The role of the serotonin receptor subtypes 5-HT\textsubscript{1A} and 5-HT\textsubscript{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\textsubscript{1A} and 5-HT\textsubscript{7} 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\textsubscript{1A} and 5-HT\textsubscript{7} as well as their interplay at the molecular, neurochemical and behavioral level. Activation of postsynaptic 5-HT\textsubscript{1A} Rs impairs emotional memory through attenuation of neuronal activity, whereas presynaptic 5-HT\textsubscript{1A} R activation reduces 5-HT release and exerts pro-cognitive effects on PA retention. Antagonism of the 5-HT\textsubscript{1A} R facilitates memory retention possibly via 5-HT\textsubscript{7} R activation and evidence is provided that 5HT\textsubscript{7} R can facilitate emotional memory upon reduced 5-HT\textsubscript{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\textsubscript{1A} Rs and 5-HT\textsubscript{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-HT subtypes.

Keywords: emotional learning, fear conditioning, fear memory, 5-HT\textsubscript{1A} receptor ligands, 5-HT\textsubscript{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 has 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-HT 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-HT1AR 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 (Lucky, 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 (Tunniciuff, 1991; Den Boer et al., 2000; Blier and Ward, 2003). The most common antidepressants, the SSRIs, act by targeting the 5-HT1AR (Hervas and Artigas, 1998; Artigas, 2015), supporting the key role of the 5-HT1AR in the pathophysiology of mood disorders.

The 5-HT7Rs 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-HT7R and 5-HT1AR 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-HT1A and 5-HT7R (distribution, signaling, and ligands), this review will focus on the role of 5-HT1AR, 5-HT7R as well as its interplay in emotional learning processes. The interaction between the 5-HT1AR and 5-HT7R signaling will be discussed and results of studies using different available 5-HT1AR and 5-HT7R 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-HT1AR and 5-HT7R, via local rather than systemic administration. Overall, the aim of this review is to draw general conclusions about the role of both 5-HT1AR and 5-HT7R 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-HT1A and 5-HT7 Receptors

All the 5-HTR subtypes belong to the G protein-coupled receptor superfamily, with the exception of the 5-HT3R 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-HT1Rs (5-HT1AR, 5-HT1BR, 5-HT1DR, 5-HT1ER, 5-HT1FR) couple to Gα/γ proteins, whereas the 5-HT2Rs (5-HT2AR, 5-HT2BR, 5-HT2CR) couple to Gαq proteins, and the 5-HT4R, 5-HT5R, and 5-HT7R couple to Gαi proteins. For the 5-HT3Rs (5-HT3AR and 5-HT3BR) G-protein coupling is not established yet (Bockaert et al., 2006).

5-HT1A Receptor Localization

5-HT1AR was the first 5-HTR subtype to be cloned and is characterized by its high affinity for 5-HT (Nichols and Nichols, 2008). 5-HT1ARs are widely distributed throughout the CNS and are present in both pre- and postsynaptic sites. Presynaptically, 5-HT1ARs 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-HT1AR autoreceptors which tightly regulate 5-HT neuronal activity.

Postsynaptically, the highest level of 5-HT1AR is found in the limbic system based on receptor autoradiography and mRNA expression. Both techniques showed the distribution of the 5-HT1AR 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-HT1AR 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-HT1AR is located on somata and dendrites of pyramidal and granular neurons, as well as on the dentritic spines of pyramidal neurons (Riad et al., 2000). Moreover, 5-HT1AR 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 (Lüttingen et al., 2005a). This indicates that systemic administration of 5-HT1AR 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-HT1A Receptor Signaling

Activation of 5-HT1AR leads to neuronal hyperpolarization, an effect mediated by pertussis-toxin-sensitive G proteinαi/o proteins. G proteinαi/o proteins are negatively coupled with the signaling pathway of adenylyl cyclase and thereby decrease the cAMP formation (De Vivo and Maayan, 1986; Weiss et al., 1986). Despite their high density in the dorsal raphe nucleus, 5-HT1A autoreceptors do not seem to inhibit AC, but mediate neuronal inhibition through different signaling pathways (Clarke et al., 1996). Both post- and presynaptic 5-HT1ARs inhibit neuronal firing via the activation...
of G protein-coupled inwardly rectifying potassium channels as well as the inhibition of Ca²⁺ 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-HT₁₅R (reviewed by Raymond et al., 2001; Bockaert et al., 2006).

**5-HT₇R Localization**

The 5-HT₇R 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-HT₇Rs 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-HT₇R 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-HT₁₅R, the 5-HT₇R 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-HT₇R 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-HT₇R in cortical neurons, where it is suggested that 5-HT₇R may have a role during the developing stages of cortical circuits (Béïque et al., 2007; Celada et al., 2013).

**5-HT₇ Receptor Signaling**

5-HT₇R activation activates adenylyl cyclase signaling and consequently the conversion of ATP to cAMP through coupling to Gα₃ (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-HT₇Rs 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-HT₁₅R and 5-HT₇ Receptors**

The expression of 5-HT₁₅R and 5-HT₇R 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-HT₁₅R has been proposed to modulate anxiety based on studies with 5-HT₁₅R 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-HT₁₅R 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-HT₁₅R 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-HT₁₅R 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-HT₇R 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-HT₇R 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-HT₇R in the action of antidepressant drugs, whereas the results from anxiety studies are contradictory (Leopoldo et al., 2011). Interestingly, studies using 5-HT₇R knockout mice revealed the crucial role of this receptor in hippocampus-dependent memory (Roberts et al., 2004; Sarkisyan and Hedlund, 2009).

**5-HT₁₅A and 5-HT₇ 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-HT₁₅R) 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-HT₁₅R and 5-HT₇R 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-HT₁₅R and 5-HT₇R 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-HT₁₅R and 5-HT₇R, 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-HT₁₅R 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 GABAₐR 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 physicochemical 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-HT₁₅R Receptor Agonists

The prototypic 5-HT₁₅R 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-HT₁₅R in behavioral manipulations (Barnes and Sharp, 1999). Despite its high selectivity for the 5-HT₁₅R, 8-OH-DPAT also acts as a 5-HT₇R 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

| Function        | Compound                  | Receptor Specificity                                      | MW   | Solvent | BBB penet. | Behavior | Reference                  |
|-----------------|---------------------------|----------------------------------------------------------|------|---------|------------|----------|----------------------------|
| Full/partial    | Alinespironine (S-20499)  | 5-HT₁₅R >> D₂ >> 5-HT₁B₂ >> α₂β >> D₁ >> H₁ (pre-synaptic) | 479  | W       | n.a.       | A        | Griebel et al. (1992)      |
| Partial         | Buspirone                 | 5-HT₁₅R = D₂ >> α₁,α₂                                      | 385.5| W       | n.a.       | A, L     | Hjorth and Carlsson (1982), Quartermain et al. (1993) |
| Full            | F-13640                   | 5-HT₁₅R >> n.a.                                           | 393.1| W, Yes  | N          |          | Desue et al. (2002), Heusler et al. (2010); Gtp     |
| Partial         | F-13714                   | 5-HT₁₅R >> 5-HT₁B₂-F₂-2                                    | n.a. | W, n.a. | PPI        |          | Assié et al. (2006)        |
| Full            | F-15599                   | 5-HT₁₅R (post-synaptic) >> 5-HT₁B₂-F₂-2                   | 394.1| n.a.    | Yes        | FST      | Maurel et al. (2007), Newman-Tancrèdi et al. (2009); Gtp |
| Full            | Flesinoxan                | 5-HT₁₅R >> α₁ (agonist) >> D₂                              | 415.5| W, Yes  | A          | A        | Ahlenius et al. (1991), Hadrava et al. (1995)         |
| Partial         | Ipsapirone (TVX Q 7821)   | 5-HT₁₅R >> α₁ (agonist)                                   | 401.5| W, Yes  | A          | A        | Traber et al. (1984)       |
| Partial/full    | LY-228729                 | 5-HT₁₅R >> 5-HT₁B                                      | n.a. | w, n.a. | L, FST     |          | Swanson and Catlow (1992)  |
| n.a.            | NDO-008                   | 5-HT₁₅R >> n.a.                                          | n.a. | w, n.a. | L          | A        | Misane et al. (1998)       |
| Full            | 8-OH-DPAT                 | 5-HT₁₅R >> 5-HT₁B                                      | 382.3| w, Yes  | A, L       |          | Arvidsson et al. (1981), Hadrava et al. (1995)        |
| Full/Partial    | Osmozotan (MKC-242)       | 5-HT₁₅R >> α₁                                           | 379.8| w, n.a. | A          | A        | Matsuda et al. (1999), Sakaue et al. (2003)           |
| Partial         | PRX-00023                 | 5-HT₁₅R >> 5-HT₁B,α₁,a₂                                  | n.a. | w, n.a. | A          | A        | Becker et al. (2006)       |
| Full            | Repinotan (BAY x 3702)    | 5-HT₁₅R >> 5-HT₁B,α₁,a₂                                 | 400.5| HC1     | Yes        | L        | De Vry et al. (1998), Schwarz et al. (2005)          |
| Partial         | Tandospirone (SM-3997)    | 5-HT₁₅R >> D₂                                           | 385.5| w, n.a. | A          | L        | Shimizu et al. (1987)      |

| Mixed profile   | S-15535                   | n.a.                                                     | 432.5| w, Yes  | A, L       |          | Milan et al. (1993), Carli et al. (1999)             |
| Mixed profile   | MDL-73005                 | n.a.                                                     | 432.5| 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; penet., penetration; 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 D2 receptor (Witkin and Barrett, 1986). Tandospirone (SM-3997) is a 5-HT1AR 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-HT1AR (Shimizu et al., 2010). MC18 fumarate and VP08/34 fumarate (Siracusa et al., 2015) were also studied for its anxiolytic properties in rats and mice (Shimizu et al., 1987). Similar to buspirone, tandospirone and was initially studied for its anxiolytic properties in rats and mice (Shimizu et al., 1987). An overview of currently available 5-HT1AR antagonists is provided in Table 1.

5-HT1A Receptor Antagonists
WAY-100635 and NAD-299 are the most commonly used selective antagonists in the study of the 5-HT1AR. Both ligands have high potencies and penetrate easily into the brain (Fletcher et al., 1996; Johansson et al., 1997; Stenfor et al., 1998). However, NAD-299 was found to have higher selectivity for the 5-HT1AR 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-HT1AR in emotional learning, such as the potent and selective 5-HT1AR 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-HT1AR 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-HT1AR antagonist with antagonist properties only at the postsynaptic HT1AR and lower affinity for the α-adrenergic receptors (Greuel and Glaser, 1992).

Finally, S-15535 is reported to act as a postsynaptic 5-HT1AR antagonist while also behaving as an agonist on presynaptic 5-HT1A 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-HT1ARs (Youn et al., 2009). An overview of currently available 5-HT1AR antagonists is provided in Table 2.

5-HT7 Receptor Agonists
The lack of selective and potent 5-HT7R agonists (Misane and Carlsson, 1982) and acts also as antagonist with high specificity for the dopamine D2 receptor (Witkin and Barrett, 1986). Tandospirone (SM-3997) is a 5-HT1AR 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-HT1AR (Shimizu et al., 2010). MC18 fumarate and VP08/34 fumarate (Siracusa et al., 2015) were also studied for its anxiolytic properties in rats and mice (Shimizu et al., 1987). Similar to buspirone, tandospirone and was initially studied for its anxiolytic properties in rats and mice (Shimizu et al., 1987). An overview of currently available 5-HT1AR antagonists is provided in Table 1.

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, 1995; 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-HT1AR and 5-HT7R 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-HT1A Receptor Ligands in Emotional Learning and Memory
An overview of the behavioral effects of various 5-HT1AR ligands is provided in Table 4.
Systemic 5-HT\textsubscript{1A} Receptor Ligand Effects

Despite the differences among the 5-HT\textsubscript{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\textsubscript{1A}R activation on fear memory. Systemic, pretraining administration of the full 5-HT\textsubscript{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; Lüttingen 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; Lüttingen et al., 2005b) linking the postsynaptic 5-HT\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{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\textsubscript{1A} Receptor Ligand Effects

Intracranial administration of 5-HT\textsubscript{1A}R agonists and/or antagonists was used to further elucidate the distinct function of pre- versus postsynaptic 5-HT\textsubscript{1A}Rs in fear learning. Pre-
but not post-training intra-hippocampal infusion of 8OH-DPAT impairs contextual FC (Stiedl et al., 2000a), pointing at the important role of the postsynaptic 5HT1A R in acquisition processes as observed after systemic administration.

### Effects of 5-HT1A Receptor Agonists and Antagonists on Memory Recall

#### Systemic 5-HT1A Receptor Ligand Effects

Unlike the unambiguous implication of the postsynaptic 5HT1A R in memory acquisition, its role in fear retrieval and expression is less clear. The systemic 5-HT1A 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-HT1A 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-HT1A 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 5HT1A R activation (Youn et al., 2013). Thus, the claims of anxiolytic actions of pretest injection of 5-HT1A R agonists as initially reported in human studies and partly in animal models cannot be supported unambiguously at least in learned fear experiments.

### TABLE 3 | Selected overview on available 5-HT7 receptor agonists and antagonists.

| Function | Compound | Receptor specificity | MW | Solvent | BBB penetr. | Behavior | Reference |
|----------|----------|----------------------|-----|---------|-------------|----------|-----------|
| **Agonists** | | | | | | | |
| Partial | AS-19 | 5-HT7 >> n.a. | 283.41 | PG | n.a. | L, N | Brenchat et al. (2009), Eriksson et al. (2012) |
| Full | E-65888 | n.a. | 257.4 | W | n.a. | N | Brenchat et al. (2009); http://pubchem.ncbi.nlm.nih.gov/ |
| n.a. | LP-211 | 5-HT7 >> D2 > 5-HT1A | 466.6 | DMSO | Yes | L | Leopoldo et al. (2008), Meneses et al. (2015); http://pubchem.ncbi.nlm.nih.gov/ |
| Partial | LP-44 | 5-HT7 >> 5-HT1A (agonist function) | 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-HT7 >> 5-HT1A >> 5-HT2A >> D2 | n.a. | W | n.a. | N | Thomson et al. (2004), Brenchat et al. (2009) |

| **Antagonists** | | | | | | | |
| DR4004 | 5-HT7 >> 5-HT2 > D2 > 5-HT1A > HT6 > HT4 | 382.5 | T80 | A, L | n.a. | Kikuchi et al. (1999); http://pubchem.ncbi.nlm.nih.gov/ |
| SB-258719 | 5-HT7 >> 5-HT1D >> D2, D3 >> 5-HT1B,5-HT2B >> HT1A | 338.5 | W | n.a. | N | Forbes et al. (1998), Brenchat et al. (2009); http://pubchem.ncbi.nlm.nih.gov/ |
| SB-269970** | 5-HT7 >> 5-HT5A >> D2 > 5-HT1B > HT1D | 352.5 | T80 | Yes | A, FST, L | Lovell et al. (2002), Thomas et al. (2003), Wesolowska et al. (2006), Eriksson et al. (2012); http://pubchem.ncbi.nlm.nih.gov/ |
| SB-656104-A | 5-HT7 >> 5-HT1D >> 5-HT2A >> HT2B >> HT1A >5-HT5A | n.a. | MC | Yes | L, REM Sleep | Thomas et al. (2003), Horisawa et al. (2011) |
| SB-258741** | 5-HT7 >> 5-HT1A >> D3 > HT1B, D2 > 5-HT1D | 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., penetration; 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).
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 | Quarterman 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 x 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 | Quarterman et al. (1993) |
| | M: Swiss-W. | 30 min pretr. | 2 and 5 | s.c. | FC: no effect at 1-h test | Quarterman et al. (1993) |
| | M: Swiss-W. | 30 min pretest | 2 and 5 | s.c. | FC: no effect | Quarterman 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. | 0.01–0.05 | 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 | Madjd et al. (2008) |
| | M: NMRI | 15 min pretr. | 0.1–3 | s.c. | PA: U-shaped PA retention facilitation (maximum at 1 mg/kg) | Madjd et al. (2008) |
| 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 | Hirst et al. (2011) |
| R: Wistar | 0 min post-tr. | 0.01 | i.v. | PA: reversed MK-801-induced memory impairment | Hirst et al. (2011) |
| R: Sprague-D. | 120 min pretr. | 3 | p.o. | FC: Reversed scopolamine-induced memory deficits | Hirst et al. (2011) |

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-HT₁₅ Receptor Agonists and Antagonists on Memory Extinction

In contrast to the well-studied implication of 5-HT₁₅Rs on memory acquisition and recall, there is only one study with 5-HT₁₅R ligands on fear extinction. The systemic 5-HT₁₅R agonist buspirone abolishes the fear extinction in mice (Quartermain et al., 1993). Similarly, the systemic 5-HT₁₅R 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-HT₁₅Rs 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-HT₇ Receptor Agonists and Antagonists on Emotional Learning

Systemic 5-HT₇ Receptor Ligand Effects

The paucity of studies 5-HT₇ receptors 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-HT₇R agonist, LP-211, when administered systematically after the training session, reversed scopolamine-induced amnesia, in rats (Meneses et al., 2015). The same group also shows a facilitating effect on memory formation by the 5-HT₇R agonist AS-19 administered after an autoshaping training session (Perez-Garcia and Meneses, 2005). The enhancing effect of 5-HT₇Rs on memory consolidation was blocked by pre-injection of the 5-HT₇R antagonist SB-269970 (Perez-Garcia and Meneses, 2005; Meneses et al., 2015) indicating the specific involvement of the 5-HT₇R.

Eriksson et al. (2008) investigated the role of 5-HT₇R on emotional learning in mice using a step-through PA paradigm. Pretraining systemic administration of the 5-HT₇R 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-HT₇R activation has a beneficial modulatory role in learning opposing the function of 5-HT₁₅R activation. Accordingly, pretraining 5-HT₇R activation by the combined use of the 5-HT₁₅R antagonist NAD-299 with the 5-HT₁₅R and 5-HT₇R 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-HT₇R antagonist SB-269970 indicating a procognitive effect of 5-HT₇R activation by this drug combination. However, the 5-HT₇R 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-HT₇R 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-HT₇ Receptor Ligand Effects

To further address the role of 5-HT₇Rs on emotional learning, Eriksson et al. (2012) performed hippocampal infusions with the 5-HT₇R agonist AS-19 in mice. Since they failed to find clear facilitatory effects, as observed after systemic treatment, they concluded that "5-HT₇Rs 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-HT₇R antagonist fully blocked the PA facilitation observed after 5-HT₁₅R blockade. Hence, the hippocampus-dependent involvement of the 5-HT₇Rs needs to be re-investigated with selective highly potent 5-HT₇R agonists, because also the low potency of AS-19 (Eriksson et al., 2012) may have contributed to the lack of effects by dorsohippocampal 5-HT₇R agonist application on PA. Finally, although the role of 5-HT₇R 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-HT₇R in memory extinction and reconsolidation, which are both essentially unexplored.

The Interplay of the 5-HT₁₅A and 5-HT₇ 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-HT₁₅ARs and 5-HT₇R. To dissect the function of these 5-HTRs, pre-treatment with selective 5-HT₁₅AR agonists is used to exclusively activate 5-HT₇R. 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-HT₇R counteracted the 5-HT₁₅AR-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-HT₇R activation and concomitant 5-HT₁₅AR blockade leads to PA facilitation (Eriksson et al., 2012). The facilitatory effect on emotional memory by the 5-HT₁₅A antagonist NAD-299 was related to stimulation of 5-HT₇Rs under conditions with reduced 5-HT₁₅AR transmission. These findings suggest that the states of 5-HT₁₅ARs and 5-HT₇Rs 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-HT₁₅AR and change the relative balance between 5-HTR subtypes, which together will...
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 postsynaptic 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_{

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**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) |
| | | | | PA: only 0.5 mg/kg had a positive effect on memory consolidation, when tested 24 h after training | Meneses et al. (2015) |
| LP-211 | R: Wistar | 0 min post-tr. | 0.1–10.0 i.p. | PA: Enhanced memory consolidation, 24 h after training | Perez-Garcia and Meneses (2003) |
| | | | | PA: no effect in retention latencies, 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. | PA: no effect | Meneses (2004) |
| SB-269970 | R: Wistar | 0 min post-tr. | 1–20 i.p. | PA: 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.
protein coupling of the 5-HT1A receptor and by reducing the ability of 5-HT1A to activate potassium channels, without affecting the G\textsubscript{i} protein coupling of the 5-HT\textsubscript{7}. The heterodimerization additionally contributes to the desensitization of the 5-HT1A receptor through facilitated internalization (Renner et al., 2012).

5-HT1A and 5-HT\textsubscript{7} are co-localized in the cell membrane of hippocampal neurons, where their heterodimerization induces an inhibitory effect on the 5-HT1A-mediated activation of potassium channels in hippocampal neurons (Renner et al., 2012). As mentioned above the post-synaptic levels of 5-HT\textsubscript{7} are lower compared to the expression levels of post-synaptic 5-HT1A, whereas this is not the case for the pre-synaptic 5-HT1A (Renner et al., 2012). These regional differences in the 5-HT\textsubscript{7} levels and therefore in the concentration of the heterodimers, can explain the preferential desensitization of 5-HT1A autoreceptors by SSRIs and more generally the region-specific differences in the signaling pathway mediated by the 5-HT1A 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-HT1A receptor, 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 relative level of 5-HTR expression and, its efficacy in activating different receptors with their downstream signaling pathways.

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-HT1A 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-HT1A activation in the basolateral amygdala and the prelimbic area of the prefrontal cortex in low-anxious rats reduced fear potentiated startle, whereas 5-HT1A 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-HT1A-mediated response differences. In line with this, single-housed mice display a stronger hypothermic effect upon 5-HT1A 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\textsubscript{7} polymorphisms on serotonergic signaling or the interaction between polymorphisms of 5-HT\textsubscript{7} and 5-HT1A. Therefore, to elucidate the functional interaction between 5HT1A and 5-HT\textsubscript{7}, 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-HT1A and 5-HT\textsubscript{7}, 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-HT1A 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-HT1A 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-HT1A inhibition and 5-HT\textsubscript{7} 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-HT1A inhibition.
FIGURE 1 | Simplified overview of 5-HT1A- and 5-HT7R-mediated modulation of fear learning in pre- and postsynaptic neurons under conditions of high (A) and low presynaptic 5-HT1A 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-HT7R activation (C), e.g., by 8-OH-DPAT at the postsynaptic dose of 1 mg/kg in combination with the 5-HT1A 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-HT1A R and 5-HT7Rs with so far unknown conditions that bias toward impaired (B) or facilitated fear memory (C). The specific functions of GABAergic interneurons in 5-HT1A R- and 5-HT7R-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
and cognitive functions. However, the complexity of the serotonergic signaling due to the existence of at least 14 pre- and postsynaptic 5-HTR subtypes with multiple transduction mechanisms makes it exceedingly difficult to assign unambiguously the physiological and behavioral role of a single 5-HTR subtype. However, the use of specific ligands in combination with systemic and intrahippocampal administration, receptor autoradiography and in vivo neurochemical measurements are powerful tools in identifying the action of specific ligands in local networks of the brain including subareas of the hippocampus. This approach, combined with in vivo electrophysiology and genetic tools, can also better define the functional role of 5-HT in the neuronal circuitry underlying cognitive function.

Overall a number of open questions need to be answered to further improve our understanding of the role of serotonergic signaling via the different 5-HTRs in health and disease:

1. How do 5-HTRs modulate hippocampal and cortical glutamatergic transmission with a focus on activation and inhibition of 5-HT1ARs and 5-HT7Rs? This needs to be determined with newly developed amperometry methods in in vivo recordings.

2. What are the roles of 5-HT1ARs and 5-HT7Rs in defined hippocampal subregions for emotional and cognitive functions? This requires the development of new ligands with low lipophilicity for local actions tested in vivo. Alternatively, is should be possible to shut down the second messenger coupling of neurons selectively expressing 5-HT1ARs and 5-HT7Rs by Designer Receptors Exclusively Activated by Designer Drugs (DREADD) technology.

3. What are the roles of 5-HT1ARs and 5-HT7Rs in different memory phases? As indicated there are considerable inconsistencies about the role of 5-HT1ARs and 5-HT7Rs in the consolidation process. In addition, extinction and reconsolidation are so far poorly explored.

4. The regulation of 5-HTR expression has so far focused on the 5-HT1AR. This needs to be extended to other 5-HTRs including the 5-HT7R. Besides the use of radio-ligands in imaging studies, the subcellular immunohistochemical analyses of 5-HTR protein levels requires the development of specific antibodies.

5. Finally, despite the evidence of the beneficial effects of 5-HT1AR antagonists in preclinical models, the therapeutic potential to facilitate cholinergic and/or glutamatergic neurotransmission for improved cognitive function in human neuropathology (e.g., Alzheimer’s disease) or in aging is so far not explored.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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