and therefore, it is characterized as a mixed profile ligand (Millan et al., 1993; Carli et al., 1999). However, a more recent study indicates predominantly weaker agonist activity of S-15535 at postsynaptic 5-HT$_{1A}$Rs (Youn et al., 2009). An overview of currently available 5-HT$_{1A}$R antagonists is provided in **Table 2**.

5-HT$_7$ Receptor Agonists

The lack of selective and potent 5-HT$_7$R agonists (Misane and Ögren, 2000; Leopoldo, 2004; Leopoldo et al., 2011) is one of the major limitations to study the role of 5-HT$_7$R in learning and memory. Currently, only a few selective 5-HT$_7$R agonists exist and even less has been used in learning and memory studies. AS-19 and LP-44 are highly selective but low efficacy (partial) HT$_7$R agonists whose functional role in fear learning was recently assessed (Eriksson et al., 2012). LP-211 is a novel highly selective 5-HT$_7$R agonists (Leopoldo et al., 2008) but it has so far only been tested in an autoshaping Pavlovian/instrumental learning task (Meneses et al., 2015). An overview of currently available 5-HT$_7$R agonists is provided in **Table 3**.

5-HT$_7$ Receptor Antagonists

SB-258719 is the first selective 5-HT$_7$R antagonist described (Forbes et al., 1998) but has not yet been used to investigate the role of 5-HT$_7$R in the modulation of emotional learning. Both SB-656104-A and SB-269970 possess high potency and selectivity for 5-HT$_7$R (Lovell et al., 2000; Thomas et al., 2002, 2003). These are the most commonly used 5-HT$_7$R antagonists in behavior studies. An overview of currently available 5-HT$_7$R antagonists is provided in **Table 3**.

Behavioral Tasks for the Assessment of Emotional Learning and Memory

The experimental studies on emotional learning and memory in animals are based originally on psychological analysis of conflict behavior involving approach and avoidance of conditioned stimuli. Traditionally, the assays used to investigate animal behavior are based on the association of pleasant (i.e., motivationally related reward like food) or aversive stimuli (i.e., conditions related to negative feelings like pain and danger) to environmental cues involving classical (Pavlovian) or instrumental conditioning (Ögren and Stiedl, 2015).

The FC and the PA tasks are the most commonly used associative learning paradigms based on contextual fear learning. This type of learning is dependent on the operation of neuronal circuits in the limbic system, such as hippocampus and amygdala (Cahill and McGaugh, 1998; LeDoux, 2000) as demonstrated by us in mice (e.g., Stiedl et al., 2000a,b; Baarendse et al., 2008). Unlike FC, PA also includes instrumental learning. In the step-through PA test, the animal needs to suppress its innate preference for the dark compartment (where it previously received a foot shock) and remain in the bright compartment. In the step-down PA paradigm, however, the retention is examined in the dark compartment, where the animal received the foot shock (unconditioned stimulus) after stepping down from an elevated platform. The PA test procedure can be modified to examine any facilitating effect of the treatment on PA retention (Madjid et al., 2006). More specific information on the PA task is provided elsewhere (Ögren and Stiedl, 2015). A refined version of this task may provide for better translational aspects to assess pathological fear states such as post-traumatic-like responses based on deliberate choice of mice (Hager et al., 2014).

The single-trial learning design of FC and PA, which is sufficient to establish long-term and remote memory, allows the exact timing of the drug treatment in relation to training and retention test. Thereby, unlike multi-session tasks, one-trial tasks provide a unique advantage to study learning mechanisms as well as drug effects (here 5-HT$_{1A}$R and 5-HT$_7$R ligands) on the different phases of learning and memory, i.e., the acquisition phase that consists of encoding and early consolidation, consolidation, the recall (retrieval and expression) phase as well as the extinction phase and reconsolidation.

Effects of 5-HT$_{1A}$ Receptor Ligands in Emotional Learning and Memory

An overview of the behavioral effects of various 5-HT$_{1A}$R ligands is provided in **Table 4**.

TABLE 2 | Selected overview on available 5-HT_{1A} receptor antagonists.

Function	Compound	Receptor specificity	MW	Solvent	BBB penetr.	Behavior	Reference
Partial	BMY-7378	5-HT_{1A} >> α_1 >> α_2 (partial agonist function) >> 5-HT_7 >> 5-HT_{1D}	385.9	W	+	A, L	Greuel and Glaser (1992), Grasby et al. (1992)
Partial	LY-426965	HT_{1A} >> 5-HT_{1B} (partial agonist function)	471.1	W	n.a.	A	Rasmussen et al. (2000); http://sis.nlm.nih.gov/
	MC18 fumarate	5-HT_{1A} >> n.a	515.7	W	n.a.	L	Pittalà et al. (2015)
	MP3022	5-HT_{1A} >> α_1 >> 5-HT_{2A}, α_2, β, D_1 and D_2	351.5 354.9	n.a.	n.a.	n.a.	Filip and Przegaliński (1996)
	NAD-299 (Robalzotan	5-HT_{1A} >> α_1,α_2, β		W	+	A, L	Johansson et al. (1997), Madjid et al. (2006); http://chem.sis.nlm.nih.gov/
Partial	NAN-190	HT_{1A} >> α_1 (partial agonist function reported) >> 5-HT_R, D	393.5	W	n.a.	A, L	Raghupathi et al. (1991), Greuel and Glaser (1992)
	p-MPPI	5-HT_{1A} >> α_1	542.4	W	+	A	Kung et al. (1994), Allen et al. (1997); http://pubchem.ncbi.nlm.nih.gov/
	p-MPPF	5-HT_{1A} >> α_1	507.4	n.a.	+	n.a.	Kung et al. (1996), Passchier et al. (2000); http://pubchem.ncbi.nlm.nih.gov/
	SB-649915	n.a., combined function as 5-$HT_{1A/B}$ autoreceptor antagonist and SSRI	n.a.	MC	n.a.	A	Starr et al. (2007)
	Spiperone	5-HT_{1A} >> 5-$HT_{2A/c}$ >> D_2 antagonist and α_{1b} antagonist	n.a.	MC	n.a.	A	Starr et al. (2007)
	SRA-333 (Lecozotan)	5-HT_{1A} >> α_1 >> D_2 >> D_3 >> D_4 (α and D agonist)	n.a.	W	n.a.	A, L	Schechter et al. (2005)
	(S)-UH-301	5-HT_{1A} >> D_2, D_3 (agonist)	301.8	W	Yes	A, L	Moreau et al. (1992), Jackson et al. (1994)
	VP-08/34 fumarate	5-HT_{1A} >> n.a	513.6	W	Yes	L	Pittalà et al. (2015)
	WAY-100635	5-HT_{1A} >> α_1 >> D_2 >> D_3 >> D_4	538.6	W	Yes	A, L	Fletcher et al. (1996), Pike et al. (1996)
	WAY-405	5-HT_{1A} >> α	n.a.	MC	Yes	A, L	Minabe et al. (2003), Villalobos-Molina et al. (2005)
	WAY-101405	5-HT_{1A} >> n.a	n.a.	W	Yes	L	Hirst et al. (2008)

A, anxiety; BBB, blood–brain barrier; D: FST, forced swim test; L, learning and memory tests; MC, methylcellulose; n.a., not available; penetr., penetrance; PPI, pre-pulse inhibition; S, 2-hydroxypropyl-β-cyclodextrin; W, soluble in water and/or saline.*

Systemic 5-HT_{1A} Receptor Ligand Effects

Despite the differences among the 5-HT_{1A}R ligands in their chemical and pharmacological features (e.g., receptor selectivity and partial or full agonist properties; see **Tables 1** and **2**), there is strong evidence for the impairing effect of postsynaptic 5-HT_{1A}R activation on fear memory. Systemic, pretraining administration of the full 5-HT_{1A}R agonist 8-OH-DPAT shows a biphasic effect on PA performance, with the low dose range (0.01, 0.03 mg/kg) facilitating and the high dose range (0.1–1 mg/kg) impairing PA retention 24 h after training in both rats (Misane and Ögren, 2000; Lüttgen et al., 2005b) and mice (Madjid et al., 2006). The impairing dose of 8-OH-DPAT (0.2 and 0.3 mg/kg) also induces signs of the serotonin syndrome (Carli et al., 1992; Lüttgen et al., 2005b) linking the postsynaptic 5-HT_{1A}R to the learning deficits. In line with these results, FC studies demonstrated that pretraining systemic injections of high doses (0.1–0.5 mg/kg) of 8-OH-DPAT impair fear learning (Stiedl et al., 2000a; Youn et al., 2009). Pretreatment with the selective 5-HT_{1A}R antagonist WAY-100635 (0.03–1 mg/kg) blocked the impairment in freezing (FC) and transfer latency (PA), confirming and extending the detrimental role of the postsynaptic 5-HT_{1A}R activation on memory acquisition.

The observed memory deficit was already present in short-term memory tests performed 1 h after training for FC retention (Stiedl et al., 2000a) and 5 min after PA training (Misane and Ögren, 2000). Thus, postsynaptic 5-HT_{1A}R activation specifically impairs memory encoding of the aversive experience and not memory consolidation. In agreement to that observation, immediate 8-OH-DPAT post-training administration did not alter PA or FC retention (Misane and Ögren, 2000; Madjid et al., 2006).

Local 5-HT_{1A} Receptor Ligand Effects

Intracranial administration of 5-HT_{1A}R agonists and/or antagonists was used to further elucidate the distinct function of pre- versus postsynaptic 5-HT_{1A}Rs in fear learning. Pre-

TABLE 3 | Selected overview on available 5-HT_7 receptor agonists and antagonists.

Function	Compound	Receptor specificity	MW	Solvent	BBB penetr.	Behavior	Reference
Agonists							
Partial	AS-19	5-HT_7 >> n.a.	283.41	PG	n.a.	L, N	Brenchat et al. (2009), Eriksson et al. (2012)
Full	E-55888	n.a.	257.4	W	n.a.	N	Brenchat et al. (2009); http://pubchem.ncbi.nlm.nih.gov/
n.a.	LP-211	5-HT_7 >> D_2 > 5-HT_{1A}	466.6	DMSO	Yes	L	Leopoldo et al. (2008), Meneses et al. (2015); http://pubchem.ncbi.nlm.nih.gov/
Partial	LP-44	5-HT_7 >> 5-HT_{1A} (agonist function) >> 5-HT_{2A}	488.1	PG	Yes	L, REM Sleep	Monti et al. (2008), Eriksson et al. (2012); http://pubchem.ncbi.nlm.nih.gov/
Partial	MSD-5a	5-HT_7 >> 5-HT_{1A} >> 5-HT_{2A} >> D_2	n.a.	W	n.a.	N	Thomson et al. (2004), Brenchat et al. (2009)
Antagonists							
	DR4004	5-HT_7 >> 5-HT_2 > D_2 > HT_{1A} > HT_6 > HT_4	382.5	T80	A, L	n.a.	Kikuchi et al. (1999); http://pubchem.ncbi.nlm.nih.gov/
	SB-258719	5-HT_7 >> 5-HT_{1D} >> D_2, D_3 >> 5- >> 5-HT_{1B},5-HT_{2B} >> HT_{1A}	338.5	W	n.a.	N	Forbes et al. (1998), Brenchat et al. (2009); http://pubchem.ncbi.nlm.nih.gov/
	SB-269970*	5-HT_7 >> 5-HT_{5A} >> D_2 > 5-HT_{1B} > HT_{1D}	352.5	T80	Yes	A, FST, L	Lovell et al. (2000), Thomas et al. (2002), Wesolowska et al. (2006), Eriksson et al. (2012); http://pubchem.ncbi.nlm.nih.gov
	SB-656104-A	5-HT_7 >> 5-HT_{1D} > 5-HT_{2A} >HT_{2B}>D_2 >5-HT_{5A}	n.a	MC	Yes	L, REM Sleep	Thomas et al. (2003), Horisawa et al. (2011)
	SB-258741**	5-HT_7 >> 5-HT_{1A} > D_3 > HT_{1B}, D_2 > 5-HT_{1D}	350.5	W	n.a.	SZ	Lovell et al. (2000), Pouzet et al. (2002); http://pubchem.ncbi.nlm.nih.gov/

*A, anxiety; BBB, blood–brain barrier; DMSO, dimethyl sulfoxide; FST, forced swim test; L, learning and memory tests; MC, methylcellulose; n.a., not available; penetr., penetrance; PG, propylene glycol; PPI, pre-pulse inhibition; SZ, schizophrenia assays; T80: Tween 80; W: soluble in water and/or saline; *behaves as quasi-full inverse agonist (Mahé et al., 2004); **behaves as partial inverse agonist (Mahé et al., 2004).*

but not post-training intra-hippocampal infusion of 8-OH-DPAT impairs contextual FC (Stiedl et al., 2000a), pointing at the important role of the postsynaptic 5-HT_{1A}R in acquisition processes as observed after systemic administration.

Effects of 5-HT_{1A} Receptor Agonists and Antagonists on Memory Recall

Systemic 5-HT_{1A} Receptor Ligand Effects

Unlike the unambiguous implication of the postsynaptic 5-HT_{1A}R in memory acquisition, its role in fear retrieval and expression is less clear. The systemic 5-HT_{1A}R agonist NDO-008 (0.5 mg/kg) administered before the retention test to rats impairs slightly PA performance (Misane et al., 1998). In contrast, systemic administration of buspirone at the dose of 1 and 3 mg/kg had no effect on fear expression in mice (Quartermain et al., 1993). These different effects may partly depend on the readouts and the side effects elicited by higher 5-HT_{1A}R dosages, such as the hypolocomotion induced together with the serotonin syndrome (Stiedl et al., 2000a). The hypolocomotion confounds the interpretation of fear expression results in mice when based on freezing. Moreover, it also possible that differences exists between rats and mice, although our own data shows high similarity of results in these two species.

Therefore, a recent study tried to clarify the role of the 5-HT_{1A}R in fear recall, by assessing the effect of 8-OH-DPAT on fear-conditioned HR responses (reviewed by Stiedl et al., 2009) upon training and 24 h after training, in mice (Youn et al., 2013). Systemic pretest administration reduced the conditioned maximum HR as a consequence of the significantly reduced baseline HR before the presentation of the conditioned stimulus (tone). However, the tone-induced HR increase was preserved during the retention of auditory fear in mice with similar magnitude as compared to that in controls. Additionally, 8-OH-DPAT reduced the unconditioned tachycardia elicited by novelty exposure as a consequence of altered HR dynamics indicating autonomic dysregulation with enhanced parasympathetic function through postsynaptic 5-HT_{1A}R activation (Youn et al., 2013). Thus, the claims of anxiolytic actions of pretest injection of 5-HT_{1A}R agonists as initially reported in human studies and partly in animal models cannot be supported unambiguously at least in learned fear experiments.

TABLE 4 | Overview of the behavioral effects of 5-HT$_{1A}$ receptor agonists, ligands with mixed profile and antagonists in fear learning tasks.

Compound	Species: Strain	Time of injection	Dose (mg/kg)	Admin. route	Behavior assay and behavioral consequences	Reference
Agonists						
Buspirone	M: Swiss-W.	30 min pretr.	1	s.c.	FC: reduced freezing in 24-h delay	Quartermain et al. (1993)
NDO-008	R: Sprague-D.	15 min pretr.	0.25–1.0	s.c.	PA: impaired PA retention at 24-h test	Misane et al. (1998)
8-OH-DPAT	M: C57BL/6J	15 min pretr.	0.05 and 1	s.c.	FC: impaired freezing at 1-h and 24-h test	Stiedl et al. (2000a)
		0 min post-tr.	0.05 and 1	s.c.	FC: no effect	Stiedl et al. (2000a)
		15 min pretr.	2 × 2.5 μg	i.h.	FC: impaired freezing at 24-h test	Stiedl et al. (2000a)
	M: C57BL/6J	15 min pretr.	0.3	s.c.	PA: impaired PA retention at 24-h test	Eriksson et al. (2012)
Tandospirone	M: Swiss-W.	30 min pretr.	2 and 5	s.c.	FC: reduced freezing at 24-h test	Quartermain et al. (1993)
	M: Swiss-W.	30 min pretr.	2 and 5	s.c.	FC: no effect at 1-h test	Quartermain et al. (1993)
	M: Swiss-W.	30 min pretest	2 and 5	s.c.	FC: no effect	Quartermain et al. (1993)
	M: Swiss W.	30 min pretr.	2.5 and 5	s.c.	PA: DD PA retention impairment	Mendelson et al. (1993)
Mixed profile						
MDL-73005	R: Long-E.	15 min pretr.	2	i.p	MWM: no effect alone but prevented the memory impairment induced by scopolamine (0.25 mg/kg)	Bertrand et al. (2001)
S15535	M: C57BL/6J	20 min pretr.	0.01–05	s.c.	FC: impairment at higher dose (>2 mg/kg)	Youn et al. (2009)
Antagonists						
BMY-7378	M: Swiss-W.	30 min pretr.	0–5	s.c.	PA: no effect	Mendelson et al. (1993)
MC18	M: C57BL/6J	15 min pretr.	0.1–1	s.c.	PA: U-shaped PA retention facilitation (maximum at 0.3 mg/kg)	Pittalà et al. (2015)
NAD-299	M: C57BL/6J	20 min pretr.	0.3 and 1	s.c.	FC: increased freezing at 24-h test	Youn et al. (2009)
	M: C57BL/6J	15 min pretr.	0.1–3	s.c.	PA: DD PA retention facilitation at 24-h test	Madjid et al. (2006)
	M: NMRI	15 min pretr.	0.1–3	s.c.	PA: U-shaped PA retention facilitation (maximum at 1 mg/kg)	Madjid et al. (2006)
SRA-333	R: Sprague-D.	30 min pretr.	0.3–2	s.c.	PA: DD PA retention facilitation	Skirzewski et al. (2010)
(S)-UH-301	R: Sprague-D.	30 min pretr.	0–3	s.c.	PA: no effect	Jackson et al. (1994)
VP-08/34	M: C57BL/6J	15 min pretr.	0.3 and 1	s.c.	PA: no effect	Pittalà et al. (2015)
WAY-100635	R: Sprague-D.	30 min pretr.	0.003–0.3	s.c.	PA: attenuated the PA retention deficit by PC A (0.03–0.1 mg/kg)	Misane and Ögren (2000)
	R: Wistar	30 min pretr.	1	i.p.	PA: reversed MK-801-induced memory impairment	Horisawa et al. (2011)
	R: Wistar	0 min post-tr.	0.01	i.v.	PA: reversed MK-801-induced memory impairment	Horisawa et al. (2011)
	R: Sprague-D.	120 min pretr.	3	po.	FC: Reversed scopolamine-induced memory deficits	Hirst et al. (2008)

A, anxiety tests; DD, dose-dependent, FC, fear conditioning; i.h., intrahippocampal; i.p., intraperitoneal, i.v., intravenous; M, mice; n.a., not available; PA, passive avoidance; post-tr., post-training; p.o., per os; pretr, before training; R, rats; s.c., subcutaneous.

Local 5-HT$_{1A}$ Receptor Ligand Effects

Local administration approaches tried to distinguish the role of the post- versus the presynaptic 5-HT$_{1A}$R in the different aspects of fear expression. Bilateral microinjections of a selective 5-HT$_{1A}$R agonist flesinoxan decreased the expression of conditioned contextual freezing when injected into the hippocampus or amygdala but not in the medial prefrontal cortex (Li et al., 2006), as well as the fear-potentiated startle responses when infused into the central amygdala (Groenink et al., 2000).

The role of 5-HT$_{1A}$ autoreceptors in fear expression was also studied by pretest infusion of 8-OH-DPAT into the median raphe nuclei. This resulted in impaired contextual freezing responses (Borelli et al., 2005; Almada et al., 2009), but not fear-potentiated startle (Groenink et al., 2000; Almada et al., 2009) suggesting the existence of raphe-dependent serotonergic regulation that appears to modulate the freezing response to the aversive context. In contrast, hippocampal 8-OH-DPAT impaired the expression of both contextual freezing and fear-potentiated startle (Almada et al., 2009). However, 8-OH-DPAT mediates hyperlocomotion in rats (but hypolocomotion in mice) leading to a similar problem of potentially confounded interpretation of freezing performance during the drug state as mentioned before for mice.

Effects of 5-HT$_{1A}$ Receptor Agonists and Antagonists on Memory Extinction

In contrast to the well-studied implication of 5-HT$_{1A}$Rs on memory acquisition and recall, there is only one study with 5-HT$_{1A}$R ligands on fear extinction. The systemic 5-HT$_{1A}$R agonist buspirone abolishes the fear extinction in mice (Quartermain et al., 1993). Similarly, the systemic 5-HT$_{1A}$R antagonist WAY-100635 before a second sampling trial impaired the extinction of object recognition memory in rats (Pitsikas et al., 2003). Further studies are needed to determine the precise role of 5-HT$_{1A}$Rs in memory extinction and/or reconsolidation in emotional learning tasks. Furthermore, local rather than systemic approaches are necessary to identify the neurocircuitry involved in these processes. The roles of other 5-HTRs in fear learning and the consequences of altered 5-HT neurotransmission on fear extinction are reviewed by Homberg (2012).

Effects of 5-HT$_7$ Receptor Agonists and Antagonists on Emotional Learning

Systemic 5-HT$_7$ Receptor Ligand Effects

The paucity of studies 5-HT$_7$R functions on emotional learning is mainly due to the lack of selective ligands, especially agonists (Misane and Ögren, 2000; Leopoldo, 2004; Leopoldo et al., 2011; see **Table 5** and text above). Recent data from an autoshaping task showing that the 5-HT$_7$R agonist, LP-211, when administered systematically after the training session, reversed scolopamine-induced amnesia, in rats (Meneses et al., 2015). The same group also shows a facilitating effect on memory formation by the 5-HT$_7$R agonist AS-19 administered after an autoshaping training session (Perez-García and Meneses, 2005). The enhancing effect of 5-HT$_7$Rs on memory consolidation was blocked by pre-injection of the 5-HT$_7$R antagonist SB-269970 (Perez-García and Meneses, 2005; Meneses et al., 2015) indicating the specific involvement of the 5-HT$_7$R.

Eriksson et al. (2008) investigated the role of 5-HT$_7$R on emotional learning in mice using a step-through PA paradigm. Pretraining systemic administration of the 5-HT$_7$R antagonist SB-269970 enhanced the impairing effect of low doses of 8-OH-DPAT (Eriksson et al., 2008). This result supports the notion that 5-HT$_7$R activation has a beneficial modulatory role in learning opposing the function of 5-HT$_{1A}$R activation. Accordingly, pretraining 5-HT$_7$R activation by the combined use of the 5-HT$_{1A}$R antagonist NAD-299 with the 5-HT$_{1A}$R and 5-HT$_7$R agonist 8-OH-DPAT facilitated PA retention (Eriksson et al., 2012). This PA facilitation by NAD-299 together with 8-OH-DPAT was again blocked by the 5-HT$_7$R antagonist SB-269970 indicating a procognitive effect of 5-HT$_7$R activation by this drug combination. However, the 5-HT$_7$R agonists LP-44 and AS-19 failed to mediate this PA facilitation, despite dose-dependent tests. Despite their high *in vitro* potency to stimulate intracellular signaling cascades (Eriksson et al., 2012), the 5-HT$_7$R agonists LP-44 and AS-19 have moderate agonist efficacy *in vivo*. This finding is in agreement with previous pharmacological characterization (Monti et al., 2008; Bosker et al., 2009; Brenchat et al., 2009) *in vivo* and may explain why the facilitatory effect of NAD-299 with 8-OH-DPAT could not be mimicked by the putative agonists LP-44 and AS-19.

Local 5-HT$_7$ Receptor Ligand Effects

To further address the role of 5-HT$_7$Rs on emotional learning, Eriksson et al. (2012) performed hippocampal infusions with the 5-HT$_7$R agonist AS-19 in mice. Since they failed to find clear facilitatory effects, as observed after systemic treatment, they concluded that "5-HT$_7$Rs appear to facilitate memory processes in a broader cortico-limbic network and not the hippocampus alone." The failure of the SB-269970 to enhance emotional memory, upon hippocampal infusions, may be the consequence of the low dose that can be locally infused due to the relatively poor solubility of SB-269970. However, systemic administration of this 5-HT$_7$R antagonist fully blocked the PA facilitation observed after 5-HT$_{1A}$R blockade. Hence, the hippocampus-dependent involvement of the 5-HT$_7$Rs needs to be re-investigated with selective highly potent 5-HT$_7$R agonists, because also the low potency of AS-19 (Eriksson et al., 2012) may have contributed to the lack of effects by dorsohippocampal 5-HT$_7$R agonist application on PA. Finally, although the role of 5-HT$_7$R in memory consolidation has been suggested, there are currently insufficient data supporting this view. More work is also required to clarify the role of 5-HT$_7$R in memory extinction and reconsolidation, which are both essentially unexplored.

The Interplay of the 5-HT$_{1A}$ and 5-HT$_7$ for Emotional Learning

The interaction of the two 5-HTR subtypes in emotional learning has been studied by using 8-OH-DPAT, which exerts agonistic effects for both 5-HT$_{1A}$Rs and 5-HT$_7$Rs. To dissect the function of these 5-HTRs, pre-treatment with selective 5-HT$_{1A}$R antagonists is used to exclusively activate 5-HT$_7$R. Eriksson et al. (2008) were the first to suggest the functional interplay between the two 5-HTRs on the behavioral level as the activation of 5-HT$_7$R counteracted the 5-HT$_{1A}$R-mediated impairments in PA performance. The interaction between the two 5-HTRs and their functional antagonism was then extended by experiments in mice, demonstrating that 5-HT$_7$R activation and concomitant 5-HT$_{1A}$R blockade leads to PA facilitation (Eriksson et al., 2012). The facilitatory effect on emotional memory by the 5-HT$_{1A}$ antagonist NAD-299 was related to stimulation of 5-HT$_7$Rs under conditions with reduced 5-HT$_{1A}$R transmission. These findings suggest that the states of 5-HT$_{1A}$Rs and 5-HT$_7$Rs play a critical role for 5-HT effects on emotional memory. Consequently, the elevation of endogenous 5-HT via SSRIs will most likely result in differential cognitive/emotional effects depending on genetic and/or epigenetic regulation and occupancy of these two 5-HTRs in health and disease. This condition will affect the expression of the 5-HT$_{1A}$R and change the relative balance between 5-HTR subtypes, which together will

TABLE 5 | Overview of the behavioral effects of 5-HT$_7$ receptor agonists and antagonists in learning tasks (not restricted to fear learning).

Compound	Species: Strain	Time of injection	Dose (mg/kg)	Admin. route	Behavior assay and behavioral consequences	References
Agonists						
AS-19	M: C57BL/6J	15 min pretr.	3–10	i.p.	DD activity reduction PA: no effect in retention latencies, 24 h after training	Eriksson et al. (2012)
	R: Wistar	0 min post-tr.	0.5–10.0	s.c	P/I-A: Enhanced memory consolidation, 24 h after training	Perez-García and Meneses (2005)
LP-211	R: Wistar	0 min post-tr.	0.1–10.0	i.p.	P/I-A: only 0.5 mg/kg had a possitive effect on memory consolidation, when tested 24 h after training	Meneses et al. (2015)
LP-44	M: C57BL/6J	15 min pretr.	1–10	i.p.	PA: DD activity reduction but no effect on PA retention latencies tested 24 h after training	Eriksson et al. (2012)
NAD-299 + 8-OH-DPAT	M: C57BL/6J	30 min +15 min pretr.	0.3 + 1	s.c.	PA: facilitates retention latencies 24 h after training serving as 5-HT$_7$R activation	Eriksson et al. (2012)
Antagonists						
DR4004	R: Wistar	0 min post-tr.	0.5–10.	i.p.	P/I-A: no effect	Meneses (2004)
SB-269970	R: Wistar	0 min post-tr.	1–20	i.p.	P/I-A: no effect	Meneses (2004)
	M: C57BL/6J	30 min pretr.	20	s.c.	PA: reversed the facilitation by 8-OH-DPAT + NAD-299	Eriksson et al. (2012)
SB-656104-A	R: Wistar	60 min pretr.	10 and 30	i.p.	PA: reversed MK-801-induced memory impairment	Horisawa et al. (2011)
	R: Wistar	60 min pretr.	0.3	i.p.	PA: Counteracted the effect of MK-801	Horisawa et al. (2011)

A, anxiety tests; DD, dose dependent, FC, fear conditioning; i.h., intrahippocampal; i.p., intraperitoneal, i.v., intravenous; M, mice, MSRAP, multiple schedule repeated acquisition performance; MWM, Morris water maze; n.a., not available; OR, object recognition task; OT, operant task; PA, passive avoidance; P/I-A, Pavlovian/instrumental autoshaping task; post-tr., post-training; p.o., per os; pretr, before training; R, rats; s.c., subcutaneous.

eventually determine the physiological actions of 5-HT and the clinical efficacy of SSRI treatment.

Mechanisms Underlying the Functional Interaction of 5-HT$_{1A}$R and 5-HT$_7$R

As described above, 5-HT$_{1A}$Rs and 5-HT$_7$Rs mediate opposing effects regarding the neuronal excitability. 5-HT$_{1A}$R activation reduces the activity of adenyl cyclase, whereas 5-HT$_7$R activation stimulates adenyl cyclase activity and thereby increases intracellular cAMP thereby increasing neuronal excitability (Bockaert et al., 2006; Nichols and Nichols, 2008; Berumen et al., 2012). Accordingly, 5-HT$_7$R stimulation in the hippocampus was found to activate pyramidal neurons, unlike 5-HT$_{1A}$R activation which inhibited pyramidal neurons (Bickmeyer et al., 2002). Both 5-HTRs are expressed in glutamatergic hippocampal pyramidal neurons (Bockaert et al., 2006; Nichols and Nichols, 2008; Berumen et al., 2012). Therefore, it is likely that 5-HT$_{1A}$R and 5-HT$_7$R stimulation decreases and increases glutamate release in the hippocampus, respectively. In line with these results, 5-HT$_7$R activation enhances the AMPA receptor-mediated synaptic currents on CA1 pyramidal neurons, whereas 5-HT$_{1A}$R activation inhibits the AMPA receptor-mediated transmission between CA3 and CA1 pyramidal neurons in both pre- and postsynaptic sites (Costa et al., 2012). However, the 5-HT$_{1A}$R-mediated inhibitory effect on glutamatergic neurotransmission was stronger than the 5-HT$_7$R-mediated facilitatory effect (Costa et al., 2012). One explanation for the increased effectiveness of 5-HT$_{1A}$R in controlling the input from the Schaffer collaterals may stem from the different localization of the two receptors on the CA1 pyramidal neurons: 5-HT$_7$Rs are found on the cell bodies (Bickmeyer et al., 2002), whereas the 5-HT$_{1A}$Rs appear to be mainly localized on dendrites (Kia et al., 1996).

Differences in the expression of the receptors could also play an essential role in their distinct activation pattern from the endogenous 5-HT. The progressive reduction of post-synaptic 5-HT$_7$R levels during postnatal development, together with the maintenance of the expression level of 5-HT$_{1A}$R (Kobe et al., 2012; Renner et al., 2012), could increase the ratio of membrane 5-HT$_{1A}$Rs over 5-HT$_7$Rs. Consequently, a model has been proposed regarding the molecular mechanisms that underlie the regulation of the 5-HT$_{1A}$Rs and 5-HT$_7$Rs. 5-HT$_{1A}$R and 5-HT$_7$R form heterodimers both *in vitro* and *in vivo* (Renner et al., 2012). This heterodimerization plays a functional role by decreasing G$_i$

protein coupling of the 5-HT_{1A}R and by reducing the ability of 5-HT_{1A}R to activate potassium channels, without affecting the G_s protein coupling of the 5-HT_7R. The heterodimerization additionally contributes to the desensitization of the 5-HT_{1A}R through facilitated internalization (Renner et al., 2012).

5-HT_{1A}R and 5-HT_7R are co-localized in the cell membrane of hippocampal neurons, where their heterodimerization induces an inhibitory effect on the 5-HT_{1A}R-mediated activation of potassium channels in hippocampal neurons (Renner et al., 2012). As mentioned above the post-synaptic levels of 5-HT_7R are lower compared to the expression levels of post-synaptic 5-HT_{1A}R, whereas this is not the case for the pre-synaptic 5-HT_7R (Renner et al., 2012). These regional differences in the 5-HT_7R levels and therefore in the concentration of the heterodimers, can explain the preferential desensitization of 5-HT_{1A} autoreceptors by SSRIs and more generally the region- and cell- specific differences in the signaling pathway mediated by the 5-HT_{1A}R activation (see Naumenko et al., 2014). In summary, the above data suggest that the positive or negative consequences of a drug on emotional memory and cognition depend on the relative level of 5-HTR expression and, its efficacy in activating different receptors with their downstream signaling pathways.

Genetic and Epigenetic Effects on 5-HT Transmission and Receptor Expression

Genetic and/or epigenetic effects regulate the receptor's state and eventually define the physiological actions of endogenous 5-HT. A characteristic example is the Ala50Val variant of the 5-HT_{1A}R, located in the transmembrane region 1, that leads to loss of response to 5-HT and consequently to the interruption of 5-HT signaling (Del Tredici et al., 2004). Moreover, the human polymorphism Gly22Ser attenuates the downregulating effect induced by long-term 8-OH-DPAT stimulation in comparison to the Val28 variant and wild-type without effect on the ligand binding capacity (Rotondo et al., 1997). It is suggested that individuals with the Ser22 variant have higher sensitivity to SSRIs treatment since its serotonergic effect depends on the efficiency of 5-HT_{1A}R transmission (Rotondo et al., 1997). Furthermore, carriers of the short (s) allele of the 5-HT transporter promotor region possess behavioral abnormalities, such as increased levels of anxiety and FC as well as stronger fear potentiated startle (Bauer, 2014) in comparison to long (l) allele carriers. Accordingly, the therapeutic efficacy of SSRIs is reduced in patients homozygous for the s-allele when compared with heterozygous or l-allele carriers (Tomita et al., 2014).

The epigenetic regulation of 5-HTR subtypes is also implicated in the differential emotional and cognitive modulation induced by the serotonergic signaling. It is widely accepted that 5-HT_{1A}R binding is reduced in the brain of depressed humans (e.g., Savitz et al., 2009) as well as in stressed rats (e.g., Choi et al., 2014) as indication of epigenetic modulation. 5-HT_{1A}R activation in the basolateral amygdala and the prelimbic area of the prefrontal cortex in low-anxious rats reduced fear potentiated startle, whereas 5-HT_{1A}R activation in the periaqueductal gray of high-anxious rats had the opposite effect (Ferreira and Nobre, 2014). These findings highlight how environmental conditions can contribute to individual differences in 5-HT_{1A}R-mediated response differences. In line with this, single-housed mice display a stronger hypothermic effect upon 5-HT_{1A}R activation by 8-OH-DPAT, which is associated with an increased depressive-like state, in comparison to their group-housed counterparts (Kalliokoski et al., 2014). However, the mechanisms underlying the inter-individual differences in serotonergic signaling and consequently in cognitive and emotional modulation are not clear yet.

A linkage disequilibrium study identified two polymorphisms (rs3808932 and rs12412496) in the human *HTR7* suggesting that it is a schizophrenia susceptibility gene (Ikeda et al., 2006). However, to the best of our knowledge, there is no evidence for the effect of 5-HT_7R polymorphisms on serotonergic signaling or the interaction between polymorphisms of 5-HT_7 and 5-HT_{1A}Rs. Therefore, to elucidate the functional interaction between 5HT_{1A}R and 5-HT_7R, it is of high importance to understand which polymorphisms influence the expression of those 5-HTRs and how these changes affect emotional and cognitive functions. This knowledge could potentially reveal the polymorphisms that modulate the endophenotypes of different affective disorders, closely linked with the function of 5-HT_{1A}R and 5-HT_7R, such as anxiety and depression.

Neurochemical Effects in the Hippocampus

In contrast to the above electrophysiological results, *in vivo* microdialysis in awake rats showed that the local blockade of 5-HT_{1A}R increased extracellular acetylcholine (ACh) levels (Madjid et al., 2006; Hirst et al., 2008; Kehr et al., 2010) but failed to show changes in hippocampal glutamate release in the ventral hippocampus and the prefrontal cortex (Kehr et al., 2010). The result with ACh is consistent with the pro-cognitive effect of (postsynaptic) 5-HT_{1A}R blockade in PA (Madjid et al., 2006). However, the expected glutamate increase may not be detectable because of the limited capacity of microdialysis to detect small transmitter changes restricted to the synaptic cleft. More sensitive techniques are required such as enzyme-based microelectrode amperometry, which is selective for the detection of extracellular glutamate with (1) spatial resolution in the μm level, (2) sub-second temporal resolution and (3) sensitivity in the μm range of glutamate (Day et al., 2006; Konradsson-Geuken et al., 2009; Mishra et al., 2015). This novel technology is suited to provide evidence for the expected enhancement of glutamatergic transmission in the hippocampus by both 5-HT_{1A}R inhibition and 5-HT_7R activation.

It is clear that the impairing effects of low dose NMDA receptor antagonists (e.g., MK-801) and cholinergic antagonist (e.g., scopolamine) can be prevented by serotonergic manipulations (Ögren et al., 2008). Thus, these two pharmacological models of cognitive impairment relevant for Alzheimer's disease are both alleviated by 5-HT_{1A}R inhibition

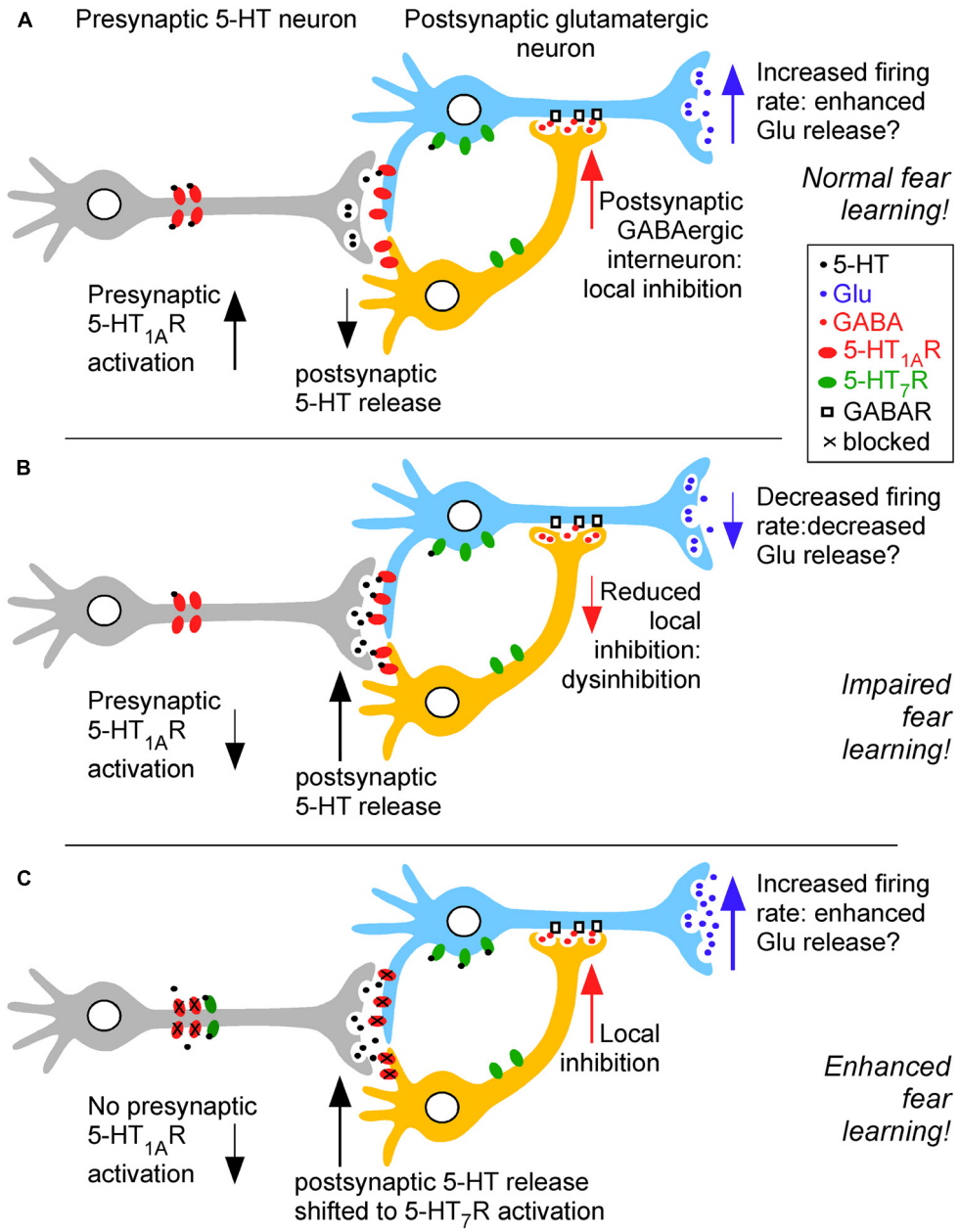

FIGURE 1 | Simplified overview of 5-HT$_{1A}$R- and 5-HT$_7$R-mediated modulation of fear learning in pre- and postsynaptic neurons under conditions of high (A) and low presynaptic 5-HT$_{1A}$R activation (B), resulting in low and high postsynaptic 5-HT release, respectively. This in turn causes increased and decreased acetylcholine (ACh) release in the hippocampus (and also the medial septum). A similar effect on hippocampal glutamate (Glu) levels is hypothesized (as shown in the medial septum). When high postsynaptic 5-HT levels are biased to 5-HT$_7$R activation **(C)**, e.g., by 8-OH-DPAT at the postsynaptic dose of 1 mg/kg in combination with the 5-HT$_{1A}$R antagonist NAD-299 at 0.3 mg/kg, a pro-cognitive effect in fear learning is observed. Thus, emotional learning and memory depend on intrasynaptic 5-HT levels, receptor availability and occupancy, genetic and epigenetic factors for 5-HTR regulation and its short- and long-term mechanisms underlying altered synaptic transmission via ACh and glutamate (Glu) release. Under conditions of higher (postsynaptic) 5-HT release, the cognitive consequences depend on the availability and occupancy of 5-HT$_{1A}$R and 5-HT$_7$Rs with so far unknown conditions that bias toward impaired **(B)** or facilitated fear memory **(C)**. The specific functions of GABAergic interneurons in 5-HT$_{1A}$R and 5-HT$_7$R-mediated fear memory modulation are currently not understood.

demonstrating a role for both enhanced glutamatergic and cholinergic transmission for improved cognitive function (e.g., Schechter et al., 2005; Madjid et al., 2006). An overview of these modulatory effects is provided in **Figure 1**.

Conclusion and Future Perspectives

During the last three decades many studies have indicated important regulatory functions of 5-HT signaling for emotional

protein coupling of the 5-HT$_{1A}$R and by reducing the ability of 5-HT$_{1A}$R to activate potassium channels, without affecting the G_s protein coupling of the 5-HT$_7$R. The heterodimerization additionally contributes to the desensitization of the 5-HT$_{1A}$R through facilitated internalization (Renner et al., 2012).

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Cronobacter sakazakii infection alters serotonin transporter and improved fear memory retention in the rat

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It is well established that *Cronobacter sakazakii* infection cause septicemia, necrotizing enterocolitis and meningitis. In the present study, we tested whether the *C. sakazakii* infection alter the learning and memory through serotonin transporter (SERT). To investigate the possible effect on SERT, on postnatal day-15 (PND-15), wistar rat pups were administered with single dose of *C. sakazakii* culture (infected group; 10^7 CFU) or 100 μL of Luria-Bertani broth (medium control) or without any treatment (naïve control). All the individuals were subjected to passive avoidance test on PND-30 to test their fear memory. We show that single dose of *C. sakazakii* infection improved fear memory retention. Subsequently, we show that *C. sakazakii* infection induced the activation of toll-like receptor-3 and heat-shock proteins-90 (Hsp-90). On the other hand, level of serotonin (5-hydroxytryptamine) and SERT protein was down-regulated. Furthermore, we show that *C. sakazakii* infection up-regulate microRNA-16 (miR-16) expression. The observed results highlight that *C. sakazakii* infections was responsible for improved fear memory retention and may have reduced the level of SERT protein, which is possibly associated with the interaction of up-regulated Hsp-90 with SERT protein or miR-16 with SERT mRNA. Taken together, observed results suggest that *C. sakazakii* infection alter the fear memory possibly through SERT. Hence, this model may be effective to test the *C. sakazakii* infection induced changes in synaptic plasticity through SERT and effect of other pharmacological agents against pathogen induced memory disorder.

Keywords: *Cronobacter sakazakii*, animal model, fear memory, Hsp-90, SERT, microRNA-16

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Introduction

Serotonin (5-hydroxytryptamine, 5-HT) has been implicated as the modulator of learning and memory with special preference to consolidation of new information into long-term memory (Kandel, 2001). 5-HT play a key role in memory formation by interacting with other neurotransmitters/exerts its effect through their seven (5-HT_1–5-HT_7) subclass of receptors (Meneses, 1999, 2007, 2013; Meneses et al., 2011; Perez-Garcia and Meneses, 2009; Hoyer et al., 2002). Although evidence from *Aplysia* to human points at a functional role of serotonergic transmission in learning and memory, the underlying mechanism is depends on the level of 5-HT (Barbas et al., 2003; Meyer et al., 2009), and the depletion of 5-HT could affect the memory formation (Kaang et al., 1993; Barbas et al., 2003; Seyedabadi et al., 2014; Stansley and Yamamoto, 2015).

Serotonin transporters (SERT) play a key role in clearance of the released 5-HT through transport across pre-synaptic membrane and maintain the homeostasis of 5-HT level. In addition, expression status of SERT protein could control the duration and intensity of 5-HT activity at synapse (Gainetdinov and Caron, 2003; Tellez et al., 2012; Yoon et al., 2013; Bravo et al., 2014). Earlier studies reported that expression of SERT protein regulated by the interacting molecules such as ribonucleoprotein (RNP) and sequence specific microRNA (miR; Standart and Jackson, 1994; Wilkie et al., 2003; Bartel, 2009; Croce, 2009; Gyawali et al., 2010; Goldie and Cairns, 2012; Hartley et al., 2012). At this point, heterogeneous nuclear ribonucleoprotein K (hnRNPK) and miR-16 appears to negotiate for the binding site at 3′-untranslated region (UTR) of SERT and regulate the repression/depression of translation (Baudry et al., 2010; Yoon et al., 2013).

Over the past few years, research has been conducted to understand the pathogenicity mechanism, genetic, nature of survival and molecular characterization of virulence in *Cronobacter* spp. (Jaradat et al., 2014). Reports have shown that source of *Cronobacter* infection was the powder infant formula (PIF; Yan et al., 2012), apart from that significant association was found with contaminated home environment (Kandhai et al., 2004), packed foods (Friedemann, 2007) and drinking water (Liu et al., 2013). In parallel, Clinical and laboratory studies reported that they have resistance to heat, desiccation and acid stress growth condition (Breeuwer et al., 2003; Edelson-Mammel et al., 2005; Dancer et al., 2009), and *Cronobacter* infection in neonates and infants cause meningitis, necrotizing enterocolitis (NEC) and sepsis with case fatality rate ranging from 40 to 80% (Muytjens et al., 1983; Block et al., 2002; Hyun-Lee et al., 2011; Yan et al., 2012; Hunter and Bean, 2013). However, *Cronobacter* infection also have been reported in elder patients or immunocompromised persons (Healy et al., 2010), among them 50% had an underlying malignancy (Lai, 2001; See et al., 2007). In addition, *Cronobacter* infection have been linked to conjunctivitis, osteomyelitis, diarrhea, acute cholecystitis, and wound infection (Gosney et al., 2006; Flores et al., 2011; Yan et al., 2012; Tsai et al., 2013). Pathogen induced neuroinflammation can alter the behavior possibly either through hypothalamus pituitary-adrenal (HPA) axis or neurotransmitter system through the interacting molecules (Pérez et al., 2009; Herrero et al., 2015). In fact, several line of studies reporting that responding to the endotoxin (lipopolysaccharide, LPS) produced by the pathogenic bacteria, the host system activate innate immune response, in which different toll-like receptors (TLRs) and heat-shock proteins (Hsp) are part of it (Pandey and Agrawal, 2006; Okun et al., 2009; Chen et al., 2014), TLRs in dendritic cells play critical role (Stanislawska et al., 2004) and 5-HT transmission (Desbonnet et al., 2008; van Heesch et al., 2014; Depino, 2015). Currently, very little information is available on the pathogen infection mediated effect on serotonergic system. Therefore, the present study is designed to examine the effect of *Cronobacter sakazakii* infection on postnatal rats' serotonergic system particularly on SERT and associated changes in learning and memory.

Materials and Methods

Bacterial Strain and Media

The bacterial strain *C. sakazakii* was obtained from American Type Cell Culture (ATCC BAA-894). The obtained bacterial strain was cultured on the selective chromogenic *Enterobacter sakazakii* agar medium (Song et al., 2008). The positive blue-green colonies were picked and grown on 1.5% Luria-Bertani (LB) agar. The overnight culture was prepared in the LB broth, which was maintained at 37°C in an incubator shaking at the rate of 145 rpm. Serial dilution and plating method was used to assess the bacterial concentration (Miller, 1972). In detail, 3 h culture of *C. sakazakii* was examined through biophotometer (Eppendorf Inc) at $O.D_{600}$. The bacterial culture was serially diluted and plated on LB agar for colony counting. Based on colony counting assay result, concentration of bacterial cells was calculated. Bacterial concentration of 10^7 CFU was fixed as infectious dose for the present study based on LC_{50} analysis.

Animals

Timed-pregnant wistar rats at gestation day-15 were acquired (Sri Venkateshwara Enterprise, Bangalore, India), acclimated and maintained under controlled ambiance (12 h light/dark cycle; temperature: 22 ± 2°C; humidity: 50 ± 5%). The pregnant rats were housed individually in a standard laboratory cage (43 cm × 27 cm × 15 cm) with saw dust as bedding material, and food and water provided *ad libitum*. This study was carried out in accordance with the recommendation of Institutional Animal Ethics Committee (IAEC), Bharathidasan University (BDU). The animal experimental protocol was approved by IAEC, BDU.

Experimental Groups

Wistar rat pups at the age of postnatal day-15 (PND-15) were used as host system for the present study. Pups from different litters were randomly divided into three different groups: naïve control (NC), medium control (MC), and infected (IF) group. Rat pups in NC groups were maintained at normal condition without treatment. MC groups were treated with single dose of LB (100 μL) and IF pups with *C. sakazakii* culture (10^7 CFU) on PND-15 by oral gavage. Then the animals were maintained at typical condition with mother.

Confirmation of Infection

On PND-30, the amygdala region was dissected out as described by Kalin et al. (1994) from NC and IF group rats ($n = 3$ from each group) and homogenized in phosphate buffer saline (PBS). The homogenate was serially diluted up to 10^{-4} with PBS and plated on specific medium to identify *C. sakazakii* (Hicrome *E. sakazakii* agar; Himedia cat. No. M1641-100G) and incubated at 37°C for overnight to observe the presence of *C. sakazakii* in brain tissue.

Behavioral Test

Passive avoidance test

Passive avoidance apparatus was constructed following the specification of Zare et al. (2015). The apparatus consisted of equally sized light and dark compartments (20 cm × 40 cm × 20 cm) made up of Plexiglas separated

by a guillotine door (12 cm × 12 cm). The floor of both chambers were made up of stainless steel rods (3 mm diameter) spaced 1 cm apart but the gridded floor of the dark chamber could be electrified using a shock generator. All the experiments were conducted between 09:00 and 18:00 h. All groups (NC, $n = 14$; MC, $n = 20$; IF, $n = 29$) were subjected to step-through passive avoidance test, in which the rats were trained to the criterion and tested for their retention 24 h post-training. Each time after removing the animal, the apparatus was wiped with 70% ethanol to remove odor. During each experiment the experimenter handle the animals for <60 s.

Exploration and training

On PND-31, each animal was placed in the light compartment of the apparatus facing away from the door and 10 s later the guillotine was raised. The animal was left for 5 min to habituate the apparatus. On PND-32, each animal was trained for the criterion. The rat was placed in the light compartment of the apparatus facing away from the door and 10 s later the guillotine was raised. When the animal had placed at all four paws in the dark compartment, entrance latency to the dark compartment was recorded. Once the animal entered into the dark compartment, the door was closed and an inescapable foot shock (0.5 mA) was applied for 5 s. After 20 s, the animal was retrieved from the dark box and placed back into their home cage. After 2 min, the procedure was repeated. The rat received foot shock each time it placed its four paws into the dark compartment. The training was terminated when the rat remained in light compartment for 120 s consecutively. Number of trials required for training the animal was recorded.

Retention test

On PND-33, retention test was performed 24 h post training. The rat was placed on the light compartment and 10 s later the door was raised. The step-through latency and time spent in dark compartment was recorded up to 300 s. If the rat did not enter the dark compartment within 300 s, a score of 300 s was assigned.

Neurotransmitter Analysis

On PND-30, group of rats from NC ($n = 5$), MC ($n = 5$), and IF ($n = 5$) were euthanized, and the amygdala region was dissected as described elsewhere (Kalin et al., 1994) and frozen on dry ice. The tissue samples were weighed and homogenized in a glass homogenizer with 0.1 M perchloric acid containing 4.5 mM Na_2EDTA and 1.6 mM reduced glutathione. The homogenates were centrifuged at 12,000 rpm for 20 min at 4°C. The supernatants were collected in a fresh tube and stored at −70°C. The level of 5-HT was estimated with a 5-HT ELISA kit (Biosource, Europe S.A., Belgium) by following the manufacturer's instructions. The concentrations of 5-HT in each tissue samples were calculated by comparing the optical density of the sample (mean for duplicates) with that of the standard curve.

Sample Preparation

On PND-30, group of rats from NC ($n = 5$), MC ($n = 5$), and IF ($n = 5$) groups were euthanized and amygdala region was dissected out from and divided into two part for the preparation of total RNA and protein. Total RNA was isolated from the tissue samples following the manufacture' instructions (Trizol method; Merck, Bangalore, India) and stored at −70°C with RNase inhibitor (1U/μL; Rnasin, Promega, Madison, WI, USA). Total RNA (1 μg) was converted into cDNA by following manufacture' instructions (QuantiTect® Reverse Transcription Kit; catalog no. 205311, Qiagen, Germany). Tissue samples were homogenized in 300–400 μL of ice cold lysis buffer (150 mM NaCl, 50 mM Tris–HCl; pH 7.5, 5 mM EDTA, 0.1% v/v NP-40, 1 mM DTT, 0.2 mM sodium orthovanadate, 0.023 mM PMSF) with protease inhibitor cocktail (10 mg/mL; Sigma-Aldrich, USA), and incubated on ice for 30 min. The homogenate was centrifuged at 10,000 g for 30 min at 4°C. The supernatant was collected in a fresh tube and again centrifuged at 12,000 g for 30 min at 4°C. The supernatant was extracted and stored at −70°C.

Quantitative Real-Time PCR

The quantitative real-time PCR (qRT-PCR) was performed in CFX-96 Touch™ Real-time PCR detection system using SSoAdvanced™ SYBR® green mix (Bio-Rad Laboratories, Inc., USA). The level of mRNA of the selected genes were assessed through qPCR using specific primers: *Tlr-3* (for 5′-ACAATGCCCAACTGAACCTC-3′ and rev 5′-CGGAGGCTGTTGTAGGAAAG-3′) and *miR-16* (for 5′-CCGCTCTAGCAGCACGTAAA-3′ and rev 5′-CCCTGTCACACT AAAGCAGC-3′). The level of *Tlr-3* and *miR-16* was normalized with internal control GAPDH (for 5′-AACATCATCCCTGCATCCAC-3′ and rev 5′-AGGAACACGGAAGGCCAT GC-3′) and U6 SnRNA (for 5′-CTCGCTTCGGCAGCACA-3′ and rev 5′-AACGCTTCACGAATT TGCGT-3′), respectively. Thermo cycling conditions for qPCR were as follows: initial denaturation at 92°C for 3 min and then denaturation at 92°C for 5 s, annealing (at 59°C for GAPDH, 62°C for *tlr-3*, 60°C for *U6 SnRNA*, and 64°C for *miR-16*), for 5 s, extension at 72°C for 5 s, and melt curve analysis at 65–95°C. Amplification of the single PCR product was confirmed by monitoring the dissociation curve followed by melting curve analysis. Each reaction was performed in triplicates with threefold serial dilution of cDNA with normalizing internal control GAPDH/U6 SnRNA. The data are presented as mean fold change of the normalized expression (CFX Manager™ version 2 software; Bio-Rad Laboratories, Inc., USA).

Western Blotting

An equal concentration of protein (40 μg) was mixed with loading buffer (glycerol, 125 mM Tris–HCl pH 6.8, 4% SDS, 0.006% bromophenol blue, 2% mercaptoethanol) and resolved on 10% polyacrylamide gel (PAGE). The separated proteins were transferred electrophoretically on to the PVDF membrane (Millipore India Pvt. Ltd., India). The membranes were then placed in the blocking solution [5% non-fat dry milk in Tris-buffered saline (TBS) containing 0.1% Tween-20: TBS-T] for 3 h at room temperature (RT). The blocking solution was discarded and the membranes incubated at 4°C overnight with one of the following primary antibodies (Santa-Cruz Biotech, Germany/BD Biosciences, USA): SERT (SC-1458, 1:200), anti-β-actin (SC-130656; 1:1000) affinity purified rabbit polyclonal antibody and

Hsp-90 (SC-5977, 1:200) mouse monoclonal antibody. β-actin was used as control for each samples. The membrane was washed and bound antibodies were detected by incubating for 3 h either with the mouse anti-rabbit (Cat # 621100180011730; 1:2000; MERCK, Bangalore, India) or goat anti-mouse (Cat # 621100480011730; 1:2000; MERCK, Bangalore, India) alkaline phosphatase conjugated antibody. The membrane was washed three times with TBS-T, and alkaline phosphatase activity was detected with 5-bromo-4-chloro-3-indolylphosphate disodium salt (BCIP)/nitro-blue tetrazolium chloride (NBT) following the instructions from the manufacturer (Invitrogen, USA). The images were acquired with Molecular Imager ChemiDoc XRS system (Bio-Rad Laboratories, Inc., USA) and the trace quantity for each band was measured using Quantity One image analysis software (Bio-Rad Laboratories, Inc., USA). The obtained Hsp90 and SERT levels were normalized to β-actin for respective samples.

Statistical Analysis

Data were presented as a mean ± standard error of the mean (SEM) and plotted with KyPlot (version 1.0) for graphical representation. The obtained data were evaluated by one-way analysis of variance (ANOVA) to detect differences between groups (SigmaStat; version 3.1) followed by Bonferroni *post hoc* test was performed. Differences were considered significant if $p < 0.05$.

Results

C. sakazakii Infection Alters the Fear Memory Retention

To determine whether the *C. sakazakii* infection affects cognitive function, we compare the fear memory retention between the experimental groups. We first assessed the performance of experimental groups during the training session of the light-dark passive avoidance task, there was no significant difference in the number of acquisition trials between NC (1.42 ± 0.17) and MC (1.15 ± 0.08) groups [$F_{(1,33)} = 2.56$; $P > 0.05$]. Similarly, *C. sakazakii* infection did not change the number of trials in IF group (1.35 ± 0.15) from NC [$F_{(1,42)} = 0.113$; $P > 0.05$] and MC group [$F_{(1,47)} = 1.00$; $P > 0.05$; **Figure 1A**]. Further, Bonferroni test revealed that the acquisition trials required by IF group was not significantly different from NC ($P = 0.739$) and MC group ($P = 0.322$). In comparison, there was no significant difference between NC and MC groups ($P = 0.011$). Similarly, there was no significant difference in entrance latency between NC (23.21 ± 3.36 s) and MC (35.10 ± 8.13 s) group [$F_{(1,32)} = 1.36$; $P > 0.05$]. When the IF group (30.62 ± 3.0 s) compared to NC [$F_{(1,42)} = 2.26$; $P > 0.05$] and MC [$F_{(1,47)} = 0.344$; $P > 0.05$], no significant difference was found (**Figure 1B**). Bonferroni test confirmed that the entrance latency of IF group was not significantly different from NC ($P = 0.144$) and MC group ($P = 0.62$). In addition, it showed that NC group was not significantly different from MC group ($P = 0.160$). However, there was a significant difference between groups in step-through latency during testing. Our analysis revealed that the IF group (237.34 ± 19.35 s) rats showed significantly higher latencies to enter the dark box compared to NC (150.92 ± 30.5 s) [$F_{(1,42)} = 4.883$; $P < 0.05$] and MC (171.8 ± 26.38 s) group [$F_{(1,47)} = 4.26$; $P < 0.05$]. When we compare the latency exhibited by the NC and MC groups, there was no significant difference between them [$F_{(1,32)} = 0.344$; $P > 0.05$]. Further, Bonferroni test showed that the IF group took significantly more time to step into the dark box than NC ($P = 0.021$) and MC group ($P = 0.044$), but there was no significant difference between NC and MC group ($P = 0.596$). The observed data showed that *C. sakazakii* infection did not alter their learning during acquisition but IF group exhibited higher step-through latency during retention test, which showed the persistence of fear memory.

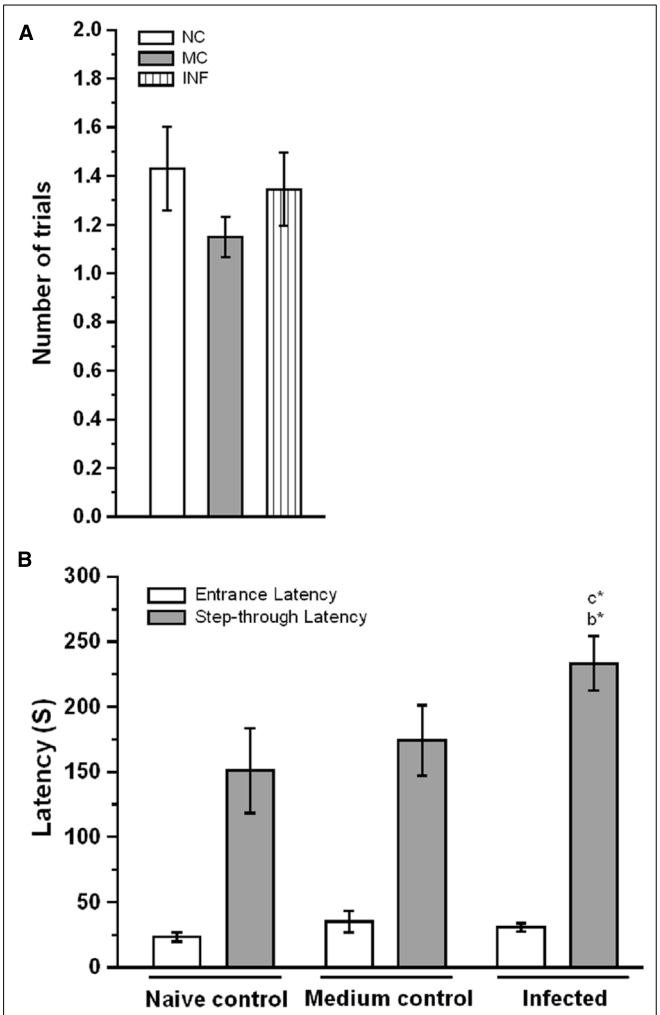

FIGURE 1 | *Cronobacter sakazakii* infection improved the fear memory retention in passive avoidance (PA) test. (A) The number of trials to acquisition, naïve control (NC), medium control (MC), and infected (IF) group did not show significant difference in the number of trials to acquisition. **(B)** Step-through latency in the retention test. IF group rats spend more in the light-chamber and taken more time to step-down into the dark-chamber considered as indices of improvement in fear memory. Values are represented as the mean ± SEM; *$P < 0.05$. Asterisk indicates significant difference respect to comparison between groups (b = NC verses IF; c = MC verses IF).

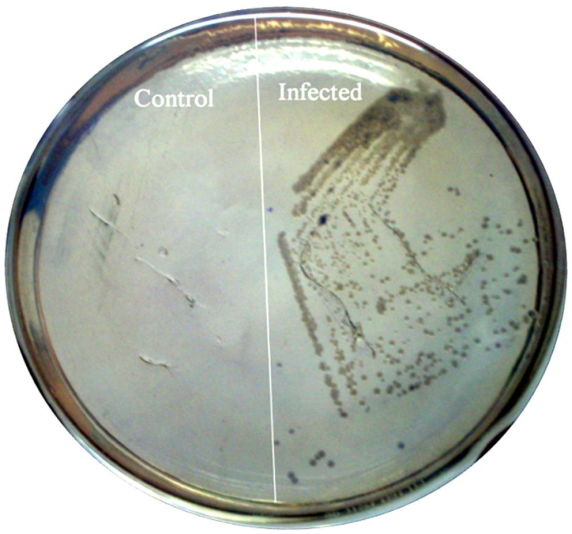

FIGURE 2 | *Cronobacter sakazakii* colonization in the brain tissue of rat pups. Brain tissues were obtained from experimental groups (Control, Infected) on PND-30. Equal weights of brain tissues were homogenized and homogenates were plated on specific medium. Specific medium showing the presence of *C. sakazakii* in infected group.

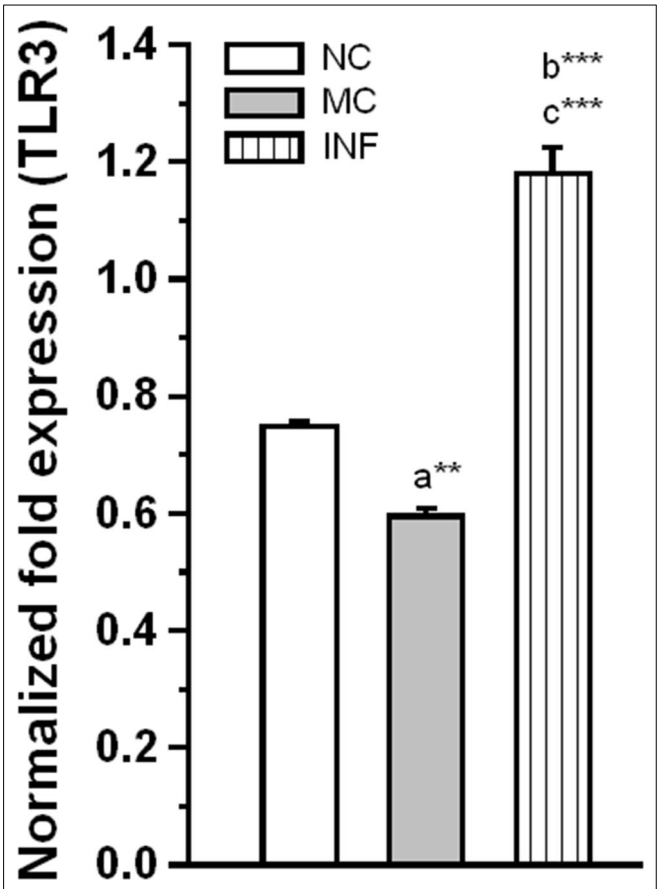

FIGURE 3 | Rats received *C. sakazakii* infection showed up-regulated expression of TLR-3. The level of TLR-3 expression was significantly increased in infected (IF) group than medium control (MC) and naïve control (NC). The normalized fold variation shown as mean ± SEM. Asterisk indicates significant difference (**$P < 0.01$; ***$P < 0.001$) respect to comparison between groups (a = NC verses MC; b = NC verses IF; c = MC verses IF).

C. sakazakii Entered into the Brain

To confirm the observed behavioral phenotype was due to the single dose of *C. sakazakii* infection on PND-15, we tested the presence of *C. sakazakii* in brain. When we plated the brain tissue homogenates in *Enterobacter* medium plate, we found the growth of blue-green color colonies from the IF group samples but not in the naïve control (**Figure 2**). This result suggested that single dose of oral treatment of *C. sakazakii* during post-natal day is enough to induce the infection at brain.

C. sakazakii Infection Activates TLR-3

To further evaluate the effect of *C. sakazakii* infection on activation of TLR-3. Our analysis revealed that *C. sakazakii* infection significantly increased the expression level of TLR-3 (**Figure 3**), the Ct values of TLR-3 for each group followed by GAPDH (NC: 23.24 ± 0.038; 12.11 ± 0.041; MC: 23.24 ± 0.026; 11.77 ± 0.064; IF: 22.36 ± 0.122; 12.12 ± 0.054). The estimated level of TLR-3 was significantly higher in IF group than MC group [$F_{(1,9)} = 167.19$; $P < 0.001$] and NC group [$F_{(1,9)} = 103.78$; $P < 0.001$]. Similarly, there was a significant difference between MC and NC groups, but the difference obtained by the reduction of TLR-3 expression was significant in MC group than NC group [$F_{(1,9)} = 23.68$; $P < 0.01$]. These results suggesting that *C. sakazakii* infection activated TLR-3 expression.

C. sakazakii Infection Up-Regulate Hsp-90

We next examined along with activation TLR-3, whether the Hsp-90 also activated following *C. sakazakii* infection. When examined the level of Hsp-90 in the experimental groups (**Figure 4**), we found that the *C. sakazakii* infection significantly alter the Hsp-90. The estimated level was significantly high in IF group than MC [$F_{(1,9)} = 188.64$; $P < 0.001$] and NC group [$F_{(1,9)} = 1424.96$; $P < 0.001$]. However, there was no significant difference between MC and NC groups [$F_{(1,9)} = 6.28$; $P = 0.052$]. Our analysis revealed that *C. sakazakii* increased Hsp-90 expression.

C. sakazakii Infection Modulates Serotonin and SERT Protein Level

In addition to the activation of TLR-3 and Hsp-90, we estimated the level of 5-HT, and expression level of SERT in experimental group rats. As shown in **Figure 5**, the basal levels of 5-HT was significantly affected by *C. sakazakii* infection [$F_{(1,9)} = 9735.27$; $P < 0.001$] compared to NC and MC [$F_{(1,9)} = 236.78$; $P < 0.001$]. In addition, levels of 5-HT was significantly lower in MC group than NC group [$F_{(1,9)} = 9.12$; $P < 0.05$]. Further, our analysis revealed that the expression of SERT was significantly reduced in IF group [$F_{(1,9)} = 51.85$; $P < 0.001$] than NC group, but not significantly different from MC group [$F_{(1,9)} = 4.8$; $P = 0.07$]. When we compare the expression level of MC and NC groups, they were not significantly different [$F_{(1,9)} = 4.1$; $P = 0.074$]. Our analysis suggests that *C. sakazakii* infection reduced the levels of 5-HT and expression of SERT protein.

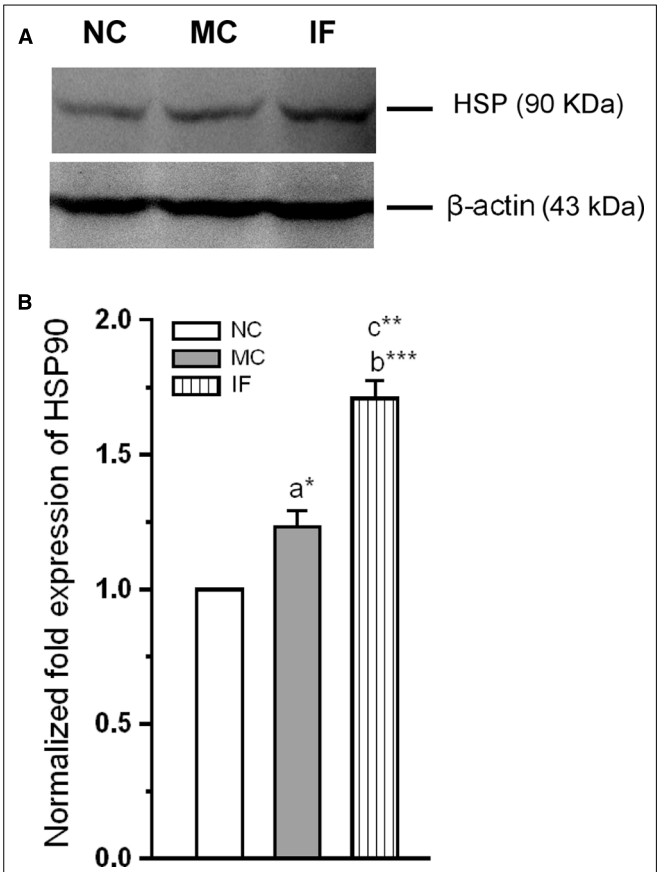

FIGURE 4 | *Cronobacter sakazakii* infection increases the expression of heat-shock protein-90. (A) Representative western blot showing variation in the expression of HSP-90 levels in experimental groups. **(B)** HSP-90 level was significantly increased in infected (IF) group than control (MC) and naïve control (NC) groups. The fold variation shown as mean ± SEM. Asterisk indicates significant difference (*$P < 0.05$; **$P < 0.01$; ***$P < 0.001$) respect to comparison between groups (a = NC verses MC; b = NC verses IF; c = MC verses IF).

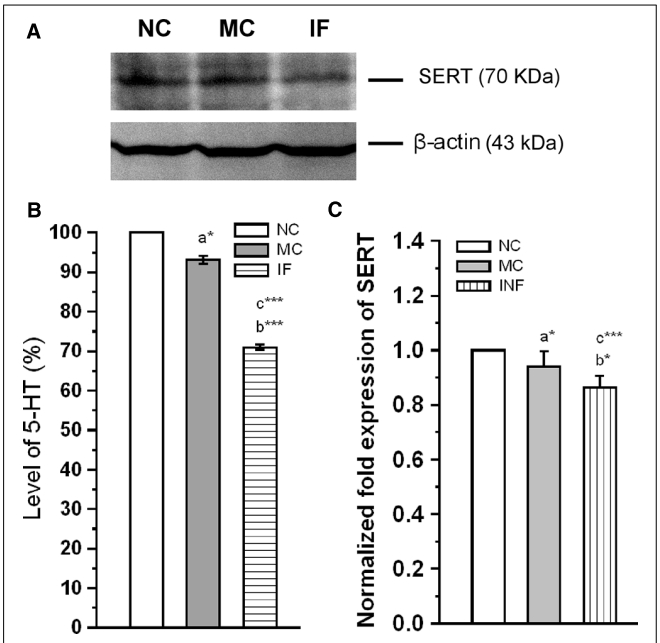

FIGURE 5 | *Cronobacter sakazakii* infection alters the serotonergic system. (A) Representative western blot showing the differential expression of SERT in experimental groups. **(B)** There was a significant decrease in 5-HT level in *C. sakazakii* infected (IF) group than medium control (MC) and naïve control (NC) groups. **(C)** SERT protein level was significantly decreased in IF group than MC and NC mice. Data were shown as mean ± SEM. Asterisk indicates significant difference (*$P < 0.05$; ***$P < 0.001$) respect to comparison between groups (a = NC verses MC; b = NC verses IF; c = MC verses IF).

C. sakazakii Infection Modulates miR-16 Expression

Subsequently, we explored the possible role of miR-16 in *C. sakazakii* mediated regulation of SERT protein expression. We observed that the level of miR-16 expression was significantly elevated in IF (Ct: 23.21 ± 0.054) group than NC [$F_{(1,9)} = 8348.15$; $P < 0.001$] and MC group [$F_{(1,9)} = 8117.98$; $P < 0.001$]. However, there was no significant difference between the MC (Ct: 24.73 ± 0.103) and NC (Ct: 25.34 ± 0.260) groups [$F_{(1,9)} = 1.68$; $P = 0.084$; **Figure 6**]. Our results suggested that up-regulation of miR-16 by *C. sakazakii* infection possibly suppressed the translation of SERT.

Discussion

Cronobacter sakazakii has been associated with human infection especially in newborn and infants (Joseph and Forsythe, 2011; Cruz-Córdova et al., 2012). Earlier, Hyun-Lee et al. (2011) demonstrated that *C. sakazakii* can cross the blood–brain barrier (BBB) in postnatal mice (PND-3.5), possibly through exploiting immature dendritic cells (Townsend et al., 2007; Mittal et al., 2009; Emami et al., 2011). In the present study, we IF the postnatal rats on PND-15, during the onset of "brain growth spurt" period (Dobbing and Sands, 1979). During this period, changes like axonal outgrowth and dendritic maturation, establishment of neuronal connections and proliferation of glial cells occurred accompanying with myelinization (Kolb and Whishaw, 1989). At first, we showed the presence of *C. sakazakii* in PND-30 rats' brain. Further, we demonstrated that single dose of *C. sakazakii* infection in postnatal rats did not alter their learning efficiency but improved the fear memory retention. Our results adding support to the earlier study and suggest that postnatal Wistar rats may be used as animal model for human neonatal *C. sakazakii* infections. Earlier studies discussed how the infection and inflammation lead to changes in brain (Goehler et al., 2007; Jaradat et al., 2014), in which TLRs are a part. TLRs are conserved from sponges to human and very much present in neuronal cells (Barton, 2007; Tang et al., 2007; Wiens et al., 2007). It has been stated that as a pro-inflammatory or a comprehensive neuroprotective response, TLR-3 is activated (Bsibsi et al., 2006; Kim et al., 2008), whereas its activation has not yet been established under normal condition (Okun et al., 2011). In brain, TLR-3 has broad effect on the cognitive function based on injury and/or disease. Studies in animal models reported that TLR-3 deficient mice showed improved contextual and extinction of fear memory (Okun et al., 2010). Interestingly, we found that rats with *C. sakazakii* infection showed elevated level of TLR-3 expression compared to other groups and they displayed improved fear memory. Supporting to

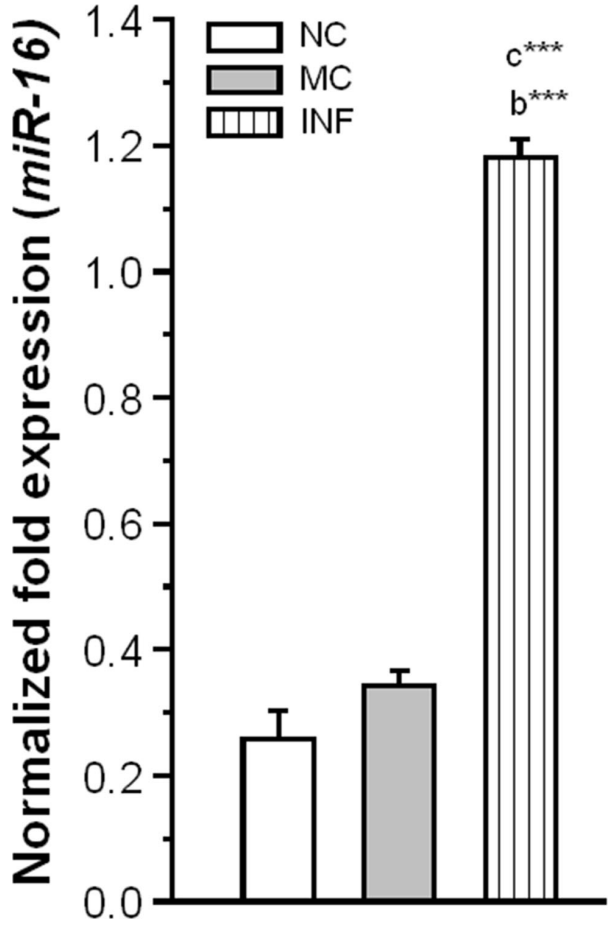

FIGURE 6 | *Cronobacter sakazakii* infection alters the miR-16 expression. The level of miR-16 expression was significantly increased in infected (IF) group than control (MC) and naïve control (NC) groups. The normalized fold variation shown as mean ± SEM. Asterisk indicates significant difference (***$P < 0.001$) respect to comparison between groups (a = NC verses MC; b = NC verses IF; c = MC verses IF).

this, earlier study demonstrated that TLR-3 activation possibly negatively regulate ERK-CREB signaling, thus, activation of TLR-3 contribute to cognitive impairment and other behavioral disorders (Okun et al., 2010).

Earlier studies reported that as a innate immune response exposure to pathogen/LPS activate TLR and Hsp90 (Stanislawska et al., 2004; Xie et al., 2015), in many observations expression of Hsp90 facilitates the pathogenesis (Qin et al., 2010; Smith et al., 2010; Shapiro et al., 2012). TLR-3 can also respond to the endogenous ligands such as Hsp-90, especially in dendritic cells during pathogenesis (Stanislawska et al., 2004). Similarly, we found that *C. sakazakii* infection induced the expression of Hsp90, the estimated level was higher than the other experimental group. Hsp90 is one of the molecules that interact with serotonergic system, especially with SERT. In fact, N- or C-terminus of SERT protein known to interact with many regulatory proteins, they play critical role in folding of SERT protein (El-Kasaby et al., 2010, 2014; Zhong et al., 2012). When we tested the expression pattern of SERT, the level of SERT protein was significantly low in IF group than other experimental groups. Although, it is interesting that the infection appears to have changed 5-HT level and SERT protein, the SERT effect appeared to be rather modest and it is unclear whether the alternation of SERT or any other interacting molecules in altering the individual's behavior in IF group. However, earlier *in vitro* report demonstrated that over expression of Hsp90 interact with SERT protein and alter the folding trajectory of SERT protein (El-Kasaby et al., 2014). On the other hand, expression of SERT could be exerted by microRNAs, particularly miR-16 (Baudry et al., 2010; Yoon et al., 2013). Specific miRNAs activation/inactivation patterns are critically regulated by the presence of bacterial effector proteins and localization of the pathogen (Zhu et al., 2010; Al-Quraishy et al., 2012; Izar et al., 2012). Although, there is a differential expression of miR-16 following pathogen infection, we found that miR-16 expression was increased after *C. sakazakii* infection. Further, our analysis suggests that *C. sakazakii* infection up-regulate the expression of miR-16, which also interact with the 3′UTR of SERT and down-regulate the translation process. Supporting to our behavioral observations, SERT knock-out animals showed impaired fear extinction (Wellman et al., 2007; Narayanan et al., 2011; Hartley et al., 2012). The down-regulated SERT expression could affect the reuptake of released 5-HT, and then the level of 5-HT. Our analysis revealed that the level of 5-HT significantly decreased following *C. sakazakii* infection. Supporting to this, *in vivo* and *in vitro* studies demonstrating that exposure to pathogens/pathogen produced endotoxin alter the level of 5-HT and behavior (Esmaili et al., 2009; Martin et al., 2009; Shin and Liberzon, 2010; van Heesch et al., 2014). In addition, we observed difference between the naïve control and MC in molecules we tested in this study but not in the behavior. The observed difference in this study is possibly by the micronutrients in the bacterial medium, which may alter the gut microbiota of the individuals. They have the capacity to can influence precursor pool for 5-HT (Desbonnet et al., 2008).

In conclusion, our results demonstrates that *C. sakazakii* infection enhanced the fear memory retention. Although, further study needed to establish the mechanism of this effect, based on our data, we hypothesize that observed changes in SERT expression may have caused this effect, possibly through the interaction of Hsp-90 and miR-16. Further, the present study suggest that *C. sakazakii* infection in postnatal rats may be used an animal model to examine the effect of bacterial infection mediated changes in synaptic plasticity through SERT and effect of other pharmacological agents against pathogen induced memory disorder.

Acknowledgments

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Post-training depletions of basolateral amygdala serotonin fail to disrupt discrimination, retention, or reversal learning

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In goal-directed pursuits, the basolateral amygdala (BLA) is critical in learning about changes in the value of rewards. BLA-lesioned rats show enhanced reversal learning, a task employed to measure the flexibility of response to changes in reward. Similarly, there is a trend for enhanced discrimination learning, suggesting that BLA may modulate formation of stimulus-reward associations. There is a parallel literature on the importance of serotonin (5HT) in new stimulus-reward and reversal learning. Recent postulations implicate 5HT in learning from punishment. Whereas, dopaminergic involvement is critical in behavioral activation and reinforcement, 5HT may be most critical for aversive processing and behavioral inhibition, complementary cognitive processes. Given these findings, a 5HT-mediated mechanism in BLA may mediate the facilitated learning observed previously. The present study investigated the effects of selective 5HT lesions in BLA using 5,7-dihydroxytryptamine (5,7-DHT) vs. infusions of saline (Sham) on discrimination, retention, and deterministic reversal learning. Rats were required to reach an 85% correct pairwise discrimination and single reversal criterion prior to surgery. Postoperatively, rats were then tested on the (1) retention of the pretreatment discrimination pair, (2) discrimination of a novel pair, and (3) reversal learning performance. We found statistically comparable preoperative learning rates between groups, intact postoperative retention, and unaltered novel discrimination and reversal learning in 5,7-DHT rats. These findings suggest that 5HT in BLA is not required for formation and flexible adjustment of new stimulus-reward associations when the strategy to efficiently solve the task has already been learned. Given the complementary role of orbitofrontal cortex in reward learning and its interconnectivity with BLA, these findings add to the list of dissociable mechanisms for BLA and orbitofrontal cortex in reward learning.

Keywords: cognitive flexibility, reward learning, serotonin, amygdala, retention, 5,7-DHT, 5-HT

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Introduction

Control over inappropriate responding plays a pivotal role in adaptive decision making. Poor inhibitory control is a characteristic of a wide range of psychiatric disorders, including obsessive-compulsive disorder (Chamberlain et al., 2005), attention-deficit-hyperactivity disorder

(Itami and Uno, 2002), addiction (Brewer and Potenza, 2008; Winstanley et al., 2010), and personality disorders (Soloff et al., 2003; Lieb et al., 2004). Reversal learning, measuring the ability to actively suppress prepotent responding, is a broadly-used assay of flexible reward learning and has been proposed as an index for some psychopathology (Izquierdo and Jentsch, 2012).

The literature on the modulation of cognitive flexibility by serotonin (5-HT) is vast. Substantial evidence from studies employing pharmacological manipulations of serotonergic neurotransmission by receptor antagonist administration (Boulougouris and Robbins, 2010), selective toxin-mediated depletions of 5-HT and destruction of 5-HT terminals (Clarke et al., 2004, 2007; Masaki et al., 2006) combined with genetic studies (Homberg et al., 2007; Brigman et al., 2010; Jedema et al., 2010) have established a prominent role for this neurotransmitter in reversal learning performance in different species. Overall, these studies suggest that global reductions of serotonin levels are associated with a higher degree of perseveration and poor response control.

Among other brain nuclei, the serotonergic system innervates prefrontal cortex (PFC), basolateral amygdala (BLA), and nucleus accumbens (Kapur and Remington, 1996; McQuade and Sharp, 1997), regions critical for flexible reward learning (Cools et al., 2002; Ghahremani et al., 2010; Izquierdo and Jentsch, 2012). Several lines of evidence point to a selective role for 5-HT within subregions of the PFC in the modulation of behavioral flexibility (Dalley et al., 2002; Clarke et al., 2004; Winstanley et al., 2006). The role of 5-HT in BLA in reward learning is not as well understood. The relative paucity of systematic examination is surprising given that the lateral amygdala receive dense serotonergic inputs from the dorsal raphé (Sadikot and Parent, 1990) and expresses several subtypes of serotonergic receptors (Xu and Pandey, 2000; Mascagni and McDonald, 2007). The activity levels of BLA neurons, the degree of inhibition, and synaptic responsiveness are modulated by 5-HT signaling (Rainnie, 1999; Yamamoto et al., 2012), suggesting an important role of this neurotransmitter in BLA function.

BLA has been implicated in the performance of tasks measuring cognitive flexibility, including reversal learning, although the results are contradictory with some studies reporting normalization (Stalnaker et al., 2007), enhancement (Izquierdo et al., 2013), or deterioration of performance (Churchwell et al., 2009) following manipulations. Given that reversal learning is a net manifestation of multiple processes, including inhibition of a previously learned association, sensitivity to reward feedback following choice (Stolyarova et al., 2014), degree of perseveration, and learning of new stimulus-outcome contingencies (Roberts, 2006; Izquierdo and Jentsch, 2012), the lack of agreement in these results likely hinge on the particular demands of the task, animals' motivational state, the order of the task presentation (pre- or post-training manipulations), or the specificity of the manipulation. Irrespective of the methodological differences, it appears that BLA is selectively important in updating responses to changes in reward value (Coleman-Mesches et al., 1996; Baxter and Murray, 2002; Liao and Chuang, 2003; Belova et al., 2008) and sensitivity to negative feedback (Rudebeck and Murray, 2008; Izquierdo et al., 2013). Rather than integrating the information across time, the BLA-lesioned animals appear to be guided by immediate outcomes, potentially leading to an enhanced win-stay/lose-shift strategy. Thus, an effect on reversal learning can be expected under some but not all experimental protocols.

A specific role for 5-HT in amygdala in reversal learning has previously been suggested (Masaki et al., 2006). In this study the levels of 5-HT in amygdala were negatively correlated with the number of sessions required by the animals to advance to both discrimination and reversal criterion on a go/no-go task. Similarly, Izquierdo et al. (2012) reported impaired stimulus-reward association learning despite intact motivation after systemic depletions of 5-HT after parachlorophenylalanine, a tryptophan hydroxylase (TPH) inhibitor. However, both of those studies employed systemic pharmacological manipulations, which also produced significant 5-HT reductions in OFC, mPFC, and hippocampus, among the brain regions examined. Therefore, the reversal learning effect reported in Masaki et al. (2006) cannot be attributed exclusively to amygdalar 5-HT depletions. To our knowledge, only one experimental study has been conducted thus far to suggest a causal role for 5-HT in BLA in mediating reversal learning. Rygula et al. (2014) observed impaired probabilistic reversal learning performance in marmosets following 5-HT depletion of amygdala. The impairment resulted from increased effectiveness of misleading feedback and decreased overall reinforcer sensitivity. However, it is not known whether 5-HT neurotransmission within BLA is necessary for deterministic, non-probabilistic two-choice reversal learning: wherein one stimulus is rewarded 100% of trials and the other stimulus rewarded 0% of trials.

To test whether the BLA-specific depletion of 5-HT produces impairments in reversal learning, we first assessed animals' performance on initial pairwise discrimination and reversal learning, then performed selective 5-HT depletions within this region to examine their subsequent performance on retention of preoperative reward contingencies, novel pairwise discrimination, and reversal learning.

Experimental Procedures

Subjects

Fifteen experimentally naïve male Long-Evans (Charles Rivers Laboratories, Hollister, CA) rats (PND 50, weighing between 280 and 300 g at the beginning of the study) were pair housed in rooms with automatically regulated lighting (12 h light/dark cycle; lights on at 06:00), maintained on rat chow (Rodent Lab Chow 50#) and water *ad libitum* until training commenced. Upon arrival, the rats were allowed to habituate for 3 days prior to being handled for 5 days (10 min per rat). Following handling, the rats were food restricted to no less than 85% of their free-feeding body weight to ensure motivation to work for sucrose pellets (Bio-Serv, Frenchtown, NJ) in the operant chambers while water was available *ad libitum*. Body weights were monitored at least 3 times per week. Behavioral testing took place between 08:00 and 16:00 h during the rats' inactive period, consistent with previous studies in our lab.

Behavioral Apparatus

Behavioral testing was done in eight operant conditioning chambers (Model 80604 Lafayette Instrument Co., Lafayette, IN) that were housed within sound- and light- attenuating cubicles. Each chamber was equipped with a house light, tone generator, video camera, and LCD touchscreen opposing the pellet dispenser. The pellet dispenser delivered single 45-mg dustless precision sucrose pellets. Software (ABET II TOUCH) controlled touchscreen stimuli presentation, tone generation, tray- and house-light illumination, and pellet dispensation.

Behavioral Pre-training

The order of training, testing, and surgical procedures is outlined in **Figure 1**. The pre-training protocol, adapted from Kosheleff et al. (2012) and Izquierdo et al. (2010), consisted of a series of phases: Habituation, Initial Touch Training (ITT), Must Touch Training (MTT), Must Initiate Training (MIT), and Punish Incorrect Training (PIT) designed to train rats to nose-poke, initiate a trial, and discriminate between stimuli. During habituation, rats were required to eat five pellets out of the pellet dispenser inside of the chambers within 15 min before exposure to any stimuli on the touchscreen. ITI began with the display of white graphic stimuli on the black background of the touchscreen. During this stage a trial could be terminated for one of two reasons: if a rat touched the displayed image, or if the image display time (40 s) ended, after which the stimulus was removed and black background displayed. The disappearance of the image was paired with the onset of a "reinforcer event": dispensation of one (image time ended) or three (image touched) sucrose pellets, a 1 s tone, and an illumination of the tray-light. Trials were separated by a 10 s ITI. In MTT, a trial could be terminated only if the rat touched the image, which then disappeared followed by reward delivery. Following successful acquisition of stimulus to reward relationship the rat had to learn to initiate a trial by nosepoking and exiting the reward magazine (MIT). Magazine entry was accompanied by auditory feedback. For all the stages, the criterion for advancement into the next stage was set to 60 rewards consumed in 45 min. During the last stage of pre-training rats were exposed to punishment (i.e., "time out" time during which a new trial could not be initiated) upon an incorrect response (PIT). The criterion for PIT was set to 60 rewards consumed in 45 min across two consecutive days.

Pre-operative Behavioral Testing

The animals were given one testing session per day until the criterion was reached and were restricted to a maximum of 60 correct responses per testing session. Rats were presented with two novel, white, equiluminescent stimuli that differed only in shape with predetermined reinforcement contingencies. The software enabled either a reward event in the form of sugar pellet dispensation, paired with house-light illumination and auditory feedback, as a result of nose poking the correct stimulus, or a punishment as a result of nose poking the incorrect stimulus; the latter consisting of a 5 s "time out" wherein rats were unable to initiate the next trial. Trials were separated by a 5 s ITI. If the rat committed an error and received a punishment, a correction trial was administered to prevent side bias formation: this consisted of the same spatial (left/right) presentation of the stimulus until the rat nose poked correctly. Spatial configuration of stimuli presentation occurred pseudo randomly, the stimulus could not have appeared on the same side of the screen more than three times in a row except during correction trial. Stimulus assignment was counterbalanced across treatment groups. Criterion for advancement was 60 correct nose pokes at 85% correct responses to the stimulus within 45 min, on each of two consecutive days. Upon reaching criterion on this phase, the rats were tested on a reversal of the reward contingencies. Parameters for the reversal phase were identical to the visual discrimination learning phase, with the exception that the reward contingencies were reversed.

Surgery

Rats were treated with Desipramine (10 mg/kg) 30 min before surgery, anesthetized with isoflurane (2–2.5%, to effect) through a nosecone and mounted on a stereotaxic apparatus (Model #963, Kopf Instruments, Tujunga CA). Respiratory rate and body temperature were monitored throughout the surgery. The skin was incised (anterior to posterior), retracted using hemostats,

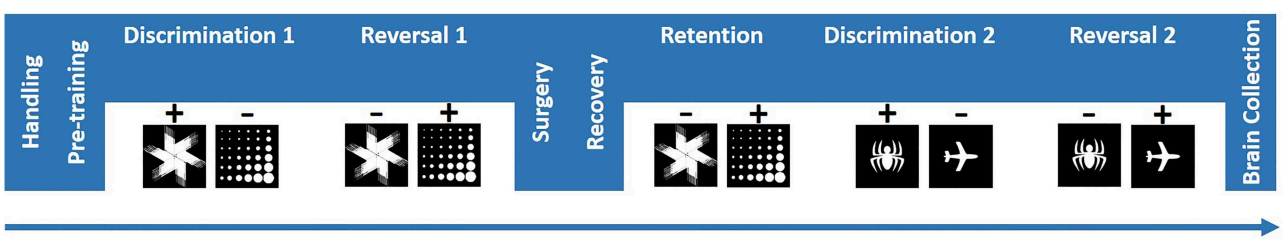

FIGURE 1 | Order of training, testing, and surgical procedures. Following a series of pre-training phases rats were presented with two novel stimuli with predetermined reinforcement contingencies. The software enabled either a reward event as a result of nosepoking the correct stimulus, or a punishment as a result of nose poking the incorrect stimulus; the latter consisting of a 5 s "time out" wherein rats were unable to initiate the next trial. Criterion for advancement was 60 correct nose pokes at 85% correct responses to the stimulus within 45 min, on each of two consecutive days. Upon reaching criterion on this phase, the rats were tested on a reversal of the reward contingencies. Once the reversal learning criterion was met rats received bilateral injections of 5,7-DHT in the BLA (Depletion) or saline (Sham). Following a 7-day recovery period, rats were tested for retention of the previously learned reversal task as well as new discrimination and reversal learning problems.

and the head position was adjusted to fit Bregma and Lambda on the same horizontal plane. Over the target area, small burr holes (2 mm diameter) were drilled bilaterally on the skull for the placement of an injection needle. A 10 μL Hamilton syringe was mounted and placed on an infusion pump and connected to an injection needle with polyethelyne tubing. Rats received two infusions per hemisphere (0.1 and 0.2 μL per site) of 5,7-DHT (5,7-dihydroxytryptamine, 20 mg/mL) bilaterally in the BLA (Depletion, $n = 8$) and sham-operated animals (Sham, $n = 7$) received 0.9% saline in the same sites. The total dose of 5,7-DHT administered was 6 μg per hemisphere or 12 μg per animal, which falls within the range of doses previously reported to produce specific and reliable 5-HT depletions associated with behavioral effects (Sommer et al., 2001; Macedo et al., 2002; Izumi et al., 2012; West et al., 2013).

The coordinates used for the injections were adapted from a previous report (Burke et al., 2007) were as follows: Site 1 (0.2 μL) AP = +2.8 mm; ML, ±5.0 mm; DV = −8.4 mm; Site 2 (0.1 μL) AP = +2.8 mm; ML, ±5.0 mm; DV = −8.1 mm from bregma. After the last infusion, the incision was sutured and warmed sterile saline (1 mL, s.c.) was administered. The rats were placed on a heating pad and kept in recovery until ambulatory before being put back into the vivarium.

Post-operative Behavioral Testing

Following a 7-day recovery period, rats were put back on food restriction and tested for retention of the previously learned reversal task, using procedures identical to pre-operative reversal learning testing. Criterion for advancement was 60 correct nose pokes at 85% correct responses to the stimulus within 45 min, on each of two consecutive days. Upon completion of the retention stage, two novel stimuli were presented in a new discrimination and reversal phase.

Immunohistochemistry

Depletions were verified using immunohistochemistry for the marker TPH. Following behavioral testing, rats were humanely euthanized with an overdose of sodium pentobarbital and perfused transcardially with 0.9% Saline buffer, followed by a 10% formaldehyde. The brains were extracted and post-fixed in 10% formaldehyde for 24 h, then transferred into a 30% sucrose solution until the brain sank to the bottom of the 5 ml scintillation vial. Thirty five micrometer coronal sections were cut on a cryostat (−20°C) and placed in 0.9% saline. Sections were rinsed in 0.9% saline for 5 min, permeabilized for 90 min at room temperature on an agitator in 0.3% Triton-X, 3% Normal Goat Serum, and 0.9% saline. After blocking, sections were washed 3 times for 5 min in 0.9% saline. Primary incubation period at 4°C on an agitator lasted for 72 h in a 1:500 rabbit anti- TPH, 0.3% Triton-X, 3% Normal Goat Serum, and PBS solution. Sections were then washed 3 times for 5 min in 0.9% saline. Secondary antibody was incubated in low light at room temperature for 3 h in a 1:400 goat-anti-rabbit FITC in 0.3% Triton-X, 3S% Normal Goat Serum in PBS. Sections were then washed 3 times for 5 min in 0.9% saline. After the slides were allowed to dry completely, they were cover slipped with 100 μL of Ultra Cruz. Zeiss Axio Observer Z1 was used for visualization and software Slidebook 5.5 for quantification of the staining.

Statistical Analyses

Software package SPSS (SAS Institute, Inc., Version 16.0) was used for statistical analyses. Statistical significance was noted when p-values were less than 0.05, and a trend toward significance was noted when p-values were 0.05–0.06. Shapiro Wilk tests of normality, Levene's tests of equality of error variances, Box's tests of equality of covariance matrices, and Mauchly's tests of sphericity were used to characterize the data structure. The learning data were analyzed with omnibus repeated-measure ANOVAs (rmANOVAs). Three parameters were considered in learning analyses: sessions to criterion, total number of committed errors, and performance accuracy (i.e., percent correct) across sessions for each testing phase. Sessions to criterion and total errors on discrimination and reversal learning phases were subjected to rmANOVA with time (pre- and post-operatively) as within- and treatment group (Depletion vs. Sham) as between-subject factors. Session performance accuracy data were analyzed with omnibus rmANOVA with time (pre- and post-operatively) and session as within- and treatment group as between-subject factors. Retention data were analyzed with independent samples t-tests (sessions to criterion and total error), and rmANOVA with session as within- and treatment group (Depletion vs. Sham) as between-subject factors (session percent correct). Immunohistochemistry data were analyzed with ANOVA with hemisphere (left vs. right) as within- and treatment group (Depletion vs. Sham) as between-subject factors. Where the assumptions of sphericity were violated, Greenhouse-Geisser p-value corrections were applied (Epsilon < 0.75).

Results

Immunhistochemical Verification of 5,7-DHT Lesions

5,7-DHT infusions produced a reliable moderate 5-HT depletion in BLA. Immunohistochemistry data were analyzed with

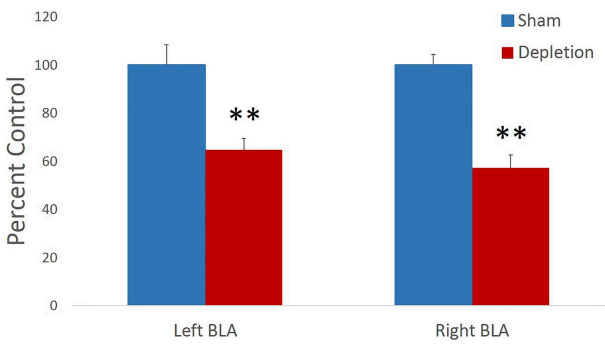

FIGURE 2 | 5,7-DHT infusions produced a long-lasting moderate 5-HT depletion in BLA. Following behavioral testing (mean number of days after the surgery = 68), TPH levels were significantly reduced in depletion compared to control group and not different between the left and right hemispheres.

ANOVA with hemisphere (left vs. right) as within- and treatment group (Depletion vs. Sham) as between-subject factors. Following the behavioral testing (mean number of days after the surgery = 68), the TPH levels were significantly reduced in depletion compared to control group: mean effect of treatment group [$F_{(1,12)} = 46.395$, $p < 0.001$ **Figure 2**]; and not different between the left and right hemispheres [$F_{(1,12)} = 0.367$, $p = 0.556$]. The mean levels of 5-HT depletion by the end of behavioral testing were 64.53% for the left and 51.01% for the right hemispheres. Individual TPH staining data are presented in **Table 1**.

5-HT-Depleted Animals had Intact Memory for Previously Learned Task Contingencies

Memory for previously learned task contingencies was assessed by retention of the pre-operative reversal task 7 days after the surgery. Sessions to criterion and total number of errors on a retention task were analyzed with independent sample *t*-tests. There was no statistical difference between treatment groups on either of the measures [sessions to criterion $t_{(13)} = 1.414$, $p = 0.181$, **Figure 3A**; total errors $t_{(13)} = -1.159$, $p = 0.267$ **Figure 3B**]. Group differences in performance accuracy on each testing day were further analyzed with rmANOVA with session as within- and treatment group (Depletion vs. Sham) as between-subject factors. All animals improved their performance with time: main effect of testing session [$F_{(7,91)} = 5.328$, $p = 0.016$]. 5-HT depletions of BLA had no effect on performance accuracy on any of the sessions of retention task (**Figure 3C**): no main effect of group [$F_{(1, 13)} = 0.017$, $p = 0.899$] or session × group interaction [$F_{(7,91)} = 0.255$, $p = 0.744$].

TABLE 1 | Individual immunohistochemical data.

Animal	Percent control		Days post-surgery
	Left	Right	
SHAM			
2Q	67.04	116.90	59
4Q	81.18	95.03	57
9Q	113.08	90.32	78
11Q	116.99	89.16	77
12Q	110.54	105.83	77
15Q	111.18	102.76	76
Average	100	100	70.67
DEPLETION			
1Q	65.59	52.57	59
3Q	81.91	56.14	58
5Q	59.85	52.34	57
6Q	63.64	69.52	55
10Q	87.96	56.86	77
13Q	50.09	56.68	76
14Q	58.27	23.98	76
16Q	48.91	39.99	76
Average	64.53	51.01	66.75

Depletions in the left and right hemisphere were verified using immunohistochemistry for the marker tryptophan hydroxylase (TPH).

5-HT Depletions of BLA do not Affect Acquisition of Visual Discrimination Learning Task

Sessions to criterion and total errors made on the pairwise visual discrimination learning task were analyzed with rmANOVA with time (pre- and post-operatively) as within- and treatment group (Depletion vs. Sham) as between-subject factors. The analyses revealed that 5-HT depletions did not impair animals' ability to acquire the discrimination task: no main effects of treatment group [$F_{(1, 13)} = 0.952$, $p = 0.347$] or time × treatment group interaction [$F_{(1, 13)} = 0.889$, $p = 0.363$] on sessions to criterion were observed. We anticipated faster acquisition of the second novel discrimination as a result of practice with the task. However, rmANOVA revealed no main effect of time [$F_{(1, 13)} = 0.416$, $p = 0.53$] on sessions to criterion (**Figure 4A**). Similarly, no practice effect was observed on total number of errors [$F_{(1, 13)} = 0.274$, $p = 0.61$]. There were also no treatment group differences [main effect $F_{(1,13)} = 0.376$, $p = 0.551$; interaction $F_{(1,13)} = 1.009$, $p = 0.334$] in the total number of committed errors (**Figure 4B**).

Some experimental manipulations are known to produce a stage-dependent effect on learning, in which case facilitated performance at a later stage may mask the impairment observed early in learning. Therefore, animals' performance was analyzed on a session-by-session basis. Performance accuracy was analyzed with rmANOVA with time (pre- and post-operatively) and session as within- and treatment group (Depletion vs. Sham) as between-subject factors. All animals improved their performance with time as evidenced by a highly significant main effect of testing session [$F_{(10,130)} = 27.493$, $p < 0.0001$]. Similarly to sessions to criterion and total errors data, the analyses revealed no improvement due to practice effect on any of the testing days: no main effect of time [$F_{(1, 13)} = 0.738$, $p = 0.406$] or time × session interaction [$F_{(10,130)} = 1.186$, $p = 0.32$] were observed. 5-HT depletion did not affect animals' learning rate: no main effect of group [$F_{(1, 13)} = 1.705$, $p = 0.214$], time × group [$F_{(1, 13)} = 0.219$, $p = 0.647$], session × group [$F_{(10,130)} = 0.416$, $p = 0.704$] or time × session × group interaction [$F_{(10,130)} = 1.07$, $p = 0.355$] (**Figure 4C**).

5-HT-Depleted Animals Adapted their Responses Following a Change in Reward Contingencies at a Rate Comparable to Controls

Sessions to criterion and total errors made on the reversal learning task were analyzed with rmANOVA with time (pre- and post-operatively) as within- and treatment group (Depletion vs. Sham) as between-subject factors. Similarly to the pairwise discrimination learning, 5HT-depleted group was indistinguishable from sham-operated animals on either of the measures. rmANOVA revealed no main effect of group [$F_{(1, 13)} = 0.346$, $p = 0.567$] or time × group interaction [$F_{(1, 13)} = 0.043$, $p = 0.839$]. In contrast to discrimination learning where there was no effect of time on any of the measures, rmANOVA detected significant main effect of time for sessions to criterion on reversal

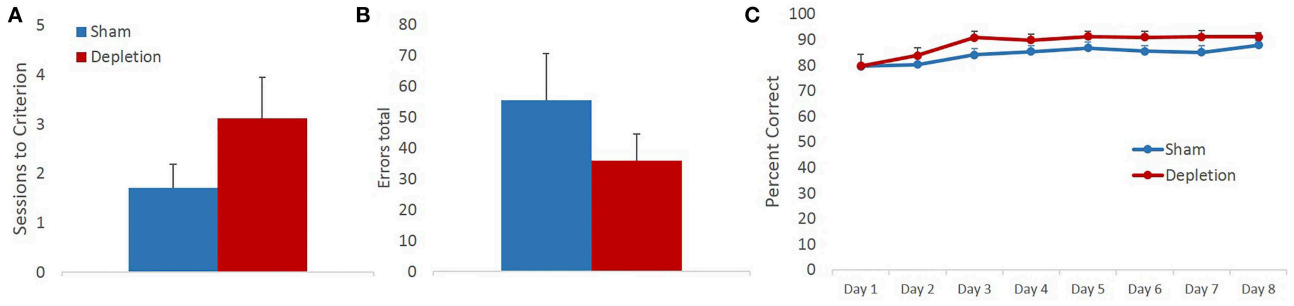

FIGURE 3 | 5-HT-depleted animals had intact memory for previously learned task contingencies. Memory for the previously learned task contingencies was assessed by retention of the pre-operative reversal task 7 days after the surgery. There was no statistical difference between treatment groups on **(A)** sessions to criterion, or **(B)** total errors. **(C)** All animals improved their performance with time. 5-HT depletions in BLA had no effect on performance accuracy on any of the sessions of retention task.

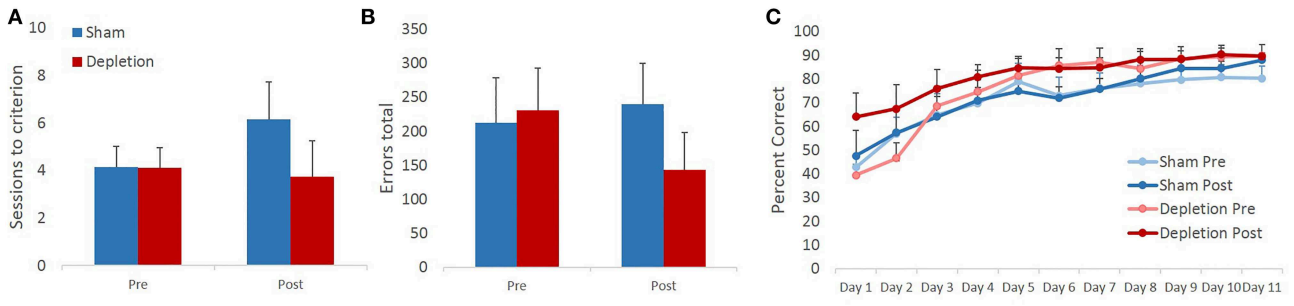

FIGURE 4 | 5-HT depletions in BLA do not affect acquisition of a visual discrimination learning task. (A) 5-HT depletions did not impair animals' ability to acquire the discrimination task. We anticipated faster acquisition of a second novel discrimination as a result of practice with the task. However, no intraproblem transfer of learning was observed. **(B)** Similarly, no practice effect was observed on total number of errors. There were also no treatment group differences in the total number of committed errors. **(C)** All animals improved their performance with time. 5-HT depletion did not affect animals' learning rate.

learning [$F_{(1,\ 13)} = 9.667$, $p = 0.008$]. Animals' performance changed in a direction opposite of the anticipated, all animals required more sessions to reach reversal learning criterion post- compared to pre-operative (**Figure 5A**). No significant effects were observed for total number of committed errors during the reversal phase: no main effect of time [$F_{(1,\ 13)} = 1.866$, $p = 0.195$], group [$F_{(1,\ 13)} = 0.123$, $p = 0.731$], or time × group interaction [$F_{(1,\ 13)} = 0.067$, $p = 0.8$] (**Figure 5B**).

To probe for between-group differences in learning rates during the reversal phase of the task, session-by-session performance accuracy data were analyzed with omnibus rmANOVA with time (pre- and post-operatively) and session as within- and treatment group (Depletion vs. Sham) as between-subject factors. There was a robust main effect of session indicating that all animals improved their performance with repeated testing [$F_{(13,169)} = 78.849, p < 0.0001$]. Minimal effects of pre-operative testing on subsequent reversal learning were observed: no main effect of time [$F_{(1,\ 13)} = 0.42$, $p = 0.528$], but a trend for time × session interaction [$F_{(13,169)} = 2.449$, $p = 0.067$]. *Post-hoc* analyses further revealed a significant difference on day 1 of reversal learning between pre- and post-operative assessments with rats obtaining higher early accuracy levels during post-operative testing [$t_{(14)} = -2.447; p = 0.027$]. 5-HT depletion of BLA had no effect on animals' reversal learning performance. There was no main effect of group [$F_{(1,\ 13)} = 0.22$, $p = 0.647$], group × time [$F_{(1,\ 13)} = 0.382$, $p = 0.547$], group × session [$F_{(13,169)} = 0.348$, $p = 0.746$], or group × time × session [$F_{(13,169)} = 0.44$, $p = 0.755$] interactions (**Figure 5C**).

Discussion

The results of the present investigation revealed intact discrimination and reversal learning following 5,7-DHT-mediated serotonergic depletions of BLA. The lesioned animals demonstrated intact memory for previously learned associations as evidenced by lack of between group differences on the retention task. These animals were unimpaired relative to controls at acquiring a novel discrimination task and flexibly adapted their responses following a change in reward contingencies, at a rate comparable to Sham animals.

The lack of group differences in retention of a previously learned stimulus-reward associations and acquisition of a novel discrimination learning problem is in line with

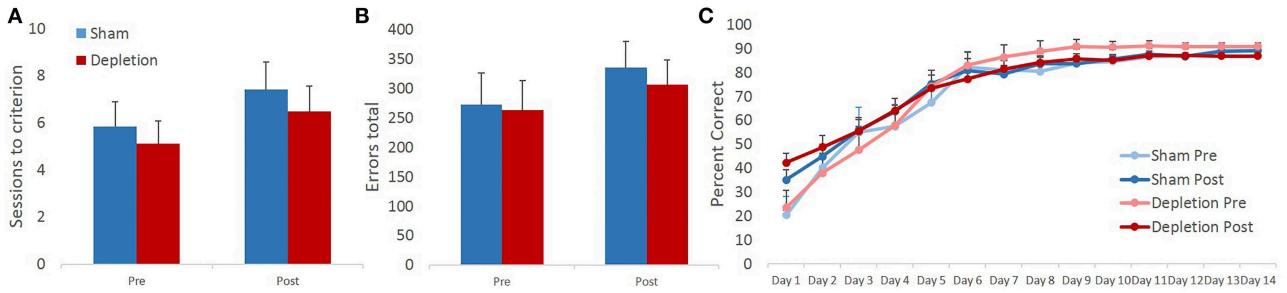

FIGURE 5 | 5-HT-depleted animals switched their responses following the change in stimulus-outcome contingencies at a rate comparable to controls. Similar to pairwise discrimination learning, 5HT-depleted were indistinguishable from sham-operated animals on all of the measures. **(A)** In contrast to discrimination learning, in reversal all animals required more sessions to reach criterion post- compared to pre-surgery. **(B)** No significant effects were observed for the total number of committed errors during the reversal phase. **(C)** There was a robust main effect of session indicating that all animals improved their performance with repeated testing. 5-HT depletion of BLA had no effect on animals' reversal learning performance.

previous observations demonstrating that amydgala lesions or inactivations have no effect on the memory for stimulus-reward associations (Izquierdo and Murray, 2007; Stalnaker et al., 2007; Izquierdo et al., 2013). It further strengthens the notion that BLA is not required in situations when the associations are stable, but instead involved in updating reward values following a change in stimulus-reward assignment.

Whereas, the lack of a stimulus-reward memory effect was expected, the lack of effect on the reversal phase of the experiment was surprising, given the prominent role of 5-HT in modulation of BLA activity and function (Rainnie, 1999; Mascagni and McDonald, 2007; Yamamoto et al., 2012), and previous results demonstrating the involvement of this brain region in cognitive flexibility (Stalnaker et al., 2007; Churchwell et al., 2009; Izquierdo et al., 2013). The mean levels of 5-HT depletion by the end of behavioral testing were 35.47% for the left and 48.99% for the right hemispheres. However, brain tissue was collected on average 68 days after the surgery, and more substantial reductions in 5-HT levels are expected at earlier time points when the behavioral assessment took place. Notably, these depletion levels are comparable to those reported in previous study where attenuated probabilistic reversal learning performance was observed (Rygula et al., 2014). The most compelling evidence for a causal role of 5-HT BLA depletions in reversal learning impairment comes from the aforementioned study by Rygula et al. (2014) that employed a probabilistic learning task. Although the explanation and analysis of the behavior presented by the authors suggesting a direct role for 5-HT in BLA in reward learning and reversal is plausible, another possibility exists. It is well established that BLA structural and functional integrity is critical for appropriate responses to reward devaluation. For example, animals with BLA-OFC disconnections are not able to update their response strategy in the face of changing reward value regardless of whether they need to rely on stored representations of reward value (i.e., during extinction) or if the devalued reward is delivered (Zeeb and Winstanley, 2013). Although not analyzed by Rygula et al. in such a manner, risk is itself a strong discounting parameter, factoring into reward valuation along with delay and effort demands. The previous studies implicating BLA in reward devaluation have suggested decreased sensitivity to probability costs. Patients with damage to amygdala frequently make disadvantageous, risky choices (Bechara et al., 1999; Brand et al., 2007), especially in tasks stressing potential gains (Weller et al., 2007). The difference in risk or uncertainty cost associated with each response options in the Rygula et al. (2014) study is the change from the initial outcome probability of 80:20 and its reversal to 20:80. In addition to the interpretation of findings provided by authors (increased responsiveness to false feedback and decreased reinforcement sensitivity), the reversal learning impairment may be explained by decreased sensitivity to changes in probability of reward associated with each option before and after reversal, and a less steep devaluation of response option with increases in uncertainty cost.

In the present investigation by contrast there is no probability component (i.e., the relationship between stimulus and outcome is deterministic); the only way in which the stimulus is devalued is by the rats' repeated experience with omission of reward delivery. Whereas, risk discounting could play a role in the former study, it is not a factor in the present investigation.

Thus, the present findings provide novel evidence for the lack of a role for 5-HT in BLA in deterministic reversal learning. However, it needs to be noted that although the methods implemented in the present investigation are similar to ones previously reported, there are several important distinctions which might have masked the effect of treatment on behavior. One of the important distinctions is animals' experience with *both* the discrimination and reversal tasks prior to lesion. Rats already acquired the knowledge of the optimal strategy to learn the task. In Izquierdo et al. (2013), where potentiated responses to negative feedback and enhanced reversal learning performance following lesions were observed, the animals were only pre-trained before the surgeries and both of the tasks were introduced only following the recovery period. In Rygula et al. (2014) monkeys had a preoperative experience with discrimination but not reversal learning. This observation is particularly interesting as it suggests that the behavioral alterations observed in the previous investigations could have

resulted from changes in strategy learning. Early studies in monkeys with amygdala lesions conducted by Schwartzbaum and Poulos (1965) showing an impairment in the transfer of learning only during initial reversals in a set, provide support for this interpretation.

BLA is critically important in detecting and updating response strategy following a change in reward-predicting rules or cues (Coleman-Mesches et al., 1996; Baxter and Murray, 2002; Liao and Chuang, 2003; Belova et al., 2008; Ostrander et al., 2011). Conflict detection is particularly important during the first experience with the reversal learning. Rygula et al. (2014) observed that the impairment on reversal learning resulted from increased responsiveness to misleading feedback and decreased overall reinforcer sensitivity, which suggests a decreased ability to integrate reward information across time. In a probabilistic learning task it is likely to manifest in an increased win-stay/lose-shift strategy, regardless of feedback veracity and lead to impaired responses after misleading feedback. Thus, in the present experiment, wherein the first experience with the reversal occurred with intact BLA, the demand of strategy learning is reduced, and the retention of the already-learned task rules may be sufficient to guide responses. Future studies need to address this question by systematically implementing different timelines of lesion or inactivation. 5-HT depletions of BLA may impair reversal acquisition only if administered after surgery, without preoperative training.

Another interesting possibility arises from the consideration of the specificity of depletions. Previous studies reporting correlations between 5-HT levels and reversal learning performance have considered global depletions, which in addition to BLA also produced significant 5-HT reductions in OFC, mPFC, and hippocampus among the brain regions examined (Masaki et al., 2006; Izquierdo et al., 2012). It is therefore plausible that the negative correlations between the reversal learning performance and 5-HT levels were driven by neurotransmitter concentrations in other brain regions, not amygdala. Future research may benefit from direct manipulation of 5-HT neurotransmission in PFC or hippocampus of animals performing a reversal learning task to understand the region-specific contribution of 5-HT in the previously reported impairment. Another possibility is that compromised 5-HT signaling in one brain region may be insufficient to produce an appreciable behavioral impairment. Instead, systems manipulations with depletions targeted in several interconnected brain regions might be necessary. Though the 5-HT depletions were restricted to BLA in the present study, this method precludes differentiation of 5-HT receptor subtype involvement in reward learning, an avenue of inquiry perhaps best pursued with more specific pharmacology or chemogenetic targeting.

Author Contributions

JO and AI designed the research; JO, AS, AK, EH, and AB performed research; JO, AS, and AI analyzed data; JO, AS, and AI wrote the paper.

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Supramammillary serotonin reduction alters place learning and concomitant hippocampal, septal, and supramammillar theta activity in a Morris water maze

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Hippocampal theta activity is related to spatial information processing, and high-frequency theta activity, in particular, has been linked to efficient spatial memory performance. Theta activity is regulated by the synchronizing ascending system (SAS), which includes mesencephalic and diencephalic relays. The supramamillary nucleus (SUMn) is located between the *reticularis pontis oralis* and the medial septum (MS), in close relation with the posterior hypothalamic nucleus (PHn), all of which are part of this ascending system. It has been proposed that the SUMn plays a role in the modulation of hippocampal theta-frequency; this could occur through direct connections between the SUMn and the hippocampus or through the influence of the SUMn on the MS. Serotonergic raphe neurons prominently innervate the hippocampus and several components of the SAS, including the SUMn. Serotonin desynchronizes hippocampal theta activity, and it has been proposed that serotonin may regulate learning through the modulation of hippocampal synchrony. In agreement with this hypothesis, serotonin depletion in the SUMn/PHn results in deficient spatial learning and alterations in CA1 theta activity-related learning in a Morris water maze. Because it has been reported that SUMn inactivation with lidocaine impairs the consolidation of reference memory, we asked whether changes in hippocampal theta activity related to learning would occur through serotonin depletion in the SUMn, together with deficiencies in memory. We infused 5,7-DHT bilaterally into the SUMn in rats and evaluated place learning in the standard Morris water maze task. Hippocampal (CA1 and dentate gyrus), septal and SUMn EEG were recorded during training of the test. The EEG power in each region and the coherence between the different regions were evaluated. Serotonin depletion in the SUMn induced deficient spatial learning and altered the expression of hippocampal high-frequency theta activity. These results provide evidence in support of a role for serotonin as a modulator of hippocampal learning, acting through changes in the synchronicity evoked in several relays of the SAS.

Keywords: supramammillary nucleus, serotonin, septum, hippocampus, theta activity, spatial learning

INTRODUCTION

Hippocampal theta activity has been related to processing of spatial information in different behavioral paradigms in various animal species (Ammassari-Teule et al., 1991; McNaughton et al., 2006) as well as in human beings (Klimesch et al., 1994; Klimesch, 1999; Caplan et al., 2001; Ekstrom et al., 2005; Lega et al., 2014). The relation of theta activity and place learning has been also studied; changes in power and/or frequency of the hippocampal theta activity have been associated with efficient learning during place learning tests in the Morris maze (Pan and McNaughton, 1997; Olvera-Cortes et al., 2002, 2004; Olvera-Cortés et al., 2012; Buzsaki, 2005; Ruan et al., 2011), conditioning (Berry and Seager, 2001; Berry and Hoffmann, 2011), working memory (Mitchell et al., 1982), and novelty detection (Aggleton and Brown, 1999; Vinogradova, 2001), among others. Moreover, deficient spatial memory has been observed after the reductions in the frequency of hippocampal theta activity (Winson, 1978; Pan and McNaughton, 1997).

Theta activity is modulated by a group of mesencephalic-diencephalic structures called the synchronizing ascending system (SAS) (Bland et al., 1990; Kirk et al., 1996; Leranth et al., 1999; Woodnorth et al., 2003). Theta activity can be generated in the hippocampus by stimulation of the nucleus *reticularis pontis oralis* (RPOn) both in anesthetized and in awake animals (Vertes, 1982, 1986). It was proposed that the RPOn theta modulation spreads through the tegmental pedunculopontine nucleus (TPPn) to the hypothalamic relays, the supramammillary (SUMn) and posterior hypothalamic (PHn) nuclei (Takano and Hanada, 2009). Because of to the tonic firing of RPOn neurons, the rhythmical firing of SUMn cells, and the result from inactivating SUMn, it was proposed that SUMn convert the tonic input received from the RPOn into a rhythmical pattern, which is relayed to the medial septum (MS), considered the pacemarker of the theta activity (Gogolak et al., 1968; Petsche et al., 1968; Andersen et al., 1979; Kirk and McNaughton, 1991; Kirk and Mackay, 2003). In support of this hypothesis, procaine infusions into (medial) SUMn induce a decrease in the frequency of hippocampal theta activity elicited by stimulation of RPOn in awake or in anesthetized rats (Kirk and McNaughton, 1993; McNaughton et al., 1995). Moreover, the rhythmic activity in the SUMn elicited by infusing carbacol into the RPOn persists after either the infusion of procaine into the MS or the bilateral transection of the communication pathways between SUMn and the MS (Kirk et al., 1996; Kirk, 1997). Additionally, an efferent influence from MS, which induce the deceleration of theta frequency-related firing in SUMn neurons, was observed (Kocsis, 2006; Kocsis and Kaminski, 2006); this influence could originate in the reciprocal connections between the two nuclei (Vertes, 1992), possibly through a GABAergic, input from the lateral septum (LS) on the (lateral) SUMn (Leranth and Kiss, 1996).

The SUMn has been related to information processing in memory. SUMn c-fos activity increases in spatial tasks (exploration, reference memory, and working memory) in the Morris water maze (Santin et al., 2003). Additionally, SUMn inactivation through the micro infusion of TTX induces deficiencies in reference memory retrieval (when TTX is applied in the seventh day of training, but not in the fourth day of training) and deficiencies in spatial working memory (Aranda et al., 2008). Furthermore, inactivation of SUMn with lidocaine impairs memory retrieval and consolidation in spatial memory tasks (Shahidi et al., 2004a). These results remarkably suggest that the SUMn functions in spatial information processing, although a relationship between SUMn and spatial learning is less clear (Santin et al., 2003). One study explored the relation between the SUMn, hippocampal theta activity and learning. Infusion of cholrdiazepoxide (CDP) into the (medial) SUMn had modest effects on theta activity and place learning in Morris water maze (Pan and McNaughton, 1997). However, after lidocaine inactivation of MS and the concomitant lack of hippocampal theta activity, both place learning and the rhythmicity of hippocampal theta activity (7.7 Hz) were restored by using the SUMn oscillation to rhythmically stimulate the fornix (McNaughton et al., 2006). This study demonstrated the relevance of both the SUMn and theta activity for place learning.

Similarly to the other relay nuclei of the SAS and the hippocampus, the SUM receives serotonergic axons both from medial and dorsal raphe nuclei (Vertes, 1988, 1992). The role of the serotonin originated in the raphe nuclei in desynchronizing of the hippocampal EEG is well documented. Briefly, stimulation of the medial raphe nucleus (MRn) desynchronizes the hippocampal EEG through the action of serotonin, whereas the electrolytic lesions of the same nucleus induce hippocampal EEG with a higher magnitude and longer duration, which is also present during immobility, in rats (Assaf and Miller, 1978; Maru et al., 1979). Furthermore, mucimol, buspirone and 8-hydroxy-2-(di-*n*-propyl-amino)-tetralin (8-OH-DPAT), a 5-HT1$_A$ agonist, injections in MRn, induce persistent theta activity in the hippocampus of anesthetized rats, through the inhibition of serotonergic neurons (Vertes et al., 1994; Kinney et al., 1995). Thus, the serotonin can act on the SAS through many relays, or directly on the hippocampus to regulate theta activity; as a negative regulator of theta rhythmicity, serotonin could contribute to the fine-tuning of theta activity in the SUMn and thus influence on the upper relays, principally the MS and the hippocampus.

The role of serotonin as a modulator of learning has been extensively studied, although a complex picture emerges from the various papers possibly due to differences in learning tasks as well as differences in experimental strategies to manipulate the cerebral or regional serotonin activity, because of these factors, impairment, no effect or improvement in learning tasks has been reported after serotonin manipulations. Impairment in water maze tests was observed both after intra-septal or intra-hippocampal infusions of 8-OH-DPAT (Carli et al., 1992; Carli and Samanin, 1992; Bertrand et al., 2000). It has also been reported that intra-septal infusion of 8-OH-DPAT causes deficient spatial working memory (Jeltsch et al., 2004). In contrast, improvement in working memory and conditioning as well as improvement in place learning has been reported after reductions in cerebral, prefrontal and hippocampal serotonin (Altman et al., 1989; Pérez-Vega et al., 2000; Sarihi et al., 2000; Gutiérrez-Guzman et al., 2011). Additionally, a relation

between the serotonergic modulation of theta and hippocampal-dependent place learning has been found (Gutiérrez-Guzman et al., 2011; Lopez-Vazquez et al., 2014). Moreover, reduction of serotonin content in the SUMn/PHn induced place learning deficiencies associated with a lack of learning-related increases in high-frequency hippocampal theta activity through the training (Gutiérrez-Guzman et al., 2012). Thus, the SUMn is a relay of the SAS participating in the modulation of hippocampal theta activity, and it is at least partially involved in place learning consolidation and/or recovery; it also receive serotonergic inputs, which could modulate the fine-tuning of hippocampal theta activity. However, despite the above, the effects of serotonin SUMn depletion alone on both spatial learning and on the characteristics of hippocampal, septal and SUMn theta activity during place learning have not been evaluated. The aim of the present work was to evaluate the consequences of serotonin depletion in the SUMn on place learning and the concomitant theta activity recorded from the SUMn, medial septum (MS), dentate gyrus (DG), and CA1, during the training in the Morris maze, in the rat.

METHODS

Animals

Seventeen male, 4-months-old Sprague Dawley rats were used. The rats were maintained under standard facility conditions, and all of the experiments were conducted in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publication No. 80-23) and for the "Norma Oficiál Mexicana" for the use of experimental animals (NOM-062-ZOO-1999). All of the experiments were and approved by the Research Ethics Committee of the Instituto Mexicano del Seguro Social.

Surgery

The rats were divided in two groups, one control group (CTR, $n = 7$), and one experimental group (EXP, $n = 10$). Both groups of rats were anesthetized under ketamine/pentobarbital anesthesia (60 mg/kg im, 20 mg/kg ip) and chronically implanted with bipolar, concentric electrodes in the MS (coordinates: 0.6 mm anterior to the bregma, 1.5 mm lateral to the midline, 15° from the vertical, and 6.8 mm ventral to the cranial surface), DG (coordinates 3.5 mm posterior to the bregma, 1.5 mm lateral to the midline, and 3.4 mm ventral to the cranial surface), CA1 (coordinates: 4.5 mm posterior to the bregma, 2.4 mm lateral to the midline, and 2.7 mm ventral to the cranial surface), and SUM (coordinates: 4.7 mm posterior to bregma, 0.2 mm lateral to the midline, and 8.7 mm ventral to the cranial surface); all coordinates were taken from the Atlas of Paxinos and Watson (1998). The electrodes were made of nichrome wire with a diameter of 60 μm fastened inside a stainless steel # 27 caliber cannula isolated with epoxy resin, with a small surface exposed at the tip. The electrodes were fixed to the skull with dental acrylic. Two screws were used, one placed in the frontal bone served as ground and the other placed in the posterior skull served to fix the implant. In the same surgery, rats in the EXP group received an intra-SUM infusion of 5 μg of 5,7-DHT (2 μg dissolved in 0.1 μl of 0.1% ascorbic acid in saline solution) at an infusion rate of 0.1 μl/min for 4 min. One injection was placed into the SUMn (4.7 mm posterior to the bregma, 0.2 mm lateral to the midline, and 8.24 mm ventral from the cranial surface) using a Hamilton syringe and an infusion pump. Thirty minutes before the 5-HT lesion, the rats received desipramine (30 mg/kg, ip) to protect the noradrenergic terminals. The rats of the CTR group only received an infusion of vehicle solution, similar in volume and rate to the EXP group.

Behavioral Test

Two weeks after the surgery, the rats were trained in a place-learning test using the Morris water maze. This maze consisted of a circular pool (1.5 m of diameter and 45 cm of height wall) filled with water made blue by adding gentian violet, which contained a submerged circular platform (9 cm of diameter) placed in a fixed position in one of the four virtual quadrants of the maze.

The rats were submitted to four daily trials during six consecutive days; each trial was initiated by placing the rat into the pool facing the wall in one of the quadrants (the starting quadrants were randomly chosen each day but were similar for all rats in one day). The trial continued until either the rat located the platform or 60 s elapsed. If the rat failed to locate the platform in this time, it was guided to the platform by the experimenter and left there for 15 s. After this time, the rat was retired and placed in a home cage during 2 min (inter-trial period) before beginning the next trial. On the seventh day, all rats received one 30 s probe trial that consisted of searching the maze after the escape platform had been removed. The behavioral tests were video recorded and stored on a computer for later analysis, when the escape latencies, distances traveled and swimming velocity achieved by the rats and also the distance swam for each quadrant in the probe trial were obtained. Recordings and analysis were performed using the Data-Wave Inc. software (VideoBench 5.1). The mean swim distances from the four daily trials as well as the mean daily latencies were compared. In the probe trial, the distance swam by the rats in each quadrant was obtained and compared.

EEG Records

Each training day the rats were connected to a commutator (Neuro-Tek, CA. IT,) using a cable with a male connector. The commutator was connected to one amplifier (Neurodata acquisition system, GRASS Mod 15, Astro Med Inc. 600 E. Greenwich Ave., W. Warwick, RI 02893, USA) and the EEG was digitalized to 1024 Hz with a DataWave Technologies data acquisition system, and the EEG was stored in a PC to be analyzed of line. A bipolar recording was taken using the nichrome wire as G1 and the cannula as G2 (A bipolar derivation was made using the G1–G2), the filters were set to 1–100 Hz, the EEG recording were synchronized to the VideoBench software, which tracked a small light-emitting diode attached to animal implant. A baseline recording was taken from the awake-immobile rat in the cage (60 s), and then, all time that the rat searched for the platform was recorded, including the final 15 s that the rats remained onto the escape platform. The data were imported into MATLAB

(Mathworks, Inc.) (Delorme and Makeig, 2004) and the software EEGLAB was used to eliminate artifact by visual inspection.

The EEG from basal and searching conditions was submitted to the Fast Fourier Transform (FFT) and absolute power was obtained as the mean spectrum of 2-s samples, to ensure a resolution of 0.5 Hz, from 4 to 12 Hz. The relative power (RP) was obtained for each behavioral condition and 0.5 Hz of frequency as the percent of the total 4–12 Hz absolute power band. Comparisons were made of the RP in the range of 5–0 Hz, in each brain region, between days and frequency for each group (intra-group comparisons) and between day, group and frequency (inter-group comparison); using an ANOVA for repeated measures and paired *t*-test with a Bonferroni correction. Additionally, coherence values were computed for pairs of recording sites and compared in manner similar to the RP values. The analyses of both EEG power and coherence were conducted using custom programs adapted from Ken's MATLAB library written by Ken Harris and available at http://osiris.rutgers.edu/Buzsaki/software.

HPLC

The serotonin content was determined using HPLC as follows, after the euthanasia of the animals, samples including SUMn were dissected from a slice containing the region of interest and a sample of the tissue was punched using a 25 G cannula. The tissue samples were homogenized in 1N HCl and centrifuged. The content of serotonin and 5HIAA (pg/mg of fresh tissue) of the supernatant was determined using a LiChroCart purospher star column (150 – 4.6, RP – 18 end caped, 5 mm, MERK KGa A, Darmstadt; Germany) with a mobile phase (pH 3.1) composed of citric acid (50 mM), H_3PO_4 (50 mM), EDTA (20 mg), octanesulfonic acid (120 mg/L), and methanol (8 %). The flow rate was 1.3 mL/min. An electrochemical detector (AtecLydenVT-03) with a work potential of 0.800 mV adjusted to the pH of the mobile phase was used. The data were compared using the Student *t*-test.

The SUMn was visually inspected to verify the electrode position, during the dissection of the tissue for HPLC. The tract of the electrode in the remaining tissue after the dissection of SUMn for HPLC and the position of the other electrodes was verified using a light microscope after the brain was sliced at 5 μm and the slices were stained with cressyl violet (**Figure 1**). After histological verification of the position of the electrodes in the MS, the DG and the CA1; the EXP group of rats included only those rats with reductions of serotonin greater than 50% from the CTR group mean content in the SUMn; thus, four rats were excluded because they showed a reduction of serotonin less than 50%, and the EXP group included 6 rats in the final analysis.

RESULTS

Serotonin Content

The EXP group had significantly lower serotonin (5-HT) and 5-hydroxyindoleacetic acid (5-HIAA) concentrations that the CTR group (Paired one tailed $t = 4.274$, $df = 5$, $p = 0.004$ and $t = 7.293$, $p = 0.0004$, $df = 5$; for 5-HT and 5HIAA, respectively) (**Figure 2A**).

Behavior

Escape latencies were compared between training days within the two groups of animals using a Friedman ANOVA, and a *post-hoc* Wilcoxon test. The CTR group significantly reduced their escape latencies (X^2_r= 23.245, $P > 0.001$), by day 3–6 ($p = 0.018$); whereas the EXP group only significantly reduced their escape latencies (X^2_r = 11.429, $P = 0.044$), on day 6 ($p = 0.028$). Intergroup comparisons (Mann Whitney U test) showed both a main effect ($\sum R_x = 391$, $P < 0.001$) and differences in the escape latencies on days 3 ($p = 0.004$), and 5 ($p = 0.003$) with a bias toward day 4 ($p = 0.063$); the escape latencies were longer for the EXP than for the CTR group (Data not showed).

Intra-group comparisons of the distances traveled by the rats were made using an ANOVA for blocks and Tukey *post-hoc*; the CTR group significantly reduced their distances traveled [$F_{(5, 30)}$ 24.112, $p < 0.001$], on days three to six of training ($p < 0.001$). The EXP group did not show significant reduction of distance traveled over the training days [$F_{(5, 25)} = 2.018$, $p = 0.111$]. Inter-group comparisons using two factors, group and day of training, were made using an ANOVA for repeated measures. The distances traveled by the EXP group were higher than the distances traveled by the CTR group [$F_{(1, 11)} = 11.232$, $p = 0.006$, main effect], however, there was no significant interaction of day and group [$F_{(5, 55)} = 2.242$, $p = 0.062$] (**Figure 2B**). The swimming velocities were compared similarly to the distances, but no changes over the training days were observed for the CTR [$F_{(5, 30)} = 1.472$, $P = 0.228$] or the EXP [$F_{(5, 25)} = 1.981$, $P = 0.116$] groups (**Figure 2D**).

Finally, the distance traveled in each quadrant during the probe trial (day seven) was compared between quadrants and groups, using a Two-Way ANOVA (group and quadrant). No significant differences between groups were observed [$F_{(3, 48)} = 2.452$, $P = 0.074$]. However, the CTR group swam significantly different distances between quadrants [$F_{(3, 24)} = 6.285$, $p = 0.002$]; the distance on the quadrant that had contained the platform in the training (N) was higher than in the S and W quadrants. The EXP group of animals swam similar distances in all quadrants (**Figure 2C**).

Theta Activity

The row EEG from the four regions recorded, under basal conditions (awake, immobile, wet rat) in the cage and during the searching for the platform on days one and six of representative rats, is shown in **Figure 3**. The natural logarithm (nl) of the absolute power of the theta band from each cerebral region and group was compared by day and frequency using Two-Way ANOVA. No significant differences were observed in any group regarding this comparison (data not shown). Intergroup comparisons of the absolute power of the nl recorded from each cerebral region were performed using ANOVA for repeated measures of two factors (group and frequency), with days as a repeated measure; no significant differences were observed for any of the regions studied.

Relative power, expressed as a percentage of the contribution of each specific frequency to the total power of the theta band, had a beneficial effect of reducing the inter-subject variance. In addition, it is possible that the changes associated with learning

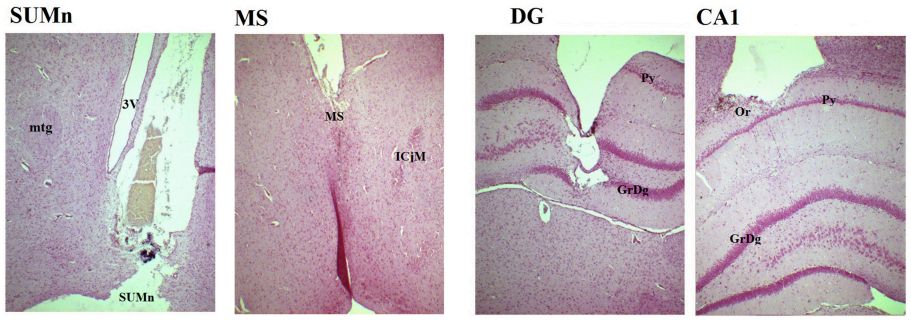

FIGURE 1 | Photomicrography of representative slices showing the position of the electrodes in the four regions. In the SUMn note that tract of the electrode reaches the zone in which the tissue was punched out for HPLC measures. SUMn, supramammillary nucleus; mtg, mamillotegmental tract; 3V, 3rd ventricle; MS, medial septum; ICjM, Major island of Calleja; DG, dentate gyrus; GrDg, granular layer of dentate gyrus; Py, pyramidal layer of CA1; CA1, field CA1 of hippocampus; Or oriens layer. Magnification 4X.

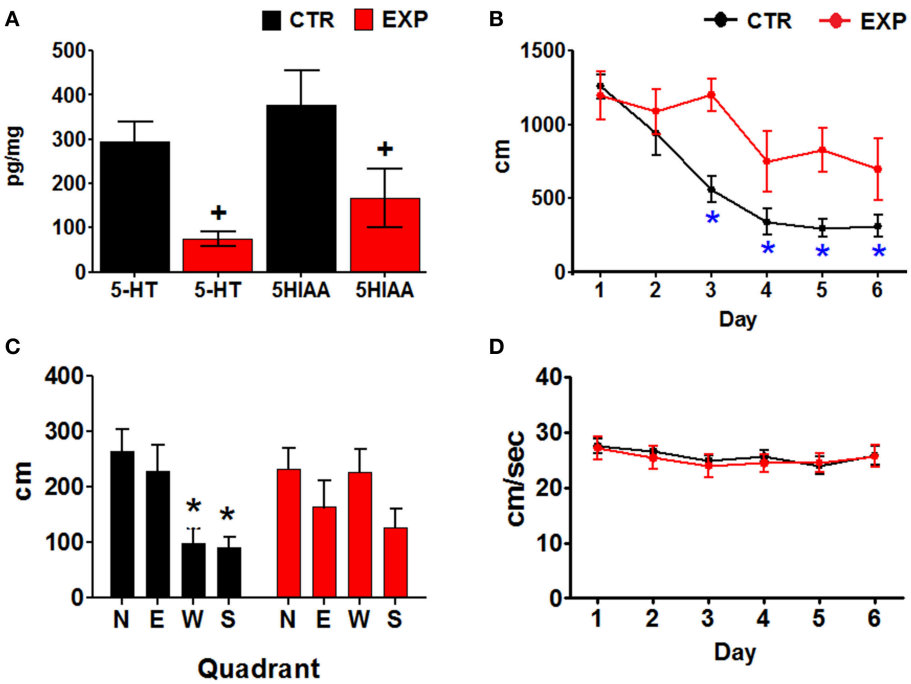

FIGURE 2 | (A) Serotonin and 5-HIAA concentrations. Mean ± SEM, $p < 0.05$. **(B)** Distances traveled by the two groups of animals, through the training days. Mean ± SEM. *Day 1 vs. subsequent days, $p < 0.001$. **(C)** Distance traveled in each quadrant during the probe test of the two groups of rats. The escape platform was placed in the north quadrant during the training. North (N), South (S), East (E), and West (W) quadrants. Mean ± SEM. *N quadrant vs. all other quadrants; +, group CTR vs. EXP, $p < 0.05$. **(D)** Swimming velocities displayed by the two groups of animals through the training days. Mean ± SEM.

on EEG could be sufficiently subtle to reflect absolute power changes; moreover, the consequences of a reduction of one neurotransmitter in one discrete nucleus from the SAS could be quite subtle and could induce changes in the expression of absolute power in the theta band. Thus, more subtle changes were expected than those observed in studies in which the cerebral reduction of serotonin or RM lesions was induced. Using this rationale, in previous studies, learning-related changes were observed in the relative power of the theta activity recorded in CA1 during the training of rats in the Morris water maze (8–10).

The relative power (RP) of each cerebral region of the CTR group was compared by day and frequency using a Two-Way ANOVA for repeated measures. The RP recorded in the SUMn for the CTR group changed across the training days [$F_{(50,\ 330)} = 2.977$, $p < 0.0001$]. The RP for high frequencies (7.5–8.5 Hz) RP increased with the training days whereas low frequencies (6.5 and 7 Hz) decreased when compared with the first and second days. RP from MS showed significant changes across the training days [$F_{(50,\ 330)} = 1.477$, $p = 0.025$]; particularly an increase for the 8 Hz frequency the lasts days of training. The RP from

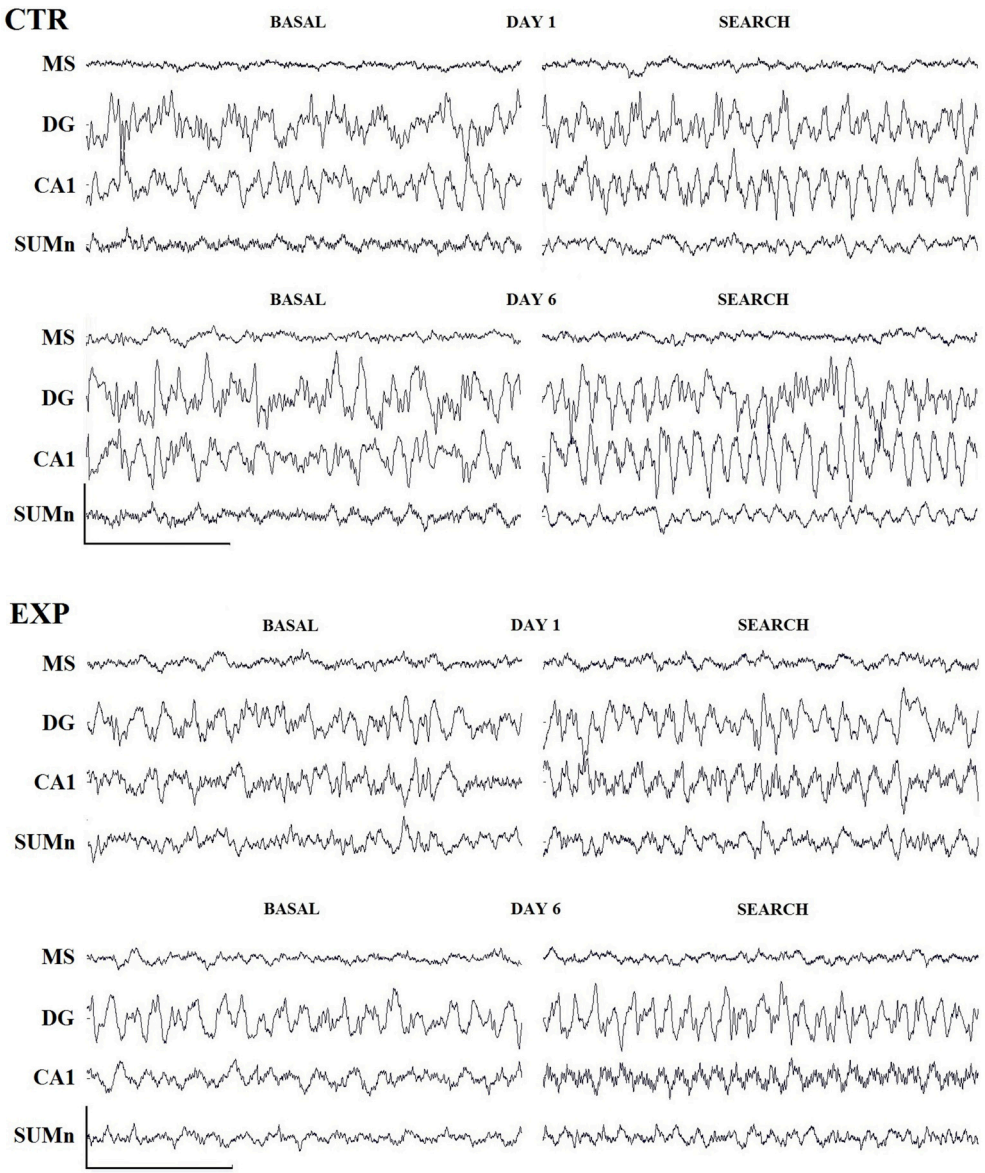

FIGURE 3 | Row traces of one representative rat from each group. Three second samples recorded during basal conditions (awake, wet rat in a holding cage) and during the search for the platform on days 1 and 6 of training. Cal. 500 mV/1 s. Abbreviations are as in the text.

the DG showed increased theta activity over the course of the training days [$F_{(50,\ 330)} = 2.689$, $p < 0.0001$] for the 7–8.5 Hz frequencies. Finally, the RP of the theta activity recorded in the CA1 showed changes with regard to training days [$F_{(50,\ 30)} = 2.729$, $p < 0.0001$], and the RP for the 6.5 and 7 Hz frequencies was reduced, whereas the RP for 8.0 and 8.5 Hz increased over the training days. **Figure 4** shows the RP only for days 1, 2, 5, and 6 when the differences between RP were maximal, and **Table 1** shows the significant differences between all of the training days from the four regions. Thus, the RP in the higher frequencies (7–5–10 Hz) increased across the training days in the different regions, and some regions showed a concomitantly reduction in low frequencies RP (6.5–7 Hz).

The RP recorded in the four cerebral regions from the EXP group did not show significant effects of training across training days [$F_{(50,\ 275)} = 1.272$, $p = 0.1181$ for MS; $F_{(50,\ 275)} = 1.010$, $p = 0.4622$ for DG; $F_{(50,\ 275)} = 1.272$, $p = 0.1178$; and $F_{(50,\ 275)} = 1.368$, $P = 0.0616$ for SUMn]. However, there were days in which the information processing was putatively different, that is, acquisition of information is prominent on days 1 and 2, whereas the consolidation and recovery of memory is prominent on days 5 and 6; therefore, an ANOVA including only days 1, 2, 5, and 6 for the EXP group was performed to determine whether the differences in processing would be expressed as differences in EEG in this group. The SUMn RP showed significant changes across training days when only the

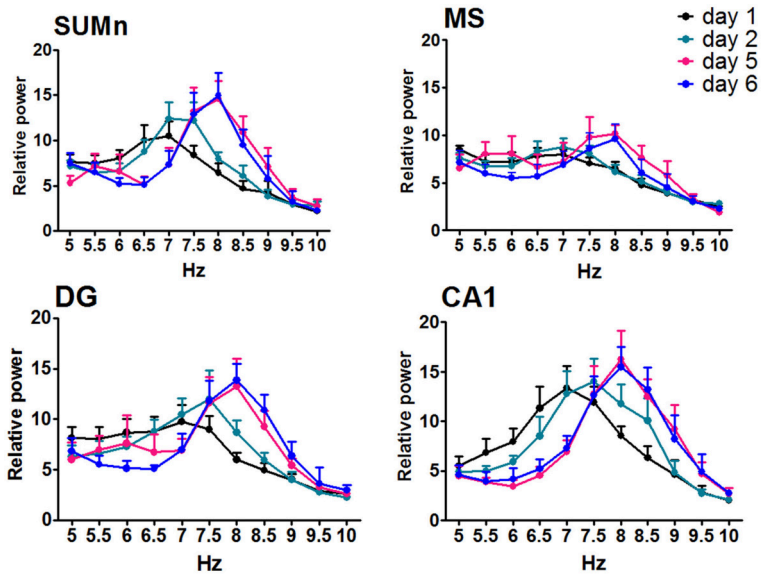

FIGURE 4 | Relative power in the theta band (5–10 Hz) recorded in the four regions of the CTR group during the searching of the platform across days of training. Only days 1, 2, and 5, 6 are showed. Mean ± SEM. Significant differences are listed in **Table 1**. $p < 0.05$.

TABLE 1 | Comparison between training days of the relative power recorded during the searching for the platform in the Morris water maze task in the CTR group.

Hz\Day	1	2	3	4	5	6	Region
6.5	10.00 ± 1.71	8.74 ± 1.32	6.97 ± 0.92	6.78 ± 0.97	5.16 ± 0.85^{A}	5.11 ± 0.85^{A}	
7	10.49 ± 1.62	12.40 ± 1.85	11.25 ± 1.98	10.17 ± 2.13	7.30 ± 1.84^{B}	7.31 ± 1.53^{B}	
7.5	8.40 ± 0.89	12.2 ± 2.02	14.23 ± 2.30^{A}	13.98 ± 3.21^{A}	13.23 ± 2.59^{A}	12.87 ± 2.40^{A}	SUM
8	6.45 ± 1.00	7.98 ± 0.73	10.19 ± 0.96	11.73 ± 1.67^{A}	14.57 ± 1.97^{ABC}	14.96 ± 2.47^{ABC}	
8.5	4.70 ± 0.84	6.07 ± 1.15	7.02 ± 1.70	8.55 ± 2.30	10.86 ± 1.80^{AB}	9.48 ± 1.74^{A}	
8	6.50 ± 0.72	6.13 ± 0.73	6.23 ± 0.86	7.39 ± 0.60	10.13 ± 0.91^{A}	9.62 ± 1.54^{A}	MS
7.5	11.90 ± 1.59	14.04 ± 2.29	16.47 ± 3.02^{A}	13.08 ± 3.09	12.73 ± 2.84	12.60 ± 1.91	
8	8.51 ± 0.97	11.76 ± 1.95	12.64 ± 2.73^{A}	10.62 ± 1.57	16.27 ± 2.87^{A}	15.48 ± 2.01^{ABCD}	DG
8.5	6.32 ± 1.20	10.05 ± 2.23	9.01 ± 2.26	8.43 ± 2.15	12.51 ± 1.74^{A}	13.20 ± 2.20^{ABD}	
6.5	11.32 ± 2.17	8.49 ± 1.96	6.43 ± 1.36	6.66 ± 1.28	4.55 ± 0.89	5.19 ± 0.96^{A}	
7	13.36 ± 2.25	12.75 ± 2.33	12.15 ± 2.40	9.74 ± 1.68	6.93 ± 1.13^{BC}	7.29 ± 1.24^{AB}	
8	8.51 ± 0.97	11.76 ± 1.95	12.64 ± 2.73	10.62 ± 1.57	16.27 ± 2.87^{AD}	15.48 ± 2.012^{A}	CA1
8.5	6.32 ± 1.20	10.05 ± 2.23	9.01 ± 2.26	8.43 ± 2.15	12.51 ± 1.74^{A}	13.20 ± 2.20^{A}	

ANOVA including the 6 days of training was significant. Values are the mean ± SEM. A, B, C, and D show significant differences compared with days 1, 2, 3 and 4, respectively. P < 0.05.

mentioned days were considered [$F_{(30,\ 165)} = 2.194, P = 0.0009$]; with increases in the RP for the 7.5 and 8 Hz frequencies. In addition, in the CA1 region, the RP showed significant changes across days [$F_{(30,\ 165)} = 1.750, p = 0.0146$] for the frequencies 6.5, 7.5, and 8 Hz (**Figure 5**), the **Table 2** shows the significant differences in the two regions. In summary, the EXP group had minimal changes related to the process of leaning evident only when the comparisons included only the days 1, 2, 5, and 6. Moreover, the increased RP observed was limited to SUM and CA1 and occurred at 7.5 and 8 Hz, whereas no change was evident in this group at 8.5 Hz.

The mean peak frequency of each day of training from the four daily trials was obtained, and intra-group comparisons were made using ANOVA for blocks. The CTR group significantly increased the peak frequency in the SUMn [$F_{(5,\ 30)} = 60.061$, $p < 0.001$]; however, paired comparisons (Tukey's test) did not show significant differences compared with day 1. Additionally, the peak frequency in the DG increased with the day of training [$F_{(5,\ 30)} = 4.611$, $p = 0.003$]; the peak frequency increased on days 5 ($p = 0.004$) and 6 ($p = 0.034$) compared with the first day of training. The EXP group did not show increase in the peak frequency across training days in any region. Finally,

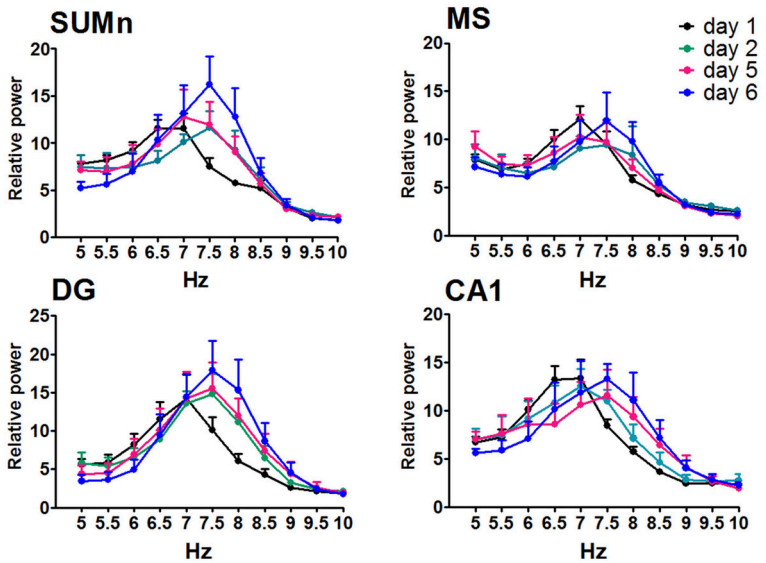

FIGURE 5 | Relative power in the theta band (5–10 Hz) recorded in the four cerebral regions of the EXP group during the searching of the platform across training days. The ANOVA was significant only when compared the days 1, 2, 5, and 6. Mean ± SEM. Significant differences are listed in **Table 2**. $p < 0.05$.

TABLE 2 | Comparison between training days of the relative power recorded during the searching for the platform in the Morris water maze task in the EXP group.

Hz\Day	1	2	3	4	5	6	Region
7.5	7.49 ± 0.93	11.60 ± 1.81	12.96 ± 2.20	10.18 ± 1.75	11.92 ± 2.47	16.25 ± 2.96^{AB}	SUM
8	6.72 ± 0.32	9.28 ± 2.07	9.35 ± 1.84	9.31 ± 2.80	9.01 ± 1.71	12.81 ± 3.01^{A}	
6.5	13.22 ± 1.45	10.86 ± 1.76	10.88 ± 1.94	9.09 ± 2.50	8.60 ± 2.15^{A}	10.18 ± 2.75	
7.5	8.46 ± 0.66	10.99 ± 1.20	11.36 ± 2.13	11.13 ± 1.70	11.57 ± 2.68	13.30 ± 1.53^{A}	CA1
8	5.79 ± 0.47	7.16 ± 1.42	8.26 ± 1.94	8.13 ± 1.70	9.41 ± 2.68	11.14 ± 2.83^{A}	

ANOVA including the days 1, 2, 5, and 6 of training was significant. Values are the mean ± SEM. A, B, C, and D, show significant differences compared with days 1, 2, 3, and 4; respectively. P < 0.05.

the Pearson correlation of the peak frequency between pairs of regions across all training days was calculated, to establish whether the changes in peak frequency were similar between them, both in control conditions and after serotonin depletion in the SUMn. In the CTR group, the peak frequencies were positively and significantly correlated between the SUMn and hippocampus (both the CA1 and the DG), between the MS and the hippocampus (both the CA1 and the DG), and between the SUMn and the MS; although no significant correlation in peak frequency was observed between the CA1 and the DG (**Figure 6**). The EXP group, however, showed high positive correlations between peak frequencies of the SUMn and the hippocampus (both the CA1 and the DG), but no significant correlations were observed between the MS and the hippocampus nor between the SUMn and the MS; moreover, this group showed significant correlation in the peak frequency within the hippocampus (the DG and the CA1) (**Figure 7**). These results imply that the peak frequency of the EEG in the CTR group is related in the three structures (SUMn, MS, and hippocampus), but no relation exists within the hippocampus; in contrast, in the EXP group a closer relation occurs between the SUMn and the hippocampus with a disengagement of MS.

Coherence was compared between training days and frequency using ANOVA for repeated measures, for each group. In the CTR group no significant change was observed in the coherence between regions regarding the training days when all 6 days of training were included [$F_{(50,\ 330)} = 1.052$, $p = 0.3852$ for MS-DG; $F_{(50,\ 330)} = 1.335$, $p = 0.741$ for MS-CA1; $F_{(50,\ 330)} = 0.8018$, $p = 0.8281$ for MS-SUMn; $F_{(50,\ 330)} = 1.036$, $p = 0.4131$ for DG-CA1; $F_{(50,\ 330)} = 0.8514$, $p = 0.7519$ for DG-SUMn; and $F_{(50,\ 330)} = 1.030$, $p = 0.4236$ for CA1-SUMn]. Using the same rationale used in the RP comparisons, ANOVA tests were applied for the days 1, 2, 5, and 6 of training, and significant effects of the training days over time were thus observed for the coherence between MS-DG [$F_{(30,\ 198)} = 1.788$, $p = 0.0104$] and MS-CA1 [$F_{(30,198)} = 1.609$, $p = 0.0300$]. Paired comparisons (t-test with Bonferroni correction) showed significant increased coherence for both MS-DG MS-CA1 coherence on days 5 and 6 principally in the higher frequencies of the theta band, the coherences of MS-CA1 and MS-DG, for the

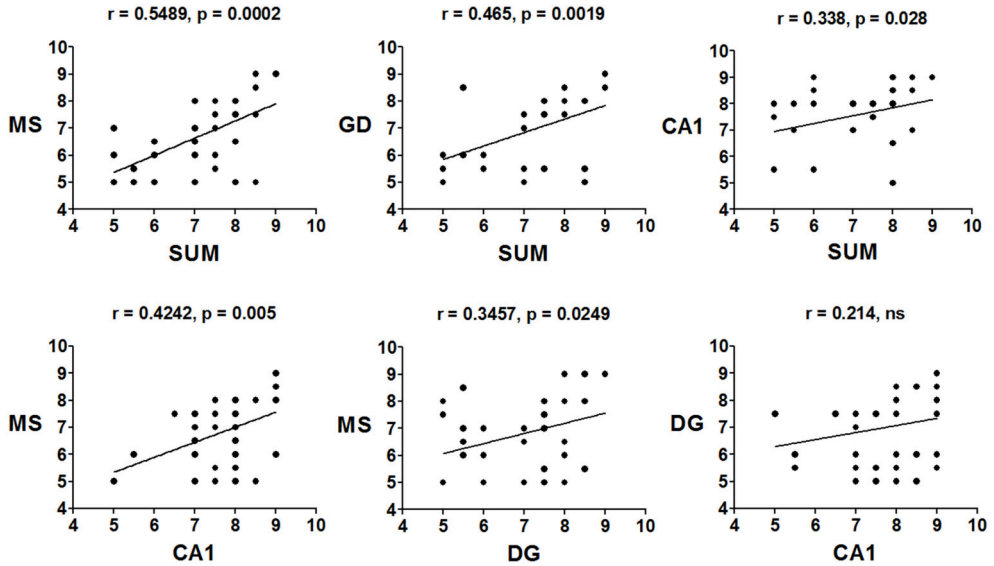

FIGURE 6 | Correlations of the mean frequency peak of the RP in the theta band (5–10 Hz) between cerebral regions, across training days, in the CTR group. Significant positive correlations between the three regions (MS, SUMn and Hippocampus), but not within the hippocampus (DG and CA1) were observed (ns, no significant).

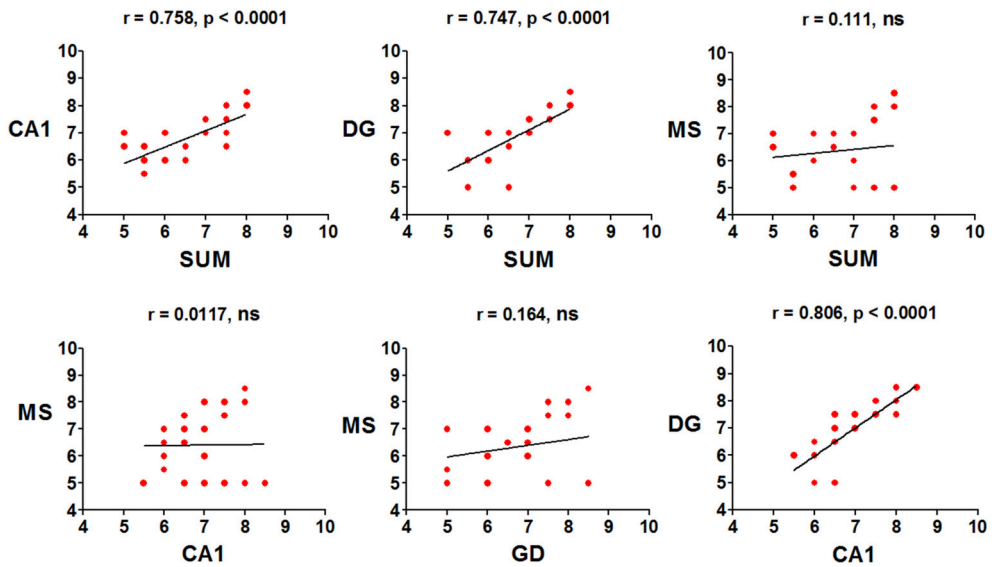

FIGURE 7 | Correlations of the mean frequency peak of the RP in the theta band (5–10 Hz) between recorded regions, across training days, in the EXP group. Significant positive correlations between the SUMn and the hippocampus and within the hippocampus (DG and CA1), were observed. No correlation between the MS and the other two regions occurred in this group (ns, no significant).

CTR group are presented in the **Figure 8**, means and significant differences are presented in the **Table 3**.

The EXP group coherences were also compared considering day and frequency using ANOVA for repeated measures. No significant effects of the training in the inter-region coherences were observed for the EXP group when all six training days were considered [$F_{(50,\ 275)} = 0.5824$, $p = 0.9887$ for MS-DG; $F_{(50,\ 275)} = 0.6685$, $p = 0.9567$ for MS CA1; $F_{(50,\ 275)} = 0.4951$, $p = 0.9983$ for MS-SUM; $F_{(50,275)} = 0.8108$, $p = 0.8130$ for DG-CA1; $F_{(50,\ 275)} = 0.5119$, $p = 0.9974$ for DG-SUM; $F_{(50,275)} = 0.4132$, $p = 0.9998$ for CA1-SUM], nor when only days 1, 2, 5, and 6 were considered. Coherences of MS-DG and MS-CA1 EEG, from the EXP group are shown in **Figure 8**.

In order to know if a shift occurred through the training days in the frequency in which the peak of coherence occurred (frequency of the coherence peak, FCP), the FCP and the

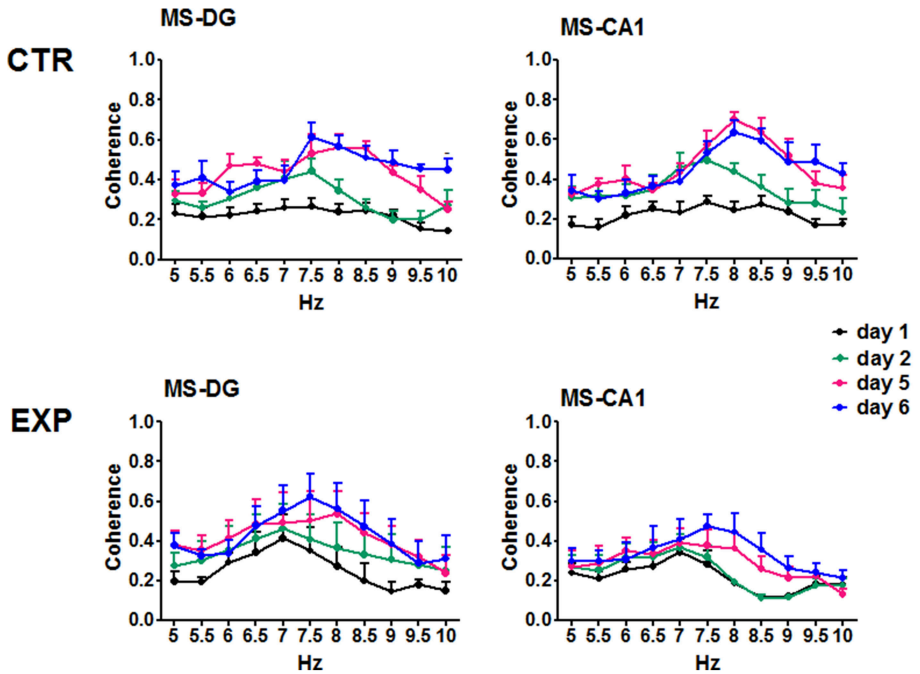

FIGURE 8 | Coherences of the EEG recorded in the MS, DG and CA1 in the two groups across training days. For the CTR group the ANOVA was significant only considering the days 1, 2, 5, and 6. Only CTR group showed increased coherence between MS-DG and MS-CA1. Mean ± SEM. Significant differences are listed in **Table 3**. $p < 0.05$.

TABLE 3 | Comparison between training days of the coherence between the EEG recorded during the searching for the platform in the Morris water maze task in the CTR group.

Hz\Day	1	2	3	4	5	6	Regions
5.5	0.214 ± 0.043	0.260 ± 0.027	0.311 ± 0.056	0.376 ± 0.070	0.331 ± 0.050^{A}	0.409 ± 0.084	
6	0.222 ± 0.037	0.306 ± 0.029	0.295 ± 0.053	0.352 ± 0.040	0.469 ± 0.059^{A}	0.338 ± 0.048	
6.5	0.241 ± 0.036	0.360 ± 0.085	0.336 ± 0.055	0.290 ± 0.035	0.480 ± 0.029^{A}	0.391 ± 0.053	
7.5	0.266 ± 0.038	0.442 ± 0.062	0.405 ± 0.074	0.438 ± 0.100	0.530 ± 0.094^{A}	0.613 ± 0.068^{A}	
8	0.238 ± 0.039	0.344 ± 0.056	0.374 ± 0.089	0.465 ± 0.082	0.561 ± 0.062^{AB}	0.565 ± 0.056^{AB}	MS-DG
8.5	0.246 ± 0.034	0.259 ± 0.043	0.368 ± 0.068	0.429 ± 0.063	0.558 ± 0.029^{AB}	0.510 ± 0.056^{AB}	
9	0.221 ± 0.027	0.198 ± 0.043	0.317 ± 0.072	0.332 ± 0.060	0.433 ± 0.035^{AB}	0.486 ± 0.060^{AB}	
9.5	0.156 ± 0.028	0.200 ± 0.043	0.228 ± 0.050	0.351 ± 0.067	0.349 ± 0.067^{A}	0.454 ± 0.020^{AB}	
10	0.144 ± 0.017	0.271 ± 0.073	0.195 ± 0.044	0.371 ± 0.058	0.251 ± 0.038	0.448 ± 0.056^{A}	
5.5	0.159 ± 0.038	0.319 ± 0.062	0.295 ± 0.084	0.349 ± 0.067	0.377 ± 0.026^{A}	0.301 ± 0.035	
7	0.235 ± 0.049	0.461 ± 0.071^{A}	0.533 ± 0.050	0.411 ± 0.084	0.432 ± 0.045	0.389 ± 0.055	
7.5	0.288 ± 0.028	0.494 ± 0.055	0.611 ± 0.045	0.529 ± 0.088	0.572 ± 0.070^{A}	0.530 ± 0.060^{A}	
8	0.246 ± 0.040	0.437 ± 0.039	0.563 ± 0.048	0.532 ± 0.075	0.700 ± 0.037^{AB}	0.635 ± 0.059^{A}	
8.5	0.275 ± 0.043	0.363 ± 0.057	0.458 ± 0.058	0.494 ± 0.076	0.634 ± 0.076^{AB}	0.594 ± 0.062^{AB}	MS-CA1
9	0.238 ± 0.045	0.280 ± 0.069	0.451 ± 0.092	0.455 ± 0.080	0.517 ± 0.085^{AB}	0.486 ± 0.096^{AB}	
9.5	0.168 ± 0.028	0.279 ± 0.065	0.328 ± 0.088	0.385 ± 0.069	0.379 ± 0.059^{A}	0.489 ± 0.083^{A}	
10	0.175 ± 0.023	0.235 ± 0.066	0.440 ± 0.071	0.375 ± 0.060	0.357 ± 0.061	0.429 ± 0.051^{A}	

ANOVA including the days 1, 2, 5 and 6 of training was significant. Values are the mean ± SEM. A and B, show significant differences compared with days 1, and 2; respectively. P < 0.05.

magnitude of the peak of coherence were compared between days of training in both groups of animals. The CTR group MS-DG, MS-CA1, and DG-SUMn FPCs, showed increases, whereas the EXP group did not show changes. In the magnitude of the peak of coherence all pairs of regions showed increases in the CTR group, whereas in the EXP group only MS-CA1, MS-SUMn, and DG-SUMn showed increase with the training. Intergroup comparison showed higher FCP in CA1-SUM and MS-CA1, and

higher magnitude of the peak for MS-CA1 for the CTR group (Figure S1). Thus, the EEG of the MS and hippocampus increased in coherence with the establishment of the memory in the CTR group but not in the EXP group.

Inter group comparisons of the coherence between pairs of regions were made using an ANOVA for repeated measures considering the factors group and frequency as independent and the training days (1–6) as repeating. MS-CA1 coherences showed significant effects both for the interaction of frequency and group [$F_{(1,\ 138)} = 17.726$, $p < 0.0001$] and for the interaction of frequency, group and day [$F_{(5,\ 690)} = 2.478$, $p = 0.031$]. Paired comparisons between frequency and group showed higher coherence between MS-CA1 regions for the CTR group from 7.5 to 10 Hz compared with the EXP group. When paired comparisons considering the training day were made, the EXP group showed lower coherences across days one to five, on day 1 in the 8.5 Hz frequency, on day 2 in the 8 and 8.5 Hz frequencies, on day 3 in the 7.5–10 Hz frequencies, on day 4 in the 9 Hz frequency and on day 5 in the 8–9 Hz frequencies. Moreover, CA1-SUMn coherences showed a significant effect of the interaction between frequency and group [$F_{(1,\ 138)} = 7.182$, $p = 0.008$], paired comparisons showed lower coherences for the EXP group in the 8.5 and 9 Hz frequencies than for the CTR group (**Figure 9**). The EXP group differed from the CTR group in both the pattern and degree of coherence.

DISCUSSION

The participation of the SUMn in place learning and memory has been controversial, with some studies reporting no or minimal effect on spatial learning and memory, after inactivation or inhibition of SUMn, and other studies implying the participation of the SUMn in retention and consolidation of spatial reference memory (Pan and McNaughton, 1997; Santin et al., 2003; Shahidi et al., 2004a; Aranda et al., 2008; Gutiérrez-Guzman et al., 2012). It was reported that lidocaine infusion into the SUMn did not affect the acquisition of an avoidance task although retention was impaired when lidocaine was infused before training. Additionally, post-training infusion caused impairments in consolidation of memory in this task (Shahidi et al., 2004b). Shahidi et al. (2004b) evaluated the effects of SUMn inactivation on spatial reference memory and spatial working memory using a Morris maze with a training schedule of 8 daily trials for 3 days. The authors did not observe alterations in reference memory when inactivation was performed before training; this implies that participation of SUMn is not crucial in the acquisition of spatial reference information. However, the author observed deficiencies when the SUMn was inactivated after the training buy prior to the probe trial. The present results showed severe impairment in spatial reference memory after SUMn serotonin depletion such that no significant reduction in the distances traveled was achieved by this group, and although the animals eventually attained a significant reduction in their escape pathways, this group searched similarly throughout the four quadrants in the probe trial. It could be interpreted that no learning was achieved by these animals based on the absence of reductions in the path lengths over the six training days; however, it was evident from the latencies in escape and the lengths of the pathways that these animals performed intermittently, presenting good performance on one trial or day and on the next trial or day and performing as badly as on the first day of training (see **Figure 2**). Thus, in spite of the severe deficiencies, the serotonin depletion did not appear to completely impair the acquisition of the spatial reference information. The deficiencies in spatial learning in the present work could be related to the impaired consolidation across days of training, according to the Shahidi results; however, some information could be acquired, although it is uncertain if the SUMn serotonin-depleted rats would have reached the control performance level with more training days. Together with the previous report in which serotonin depletion of both the SUMn and the PHn induced deficient but not absent place learning, these results support the participation of SUMn in spatial memory consolidation. Although we cannot exclude the participation of the PH, the present results show that only SUMn serotonin depletion produced deficiencies in place learning, similar to the results observed after the simultaneous serotonin depletion of the two nuclei, supporting a principal role for the SUMn in place learning.

In the present work, changes in RP were observed in CA1 theta activity, consistent with the previous studies. In addition, in the present work, evidence showed similar changes in the SUMn, that is, the decrease in RP at low frequencies (6–7 Hz) and the increase in RP at high frequencies (7.5–8.5 Hz) across training days; these changes were possibly related to the consolidation of spatial information. Furthermore, extending the brain regions previously recorded, the RP also increased in the DG (8 and 8.5 Hz), although no reduction of low frequencies was observed in the RP on this region comparing all days of training. In order to explore a possible difference in the last day compared with the first, in low frequencies, these 2 days were compared, and significant reduction was observed at 6 and 6.5 Hz frequencies the day six with respect to the first day of training. Finally, MS RP increased only in the 8 Hz frequency, this last could be an effect of broadening of the spectrum. Minor changes were evident in the EXP group only when days 1, 2 and 5, 6 were compared.

Recently a reduction in CA1 theta activity was reported in rats exposed to unexpected environmental changes, whereas they developed foraging activity (Jeewajee et al., 2008). As mentioned previously, in earlier studies assessing the relationship of theta activity with place learning ability, it was reported that the RP of the high frequency theta band (6.5–9.5 Hz) recorded in the CA1 region increased over training days in intact rats trained in the Morris spatial test, and these increases were absent in rats trained in egocentric and cue versions of the task and in aged inefficient rats (8–10). If the increased RP at high frequencies observed in the present work were due to the novelty effect for exposure of the rats to the new environment, the three groups of animals trained in the aforementioned study would have shown similar changes in their theta RP throughout the training, but only rats trained in the tasks demanding hippocampal participation presented changes in theta expression. Moreover, the changes observed in the RP in the CTR group in this study were prominent on days 5 and 6 of training, whereas changes associated with familiarity with the environment must be evident

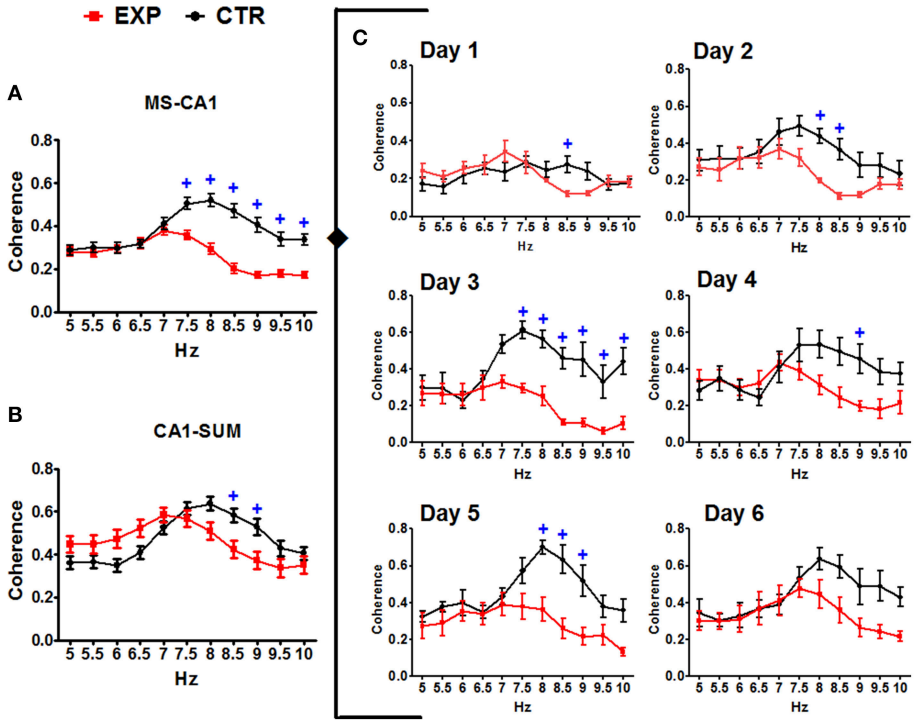

FIGURE 9 | Coherences between the EEG recorded in the MS-CA1 and CA1-SUMn, in the two groups. Inter-group comparisons in the MS-CA1 coherence (A) and in CA1-SUMn coherence (B), were observed (main effect). MS-CA1 coherence was also different through the days of training **(C)**. Mean ± SEM. +, Group CTR vs. group EXP. $p < 0.05$.

on the first days of training. Although learning could be divided into stages (essentially for the purposes of the study), learning and the consolidation of learning could occur throughout the entire training of the rats in long-term paradigms, such as the water maze. In this paradigm, there is no clear threshold indicating when the animal is learning and when it is recovering information learned in previous trials or on previous days of training; although the acquisition of more precise information continues occurring and allows the animal to develop over the days direct pathways toward the platform, it is logical to suppose that, over the training days, both consolidation of some information (spatial, motor, proprioceptive) acquired in the first trials or days could occur, whereas other information is acquired. Moreover, the recovery of the previously acquired information could occur from trial to trial or day to day of training. Thus, the different weights of the place learning processes presumably occurred at different times; it is reasonable to assume that higher acquisition of information occurred during the first 2 days, and higher consolidation and recovery of information occurred on the last 2 days, supporting the view that the processes occurred simultaneously. As in the previous work, in which depletion of SUMn/PH was realized (Gutiérrez-Guzman et al., 2012), a lack of learning-related changes in theta activity for RP was observed during processing of spatial information, not only in CA1 but also in all of the regions recorded.

The SUMn serotonin-depleted rats failed to show the increase in high-frequency theta RP related to changes over time during training, this was evident both in the peak frequency and in the RP. This failure could imply that serotonin participates in the SUMn-driven regulation of hippocampal frequency. In anesthetized rats, it was observed that neuronal SUMn theta-related firing predicted the changes in theta activity in hippocampus when sensorial stimulation occurs and also in brief episodes of theta when acceleration in frequency occur; however, the hippocampus drives the SUMn activity during spontaneous theta trains (Kocsis and Kaminski, 2006). Additionally, it has been previously observed that the ascending influence of the SUMn on hippocampal theta is not required for the occurrence theta, but it was proposed that the SUMn coding of theta frequency becomes relevant when there is a high degree of processing of information (Kirk and Mackay, 2003). Based on the absence of differences in AP through the days, we can hypothesize that changes in RP associated to learning may be caused by the same population of neurons tuning their synaptic oscillations within the range of the theta band, from lower to higher frequencies, effect that was absent in the EXP group. Thus, an increase in RP of one hertz (e.g., 8 Hz) could occur when, in fact, their power increased or when the power of all of the other frequencies decreased, or the two phenomena occurred simultaneously whatever the mechanism, it implies the predominance of high frequency activity.

The changes in RP coherence in CTR rats could reflect increased communication between the MS and hippocampus possibly related to consolidation of spatial information and

recovery of the same. In accordance, it was reported that hippocampus weakly conduced SUMn activity during the initial training, in a 1-day test of spatial learning (16 trials), whereas during the last trials of training the direction of the influence inverted so that the SUMn directed the hippocampal activity, which was also associated with an increase in coherence between the two regions during the last training trials, when the consolidation of information takes place (Ruan et al., 2011). Differences in the training paradigm could account for this because in the present work, 4 daily trials were given to the rats and more gradual process of consolidation could be occurring in comparison with the collapsed training (16 trials) in 1 day. However, in the present work, we did not observe increased coherence between the SUMn and hippocampus across training days in control rats, but the frequency of the peak of coherence for DG-SUMn increased with the days, to be significant on day 6; moreover increased coherence was evident between the MS and the hippocampus (DG and CA1) on days in which consolidation occurred more preferentially (days 3–6); and increase in the frequency of the coherency peak occurred for MS-DG (gradual but significant on day 6).

Thus, the learning of the spatial task was accompanied by changes in power in all regions recorded and increased coherence between MS and the hippocampus across training days in CTR animals; these changes were absent in the EXP group. Surprisingly, MS theta activity did not show changes in relation to the SUMn in coherence, and the RP increased only in the 8 Hz frequency across training days, this was unexpected in view of the modulator role of the SUMn on MS activity. The absence of increases in the RP of high-frequency theta in the SUMn serotonin-depleted rats as well as the absence of increases in coherence between the MS and the hippocampus could underlie the inefficient performance of these animals. In support of this idea, the peak frequency showed in each region was highly correlated in CTR animals (even though scant direct connection has been reported between the SUMn and the CA1) (Haglund et al., 1984), whereas in the EXP group the RP peak frequency between MS and the other two regions was unrelated, and there were highly correlated peak frequencies within the hippocampus (DG with CA1) and between hippocampus and the SUMn. This result, together with the minor coherence between the MS and the CA1 and the MS and the DG in SUMn serotonin-depleted animals (compared with the CTR group) would imply a reduced communication between the MS and the hippocampus caused by the withdrawal of the SUMn serotonin influence. The influence of SUMn could be necessary to entrain the information flux in the MS-hippocampus circuit, during consolidation of memory, and the absence of serotonin appears to alter the fine-tuning of the SUMn activity required for this purpose. Instead, the EXP animals appear to be entrained in a closed circuit between the hippocampus and SUMn, and this would impair the consolidation of memory (**Figure 10**).

The SUMn receives projections from medial and lateral mammillary (MM) nuclei (Gonzalo-Ruiz et al., 1992), and the MM theta-related rhythmic firing originates from a descendent influence from the hippocampus, whereas SUMn theta influences ascend through the input received from the RPOn and TPPn (Kocsis and Vertes, 1994; Kirk et al., 1996; Kirk, 1997). Although in the present work we cannot rule out the possibility of some leakage of 5,7-DHT to the adjacent MM region, the descendent origin of MM theta supports the idea that the changes observed in the EXP group are mainly due to SUMn serotonin depletion. Moreover, the role of the MM in place learning has been evaluated, and no changes in a spatial reference memory similar to those reported in the present work were observed, although, mild to severe deficiencies occurred after total or partial MM lesions when spatial working memory was implicated, e.g., in T maze delayed tasks and in Morris maze and radial arm maze working memory tasks (Santin et al., 1999; Vann and Aggleton, 2003; Vann, 2005). A previous study reporting place reference memory deficits after MM lesions included animals that suffered bilateral destruction of the SUMn in addition of the MM damage (Sutherland and Rodriguez, 1989); moreover, increased cFos expression occurred in the medial MM nucleus after a working memory task but not after a spatial reference memory task (Santin et al., 2003). Thus, it is unlikely that the deficiencies observed in spatial reference memory in Morris maze in the present work were due to serotonin depletion that extended into the MM.

The SUMn is monosynaptically connected to the DG in a segregated pattern, the lateral SUMn synapses with the dorsal DG and the medial SUMn synapses with the ventral DG (Ohara et al., 2013), where it makes glutamatergic and GABAergic/Glutamatergic contacts both on granule cells and on GABAergic neurons (Nitsch and Leranth, 1996). The SUMn also sends glutamatergic afferents to the CA2/CA3 regions of the hippocampus (Soussi et al., 2010), and is also reciprocally connected to the MS (Vertes, 1988, 1992), through a glutamatergic input onto cholinergic and GABAergic MS neurons and a GABAergic descending input from the lateral septum (LS) onto the lateral SUMn (Leranth and Kiss, 1996). Unlike the abundance of knowledge about the connectivity of the SUMn, there is scant information about the serotonergic projections to and receptors, through which serotonin influences the neuronal activity, on the SUMn. A moderate concentration of serotonergic terminals was reported to project to the lateral SUMn and slightly denser concentration was reported in the medial SUMn (Moore et al., 1978; Vertes and Martin, 1988; Vertes et al., 1999). In addition, the presence of $5HT_{1C}$ and $5\text{-}HT_2$ receptors, particularly the 5-HT2A receptor with a moderate density both on the soma and on dendrites of neurons, has been reported on the SUMn (Wright et al., 1995; Cornea-Hébert et al., 1999). Whereas the effect of SUMn manipulations on CA2/CA3 is relatively unexplored, CA1 pyramidal excitability is suppressed and theta activity activated by SUMn carbacol microinjections (Jiang and Khanna, 2006) and SUMn stimulation increases the population of spikes in the DG evoked by stimulation of the perforant pathway in anesthetized rats (Mizumori et al., 1989). Additionally, the SUMn is known to modulate septal cell firing and hippocampal theta frequency in anesthetized rats, in which procaine injection into SUMn produced the attenuation of both frequency and amplitude of hippocampal theta (Kirk and McNaughton, 1993);

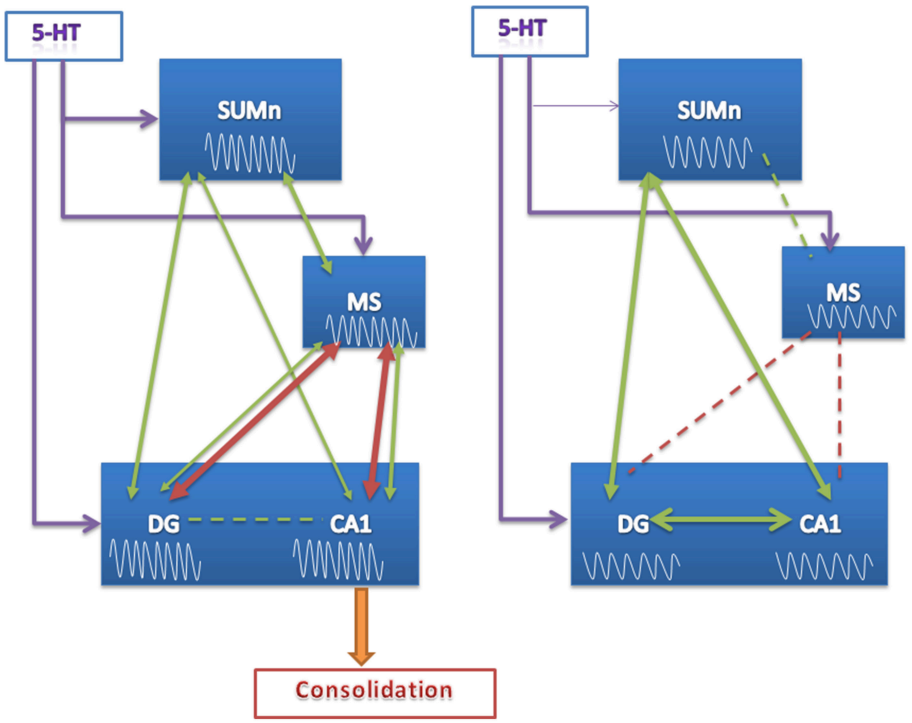

FIGURE 10 | Proposed model of function of the SUMn, the MS and the hippocampus circuit during learning after SUMn serotonin-depletion. The model represents the results observed principally the last days of training. EEG RP values are represented into the boxes, showing that no increase in high frequency RP was observed in the EXP group. Green arrows represent values of frequency peak; red arrows represent values of coherence. The thickness of the arrows represent the grade of coherence or correlation; broken lines represent the lack of coherence or correlation. When 5-HT was reduced in the SUMn, the circuit appear to entrain into a closed mode between SUMn and hippocampus. The lack of communication between MS and CA1 would impair the consolidation of memory.

however, it was observed that the electrolytic lesioning of the SUMn did not affect the movement-related theta frequency in behaving rats (Thinschmidt et al., 1995). In this manner, it is highly speculative attempt to explain what could be the consequence of reduced serotonin on the electrical activity at the neuronal level in the SUMn and the repercussion on the MS. Although it is possible support that the tuning of theta during movement-related information processing (e.g., place learning) and not the movement-related theta could be disrupted in SUMn serotonin-depleted rats, this remains speculative. To our knowledge, no other evidence of SUMn modulation of theta activity during learning in awake rats exists; however, whatever the effect, the present results support the role of the serotonin acting on the SUMn, in the modulation of the hippocampal theta activity underlying the processing of spatial information and in the consolidation of this information.

In conclusion, reduction of serotonin in the SUMn produced deficiencies in place learning ability and altered pattern of hippocampal, septal, and SUMn theta learning-related activity, in the rat.

AUTHOR CONTRIBUTIONS

All authors participated in the experimental design, experimental work and data analysis. In addition, JH and MO participated in the redaction of the final article and all four authors discussed the contents and interpretations of the work.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: http://journal.frontiersin.org/article/10.3389/fphar.2015.00250

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Serotonin, neural markers, and memory

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Diverse neuropsychiatric disorders present dysfunctional memory and no effective treatment exits for them; likely as result of the absence of neural markers associated to memory. Neurotransmitter systems and signaling pathways have been implicated in memory and dysfunctional memory; however, their role is poorly understood. Hence, neural markers and cerebral functions and dysfunctions are revised. To our knowledge no previous systematic works have been published addressing these issues. The interactions among behavioral tasks, control groups and molecular changes and/or pharmacological effects are mentioned. Neurotransmitter receptors and signaling pathways, during normal and abnormally functioning memory with an emphasis on the behavioral aspects of memory are revised. With focus on serotonin, since as it is a well characterized neurotransmitter, with multiple pharmacological tools, and well characterized downstream signaling in mammals' species. 5-HT_{1A}, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors as well as SERT (serotonin transporter) seem to be useful neural markers and/or therapeutic targets. Certainly, if the mentioned evidence is replicated, then the translatability from preclinical and clinical studies to neural changes might be confirmed. Hypothesis and theories might provide appropriate limits and perspectives of evidence.

Keywords: memory, drugs, neural markers

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Introduction

It should noted that while, memory formation and forgetting are functions of the brain (e.g., Fioravanti and Di Cesare, 1992; Wagner and Davachi, 2001; Wixted, 2004; Mansuy, 2005; Hardt et al., 2013; Hupbach, 2013; Callaghan et al., 2014; Li et al., 2015a); in contrast, diverse neuropsychiatric disorders present dysfunctional memory (Meyer-Lindenberg et al., 2012; Millan et al., 2012, 2014). AD is popular brain alteration presenting memory deficits and dementia and the leading cause of dementia, and a major public health priority; but dysfunctional memory is observed in other age-related neurodegenerative disorders, schizophrenia, post-traumatic stress disorder, strokes, etc. (Millan et al., 2014; Hashimoto, 2015). Certainly, no effective treatment for dysfunctional memory exists (e.g., Millan et al., 2012, 2014; Sun et al., 2015); likely due to the absence of neural markers associated to memory. Hence, memory, amnesia, forgetting (e.g., Tellez et al., 2012b) and AD (e.g., McConathy and Sheline, 2015; Muenchhoff et al., 2015; also Scarr et al., 2015) as well as mild cognitive impairment (MCI) (Eshkoor et al., 2015) require neural markers.

Certainly, AD is a very complex neuropsychiatric disorder, where memory becomes progressively dysfunctional (e.g., Solodkin and van Hoesen, 1997; Rodríguez et al., 2012) resulting in amnesia and dementia. In contrast, forgetting is unintentional process characterized as a failure

to remember information or a rather strategic function of the brain that helps to reduce interference in the processing or retrieval of relevant information (Ludowiq et al., 2010). Likewise, forgetting as a physiological phenomenon occurs all the time (see McGaugh, 2013; see also Davis, 2010; Berry et al., 2012; Hardt et al., 2013; Kaku et al., 2013; Li and Richardson, 2013; Papenberg et al., 2013). However, the pharmacological and neuroanatomical bases of forgetting or memory have been little explored and as diverse neuropsychiatric disorders present dysfunctional memory, we are aiming potential neural markers.

For instance, phrasing neural markers and brain functions in PubMed (May 7, 21 and 29 or June 2, 2015) yield 318 or 319 (including 50 review papers) publications. Hence, herein, aiming clues about mapping neural markers link to cerebral functions and dysfunctions. Mainly memory formation, dysfunctional memory, and as forgetting, which has been little explored respect to neural markers. In spite of promissory findings, to our knowledge, no previous systematic works have been published addressing these issues. It should be noted that of the revised papers, several are rich in backgrounds and perspectives.

Examples illustrating the interaction among behavioral tasks (**Box 1**), control groups and molecular changes and/or pharmacological effects are mentioned in the following lines. Importantly, behavioral parameters, drug-treatment and cognitive processes interact in mammals (see below) and invertebrate species (e.g., Chen et al., 2014). Particularly the role of serotonin in memory: interactions with neurotransmitters and downstream signaling might be useful (e.g., Seyedabadi et al., 2014; Eskenazi et al., 2015). Although the focus herein are adult mammal animals; notwithstanding, important recent advances in invertebrate species, include Monje et al. (2013) reporting that flotillin-1 is an evolutionary-conserved memory-related protein up-regulated in implicit and explicit learning paradigms; thus, translational approach—from invertebrates to rodents—led to the identification of flotillin-1 as an evolutionary-conserved memory-related protein.

Actually, serotonin has pharmacological tools and well characterized downstream signaling in mammals' species (e.g., Marin et al., 2012; Borroto-Escuela et al., 2015; McCorvy and Roth, 2015); then serotonin and other neural markers are used for studying cerebral functions and dysfunctions (e.g., Tomie et al., 2003; Wellman et al., 2007; Cavallaro, 2008; Marcos et al., 2008; Da Silva Costa-Aze et al., 2012; Ménard and Quirion, 2012; Reichel et al., 2012; Rodríguez et al., 2012; Woods et al., 2012; Haahr et al., 2013; Alabdali et al., 2014; Freret et al., 2014; Kitamura et al., 2014; Kondo et al., 2014; Lecoutey et al., 2014; Leger et al., 2014; Seyedabadi et al., 2014; Leiser et al., 2015; Suzuki and Lucas, 2015; Westrich et al., 2015; Zilles et al., 2015). Evidence is organized according with 5-HT markers (i.e., receptors and transporter) but markers of other neurotransmission systems are included. Importantly, using well-established 5-HT neural markers (Blenau and Baumann, 2015; Lau et al., 2015; Müller and Homberg, 2015) might provide insights about known and novel markers and therapeutic targets. Müller and Homberg (2015) are providing an excellent analysis regarding 5-HT markers, drug use and addiction.

Memory Tasks and Molecular Changes

Memory Decline across Aging

Ménard and Quirion (2012) using the Morris Water Maze (MWM) task, distinguish aged rats in two groups—memory-impaired (AI) and memory-unimpaired (AU) relative to 6-months old adult animals. Dysfunctional memory was associated to increased metabotropic glutamate receptors 5 (mGluR5) in hippocampal post-synaptic densities (PSD) (**Table 1**); Ménard and Quirion (2012) conclude that in successful cognitive aging (i.e., AU animals) present a critical role for mGluR5, Homer 1 proteins and downstream signaling pathways. Certainly, in terms of signaling respect to cognition-enhancing drug targets, insights are emerging (e.g., Seyedabadi et al., 2014; Gyurko et al., 2015; Ménard et al., 2015; Sun et al., 2015).

Autism: Neuro-inflammation and Neurotransmission Impairment

Although, Alabdali et al. (2014) did find that serotonin or dopamine in platelet-free plasma not correlated with social and cognitive dysfunction. It should be noted that serotonin has multiple markers (see below). And, several neurochemical parameters might show sensitivity and specificity; thus contributing to earlier and more accurate diagnosis of dysfunctional memory in disease such autism, AD, and the identification of effective treatments (e.g., Sheline et al., 2014a,b; Strac et al., 2015).

5-HT Systems

As already mentioned, serotonin (5-hydroxytryptamine, 5-HT) is one of the neurotransmitter well characterized in mammal species (e.g., Hoyer et al., 1994; Saulin et al., 2012; Borroto-Escuela et al., 2015; McCorvy and Roth, 2015), it has multiple neural markers, including receptors (i.e., 5-$HT_{1A/1B/1D}$, 5-$HT_{2A/2B/2C}$, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors) and transporter (named SERT) as well as volume transmission. These 5-HT markers are present in brain areas involved in memory (e.g., Buhot et al., 2003a,b; Puig and Gulledge, 2011; Rodríguez et al., 2012; Barlow et al., 2015; Leiser et al., 2015), sentence compression (Zilles et al., 2015) and drug addiction (Müller and Homberg, 2015).

Serotonergic Gene Regulation during Learning and Memory

In an elegant work, Cavallaro (2008) using DNA microarrays analyzed hippocampal 5-HT receptors in two behavioral memory tasks and different times (**Table 2**); observing differential expressions in 12 receptors (Htr1a, Htr1b, Htr1d, Htr1f, Htr2a, Htr2c, Htr3a, Htr4, Htr5a, Htr5b, Htr6, and Htr7). At least Htr2c, Htr3a and Htr6 receptors had significant changes relative to swimming control animals and water maze trained animals. Htr2c expression was reduced at 1 h and increased at 24 h following training. Htr3a-mRNA was increased at 24 h, whereas Htr6 was decreased at 6 h; as observed in autoshaping (see below). In passive avoidance task, three 5-HT receptors showed changes in expression respect to naive and trained animals (i.e., conditioned animals, CA). Indeed, the expression of Htr3a was

increased, whereas those of Htr1b and Htr4 were decreased. Certainly, expression of 5-HT receptors were also observed in control groups subjected to physical activity and mild stress (naive vs. swimming controls in the water maze; naive vs. CSTA and USTA in passive avoidance); notwithstanding, memory consolidation produced different magnitudes (e.g., Htr2c in the water maze) often opposite trends than in control animals (e.g., Htr3a in both water maze and passive avoidance). Producing cumulative patterns of gene expression, associated to time and 5-HT subtype receptor (see Cavallaro, 2008). Importantly, apparently water maze memory requires slight 5-HT_7 receptor expression within 1-h; and passive avoidance memory involves expression of 5-HT_{1A-1F}, 5-HT_{2A}, and 5-HT_{5A} receptors. Of course, remaining to determine if the suppression of the other 5-HT receptors is necessary. Certainly, the molecular requirements differ between water maze and passive avoidance.

TABLE 1 | Memory task and molecular changes: unimpaired vs. impaired aging vs. adult rats.

Function/ dysfunction	Major findings	References
Following MWM training	Brain area: hippocampal (CA1)	Ménard and Quirion, 2012
Groups		
AI	-AI dysfunctional memory, ↑ in hippocampal (CA1) mGluR5 in PSD -Hippocampal up-regulated Homer 1a and 1b/c levels PSD	
AU	-AU had enhanced mGluR5 as well as Homer 1b/c stainings. - AU had higher PKCc, ERK, p70S6K, mTOR, and CREB activation. - AU higher expression of immediate early gene Arc/Arg3.1.	

MWM, Morris Water Maze; Aging AU, memory unimpaired; aging impaired memory (IM), PSD, post-synaptic densities.

Notably, Zaldivar and Krichmar (2013) observe in behaviorally naïve (i.e., untrained) animals, neurotransmitters changing including 5-HT receptors expression in areas regarded to neuromodulation or memory (amygdala); revealing connectivity and receptor localization, and patterns of expression among neurotransmission systems, receptors and brain areas.

5-HT_{1A} Receptor

Although 5-HT_{1A} receptor may serve as a biomarker for cognitive functioning and target for treatment of cognitive impairment; notwithstanding hitherto evidence remains sparse and inconsistent (Borg, 2008; Borg et al., 2009). Certainly, the situation is changing; e.g., Yoshimi et al. (2014) report that brexpiprazole, presents serotonin-dopamine activity, and 5-HT_{1A} receptor partial agonism, attenuates phencyclidine-induced cognitive deficits; an effect blocked by the selective 5-HT_{1A} receptor antagonist WAY-100,635 (which alone has no effect). Yoshimi et al. (2014) conclude that brexpiprazole could ameliorate cognitive deficits in schizophrenia and other neuropsychiatric diseases. Contrasting findings exist regarding the 5-HT_{1A} partial agonists (e.g., buspirone), which alone impair memory in normal subjects (Meneses, 1999) but some of them (e.g., tandospirone) might be useful in the treatment of schizophrenia pathophysiology (Sumiyoshi et al., 2008). And, as tandospirone (e.g., Baba et al., 2015) also has anti-amnesic effects or facilitate performance in difficult memory tasks; hence, 5-HT_{1A} partial agonists might be useful in the treatment of dysfunctional memory.

Certainly, while if 5-HT_{1A} receptor agonists, partial agonists, or antagonists might be used for memory alterations (e.g., Meneses and Perez-Garcia, 2007; Pittalà et al., 2015); functional selectivity or biased agonism is revealing important insights regarding 5-HT_{1A} and 5-HT_{3A} receptors (e.g., Vardy and Kenakin, 2014; McCorvy and Roth, 2015). For instance, van Goethem et al. (2015) study "biased," 5-HT_{1A} receptor agonists in a novel object pattern separation task (relative to episodic memory); showing that by preferentially activating post-synaptic

TABLE 2 | 5-HT_{1A} receptor.

Function/dysfunction	Findings:			
Memory tasks	**Water maze**	**Passive avoidance**	**Fear conditioning[h, i]**	**Pavlovian autoshaping[a]**
			genetic variability within 5-HTR_{1A} (rs6295)	Pavlovian/Instrumental autoshaping[b,d]
	memory impairment and variations in expression[e]	expression[c]	modulation of expression[i]	expression
recovery from dissociative amnesia	increase of 5-HT_{1A} receptor in cortical regions[g]			
object-location associations	lower right than left hippocampal binding potential is related to better memory performance[j]			
Morris water maze memory retrieval	expression[f]			

Tomie et al., 2003[a]; Luna-Munguía et al., 2005[b]; Cavallaro, 2008[c]; Perez-Garcia and Meneses, 2009[d]; Li et al., 2015b[d]; Saroja et al., 2014[f]; Kitamura et al., 2014[g]; Baas and Heitland, 2014[h]; Sase et al., 2015[i]; Glikmann-Johnston et al., 2015[j].

TABLE 3 | 5-HT $_{1B}$ receptor.

Function/dysfunction	Findings	References
Following Groups		
5-HT$_{1B}$ receptor KO	Exhibit a task-dependent selective learning facilitation; indeed, selective facilitation/impairment depending on the cognitive demand and/or age-related decline in spatial learning (water maze) abilities	Buhot et al., 2003a,b; Wolff et al., 2003
Aggressive social model	High 5-HT1B receptor density in the BLA to predict high levels of aggression in observer rats	Suzuki and Lucas, 2015
Expression	Positive correlations in control subjects between creative ability and average 5-HT$_{1B}$ receptor availability in gray matter	Varrone et al., 2015

BLA, basolateral amygdala.

5-HT$_{1A}$ heteroreceptors, or raphe-nuclei autoreceptors are potential novel molecular targets for improving memory. Likewise, Stroth et al. (2015) report that arylpiperazine ligands of 5-HT$_{1A}$ receptor preferentially affect cAMP signaling vs. β-arrestin-2 recruitment; proposing the development of signaling pathway-selective drugs targeting this receptor.

Notably, recovery from dissociative amnesia increases cortical 5-HT$_{1A}$ receptor (Kitamura et al., 2014; **Table 3**). Likewise, memory in autoshaping task (see **Box 2**) also increases 5-HT$_{1A}$ receptor expression in 14 brain areas, but decrements in 7 and no changes in 12 (**Table 3**); suggesting that upregulated, down-regulated, and "silence" 5-HT$_{1A}$ receptor in brain areas form part of neural circuits engaged in memory formation; thus demonstrating a high degree of specificity and memory mapping.

Importantly, Glikmann-Johnston et al. (2015) report that hippocampal human asymmetry in 5-HT$_{1A}$ receptor expression (using [^{18}F] MPPF binding), accompanies memory for object-location associations; lower right than left hippocampal binding potential is related to better memory performance (**Table 2**). Aubert et al. (2013) also report that the dual 5-HT$_{1A/7}$ receptor agonist 8-OH-DPAT increased transcription of adenylate cyclase 1 in the hippocampus (CA1), suggesting that memory function could play a role in altered pairmate interaction dynamics; and these changes might be caused by 8-OH-DPAT-induced up- or down-regulation of 5-HT$_{1A}$ and 5-HT$_7$ receptor in the medial prefrontal cortex and in the hippocampus (CA1), respectively; and according with Aubert et al. (2013); and such as hypothesis is supported by rodent studies that implicate 5-HT$_7$ function in contextual learning and memory consolidation.

On the other hand, genetic variability within 5-HT$_{1A}$ receptor (rs6295) is associated with contextual fear independent (**Table 3**) (Baas and Heitland, 2014). Likewise, Weber et al. (2015) report that conditional inactivation of the GLUA1-encoding Gria1 gene selectively in 5-HT neurons of adult mice (i.e., Gria1 5-HT-/- mice) exhibited a distinct anxiety phenotype but showed no alterations in locomotion, depression-like behavior, or learning and memory. Importantly, contextual fear task increases hippocampal AMPA-, GluN1- and 5-HT$_{1A-}$ containing receptor complexes (Sase et al., 2015) (**Table 3**). In addition, Saroja et al. (2014) studied spatial memory retrieval and hippocampal monoamine receptor (MAR) complexes (including 5-HT$_{1A}$ and 5-HT$_7$ receptors, and dopamine D1 and D2 receptors and colocalizations) in mice of 3–12 and 18 months. D1, D2, and 5-HT$_7$ containing receptor complex levels were decreasing with age while 5-HT$_{1A}$ receptor-containing complex was increased. In addition, the time spent in the target quadrant (i.e., memory retrieval) correlated with D1, 5-HT$_7$, and 5-HT$_{1A}$ receptors complex expression. Saroja et al. (2014) conclude that individual monoamine receptors are linked to spatial memory retrieval and are modulated by age. This same group (Subramaniyan et al., 2015) reports that the receptor complex levels containing hippocampal GluN1 and GluN2A of NMDARs, GluA1 and GluA2 of AMPA receptors, nAch7 and the D1A dopamine receptors were elevated during spatial learning, whilst levels of GluA3 and 5-HT$_{1A}$ receptor containing complexes were reduced. Thus, supporting that 5-HT$_{1A}$ receptor is useful neurobiological marker of memory.

Pavlovian Autoshaping: 5-HT$_{1A}$ and 5-HT$_2$ Receptors (Binding Sites)

Interestingly, Tomie et al. (2003), studied the effects of experience with Pavlovian autoshaping procedures (**Box 2**) on lever-press conditioned response (CR) performance and ^{3}H-8-OH-DPAT-labeled binding of 5-HT$_{1A}$ and probably 5-HT$_7$ (it should be noted that this drug has affinity for 5-HT$_7$, see below; **Table 3**); as well as ^{125}I-LSD-labeled binding of 5-HT$_{2A}$ receptors were evaluated in four groups of rats. The groups (Paired High CR and Paired Low CR) received Pavlovian autoshaping procedures wherein the presentation of a lever (conditioned stimulus, CS) was followed by the response-independent presentation of food (unconditioned stimulus, US). Group Paired High CR showed more rapid CR acquisition and higher asymptotic levels of lever-press autoshaping CR performance relative to Group Low CR. Group Omission received autoshaping with an omission contingency, such that performing the lever-press autoshaping CR resulted in the cancelation the food US, while Group Random received presentations of lever CS and food US randomly with respect to one another. Though Groups Omission and Random did not differ in lever-press autoshaping CR performance, Group Omission showed significantly lower levels of 5-HT$_{1A}$ binding in post-synaptic areas (frontal cortex, septum, caudate putamen), as well as significantly higher plasma corticosterone levels than Group Random. In addition, Group Random showed higher levels of 5-HT$_{1A}$ binding in pre-synaptic somatodendritic autoreceptors on dorsal raphe nucleus relative

to the other three groups. Autoradiographic analysis of 5-HT_{2A} receptor binding revealed no significant differences between Groups Paired High CR and Paired Low CR or between Groups Omission and Random in any brain regions. Notably, although extensive Pavlovian autoshaping training (Tomie et al., 2003) failed to produce any correlation between 5-HT_{1A} or 5-HT_{2A} receptor expression and CR; however, regardless the number of CR, Tomie et al. (2003) demonstrated correlation between both receptors expression and paired CS–US presentations. These data are also indicating that the neuroanatomical, neurochemical, and behavioral basis of Pavlovian and Pavlovian/Instrumental Autoshaping (P/I-A) are different (see **Box 2**). Although the latter could be considered as an instance of system processing styles (i.e., S-S, S-R, and stimulus-reinforcer [S-Rf] learning; see White and McDonald, 2002); nevertheless, the association of CR and 5-HT markers (Tomie et al., 2003) is replicated (Pérez-García et al., 2006; Pérez-García and Meneses, 2008). Notably, similar associations are observed in the Morris Water Maze and passive avoidance tasks (Cavallaro, 2008). Hence, the evidence supports the notion that 5-HT_{1A} receptor provides diverse neurobiological markers, pharmacological and genetic tools that have been used to investigate a variety of functions and dysfunctions (for references Meneses and Liy-Salmeron, 2012). Likewise, 5-HT_{1A} receptor also is therapeutic target, it seems to be useful for detecting functional and dysfunctional memory, and co-expression with other neurotransmission systems and serotonergic receptors.

5-$HT_{1B/1D}$ Receptor

The Buhot et al. (2003a,b; Wolff et al., 2003) seminal work (see also Drago et al., 2010) showed that 5-HT_{1B} receptor knockout mice exhibit a task-dependent selective learning facilitation; depending on the cognitive demand and/or age-related decline of spatial learning abilities (**Table 4**). In addition, pharmacological evidence indicates a possible involvement of hippocampal CA1 5-$HT_{1B/1D}$ and 5-$HT_{2A/2B/2C}$ receptors in harmaline-induced amnesia (Nasehi et al., 2014a). And 5-HT_{1B} receptor activation disrupts delayed alternation (DAL) performance in mice (Woehrle et al., 2013) and chronic fluoxetine pretreatment blocks 5-HT_{1B} receptor- induced deficits; suggesting a 5-HT_{1B} receptor modulation in orbitofrontal-dependent DAL. The 5-HT_{1B}-induced DAL deficits may provide a model for obsessive compulsive disorder (OCD; Woehrle et al., 2013). The above evidence is consistent with the possibility that 5-HT_{1B} receptor inverse agonists might be useful for reversing memory deficits (e.g., Meneses, 2001; Meneses and Tellez, 2015). Importantly, 5-$HT_{1B/1D}$ receptor expression in the frontal cortex is correlated to memory impairment (Garcia-Alloza et al., 2004). Certainly, Drago et al. (2010) highlight that 5-HT_{1B} receptor is a candidate modulator of the mnemonic and motivationally related symptoms in psychiatric illnesses. Moreover, positive correlations exist between creative ability and 5-HT_{1B} receptor expression in gray matter of control subjects; as well as in Parkinson disease (PD) patients between depression and creative ability (Varrone et al., 2015); importantly, PD patients have poor semantic memory and creative ability (Varrone et al., 2015).

Neurobiological Mechanisms in the Observational Learning of Aggression

Suzuki and Lucas (2015) report that chronic passive exposure to aggression modifies expression of D2 receptor in the nucleus accumbens core (AcbC) and shell (AcbSh), and 5-HT_{1B} receptor in the medial (MeA), basomedial (BMA), and basolateral (BLA) amygdala. And increased aggressive behavior reduced D2 receptor in bilateral AcbSh. Likewise, regardless of exposure aggression length 5-HT_{1B} receptor was augmented in bilateral BLA. Finally, low D2 receptor expression in the AcbSh significantly interacted with high 5-HT_{1B} receptor density in the BLA, predicting high levels of aggression in observer animals (**Table 4**). Suzuki and Lucas (2015) conclude that the dopamine-serotonin or AcbSh-BLA interactions; may be risk factors for aggression in observers chronically witness aggressive interactions (Suzuki and Lucas, 2015). Clearly, 5-HT_{1B} receptor expression was useful in detecting learning and memory of aggression.

5-$HT_{2A/2B/2C}$ Receptors

Li et al. (2015a) report that 5-HT_{2A} receptor is highly expressed in the medial septum-diagonal band of Broca complex (MS-DB), especially in parvalbumin (PV)-positive neurons linked to hippocampal theta rhythm (involved in normal and dysfunctional memory of PD). The medial forebrain bundle (MFB) lesions impaired working memory, hippocampal theta, decreased firing rate and density of MS-DB PV-positive neurons, rhythm, and DA levels in septohippocampal system and medial prefrontal cortex (mPFC). Intra-MS-DB injection of the 5-HT_{2A} receptor agonist 4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl) methylamine hydrobromide (TCB-2) enhanced working memory, producing the opposite effects in control and lesioned and shortening TCB-effects; implicating dysfunctional 5-HT_{2A} receptor. Li et al. (2015a) conclude that unilateral lesions of the MFB induced working memory deficit, and activation of MS-DB 5-HT_{2A} receptor enhanced working memory, and involve monoamine levels in the hippocampus and mPFC. In addition, in a controlled cross-over PET study using a delayed match-to-sample task and the 5-HT_{2A} receptor antagonist [^{18}F] altanserin, Hautzel et al. (2011) report a cognition-induced modulation of serotonin in the orbitofrontal cortex (OFC). Importantly, Tomie et al. (2003) demonstrated an association between 5-HT_{2A} receptor expression and memory formation in Pavlovian autoshaping task. In addition, individual differences in impulsive action and 5-HT_{2A} receptor cortical variations have been noted (Fink et al., 2015). Also, D2 and 5-HT_{2A} receptors present genetic variants and modulate physiological prefrontal cortex efficiency during working memory and response to antipsychotics (Blasi et al., 2015). Moreover, although an association between 5-HT_{2A} receptor polymorphism (his452tyr) and memory performances in AD has been proposed; no differences in verbal memory were identified by Guglielmi et al. (2015).

Importantly, Barlow et al. (2015) report markers of serotonergic function in the orbitofrontal cortex and dorsal raphé nucleus predicting individual variation in spatial-discrimination serial reversal learning. These authors conclude

TABLE 4 | 5HT $_{2A/2B/2C}$ receptor.

Function/dysfunction	Findings		References
Parkinson disease	MS-DB 5-HT$_{2A}$ receptor activation enhanced WM, which may be due to changes in the activity of septohippocampal network and monoamine levels in the hippocampus and mPFC		Li et al., 2015a
Memory (match-to-sample task)	Cognition-induced modulation of serotonin in the OFC: PET study of 5-HT$_{2A}$		Hautzel et al., 2011
Memory (Pavlovian autoshaping)	5-HT$_{2A}$expression and CR(Pavlovian autoshaping) association		Tomie et al., 2003
Spatial-discrimination serial reversal learning	Individual variations of 5-HT$_{2A}$ in the OFC and dorsal raphé nucleus		Barlow et al., 2015
Dopamine2 and 5-HT$_{2A}$ receptor variants	DRD2 and HTR2A genetic variants together modulate physiological prefrontal efficiency during working memory and also modulate the response to antipsychotics		Blasi et al., 2015
Fmr1 KO mice (model of fragile X syndrome);	Combinations of 5-HT$_{2B}$ or D1-Rs or 5HT$_{2A}$ or D2-Rs (low doses)	Enhance Ras-PI3K/PKB signaling input, GluA1-dependent synaptic plasticity and learning in Fmr1 KO mice; without causing anxiety related side effects	Lim et al., 2014
5-HT$_{2B}$ receptor expression	*Htr2B* $^{-/-}$ mice, as shown by deficits in sensorimotor gating, in selective attention, in social interactions and in learning and memory processes		Pitychoutis et al., 2015
Against epilepsy induced memory decline	Combined action at MT1/2 and 5HT$_{2C}$ receptors, reduced the depolarization-evoked release of glutamate, strong neuroprotective action and possible antioxidant properties of agomelatine		Vimala et al., 2014
Chronic microwave-induced cognitive deficit	Variations of 5-HT$_{1A}$ and 5-HT$_{2C}$ receptors expressions		Li et al., 2015b

WM, working memory; OFC, orbitofrontal cortex; mPFC, medial prefrontal cortex; CR, conditioned responses; MT, melatonin.

that rats in the upper quintile of the distribution of perseverative responses during repeated S-R reversals have significantly reduced levels of the 5-HT metabolite, 5-hydroxy-indoleacetic acid, in the OFC. Additionally, 5-HT$_{2A}$ receptor expression in the OFC of mid- and high-quintile rats was significantly reduced compared with rats in the low-quintile group. These perturbations were accompanied by an increase in the expression of monoamine oxidase-A (MAO-A) and MAO-B in the lateral OFC and by a decrease in the expression of MAO-A, MAO-B, and tryptophan hydroxylase in the dorsal raphé nucleus of highly perseverative rats. Barlow et al. (2015) found no evidence of significant differences in markers of DA and 5-HT function in the DMS or MAO expression in the ventral tegmental area of low- vs. high-perseverative rats; indicating that diminished serotonergic tone (probably, at least via 5-HT$_{2A}$ receptor) in the OFC may be an endophenotype that predisposes to behavioral inflexibility and other forms of compulsive behavior (Barlow et al., 2015).

Moreover, Lim et al. (2014) investigated mechanisms of action of psychoactive drugs that modestly benefit the cognitive performance in fragile X patients (the most common form of inherited mental retardation); reporting that compounds activating 5HT$_{2B}$ receptor (5HT$_{2B}$) or dopamine (DA) subtype 1-like receptors (D1-Rs) and/or those inhibiting 5HT$_{2A}$ or D2 receptors moderately enhance Ras-PI3K/PKB signaling input, GluA1-dependent synaptic plasticity, and learning in Fmr1 knockout mice (Lim et al., 2014). Unexpectedly, combinations of these 5-HT and DA compounds at low doses synergistically stimulate Ras-PI3K/PKB signal transduction and GluA1-dependent synaptic plasticity and remarkably restore normal learning in Fmr1 knockout mice without causing anxiety-related side effects. Lim et al. (2014) suggest that properly dosed and combined psychoactive drugs may effectively treat the cognitive impairment associated with fragile X syndrome. In addition, Htr2B$^{-/-}$ mice show deficits in sensorimotor gating, selective attention, social interactions as well as in learning and memory (i.e., fear conditioning and novel object recognition: STM and LTM) (Pitychoutis et al., 2015).

Regarding 5-HT$_{2C}$ receptor, Vimala et al. (2014) highlight that epilepsy affects negatively cognitive function, producing depression, anxiety, etc. Mentioning among other issues that agomelatine is a novel antidepressant acting as melatonin MT1 and MT2 receptor agonist and 5-HT$_{2C}$ receptor antagonist; producing reduction in the depolarization-evoked release of glutamate, strong neuroprotective action and possible antioxidant effects (Vimala et al., 2014); producing hippocampal neuronal cell survival and neurogenesis, neuroprotective effect in hippocampus and frontal cortex and the antioxidant potential may contribute to the protective action of agomelatine against epilepsy induced memory decline (Vimala et al., 2014). In addition, Walker and Foley (2010) report that administration of the 5-HT$_{2C}$ inverse agonist mianserin impaired autoshaped operant response on day 2 than any other agent tested. In addition, decreasing the length of the acquisition session to 1-h augmented the difficulty of the autoshaping task further modulating the consolidation effects produced by the 5-HT$_{2C}$ ligands (Walker and Foley, 2010). Moreover, Li et al. (2015b) report that repeat exposition to 2.856 GHz microwaves (averaging 5–30 mW/cm^2) affects spatial learning and

TABLE 5 | $5HT_3$ receptor antagonist, neuroprotection.

Function/dysfunction	Findings	References
AD	Tropisetron, a potent α7 nAChR agonist and 5-HT_3 receptor antagonist, also bound to the ectodomain of amyloid precursor protein. Furthermore, tropisetron promoted greater improvements in memory current AD therapeutic drugs AD. In addition, tropisetron represents an attractive potential therapeutic drug to delay or prevent MCI and AD. This drug is also used for the treatment of chemotherapy-induced emesis	Hashimoto, 2015 Fakhfouri et al., 2014
Aβ rat model of AD in MWM	- Tropisetron might have a neuroprotective effect; tropisetron attenuated Aβ-induced hippocampal neuroinflammation	Hashimoto, 2015
	Subtypes of 5-HT_3 receptor	Thompson, 2013
KO 5-HT_{3A} receptor	Loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement	Kondo et al., 2014

AD, Alzheimer's disease; MCI, middle cognitive impairment; MWM, Morris water maze.

memory function, morphology structure of the hippocampus, electroencephalogram (EEG) and neurotransmitter content (amino acid and monoamine); including expression of 5-$HT_{1A\ 2A,\ and\ 2C}$ receptors. Li et al. (2015b) demonstrated that chronic exposure to microwave could induce dose-dependent deficit of spatial learning and memory and inhibition of brain electrical activity, the degeneration of hippocampus neurons, and the disturbance of neurotransmitters; including hippocampal and cortical expression of 5-HT_{1A} and 5-HT_{2C} receptors.

Importantly, 5-$HT_{2A/2B/2C}$ receptors are useful detecting learning and memory changes and drug effects. Aloyo et al. (2009) remind us of inverse agonism at 5-HT_{2A} and 5-HT_{2C} receptors.

5-HT_3 Receptor

5-HT_3 receptor antagonists (e.g., tropisetron, ondansetron) have a long dated antiamnesic effects, including attenuation of age-associated memory impairment (e.g., Costall and Naylor, 1992; see also Shimizu et al., 2013). Recent evidence, from preclinical studies suggests that the interaction between amyloid-β peptides (Aβ) and the α7 nicotinic acetylcholine receptor (α7 nAChR) (Hashimoto, 2015) (**Table 5**). And tropisetron is also a α7 nAChR agonist and 5-HT_3 receptor antagonist; binding to amyloid precursor protein and enhancing memory in AD patients (**Table 5**). Importantly, 5-HT_3 receptor antagonists have been useful in treatments such as chemotherapy-induced emesis to neuroprotection (Fakhfouri et al., 2014; Hashimoto, 2015). Certainly, subtypes of 5-HT_3 receptor exist (Thompson, 2013); and their mechanisms are complex. For instance, Kozuska et al. (2014) deal with the multiple salt bridges in the intracellular domain of the $5HT_{3A}$ receptor and these interactions increase the overall rigidity of the receptor, stabilize its low conducting state and affect the ligand cooperativity; suggesting that the allosteric effects of these regions on the receptor may be involved in a possible "reverse" allosteric modulation of $5HT_3$ receptor. In addition, it should be noted that agonist- and antagonist-induced up-regulation of surface 5-HT_{3A} receptor (Morton et al., 2015).

Moreover, Kondo et al. (2014) studied 5-HT_{3A} receptor subunit-deficient (htr3a-/-) mice revealing loss of exercise-induced hippocampal neurogenesis and antidepressant effects, but not of learning enhancement (**Table 5**). Kondo et al. (2014) conclude that the 5-HT_3 receptor is the critical target of 5-HT action in the brain following exercise, being indispensable for hippocampal neurogenesis and antidepressant effects induced by exercise.

5-HT_4 receptor

It should be noted that earlier evidence indicated that 5-HT_4 receptor decreased in AD (see Eglen et al., 1995). Activation of 5-HT_4 receptor has pro-cognitive effects on memory tasks (e.g., Bockaert et al., 2011; Peñas-Cazorla and Vilaró, 2014; Ramirez et al., 2014; Claeysen et al., 2015). Notably, Madsen et al. (2011) observe cerebral 5-HT_4 receptor up-regulation starts at a preclinical stage of dementia and it continues while dementia is still at a mild stage and these authors speculate that this upregulation may be a compensatory effect of decreased levels of interstitial 5-HT, increase acetylcholine release or to counteract Aβ accumulation and improved cognitive function. Hippocampal 5-HT4 receptor expression correlates inversely with human memory (Haarh et al., 2013; **Table 6**). Also, old rats have decreased 5-HT4 receptor expression and poor memory relative to adult (**Table 6**).

In addition, evidence suggests that serotonergic activity, via 5-HT4 receptors in hippocampal, striatum, and cortical areas, mediates memory function and provides further evidence for a complex and regionally specific regulation over 5-HT receptor expression during memory formation (Manuel-Apolinar et al., 2005).

Segu et al. (2010) report adaptive changes in cholinergic systems, which may circumvent the absence of 5-HT_4 receptor to maintain long-term memory under baseline conditions. In contrast, despite of adaptive mechanisms, the absence of 5-HT_4 receptor aggravates scopolamine-induced memory impairments. The mechanisms whereby 5-HT_4 receptor mediates a tonic influence on ChAT activity and muscarinic receptors remain to be determined (Segu et al., 2010). Restivo et al. (2008)

TABLE 6 | 5HT$_4$ receptor.

Function/dysfunction	Findings	References
Memory	Activation has promnesic effects in rodents and humans	Haahr et al., 2013; Peñas-Cazorla and Vilaró, 2014
Mechanisms in cognition	Increased dendritic spines in the CA1 region of the hippocampus. Neuronal activity and increased release of acetylcholine in the prefrontal cortex and hippocampus. It is not synthesized in cholinergic cells Pre-training SL65.0155 enhances olfactory memory discrimination, inducing hippocampal growth dendritic spines; suggesting that selective 5-HT$_4$ stimulation increases structural plasticity in learning activated hippocampal circuits	Restivo et al., 2008; Marchetti et al., 2011; Peñas-Cazorla and Vilaró, 2014
Changes with age	Old rats decreased 5-HT$_4$ expression and poor memory relative to adult	Waeber et al., 1996; Manuel-Apolinar et al., 2005; Marchetti et al., 2011
Memory	Hippocampal 5-HT$_4$ expression correlates inversely with memory in humans.	Haahr et al., 2013
AD	This receptor and β-amyloid protein are present in early stages of AD	Madsen et al., 2011

AD, Alzheimer's disease.

highlight that pharmacological modulation of synaptic efficacy is a prominent target in the identification of promnesic compounds and that pre-training administration of the 5-HT$_4$ receptor partial agonist SL65.0155 enhances olfactory discrimination and potentiates learning-induced dendritic spine growth in the mouse hippocampus; without affecting spine density in the pseudo-trained mice and, by itself, it does not promote spine growth. Likewise, the 5-HT$_4$ receptor antagonist RS39604 prior to SL65.0155 prevents both improved memory and additional formation of spines; thus confirming the 5-HT$_4$ receptor specificity of the observed effects (Restivo et al., 2008); and these authors conclude that 5-HT$_4$ receptor stimulation selectively increases experience-dependent structural plasticity in learning-activated hippocampal circuits.

Marchetti et al. (2011) have also highlighted that in developing rats as well as in rats ranging from 3 to 9 months of age, significant modifications of 5-HT$_4$ receptor expression have been observed (for references see Marchetti et al., 2011). These same authors propone that the poor memory formation observed in aged rats (Marchetti et al., 2011). And corresponding decreases in 5-HT$_4$ receptor expression in brain areas (e.g., hippocampus, amygdala, etc.) involved in memory formation, could explain improved memory, dendritic spines (Restivo et al., 2008), neuronal excitability and release of the neurotransmitter acetylcholine (Ach) (see Segu et al., 2010; Marchetti et al., 2011; Peñas-Cazorla and Vilaró, 2014). Clearly, 5-HT$_4$ receptor is useful neural marker of dysfunctional and memory formation as well as therapeutic target. Moreover, studying 5-HT expression during memory formation is giving new fresh insights (e.g., Haahr et al., 2013). Importantly, Haahr et al. (2013) report that hippocampal 5-HT$_4$ receptor expression correlates inversely with human memory performance.

5-HT$_5$

As mentioned above, Cavallaro (2008) reported that passive avoidance memory involves expression of several 5-HT receptors, including 5-HT$_{5A}$. 5-HT$_5$ receptor occurs in brain areas implicated in learning and memory. Post-training administration of the 5-HT$_{5A}$ receptor antagonist SB-6995516 decreased CR during short-term (STM; 1.5-h; at 0.1 mg/kg) and long-term memory (LTM; 24-h; at 3.0 mg/kg). Moreover, considering that there are no selective 5-HT$_{5A}$ receptor agonists, next, diverse doses of the serotonin precursor l-tryptophan were studied during STM and LTM, showing that l-tryptophan (5–100 mg/kg) facilitated performance, particularly at 50 mg/kg. In interactions experiments, l-tryptophan (50 mg/kg) attenuated the impairment effect induced by SB-699551 (either 0.3 or 3.0 mg/kg) (Gonzalez et al., 2013). All together this evidence suggests that the blockade of 5-HT$_{5A}$ receptor appear to be able to impair STM and LTM (24 h) in autoshaping task, while its stimulation might facilitate it. Of course further investigation is necessary, meanly with selective 5-HT$_{5A}$ compounds (Gonzalez et al., 2013). Interestingly, Yamazaki et al. (2014, 2015) reported that a 5-HT$_{5A}$ receptor antagonist ameliorates positive symptoms and cognitive impairment in animal models of schizophrenia and in aged rats and induced-amnesia. An analogous case is observed regarding 5-HT$_{1A}$ partial agonists (see above).

Returning to 5-HT$_5$ receptor, Karimi et al. (2013) report that it has long been known that hippocampal spatial memory and the ability to navigate through space are sexually dimorphic traits among mammals, and numerous studies have shown that these traits can be altered by means of sex hormone manipulation. Male and female rat pups were injected with estradiol and testosterone respectively, at early stage of their lives to examine the effect of sex hormone manipulation on mRNA expression of Slc9a4, Nr3c2, Htr5b, and Mas1; among other results, these authors report that expressions of these genes are strongly influenced by sex hormones in both the frontal cortex and hippocampus, especially in male hippocampus, in which expression of all genes were up-regulated. Htr5b was the gene that was affected only in the males (Karimi et al., 2013). Hence, considering the pharmacological evidence mentioned above, probably learning and memory might be affected in these animals.

5-HT$_6$ receptor

Diverse 5-HT$_6$ receptor antagonists produce promnesic and/or antiamnesic effects in conditions, such as memory formation, age-related cognitive impairments; memory deficits in models of diseases such as schizophrenia, PD and AD (e.g., King et al., 2008; Claeysen et al., 2015). However, not all papers report promnesic and/antiamnesic effects of 5-HT$_6$ receptor antagonists (e.g., Thur et al., 2014) (**Table 7**); probably related to timing, drug and memory task used. Memory, aging, and AD modify 5-HT$_6$ receptors and signaling cascades; and 5-HT$_6$ drugs modulate memory, which is accompanied with neural changes. Indeed, in an elegant work Eskenazi et al. (2015) manipulated selectively overexpression of 5-HT$_6$ receptor in either direct or indirect pathway striatal medium-spiny neurons (dMSN and iMSN, respectively), revealing that increased 5-HT$_6$ receptor expression in iMSNs delays instrumental learning and in DLS facilitates behavioral flexibility after habitual responding. It should be noted that 5-HT$_6$ receptor expression decreases during memory (e.g., Huerta-Rivas et al., 2010; Ramirez et al., 2014). In addition, de Bruin and Kruse (2015) suggest that cognition could be improved by 5-HT$_6$ receptor antagonists, by increasing the number of NCAM PSA-immunoreactive neurons in the dendate gyrus, inhibit mTOR and Fyn-tyrosine kinase and interact with DARPP-32.

Notably, 5-HT$_6$ receptor antagonists are, among, serotonergic therapies for cognitive symptoms in AD (e.g., Ramirez et al., 2014). Indeed, Wilkinson et al. (2014) report safety and efficacy of idalopirdine, a 5-HT$_6$ receptor antagonist, in patients with moderate AD. In addition, 5-HT$_6$ receptor is providing new insights about plasticity (Dayer et al., 2015). For example, at early stages of neuronal development, expression of 5-HT$_6$ receptor constitutively regulates the activity of the cyclin-dependent kinase (Cdk) 5 and, through this mechanism, controls cellular processes involved in circuit formation (e.g., neuronal migration, neurite outgrowth). In addition, 5-HT$_6$ receptor modulates developmental targets, including Fyn, Jab1, and mammalian target of rapamycin (mTOR). In therapeutic terms such as blockade of pathological over-activation of the mTOR pathway induced by early life insults in rodents and normalizes the associated social and episodic memory deficits. It should be noted that 5-HT$_6$ receptor and Cdk5; and the latter mediates neuronal differentiation (e.g., hippocampus, striatum) in an agonist-independent manner (Seo and Tsai, 2014). In addition, Ha et al. (2015) report that 5-HT$_6$ receptor directly interacts with SNX14 (protein-coupled receptors/regulators of G protein signaling), which regulates internalization; degradation of 5-HT$_6$ receptor and cAMP production. This finding might be related to the evidence that 5-HT$_6$ receptor agonists and antagonists modulate cAMP production and improve memory formation (e.g., Meneses et al., 2011c). We do not know yet why 5-HT$_6$ receptor agonists and antagonists (e.g., Woods et al., 2012) may facilitate memory or may reverse amnesia in some memory tasks. However, 5-HT$_6$ receptor inverse agonist might be useful (e.g., Hostetler et al., 2014; but see also Benhamú et al., 2014).

TABLE 7 | 5-HT$_6$ receptor.

Function/dysfunction	Findings	References
Memory/models of diseases	Antagonism produce promnesic and/or antiamnesic effects, including memory formation, age-related cognitive impairments; memory deficits in models for diseases such as schizophrenia, Parkinson, and AD	Meneses et al., 2011a; Ramirez et al., 2014; but always see Thur et al., 2014
Memory, aging, and AD	Modify 5-HT$_6$ receptor and signaling cascades	Ramirez et al., 2014
Expression	5-HT$_6$ decreases during memory	Huerta-Rivas et al., 2010; Ramirez et al., 2014
Expression	Overall, increased 5-HT$_6$ receptor expression in iMSNs slowed instrumental learning and in DLS facilitated behavioral flexibility after habitual responding	Eskenazi et al., 2015
Cognitive therapy	Idalopirdine antagonist administration improves memory in patients with moderate AD	Wilkinson et al., 2014; see also Ramirez et al., 2014
Mechanisms	Blocking this receptor decreases over-activation of mTOR when there are insults in early life rodent deficits associated this normalize the social and episodic memory	Dayer et al., 2015
Signaling molecules	Cdk5 activity regulated and controlled by this neuronal migration and neurite outgrowth. Cdk5 modulates the activity of Fyn, Jab1 and mTOR	Dayer et al., 2015
	SNX 14 is an endogenous negative regulator of 5-HT$_6$ receptor, modulating its signaling and trafficking Also, SNX 14 internalizes and degrades 5-HT 6 receptor	Ha et al., 2015

AD, Alzheimer's disease; Cdk5, cyclin-dependent kinase; mTOR, mammalian target of rapamycin; dMSN, direct or indirect, iMSM pathway medium-spiny neurons.

5-HT$_7$ Receptor

Recently Nikiforuk (2015) is providing perspectives of 5-HT$_7$ receptor in the search for treatments for CNS disorders: including normal and dysfunctional serotonin-induced phase shifting of the circadian rhythm control of memory as well as locomotor and exploratory activity, anxiety, depression; and Guseva et al. (2014) about molecular mechanisms responsible for the 5-HT$_7$ receptor-mediated signaling. Gasbarri and Pompili (2014) noted that 5-HT$_7$ receptor antagonism might have antiamnesic effects (see also Horisawa et al., 2013). Gasbarri et al. (2008) suggested that 5-HT$_7$ receptor blockade had procognitive effect, when the learning task implicated a high degree of difficulty. Others report that 5-HT$_7$ receptor agonists facilitate memory and have antiamnesic effects (**Table 8**); remaining clarifying why of the paradoxical effects.

Notably, Saroja et al. (2014), highlight that although evidence about monoamine receptor (MAR) biochemistry and pharmacology in aging exists, work on MAR complexes rather than subunits is limited; in consequence, MAR complexes in hippocampi of three different age groups (3–12 and 18 months) in mice and to link MAR changes to spatial memory retrieval in the water maze were determine (**Table 8**). MAR complexes were separated in order to show the pattern of dopamine and 5-HT$_{1A}$ and 5-HT$_7$ receptors and colocalizations (Saroja et al., 2014). For instance, D1-D2 and 5-HT$_7$ receptors containing receptor complex levels decreased with age while 5-HT$_{1A}$ receptor-containing complex was increasing. D1, 5-HT$_7$, and 5-HT$_{1A}$ receptor complex correlated with good retrieval memory in the water maze; hence, individual monoamine receptors are linked to spatial memory and are modulated by age. However, Beaudet et al. (2015) mention that changes in the level of transcription of the 5-HT$_7$ receptor mRNA did not account for the age-related difference observed at the protein level, at least in hippocampal CA3 region; besides, 5-HT$_7$ receptor might also be putatively subjected, across aging, to modifications in their affinity or to changes in their coupling to G-proteins or other signaling

TABLE 8 | 5-HT$_7$ receptor.

Function/dysfunction	Findings	References
Brain development, autism, depression	Contributes to networks during development and in the mature brain remodel, thus participating in emotion and cognition	Ciranna and Catania, 2014; Guseva et al., 2014; Volpicelli et al., 2014; Nikiforuk, 2015
Memory/amnesia	Apparently 5-HT$_7$ receptor agonists and antagonist might facilitate memory formation and/or have anti-amnesic effects	e.g., Nikiforuk, 2015
Amnesia	Antagonism might have antiamnesic effects	Tajiri et al., 2012; Waters et al., 2012; Horisawa et al., 2013; Nikiforuk et al., 2013; Gasbarri and Pompili, 2014; Westrich et al., 2015
Memory/amnesia	Agonism has procognitive and/or antiamnesic effects	Perez-García and Meneses, 2005; Pérez-García et al., 2006; Costa et al., 2012; Eriksson et al., 2012; Di Pilato et al., 2014; Freret et al., 2014; Ruocco et al., 2014; Meneses et al., 2015
Memory and mRNA expression	Higher level of expression of 5-HT$_7$ receptor mRNAs in autoshaping-trained relative to untrained groups	Pérez-García et al., 2006
Memory time-course	Progressive memory and mRNA 5-HT$_{1A}$ or 5-HT$_7$ receptors expression monotonically augments or declines in prefrontal cortex, hippocampus and raphe nuclei, respectively	Perez-Garcia and Meneses, 2009
Aging and memory	Hypothesis: a decreased expression of 5-HT$_7$ receptor in CA3 hippocampal could account for impairments of the shift between spatial strategies across aging	Beaudet et al., 2015
Signaling	Coupled to a G_s protein, stimulation activates the AC increased cAMP, in addition, 5-HT$_7$ is associated to G12; a small GTPase protein of the Rho family. $G_{\alpha s}$ and $G_{\alpha 12}$are involved in the regulation of TrkB expression by 5-HT$_7$, depending on the model of study	Guseva et al., 2014; Samarajeewa et al., 2014
Monoamine complex and memory	D1, D2 and 5HT$_7$ decreasing together with age, 5-HT$_{1A}$ receptors containing complex MAR increase with age. The receptors MAR, 5-HT$_7$, 5-HT$_{7A}$and D1, correlate with changes in spatial memory, which are modulated by age	Saroja et al., 2014

MWM, Morris Water Maze; MAR, monoamine receptor complex (i.e., D1, D2, and 5-HT$_7$ containing receptors).

pathways. Notably, Beaudet et al. (2015) suggest that **a** decreased expression of 5-HT_7 receptor in CA3 hippocampal could account for impairments of the shift between spatial strategies across aging (**Table 8**).

Moreover, when the time-course (0–120 h) of autoshaped CR is progressive; then mRNA 5-HT_{1A} or 5-HT_7 receptors expression is monotonically augmented or decreased in prefrontal cortex, hippocampus and raphe nuclei, respectively (Perez-Garcia and Meneses, 2009). Hence, 5-HT_{1A} and 5-HT_7 receptors expression might be regulated by the level of memory formation and to be brain areas dependent. Moreover, the cyclic adenosine monophosphate (cAMP) is a second messenger and a central component of intracellular signaling pathways that regulate a wide range of biological functions, including memory (e.g., Kandel, 2001). And progressive time-course of memory formation in an autoshaping learning task (Pérez-García and Meneses, 2008); shows that *ex-vivo* cAMP production from trained and over-trained groups compared to untrained ones, the former group had the highest levels of cAMP and the latter rats showed increased production but less relative to trained rats. Importantly these changes varied according with normal memory or amnesia and brain areas; hence cAMP production is important in the signaling case in mammalian memory formation (Pérez-García and Meneses, 2008).

The above findings should be considered in the context that apparently 5-HT_7 receptor agonists and antagonist (e.g., Nikiforuk, 2015) might facilitate memory formation and/or have anti-amnesic effects. Other interesting recent finding is that according with Rojas et al. (2014) serotonin regulates neurite outgrowth through 5-HT_{1A} and 5-HT_7 receptors in cultured hippocampal neurons. Certainly, De Filippis et al. (2015) highlight that promnesic effects of the 5-HT_7 receptor agonist LP-211 treatment strongly depend on the basal level of performance. Notably, Ruocco et al. (2014) report that 5-HT_7 receptor stimulation improves selective spatial attention and produces permanent changes in several neural markers, including expression of glutamatergic receptors and dopamine transporter (DAT).

Very importantly, 5-HT7 receptor can form heterodimers with 5-HT1A receptors both *in-vitro* and *in-vivo* (see Guseva et al., 2014) and according with these authors, from the functional point of view, heterodimerization decreases Gi-protein coupling of 5-HT1A receptor and attenuates receptor-mediate deactivation of G-protein-gated potassium (GIRK) channels, without substantial changes in the coupling of 5-HT7 receptor to the Gs-protein. Moreover, heterodimerization significantly facilitated internalization of 5-HT1A receptor, while internalization kinetics of 5-HT7 receptor was decelerated upon heterodimerization (see Guseva et al., 2014).

Factors Responsible for Inconsistencies among Laboratories

BOX 1 | Factors responsible for inconsistencies among laboratories.

Certainly, a number of factors might be produce similar results or be responsible for some inconsistencies among laboratories studying memory; which are complex and multi focal; which should provide an analytic framework offering key clues. Indeed, analysis of memory should include behavioral tasks, type of memory, the dynamic hierarchy of neural markers and brain areas involved in memory formation (e.g., Euston et al., 2012; Eskenazi et al., 2015) vs. no training, amnesia, anti-amnesic effects or forgetting (e.g., see below). Likewise, the species and the nature of behavioral task (e.g., appetitively or aversively motivated), curves of behavioral acquisition (i.e., multi-trial or two trials task) or patterns of behavioral responses (progressive vs. all or none response), cognitive demand (easy or difficult task), timing of drug administration (pre-training, post-training or pretest) and kind of drug (e.g., agonist or antagonist), protocols of training and testing together with neurobiological markers (e.g., Duewer et al., 1995; Patton, 1995) accompanying mnemonic processes deserve attention. Among the behavioral memory tasks available (e.g., Peele and Vincent, 1989; Myhrer, 2003; Lynch, 2004); importantly, the implementation of new instruments for measuring memory in behavioral tasks assists in gaining deeper insight into learning and memory processes (e.g., Cook et al., 2004; Walker et al., 2011; Markou et al., 2013; Leger et al., 2014; Wolf et al., 2014).

Neural Transporters, Memory, Forgetting and Drugs

Notwithstanding neurotransmission systems are related to memory formation, amnesia and/or therapeutic targets for memory alterations, the role of transporters γ-aminobutyric acid (GABA, GAT1), glutamate (neuronal glutamate transporter excitatory amino acid carrier; EACC1), dopamine (DAT) and serotonin (SERT) is poorly understood. Emerging evidence indicates that memory formation (short- and long-term memory; STM and LTM, respectively) in a Pavlovian/instrumental autoshaping (see **Box 1**) is associated to up-regulation of prefrontal cortex GAT1 and EAAC1, striatal SERT, DAT and EACC1; while, hippocampal EACC1, GAT1, and SERT are down-regulated (Tellez et al., 2012a,b; **Table 9**; **Figure 1**). Moreover, pharmacological analysis shows that methamphetamine (METH)- induced amnesia down-regulated SERT, DAT, EACC1, and GAT1 in hippocampus and the GAT1 in striatum; no-changes are observed in prefrontal cortex. Fluoxetine (antidepressant, 5-HT uptake inhibitor) improved memory consolidation (particularly LTM), which is associated to DAT, GAT1 (prefrontal cortex) up-regulation, but GAT1 (striatum) and SERT (hippocampus) down-regulation. Fluoxetine plus METH prevented amnesia, which was associated to DAT, EACC1 and GAT1 (prefrontal cortex), SERT and DAT (hippocampus) and EACC1 or DAT (striatal) up-regulation.

Memory Formation/Forgetting and SERT Expression

Forgetting in Pavlovian/instrumental autoshaping is associated to up-regulation of GAT1 (PFC and HIP) and DAT (PFC) while SERT (HIP) is down-regulated; no-changes are observed in striatum (**Table 9**). Methamphetamine alone not affected forgetting but up-regulates hippocampal DAT and EACC, prefrontal cortex DAT and striatal GAT1 or EACC1. Fluoxetine alone prevents forgetting, which is associated to striatal GAT1 and hippocampal DAT up-regulation, but prefrontal cortex GAT1 down-regulation. Fluoxetine plus METH prevent forgetting, which is associated to hippocampal DAT, prefrontal cortex SERT and striatal GAT1, DAT, or SERT up-regulation,

BOX 2 | Autoshaping tasks.

Autoshaping memory tasks have been focus by several research groups (e.g., Brown and Jenkins, 1968; Myer and Hull, 1974; Atnip, 1977; Oscos et al., 1988; Bussey et al., 1997, 2013; Lindner et al., 2003; Vanover et al., 2004; Ballaz et al., 2007; Rodriguez et al., 2008; Walker and Foley, 2010; Walker et al., 2011; Tomie et al., 2012; Krynetskiy et al., 2013; Markou et al., 2013; Gallistel et al., 2014; Holland et al., 2014; Lesaint et al., 2014; Talpos et al., 2014; Eskenazi et al., 2015; Talpos and Shoaib, 2015; in several animal species (e.g., Wasserman, 1981) including humans (Wilcove and Miller, 1974; Pithers, 1985). According with Holland et al. (2014), "autoshaping" or "sign-tracking" phenomenon has recently attracted considerable attention as a platform for studying individual differences in impulsivity, drug sensitization, and other traits associated with vulnerability to drug addiction. Autoshaping has been also used for detecting effects induced by memory, amnesia, drugs, genetic variations, aging and neural markers (e.g., Tomie et al., 2003, 2012; Vanover et al., 2004; Rodriguez et al., 2008; Fitzpatrick et al., 2013; Markou et al., 2013; Talpos et al., 2014). Notably, autoshaping is an associative automatized learning task (see below), and during memory consolidation of Pavlovian/instrumental autoshaping learning task, dentate gyrus, hippocampal CA1, basolateral amygdaloid nucleus and prefrontal cortex are require (see below). It should be noted that an important innovation, and growingly popular method of assessing cognitive functions is the automated touchscreen platform (e.g., Abela et al., 2013; Talpos et al., 2014; Delotterie et al., 2015), used for diverse cognitive tasks, comparable those in employ in human subjects (Horner et al., 2013), including autoshaping (e.g., Gallistel et al., 2014; Talpos et al., 2014; Silverman et al., 2015).

Autoshaping learning tasks involve classical and instrumental conditioning (i.e., stimulus-stimulus and stimulus-responding conditioning). It should be mentioned that long-lasting memories are most efficiently formed by multiple training sessions separated by appropriately timed intervals. Autoshaping meets this criterion and it allows modeling of behavioral situations requiring integration of information obtained from sign- and goal-tracking settings; representing memory of self-taught settings (Meneses, 2013, 2014). Certainly, autoshaping tasks (Pavlovian or instrumental; and Pavlovian/instrumental may produce initial modest and/or variable levels of conditioned responses (CR). Importantly, memory formation, amnesia and forgetting in Pavlovian/instrumental paradigms are accompanied by changes in neural markers, including 5-HT, glutamate, dopamine, and GABA transporters expression levels (Tomie et al., 2003; Tellez et al., 2012a,b), 5-HT receptor expression and cAMP production (Meneses, 2013). Certainly, forgetting as therapeutic targets for dysfunctional memory it has been little explored. As above mentioned, similar results, including pharmacological and neurobiological changes to those reported in autoshaping have been described in other memory behavioral tasks (for review see King et al., 2008; Marcos et al., 2008; Da Silva Costa-Aze et al., 2012; Reichel et al., 2012; Woods et al., 2012; Haahr et al., 2013; Freret et al., 2014; Nasehi et al., 2014b; Seyedabadi et al., 2014; Subramaniyan et al., 2014; Wilkinson et al., 2014; Delotterie et al., 2015; Sase et al., 2015; Westrich et al., 2015).

Behavioral parameters during STM and LTM

In addition to measuring CR in autoshaping, head-pokes (HP) during each training/testing session and head-pokes during CS (HP-CS) have recorded. These parameters provide information about exploration activity (HP) and food- intake motivation (Tellez et al., 2012a). For instance, as CR becomes progressive, HP-CS provides information on the association of CS-US and CR-US.

Maximum level of CR

It should be noted that as animals present different levels of CR, these values are normalized and the maximal CR level attained for each rat at 48 h is considered as 100% of performance. This value is then used to calculate the proportion or percentage of CR observed at 1.5, 24, and 216 h and the data of 1.5 h and 24 h are used as illustration; and multiple comparisons, including memory, forgetting, time vs. treatments for all behavioral parameters (Meneses and Tellez, 2015).

Memory, amnesia and forgetting and neural transporters analysis

As already mentioned autoshaping procedures produce variable levels of CR and a number of laboratories have been using autoshaping. It should be noted that, reproducibility among studies is important and expected that to vary (e.g., Marcus, 2014).

but prefrontal cortex GAT1 down-regulation. Together these results show that forgetting provokes primarily hippocampal and prefrontal cortex transporters changes; it represents a cognitive process hardly modifiable and its prevention could causes different transporters expression patterns. Notably, together the results suggest that: (1) memory formation, amnesia and anti-amnesic effects are associated to specific patterns of transporters expression; (2) STM and LTM, forgetting and anti-forgetting effects are associated to specific patters of transporters expression and brain areas; (3) amnesia and forgetting affect different brain areas and produce differential patters of transporter expression. Hence, in pharmacological and neuroanatomical terms, amnesia and forgetting differ.

Neural Transporters and Brain Functions and Dysfunctions

It should be noted that neural transporters regulate intra-synaptic levels of neurotransmitter, which allows a global picture of synapses. Moreover, diverse evidence indicates that memory formation, forgetting, amnesia, and/or anti-amnesic effects can also be modulated by changes in the expression of neurotransmitter transporters (e.g., Schmitt and Hiemke, 2002; Chen et al., 2011; Reichel et al., 2012; Yang et al., 2013). Hence, a brief overview of evidence involving GAT1, EAAT1, SERT, and DAT as well other neurobiological markers regarding memory and other cerebral functions is include.

GAT 1

Attention deficit/hyperactivity disorder (ADHD) is featured by hyperactivity, impaired sustained attention, impulsivity, and usually varying degrees of dysfunctional learning and memory (see also Meneses et al., 2011b) and motor incoordination (Yang et al., 2013). Importantly, Yang et al. (2013) report that GAT1 gene knockout (KO) mouse ($GAT1^{-/-}$) is hyperactive and exhibit impaired memory performance (Morris water maze). KO GAT1 mice have low levels of attentional focusing and increased impulsivity; the hyperactivity in these KO mice is reduced by both methylphenidate and amphetamine; Yang et al. (2013) suggest that GAT1 KO mouse is a new animal model for ADHD studying and GAT1 may be a new target to treat ADHD. Schmitt and Hiemke (2002) note that GABA is cleaved from the synaptic cleft by uptake (see Hu and Quick, 2008), via specific transporters and inhibition of such transporters increases the effectiveness of physiologically released GABA. Increased GABAergic neurotransmission has an impact on learning and memory. Indeed, tiagabine, a GABA-transporter inhibitor, impaired learning (Morris water-maze) and retrieval (only at the probe trial; Schmitt and Hiemke, 2002). But,

TABLE 9 | Neural transporters during STM and LTM, amnesia (methamphetamine), forgetting, (fluoxetine) improved LTM, (fluoxetine) anti-forgetting effects and anti-amnesic (fluoxetine plus methamphetamine) effects.

Cognitive process	Neural transporters expression of transporters in brain areas	References Tellez et al., 2012a,b
STM and LTM	Up-regulation of PFC GAT1 and EAAC1, striatal SERT, DAT and EACC1; while, HIP EACC1, GAT1 and SERT are down-regulated	
Amnesia	Down-regulated SERT, DAT, EACC1 and GAT1 in HIP the GAT1 in striatum; no-changes are observed in PFC	
Forgetting	Up-regulation of GAT1 (PFC and HIP) and DAT (PFC) while SERT (HIP) is down-regulated; no-changes are observed in striatum	
Improved LTM	DAT, GAT1 (PFC up-regulation), but GAT1 (striatum) and SERT (HIP) down-regulation	
Anti-forgetting effects	striatal GAT1 and HIP DAT up-regulation, but PFC GAT1 down-regulation	
Anti-amnesic effects	DAT, EACC1 and GAT1 (PFC), SERT and DAT (HIP) and EACC1 or DAT (striatal) up-regulation	

STM, short-term memory; LTM, long-term memory; GAT1, GABA transporter 1; DAT, dopamine transporter; SERT, serotonin transporter; EACC1, glutamate transporter 1; PFC, prefrontal cortex; HIP, hippocampus.

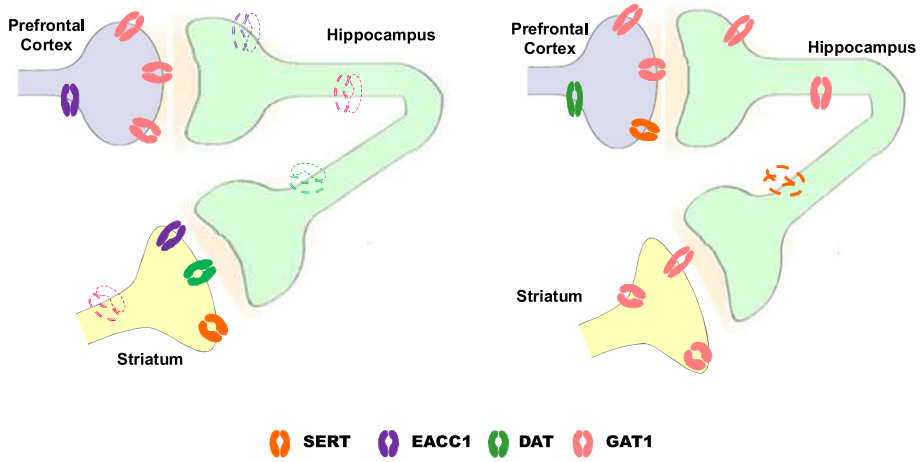

FIGURE 1 | Schematic representation of changes with Western blot analysis of neural transporters in prefrontal cortex, hippocampus and striatum during memory formation and temporal-course of forgetting. Strong color refers to up-regulation, slight color refers to down-regulation. GAT1, GABA transporter 1; EAAC1, neuronal glutamate transporter excitatory amino acid carrier-1; DAT, dopamine transporter SERT, serotonin transporter (modified from Tellez et al., 2010, 2012a,b).

Sałat et al. (2015) note that tiagabine slightly decreased memory but did not augment that induced by scopolamine. According with Shi et al. (2012), homozygous GAT1$^{(-/-)}$ mice exhibit impaired hippocampus-dependent learning and memory; and they evaluated the impact of endogenous reduced GABA reuptake on cognitive behaviors. Learning and memory of heterozygous GAT1$^{(+/-)}$ mice was determined in passive avoidance and Morris water maze; showing that GAT1(+/−) mice displayed increased learning and memory, decreased anxiety-like behaviors, and highest synaptic plasticity relative to wild-type and homozygous GAT1$^{(-/-)}$ mice; and authors conclude that a moderate reduction in GAT1 activity is associated to learning and memory facilitation (Shi et al., 2012); which is consistent, in part, with GAT1 reduced and increased expression in autoshaping amnesia, forgetting and improved memory as well as anti-amnesic and anti-forgetting effects (see **Table 10**). In addition, Pang et al. (2011) testing the GABAergic immunotoxin; GAT1-saporin (GAT1-SAP), report no alterations in spatial reference memory. But GAT1-SAP impaired the platform location in a delayed match to position test (changing daily the platform location). In the active avoidance task, intraseptal GAT1-SAP impaired extinction but not acquisition (Pang et al., 2011). In contrast, GAT1-Saporin into the medial septum/vertical limb of the diagonal band (MS/VDB) spared mnemonic function and use of environmental cues; however, self-movement cue processing was compromised (Köppen et al., 2013).

EAAT1

According with Chen et al. (2011), an imbalance of neurotransmitters (e.g., glutamate, acetylcholine, dopamine, and serotonin) has been proposed as the neurobiological basis of behavioral symptoms of AD, hence they are hypothesizing that altered reuptake of neurotransmitters by vesicular glutamate transporters (VGLUTs), excitatory amino acid transporters (EAATs), the vesicular acetylcholine transporter (VAChT), SERT or DAT. Examining protein and mRNA levels of these transporters in post-mortem prefrontal cortex from patients and matched non-AD controls, Chen et al. (2011)

TABLE 10 | GABA transporter GAT1.

Function/dysfunction	Findings	References
GAT1 KO mice and ADHD	Hyperactive behavior and memory dysfunctions in the MWM, also have low levels of attention and increased impulsivity	Yang et al., 2013
GABA-transporter inhibitor	Tiagabine, in the MWM, compared to saline treated rats, impaired learning during the acquisition trials. And retrieval only at the probe trial	Schmitt and Hiemke, 2002
GAT1$^{(-/-)}$ KO mice	Impaired hippocampus-dependent learning and memory (MWM, PA)	Shi et al., 2012
GABAergic immunotoxin: GAT1-saporin (GAT1-SAP)	Intraseptal impaired a delayed match to position task and extinction of avoidance without altering acquisition of WMWM, active avoidance acquisition or open field behavior. Also, animals were slower to update changes to previous contingencies	Pang et al., 2011; but see Köppen et al., 2013
GAT1(+/−) mice	Increased learning and memory, decreased anxiety-like behaviors, and highest synaptic plasticity compared with wild-type and homozygous GAT1(−/−) mice	Shi et al., 2012

ADHD, attention deficit and hyperactivity disorder; KO, knock out; MWM, Morris water maze; PA, passive avoidance.

TABLE 11 | Glutamate transporter 1 and markers.

Function/dysfunction	Findings	References
Glutamate and AD	Reduced mRNA levels of VGLUTs, EAAT1-3 proteins	Chen et al., 2011
MDMA	Improved expression of GluR2 receptor, mGluR1, mGluR5, NR1, NR2A, NR2B and EAAT1, EAAT2-2 transporters. Increased mRNA levels of GluR3, NR2A and NR2B in caudate putamen. GluRl is reduced in the hippocampus, in hypothalamus increases expression of GluRl, GluR3, and mGluR3 mGluR	Kindlundh-Högberg et al., 2008, 2010
Mild stress model induced	Mice heterozygous (+/− VGLUT1) VGLUT1 decrease expression relative to wild mice: dysfunctions in recognition memory (recognition new object); anhedonia (sucrose intake), hopelessness (forced swimming), anxiety (elevated plus maze)	Garcia-Garcia et al., 2009
Glutamate transporter 1 and training	The hippocampal levels of GLT-1 complex are parallel to training in the memory multiple T-maze test	Heo et al., 2012

AD, Alzheimer's disease; VGLUTs, Vesicular glutamate transporters; MDMA, methylenedioxymethamphetamine.

found that protein and mRNA levels of VGLUTs, EAAT1-3, VAChT, and SERT are reduced in AD, without changing DAT (**Table 11**). Chen et al. (2011) conclude that the reduced VAChT expression could contribute to cholinergic deficit in AD and altered neurotransmitter transporters could contribute to the pathophysiology of AD; which are potential targets for therapy (Chen et al., 2011).

Likewise, Kindlundh-Högberg et al. (2010) investigated the effect of intermittent 3,4-methylenedioxy-metamphetamine (MDMA; ecstasy) administration upon gene-transcript expression of the glutamate transporters (EAAT1, EAAT2-1, EAAT2-2), glutamate receptor subunits of AMPA (GluR1, GluR2, GluR3), glutamate receptor subunits of NMDA (NR1, NR2A, and NR2B), and metabotropic glutamate receptors (mGluR1, mGluR2, mGluR3, mGluR5); showing increased cortical expression of GluR2, mGluR1, mGluR5, NR1, NR2A, NR2B, EAAT1, and EAAT2-2 (Kindlundh-Högberg et al., 2010). In the caudate putamen, mRNA levels of GluR3, NR2A, and NR2B receptor subunits are increased; in contrast, GluR1 is reduced in the hippocampus but in the hypothalamus GluR1, GluR3, mGluR1, and mGluR3 expression is increased (Kindlundh-Högberg et al., 2010; see also Carmona et al., 2009); concluding that repeated MDMA administration is associated with changes in gene-transcript expressions of glutamatergic NMDA and AMPA receptor subunits, metabotropic receptors and transporters in brain areas mediating learning and memory (Kindlundh-Högberg et al., 2010). In addition, decreased expression of vesicular glutamate transporter 1 (VGLUT1+/−) respect to wild-type (WT) mice occur with chronic mild stress (CMS)-induced, affecting several functions and impairing recognition memory. In addition, Heo et al. (2012) detect hippocampal glutamate transporter 1 (GLT-1) complex expression during training and memory in the Multiple T-maze.

SERT

Reichel et al. (2012) report that control rats spent more time interacting with the objects in the changed locations. In contrast, contingent or non-contingent methamphetamine (meth) disrupted object-in-place (OIP) task performance as seen by similar amounts of time spent with all objects,

regardless of location. While only acute meth binge produced signs of neurotoxicity, both meth regimens decreased SERT in the perirhinal cortex and hippocampus. Only meth self-administration resulted in a selective decrease in NET. Meth-induced changes in SERT function in the OIP circuitry may underlie memory deficits independently of overt neurotoxic effects (Reichel et al., 2012). It should be noted that SERT is reduced in AD (Chen et al., 2011; Claeysen et al., 2015).

Parrott (2013) highlights that decreased SERT (hippocampus, parietal cortex, and prefrontal cortex expression) in abstinent Ecstasy/MDMA users is associated to dysfunctional declarative and prospective memory. Even the children of mothers who take Ecstasy/MDMA during pregnancy have psychomotor impairments (Parrott, 2013). In addition, Thomasius et al. (2006) report reduced SERT expression, which might be a transient effect of heavy ecstasy use, since it partially recovered as the users reduced their MDMA use; though this parameter may not necessarily be a valid indicator of the number or integrity of serotonergic neurons. Importantly, ex-ecstasy users' verbal memory show no sign of improvement even after over 2.5 years of abstinence and thus may represent persistent functional consequences of MDMA neurotoxicity; alternative causes such as pre-existing group differences cannot be excluded (Thomasius et al., 2006). In addition, AD and drugs of abuse like d-methamphetamine (METH) or MDMA have been associated to decrements in the SERT expression and memory deficits; thus supporting the notion that the SERT plays a key role in both normal and pathological states (e.g., Line et al., 2014). Particularly, the s allele of the polymorphic regulatory region of the SERT or 5-HTT gene promoter is associated with reduced 5-HTT expression and vulnerability to psychiatric disorders, including anxiety and depression. Moreover, the l allele increases 5-HTT expression and is generally considered protective (Line et al., 2014). However, Line et al. (2014) suggest that 5-HTT over-expression results in a reduced sensitivity to both positive and negative reinforcers, and produces some maladaptive effects, supporting recent suggestions that l allele homozygosity may

TABLE 12 | Serotonin transporter SERT.

Function/dysfunction	Findings	References
Methamphetamine	Memory deficits and decreased SERT function in perirhinal cortex and hippocampus	Reichel et al., 2012
MDMA (ecstasy)	During abstinence memory deficits and decreased SERT in hippocampus, parietal cortex and prefrontal cortex	Parrott, 2013
MDMA ex-users	Verbal memory dysfunction even after 2.5 years of abstinence	Thomasius et al., 2006
AD	Decreased SERT	Chen et al., 2011
5-HT re-uptake inhibition	In healthy individuals and aged transgenic AD mice model (APP/PS1 plaque-bearing mice), citalopram decreased Aβ in brain interstitial fluid in a dose-dependent manner	Sheline et al., 2014a,b
5-HT uptake inhibitor or SERT$^{(-/-)}$ KO mice	Pharmacological or genetic inactivation of the serotonin transporter improves reversal learning in mice	Brigman et al., 2010
Expression	Overexpression of SERT reduces sensitivity to both positive and negative reinforcers evidence in CER and the T-maze; this overexpression is maladaptive effects, suggesting that the homozygous allele/can cause disabling psychiatric features	Line et al., 2014
Expression	Increased 5-HTT expression reduces negative cognitive bias for stimuli with uncertain outcomes	McHugh et al., 2015

AD, Alzheimer's disease; MDMA, methylenedioxymethamphetamine; CER, conditioned emotional response.

TABLE 13 | Dopamine transporter DAT.

Function/dysfuntion	Findings	References
Cognition	Variations in DAT1 influence the improvement of working memory in preschool children after cognitive training	Söderqvist et al., 2012
Dopamine inhibition	Modafinil is dopamine inhibitor can improve cognition in people with mental disorders who use substances abuse	Mereu et al., 2013
Modulated DAT expression in animal model of ADHD	Improved selective spatial attention	Ruocco et al., 2014

ADHD, attention deficit and hyperactivity disorder.

be a potential risk factor for disabling psychiatric traits (Line et al., 2014). In contrast, increased 5-HTT expression reduces negative cognitive bias for stimuli with uncertain outcomes (McHugh et al., 2015). And Brigman et al. (2010) report that fluoxetine-treated C57BL/6J mice made fewer errors than controls during the early phase of learning reversal when perseverative behavior is relatively high and 5-HTT null mice made fewer errors than controls in completing the reversal task (**Table 12**). And these authors suggest that inactivating 5-HTT improves reversal learning, which is relevant for the pathophysiology and treatment of neuropsychiatric disorders characterized by executive dysfunction (Brigman et al., 2010) and possibly post-traumatic stress disorder.

Certainly, SERT is providing useful information as neural marker and therapeutic target. For instance, Wallace et al. (2014) report that vortioxetine, a novel, multimodal-acting antidepressant, is a 5-HT_3, 5-HT_7, and 5-HT_{1D} receptor antagonist, a 5-HT_{1B} receptor partial agonist, a 5-HT_{1A} receptor agonist, and inhibits the 5-HT transporter. This drug changes the expression of multiple genes involved in neuronal plasticity by antidepressant treatment, which is associated with improved cognitive function and a reduction in depression-like behavior in middle-aged mice (Li et al., 2015c).

Hence, the SERT expression seems to be a reliable neural marker related to memory mechanisms, its alterations and potential treatment (Meneses, 2013). Resulting crucial determining the pharmacological, neural and molecular mechanisms associated to these changes and therapeutic targets. For instance, Sheline et al. (2014a) report that serotonin signaling suppresses generation of amyloid-β (Aβ) *in-vitro* and in animal models of AD and healthy individuals. In fact, in an aged transgenic AD mouse model the antidepressant citalopram (5-HT uptake inhibitor) in dose-dependent manner decreased Aβ in cerebrospinal fluid, suggesting AD prevention trials (Sheline et al., 2014a,b).

DAT

According with Mereu et al. (2013), modafinil (MOD) and its R-enantiomer (R-MOD) are used for narcolepsy and sleep disorders; and also employed, off-label used as cognitive enhancers in individuals with mental disorders, including substance abusers that demonstrate impaired cognitive function. Their mechanisms of action include inhibition of dopamine (DA) reuptake via the DAT in diverse brain areas (Mereu et al., 2013; **Table 13**). Importantly, memantine (MEM), a dual antagonist of NMDA and alpha7 receptors, is neuroprotector against MDMA in rats, and it also prevents MDMA effect on SERT functionality and METH effect on DAT (Escubedo et al., 2009). Moreover, Söderqvist et al. (2012) have noted that dopamine plays an important role not only in dysfunctional working memory (WM) but also for improving it, including variation in DAT1, improving WM and fluid intelligence in preschool-age children following cognitive training; concluding with the role of dopamine in determining cognitive plasticity (Söderqvist et al., 2012). Ruocco et al. (2014) report that 5-HT_7 receptor stimulation (low doses) was associated to among other findings reduced horizontal activity and (at higher dose) increased selective spatial attention, the DAT levels were decreased (low dose), and modulated expression of NMDA receptors.

It should be noted that, before the perspective of the absence of effective treatments for dysfunctional memory and regardless the mechanisms; environmental interventions and exercise (physical and cognitive) seem offer feasible approaches (e.g., Mora, 2013; Mo et al., 2015).

Conclusions

Of course if the above findings are replicated over time, across countries and in different experimental settings, they might provide insights about serotonin and other neurotransmission systems presenting convergent changes in diverse neural markers and signaling; thus, allowing the study of different brain functions and dysfunctions, including memory. Hence, diverse approaches might support the translatability of using neural markers and cerebral functions and dysfunctions (e.g., memory formation, AD, MCI). Likewise, hypothesis and theories (e.g., Borroto-Escuela et al., 2015) might provide appropriate limits and perspectives of the diversity of evidence. Certainly, at least, 5-HT_{1A}, 5-HT_4, 5-HT_5, 5-HT_6, and 5-HT_7 receptors as well as SERT seem to be useful as neural markers and therapeutic targets.

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