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Serotonin 2A Receptors are a Stress Response System: Implications for Post-Traumatic Stress Disorder

Kevin Sean Murnane

Department of Pharmaceutical Sciences, Mercer University College of Pharmacy, Mercer University Health Sciences Center, Atlanta, GA USA

Abstract

Serotonin, one of the first neurotransmitters to be identified, is an evolutionarily old molecule that is highly conserved across the animal kingdom, and widely used throughout the brain. Despite this, ascribing a specific set of functions to brain serotonin and its receptors has been difficult and controversial. The 2A subtype of serotonin receptors (5-HT_{2A} receptor) is the major excitatory serotonin receptor in the brain and has been linked to the effects of drugs that produce profound sensory and cognitive changes. Numerous studies have shown that this receptor is upregulated by a broad variety of stressors, and have related 5-HT_{2A} receptor function to associative learning. This review proposes that stress, particularly stress related to danger and existential threats, increases the expression and function of 5-HT_{2A} receptors. It is argued that this is a neurobiological adaptation to promote learning and avoidance of danger in the future. Upregulation of 5-HT_{2A} receptors during stressful events forms associations that tune the brain to environmental cues that signal danger. It is speculated that life-threatening situations may activate this system and contribute to the symptoms associated with post-traumatic stress disorder. 3,4-Methylenedioxymethamphetamine, which activates 5-HT_{2A} receptors, has been successful in the treatment of PTSD and has recently achieved status as a breakthrough therapy. An argument is presented that MDMA may paradoxically act through these same 5-HT_{2A} receptors to ameliorate the symptoms of PTSD. The central thematic contention is that a central role of serotonin may be to function as a stress detection and response system.

Keywords

MDMA; PTSD; 5-HT_{2A}; stress; associative learning

Brain serotonin systems

The serotonin system is an evolutionarily old system that is highly conserved across the animal kingdom. Serotonin, which was one of the first neurotransmitters to be discovered, is found in organisms as diverse as roundworms (Sawin et al. 2000), locusts (Anstey et al.

Corresponding author: Kevin S. Murnane, Ph.D., Assistant Professor, Department of Pharmaceutical Sciences, Mercer University College of Pharmacy, Mercer University Health Sciences Center, 3001 Mercer University Dr., Atlanta, GA 30341, Phone: (678)547-6290, Fax: (678)547-6423, murnane_ks@mercer.edu.

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2009), fruit flies (Sitaraman et al. 2012), rodents (Ray et al. 2018), and primates (Murnane et al. 2012). Serotonin was variously named enteramine because it came from gastrointestinal enterochromaffin cells to induce gastric contraction (Negri 2006), serotonin because it is a serum mediator of vascular tone (Rapport et al. 1948), or 5-hydroxytryptamine (5-HT) because it is a derivative of tryptamine, which is structurally similar to the amino acid tryptophan. In addition to the blood and gastrointestinal system, 5-HT is widely expressed in the brain and, across species, has been linked to learning, mood, sleep, and appetite (Fantegrossi et al. 2008; Murnane and Howell 2011). Brain 5-HT projections arise from the dorsal raphe nucleus (DRN) and project to terminal fields within many neural regions. There are 16 distinct presynaptic and postsynaptic 5-HT receptors that are grouped into 7 families, the 5-HT₁–5-HT₇ receptors (Boess and Martin 1994; Green 2006; Hoyer et al. 2002). These receptors provide a broad diversity of functions to brain 5-HT. Indeed, ascribing a specific set of functions to brain 5-HT has been difficult and controversial. For example, one of the most widely known aspects of 5-HT pharmacology is the use of selective 5-HT reuptake inhibitors (SSRIs) to alleviate the symptoms of depression. Yet, the precise role of 5-HT in depression remains elusive despite decades of research; even repeatedly-documented observations such as the therapeutic lag time in response to SSRIs are poorly understood (Andrews et al. 2015). In addition to being linked to learning, mood, sleep, and appetite, 5-HT has also been linked to functions that are critical for sustaining life, including mating behavior, defensive mechanisms, dominance hierarchies, and directing behavior towards the acquisition of nutrients to prevent starvation (Kravitz 1988; Kristan et al. 2005).

The 2A subtype of 5-HT receptors (5-HT_{2A}) is the major excitatory 5-HT receptor in the brain, and is a likely candidate to mediate aspects of the 5-HT response to stress. 5-HT_{2A} receptors are widely expressed in the mammalian brain (Pompeiano et al. 1994) and are localized to glutamate and dopamine neurons (Cornea-Hebert et al. 1999; Jakab and Goldman-Rakic 2000; Miner et al. 2003). The highest expression of 5-HT_{2A} receptors in the brain is on the dendrites of layer 5 cortical pyramidal glutamatergic cells (Cornea-Hebert et al. 1999; Jakab and Goldman-Rakic 2000; Miner et al. 2003). These layer 5 pyramidal cells are the major type of cell in the cortex that sends subcortical projections. Excitation of these pyramidal cells by 5-HT_{2A} receptor stimulation has *in vivo* relevance. The mixed 5-HT_{2A/2C} receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) increases release of glutamate in rats (Scruggs et al., 2003). This effect is blocked by the selective 5-HT_{2A} receptor antagonist M100907, and glutamate receptor agonists and antagonists also modulate the behavioral effects of DOI (Gewirtz and Marek, 2000; Zhang and Marek 2008). Neuroimaging approaches in human subjects have produced findings that are consistent with these interactions between 5-HT_{2A} receptors and glutamate systems (Vollenweider et al., 1997, 2001; Gouzoulis-Mayfrank et al., 1999; Hermle et al., 1998). Accordingly, the localization of 5-HT_{2A} receptors in cortex provides for their input into cortical regulation of subcortical function. 5-HT_{2A} receptors are also localized to dopamine projection neurons in the ventral tegmental area (VTA) (Bubar and Cunningham 2007; Bubar et al. 2005; Ikemoto et al. 2000; Nocjar et al. 2002). This distribution of 5-HT_{2A} receptors provides these receptors with the capacity to regulate dopaminergic in addition to glutamatergic neurotransmission, providing other pathways through which 5-HT_{2A} receptors can modify behavior. It is interesting to note that both glutamate and dopamine systems have been closely tied to learning and

memory. This review will lay out the case that a major function of brain 5-HT is to respond to stressors that may signal danger. It is proposed the modulation of 5-HT_{2A} receptor expression and function tunes the brain through plastic changes that facilitate danger avoidance in the future by creating enduring associations between environments cues and stressful events. This may be mediated by direct effects of 5-HT systems, or through 5-HT-mediated activation of complex brain circuits that involve glutamate, dopamine, and other neuroactive substances. Regardless of the precise neural machinery mediating the tuning of the brain towards danger, the primary argument is that a central role of 5-HT may be to function as a stress detection and response system.

Preclinical studies of 5-HT_{2A} receptors

A large body of pharmacology research, largely commencing in the 1980s, has used laboratory animals to study the 5-HT_{2A} receptor. Some of the most widely used behavioral techniques employed in this research were assays of drug-elicited behavior. Drug-elicited behavior is a widely established approach to the study of the *in vivo* pharmacology of many psychoactive drugs (Ator and Griffiths 2003). An assay of drug-elicited behavior involves the administration of a drug and the measurement of unconditioned behavior that is known to be mediated by a discrete number of receptor subtypes. Such procedures have been used, for example, in the study of stimulant-induced locomotor and stereotyped behavior, opioid-induced Straub tail responses, and cannabinoid-induced tetrad responses. In the context of the study of 5-HT_{2A} receptors, the particular elicited effect that has been of great utility is the drug-elicited head twitch response (HTR) (Corne and Pickering 1967; Corne et al. 1963). Head twitching occurs in rodents spontaneously, but it is increased in frequency by the administration of various 5-HT_{2A} receptor agonists (Colpaert and Janssen 1983; Darmani et al. 1990; Fantegrossi et al. 2004; Goodwin and Green 1985; Green et al. 1983; Peroutka and Snyder 1981). This assay has been used both to study the direct effects of the 5-HT_{2A} receptor agonists, as well as the interactions between environmental and organismal factors (such as stress) on 5-HT_{2A} receptor expression and function. This assay is believed to be a highly selective *in vivo* assay of 5-HT_{2A} receptor function as the selective antagonist M100907 (Fantegrossi et al. 2005, 2006) or genetic ablation of the 5-HT_{2A} receptor attenuate the agonist-induced HTR. The potency with which a 5-HT_{2A} receptor antagonist blocks the HTR is highly correlated with the antagonist's affinity for 5-HT_{2A} receptors (Ortmann et al. 1982; Peroutka and Snyder 1981). Such preclinical studies have been supported by seminal clinical research in which a strong correlation ($r=0.97$) between ligand affinity at 5-HT₂ receptors and psychoactive potency in humans was established (Sadzot et al. 1989). The HTR has also been used to study interactions between environmental and organismal factors and 5-HT_{2A} receptor expression and function. Many of these studies used DOI as the prototypical 5-HT_{2A} receptor agonist.

Stress can affect the expression and function of 5-HT_{2A} receptors

Numerous previous studies have documented a relationship between stress and 5-HT_{2A} receptors. The forms of stress that have been associated with increased 5-HT_{2A} receptor expression and function can be broadly classified as physical stress, social stress, and maternal stress. These studies often employed radioligand binding studies in tissue or the

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drug-elicited HTR to relate the effects of stress to changes in the expression and function of 5-HT_{2A} receptors. In an early study of physical stress, it was demonstrated that electroconvulsive shock (ECS) increased the availability of 5-HT_{2A} binding sites as well as the HTR induced by 5-hydroxytryptophan (5-HTP), which stimulates the synthesis of 5-HT (Goodwin et al. 1984). Mice treated with a single ECS daily for up to 3 days showed a progressively enhanced HTR to the direct 5-HT_{2A} receptor agonist 5-methoxy-N,N'-dimethyltryptamine (5-MeO-DMT) 24 hours shock exposure (Metz and Heal 1986). Similarly, after 30–120 minutes of acute immobilization stress or after 6 consecutive days of 120 minutes of immobilization followed by 24 hours of rest, there was a significant increase in the number of binding sites in rat frontal cortex, based on values obtained with tritiated (3H) ketanserin (a non-selective 5-HT₂ receptor antagonist). It was speculated that this increase may be related to increased 5-HT turnover (Torda et al. 1988). In another study, chronic, but not acute, forced swim stress increased the number of 5-HT₂ receptors in rat frontal cortex and the DOI-induced HTR. Suggesting specific changes at the receptor level and not changes in 5-HT turnover, the levels of 5-HT and its major metabolite 5-hydroxyindole acetic acid (5-HIAA) in frontal cortex, in this model, were unchanged after either the acute or the chronic stress (Takao et al. 1995). Toe pinch stress results in a spectrum of behaviors, most of which are alleviated by either subcutaneous or intracerebroventricular administration of DOI, which is consistent with reduced anxiety in response to this form of stress (Hawkins et al. 2002). Likewise, chronic unpredictable stress produced an approximately 50% increase in cortical 5-HT_{2A} receptor expression. This paradigm involved repeated exposure of electric footshock, immobilization, swimming in cold water, and food deprivation, and other physical stressors (Ossowska et al. 2001). A common element across all of the physical stress paradigms used in these various studies is that they signal danger and possible existential threats. These studies, and others, clearly document that physical stress can increase the expression and function of 5-HT_{2A} receptors.

In addition to physical stress, social stress has also been repeatedly shown to increase the expression and function of 5-HT_{2A} receptors. For example, social isolation of male mice for six weeks resulted in an increased DOI-induced HTR and upregulation of 5-HT_{2A} receptors in the frontal cortex. These changes associated with isolation stress were attenuated by oral administration of the traditional Japanese natural product Yokukansan, which has been shown to reduce aggressiveness, excitability, and hallucinations in other studies (Ueki et al. 2015). Social isolation of adult rats for approximately 40 days also results in an increase in the DOI-induced HTR, as well as impaired male sexual behavior (Brotto et al. 1998). The social stress resulting from mixed-sex group housing of male rats results in subordinate animals taking on a severely stressed behavioral and hormonal phenotype, and exhibiting increased binding to 5-HT₂ receptors in cortex (McKittrick et al. 1995). Social defeat is a form of stress that involves elements of both social and physical stress that elicits a hypothalamo-pituitary-adrenal (HPA) axis response consistent with other stressors, as the HPA axis is a major stress response system. Exposure of Lewis rats to social defeat by Long-Evans rats exaggerated their basal anxious phenotype, increased 5-HT turnover in frontal cortex, and an increased availability of 5-HT₂ receptors (Berton et al. 1998). However, it is important to note that the relationship between 5-HT_{2A} receptors and social stress may depend on genetic and trait variables. This is supported by the finding that spontaneously

hypertensive rats, which exhibit lower anxiety levels as a trait variable than Lewis rats, do not show similar changes in anxiety, 5-HT turnover, or 5-HT_{2A} expression following social defeat despite showing an acute HPA response to this form of stress (Berton et al. 1998). Access to cooperative conspecifics, access to mates, and defeat by social rivals involve elements of danger and continued existence. These forms of stress also seem to increase the expression and function of 5-HT_{2A} receptors.

Stress related to prenatal development and maternal care also influences the expression and function of 5-HT_{2A} receptors. Separation of rat pups from their mothers from postnatal day 2–14 resulted in an enhanced DOI-induced HTR in rats and altered expression of the immediate early gene c-Fos, used as a marker of cellular activity, in response to DOI-treatment in frontal cortex, but not in the hippocampus, lateral septum or hypothalamus (Sood et al. 2018). Exposure to the bacteria-derived endotoxin lipopolysaccharide (LPS) is a form of physiological stress as it induces behavioral dysregulation and inflammation of many organ systems. Prenatal exposure to LPS leads to an increased DOI-induced HTR in both male and female mice (Wischhof et al. 2015). Maternal variable stress in pregnant mice has been shown to increase the expression 5-HT_{2A} in frontal cortex and the DOI-induced HTR in the adult but not prepubertal offspring born to stressed mothers. Interestingly, cross fostering demonstrated that these long-term effects of gestational stress on 5-HT_{2A} receptors are not attributable to observable differences in maternal care (Holloway et al. 2013). Disruption of maternal care is also likely to occur during times of danger and existential threats.

Overall, these studies provide convincing evidence that exposure to a variety of stressors enhances the expression and function of cortical 5-HT_{2A} receptors. An overview of previous studies examining the effects of various stressors on 5-HT_{2A} receptor expression or function is presented in Table 1.

Not all previous studies have supported the idea that stress increases either the expression or function of 5-HT_{2A} receptors. For example, in adult male Wistar rats, LPS was shown to suppress rather than enhance the DOI-induced HTR 1 and 3 hours after injection. Suppression of the HTR was reversed by co-treatment with indomethacin or naltrexone suggesting that it may be mediated by a cyclooxygenase-induced inflammatory response (Kouhata et al. 2001). Likewise, exposure to a single footshock attenuated rather than enhanced the HTR to DOI 24 hours after the footshock. Importantly, however, ketanserin binding studies showed that this decrease in the HTR was not attributable to downregulation of 5-HT_{2A} receptors in frontal cortex, suggesting changes in other neural regions in response to this form of stress were modulating the HTR (Izumi et al. 2002). Exposure of mice to ten minutes of swim stress resulted in a profound inhibition in the HTR induced by either DOI or 5-HTP (Pericic 2003). Additionally, genetic removal of the 5-HT_{2A} receptor neither ameliorated nor worsened the depression-like state induced by chronic unpredictable stress in either male or female mice, but did appear to protect against dyslipidemia induced by chronic unpredictable stress in wild-type male mice (Jaggar et al. 2017). Moreover, chronic unpredictable stress induced dyslipidemia in both wild-type and 5-HT_{2A} knockout mice that were female. In contrast, however, genetic removal of the 5-HT_{2A} was protective against dyslipidemia in males (Jaggar et al. 2017). This study suggests critical dynamics related to

sex differences may modulate the interactions between stress and the 5-HT_{2A} receptor. Despite these discrepancies and nuances, the bulk of previous research strongly supports the hypothesis that stress enhances the expression and function of 5-HT_{2A} receptors.

Why does stress increase 5-HT_{2A} receptor expression and function?

The literature described above presents an important question: What is the purpose of stress-induced increases in 5-HT_{2A} receptor expression and function? Some have described activation of 5-HT_{2A} systems as an active coping system for mitigating the consequences of stress (Carhart-Harris and Nutt 2017). This may be true, but does that mean that endogenous increases in 5-HT_{2A} receptor expression or function during times of stress reduce the intensity of the stressor and/or the consequences of exposure to the stressor? There is some evidence for this: For example, DOI can alleviate a pattern of behaviors that emerges following exposure to toe pinch stress, and which are consistent with the adverse consequences of exposure to a stressor (Hawkins et al. 2002). Similarly, microinjection of 5-MeO-DMT or the 5-HT uptake inhibitor zimelidine into the dorsal hippocampus immediately after 2 hours of restraint prevented the development of an anxious-like phenotype in the elevated plus-maze assay (Graeff et al. 1996). However, whether this has relevance for changes in cortical 5-HT_{2A} receptor expression or function remains to be elucidated. There is also evidence that activation of 5-HT_{2A} increases rather than decreases the intensity and consequences of stress. For example, 5-HT_{2A} receptor expression, as indexed by radiolabeled LSD binding, protein expression, and mRNA expression, is elevated in the frontal cortex of suicide victims (Pandey et al. 2002). This suggests that increased expression of 5-HT_{2A} receptors is associated with unmitigated stress rather than the amelioration of stress. The idea that 5-HT_{2A} receptors ameliorate the effects of stressors is also difficult to reconcile with findings that stimulation of 5-HT_{2A} receptor recapitulates many aspects of stressors, and could be viewed as a stressor in its own right. For example, administration of psilocybin dramatically increased levels of the HPA-axis stress hormone cortisol, which is released in response to a very broad range of stressors (de Veen et al. 2017). Likewise, genetic removal of the 5-HT_{2A} receptor led to a baseline state of reduced anxiety in male and female mice (Jaggar et al. 2017). Overall, then, this is a complex issue. In the literature, models have been proposed in which 5-HT can either trigger or reduce psychopathology through increased brain plasticity, depending on the nature of the experiences associated with the elevation in 5-HT (Branchi 2011).

If increased expression or function of 5-HT_{2A} receptors following stress is not related to amelioration of the consequences of stress, then what is a plausible alternative? It is here proposed that this is a learning mechanism through which 5-HT_{2A} receptors increase synaptic plasticity to tune the brain towards avoiding danger associated with stressors in the future. In support of this idea, exposure to immobilization stress and acute administration of DOI overlap in their molecular consequences: they both induce activation of the immediate early gene, activity regulated cytoskeletal-associated protein (Arc) within the cortex, which is important for both activity and experience dependent plasticity. DOI- or stress-induced activation of Arc depends on levels of brain derived neurotrophic factor (BDNF), which has important roles in structural and synaptic plasticity (Benekareddy et al. 2013). Moreover, both indirect (Edut et al. 2014) and direct (Afshar and Shahidi 2018) 5-HT_{2A} receptor

agonists are known to increase BDNF levels and thereby synaptic plasticity. This may have particular relevance for cortical modulation of subcortical function, as mice with conditional genetic ablation of BDNF, such that the neurotrophin is deleted postnatally, show reduced postsynaptic 5-HT_{2A} receptor levels and loss of stimulated induction of glutamate postsynaptic potentials (Rios et al. 2006). This suggests that increased BDNF levels may facilitate 5-HT_{2A} receptor mediated release of glutamate. As such, it does not appear to be the case the 5-HT_{2A} receptor activity decreases the intensity of stressors; rather it appears more likely that 5-HT_{2A} receptor function increases synaptic plasticity in response to a variety of stressors. Cortical-mediated processing of stress could then result in increased release of glutamate into subcortical regions such as the amygdala that respond to stress. Much research needs to be completed to assess this hypothesis and it should be recognized that confounding data have been reported (Vaidya et al. 1997).

5-HT_{2A} receptors and associative learning

In line with the idea that stimulating 5-HT_{2A} receptors facilitates synaptic plasticity, a long series of studies has convincingly demonstrated that activation of 5-HT_{2A} receptors promotes associative learning. A series of pioneering studies by Harvey and colleagues consistently showed that the 5-HT_{2A} receptor agonist *d*-lysergic acid diethylamide (LSD) enhanced acquisition of the classically conditioned nictitating-membrane response. These studies were typically conducted in rabbits. The stimuli used were often composed of tone and light conditioned stimuli presented before delivery of the unconditioned stimulus, consisting of a shock to the skin over the paraorbital region of the head or a puff of air to the eye. An early study showed that intravenous LSD administered at doses from 1–300 nmol/kg and 30 minutes before each daily conditioning session facilitated the acquisition of this form of associative learning, with a typical response of decreasing trials to criterion by close to 50%. Control experiments demonstrated that this was not due to sensitization, pseudoconditioning, changes in baseline responding, or modification of the unconditioned response, indicating that LSD selectively increased associative learning (Gimpl et al. 1979). This enhancement occurred with both aversive (e.g., shock) and appetitive (e.g., water delivery) unconditioned stimuli. The ability of LSD to facilitate classical conditioning of the rabbit nictitating-membrane response across a broad range of conditioning parameters appears to be related to increasing the salience of environmental cues that serve as either an unconditioned stimulus or a conditioned stimulus (Harvey et al. 1988). This has been corroborated many times: for example, LSD facilitated associative learning during Pavlovian eyeblink conditioning (Romano et al. 2006). Furthermore, consistent with these animal studies, LSD enhanced associative learning in humans using a paradigm wherein a tone was repeatedly paired with an aversive white noise (Hensman et al. 1991).

Although LSD has a notoriously non-selective receptor pharmacology, there is substantial evidence that its enhancement of associative learning is mediated by 5-HT_{2A} receptors. The facilitation of associative learning by LSD is not affected by depletion of 5-HT by bilateral intraventricular injections of 5,7-dihydroxytryptamine (5,7-DHT), suggesting that it is directly acting at a receptor rather than stimulating the release of 5-HT to produce this effect (Romano et al. 2006). The facilitation of associative learning by LSD is attenuated by pretreatment with the 5HT_{2A} antagonist ritanserin (Welsh et al. 1998a). Other 5-HT_{2A}

receptor agonists, such as 2,5-dimethoxy-4-methylamphetamine (DOM), increase the magnitude and rate of acquisition of associative learning (Harvey et al. 1982). In contrast, the 5-HT_{1A} receptor agonists 8-OH-DPAT and lisuride have no significant effect on associative learning, demonstrating specificity to the 5-HT_{2A} receptor (Welsh et al. 1998a). The 5-HT_{2A} receptor antagonists ritanserin and MDL11,939 retard the acquisition of conditioned responses during classical conditioning of the rabbit nictitating-membrane response when administered alone, supporting the role of the 5-HT_{2A} receptor, and suggesting they may be acting as inverse agonists at that receptor (Welsh et al. 1998b). 5-HT_{2A} receptor antagonists such as BOL (d-bromolysergic acid diethylamide), LY53,857, and ketanserin act as neutral antagonists in that they had no effect on learning, whereas MDL11,939, ketanserin, and mianserin seem to act as inverse agonists (Harvey 2003). Consistent with these animal studies, ritanserin retards associative learning in humans (Hensman et al. 1991). It is important to note that not every reported finding is in agreement with a role for the 5-HT_{2A} receptor in associative learning. For example, chronic treatment with LSD resulted in downregulation of 5-HT_{2A} receptors but no concomitant decrease in associative learning (Harvey et al. 2004). Nevertheless, the vast preponderance of evidence is supportive of this hypothesis.

The 5-HT_{2A} receptor appears to have not just a role in associative learning, but also likely a role in long-term learning associated with stressful situations, especially those that signal danger or existential threats. In addition to acute pharmacological stimulation of 5-HT_{2A} receptors, chronic upregulation of 5-HT_{2A} receptors facilitates associative learning. This has been demonstrated by chronic treatment with MDL11,939, which results in upregulation of 5-HT_{2A} receptors in frontal cortex as well as a facilitation of associative learning during classical trace eyeblink conditioning (Harvey et al. 2004). Viral-mediated reexpression of 5-HT_{2A} receptors in mice genetically devoid of 5-HT_{2A} receptors rescues the otherwise absent potentiation of glutamate signaling and deficits in associative learning, further relating associative learning and glutamate signaling (Barre et al. 2016). 5-HT_{2A} receptors have also been directly related to associative learning of fear responses to physical stressors. For example, in male C57BL/6J mice, administration of the 5-HT_{2A} receptor agonist TCB-2 enhanced contextual and cued fear memory as well as the consolidation of object memory (Zhang et al. 2013). Ayahuasca is a hallucinogenic beverage that combines the actions of the 5-HT_{2A/2C} agonist N,N-dimethyltryptamine (DMT) from *Psychotria viridis* with beta-carbonyl monoamine oxidase inhibitors (MAOIs) from *Banisteriopsis caapi*. Ayahuasca has been shown to increase a conditioned fear response (Favaro et al. 2015). These findings that 5-HT_{2A} receptor stimulation facilitates associative learning, including fear conditioning (i.e., the amount of time spent immobile in the presence of the conditioned stimulus), is again not supportive of the idea that this receptor mitigates the intensity of stress, and rather that it is involved in learning and synaptic plasticity as a response to stress. An overview of previous studies examining the role of 5-HT_{2A} receptors in associative learning is presented in Table 2.

The facilitation of learning through stimulation of 5-HT_{2A} receptors may be selective for associative learning over other forms of learning, as acute psilocin had no effect on spatial navigation, working memory, or memory consolidation in the Carousel maze or Morris water maze (MWM) tasks (Rambousek et al. 2014). Administration of ayahuasca as a daily

oral dose for 30 days by gavage, followed by a 48 hour washout, did not affect performance of animals in the MWM task (Favaro et al. 2015). By contrast, iontophoresis of the 5-HT_{2A} receptor agonists alpha-methyl-5-HT or 5-HT directly into the frontal cortex accentuated the spatial tuning of “memory field” neurons in monkeys performing a delayed-response task, and these agonist effects were reversed by the selective 5-HT_{2A} receptor antagonist M100907 (Williams et al. 2002). Administration of TCB-2 enhanced object memory in addition to contextual and cued fear memory (Zhang et al. 2013). A very recent study demonstrated that TCB-2 prevents loss of hippocampal BDNF levels, formation of A β plaques, the development of hippocampal neurodegeneration, and the presentation of cognitive impairments in a streptozotocin rat model of Alzheimer’s disease (Afshar and Shahidi 2018). Insufficient data are available to determine whether 5-HT_{2A} receptors are specifically involved in associative learning or are a general molecular mediator of learning. This should be investigated in future studies. However, regardless of which possibility turns out to be the case, it remains likely that 5-HT_{2A} receptors are involved in the kinds of learning that occur during stressful situations. This may be mediated by interactions between 5-HT_{2A} receptors and other neurotransmitter or hormonal systems. In this regard, the exogenous administration of corticosterone facilitates the associative learning of conditioned taste aversion, and the recall of this learning is attenuated by treatment with nefazodone, which has 5-HT_{2A} receptor antagonist properties (Gorzalka et al. 2003).

Post-Traumatic Stress Disorder (PTSD)

Mental health problems are a major worldwide source of morbidity and mortality. Anxiety related disorders can be largely categorized into PTSD, panic disorders, phobias, and generalized anxiety disorder. This spectrum of disorders is diverse but shares common features of threat-relevant responding (e.g., anxious apprehension, fear, and avoidance). Significant risk factors have been identified, including heredity, temperament, cardiac tone, cognitive/discrimination disruptions, and associative learning (Craske and Waters 2005). Chronic PTSD is characterized by excessive fear and anxiety resulting from previously experienced traumatic events. A key element of PTSD is the inability to overcome feelings of danger, even in safe environments. This is likely from an inability to fully process safety signals, which are environmental cues that enable healthy individuals to over-ride fear due to aversive cues (Jovanovic et al. 2012). The current model of PTSD states that the underlying cause of the symptoms related to uncontrollable fear is likely due to hyperactivity in the amygdala (Rauch et al. 2006). Recent human neuroimaging studies have focused on a top-down approach, elucidating the mechanisms by which amygdala activity is regulated via inhibitory control exerted by areas of the frontal cortex. Areas of the frontal cortex are known to exert top-down control over attentional processes, and are bi-directionally connected to the amygdala (Amaral et al. 1992; Carmichael and Price 1995) as well as other frontal areas involved in emotion regulation. The human neuroimaging data have not yielded consistent directions of abnormal activity (hyper versus hypoactivity) in all regions of the frontal cortex (Britton et al. 2012; Fani et al. 2012; Monk et al. 2006; Rauch et al. 2006; Telzer et al. 2008). However, a meta-analysis of 26 positron emission tomography (PET) and functional magnetic resonance imaging (fMRI) studies of PTSD revealed consistent hypoactivity in the anterior cingulate (Hayes et al. 2012), a region within the frontal cortex.

A reasonable hypothesis is that upregulation of 5-HT_{2A} receptors during time of stress could yield persistent increases in sensitivity to environmental cues associated with the stressor. Increased expression of excitatory 5-HT_{2A} receptors on layer V cortical pyramidal glutamatergic cells can make these cells more sensitive to stimulation, which could result in increased excitation of the amygdala through modulation of glutamatergic neurotransmission. This is supported by findings that 5-HT_{2A} receptors mediate increased excitatory post-synaptic potentials in pyramidal cells following application of 5-HT (Aghajanian and Marek 1997), the 5-HT_{2A} agonist DOI increases release of glutamate (Scruggs et al. 2003), and this release is blocked by the selective 5-HT_{2A} receptor antagonist M100907 (Gewirtz and Marek 2000; Zhang and Marek 2008). As this relates to PTSD, there may be selective upregulation of 5-HT_{2A} receptors in frontal areas that mediate release of glutamate into the amygdala. At the same time, areas of the frontal cortex that exert top down control over the amygdala may be devoid of 5-HT_{2A} receptors or may, for some unknown reason, not show similar increases in 5-HT_{2A} receptor expression or function during times of stress. As 5-HT_{2A} receptors increase the output of the frontal cortex to subcortical areas, this increased output in regions that stimulate fear circuits in the amygdala could feed back on itself to suppress activity in the frontal cortex. The facilitation of persistent learned associations between environmental cues and stressful situations may tune sensory systems to respond to stress-associated stimuli (i.e., danger signals). 5-HT_{2A} receptor mediated facilitation of associative learning is effective for a diversity of environmental stimuli, such as lights and tones. Likewise, both visual and auditory stimuli can trigger symptoms of PTSD. Increased expression of 5-HT_{2A} receptors results in specific neurobiological adaptations that tunes the brain towards selective attention to danger signals associated with times of stress and existential threats. This selective augmentation of danger signals may then override the protective effects of safety signals, particularly when the activity of the frontal cortex is suppressed, and result in the symptoms of PTSD. These claims are admittedly speculative, but the contention is that they lead to interesting and testable research questions.

3,4-methylenedioxymethamphetamine (MDMA)

If stress-induced increases in 5-HT_{2A} receptor expression and function are a learning mechanism that tunes the brain towards danger, and this mechanism induces pathological over-tuning of brain systems during life-threatening situations, it would be logical to surmise that antagonism of 5-HT_{2A} receptors would alleviate the symptoms of PTSD. This review is agnostic on that possibility as few studies have documented the efficacy of 5-HT_{2A} receptor antagonists for PTSD. The focus will instead be on the indirect 5-HT_{2A} receptor agonist MDMA and the implications of stress-induced changes in 5-HT_{2A} receptor expression and function for understanding the growing evidence that MDMA is an effective treatment for PTSD. MDMA was used as a treatment for psychiatric disorders in the 1970s and early 1980s. Some psychotherapists suggested that its so called “empathogenic” effects supported its use in counseling sessions (Greer and Strassman 1985; Greer and Tolbert 1998; Grinspoon and Bakalar 1986). Other clinicians used MDMA as an adjunct to “insight oriented” psychotherapy (Shulgin 1986). Following its scheduling in the mid 1980s, there

was over a decade in which there was a near hiatus on clinical studies with MDMA (Parrott 2007).

In the modern era, there is a growing body of research examining the potential clinical benefits of MDMA, particularly in the context of depression and PTSD. In this regard, the Food and Drug Administration (FDA) determined in 2002 that the risk/benefit ratio is favorable under certain circumstances for clinical studies investigating MDMA-assisted psychotherapy (Doblin 2002), and launched a study of the safety and efficacy of MDMA for PTSD. Twenty patients with chronic PTSD, refractory to both psychotherapy and psychopharmacology, were randomly assigned to groups of psychotherapy with MDMA (approx. 1.78mg/kg) or psychotherapy with placebo. The authors reported significantly greater decreases in PTSD scale scores from baseline in the MDMA group 4 days after each session, and at 2 months after the second session. Moreover, they reported that there were no drug-related serious adverse events, adverse neurocognitive effects, or clinically significant blood pressure increases, and they concluded that MDMA can be used safely to effectively treat PTSD (Mithoefer et al. 2011). Importantly, the FDA concurred with this interpretation, and significant further studies have been completed since that conclusion was reached. Indeed, MDMA-assisted psychotherapy for the treatment of PTSD has recently progressed to phase 3 clinical trials and received breakthrough therapy designation by the FDA, at least in part, because of durable remission rates approaching 70% (Feduccia and Mithoefer 2018).

Several reviews have suggested a number of possible mechanisms by which MDMA improves PTSD, with one of the leading hypotheses being that it increases frontal cortex activity and decreases amygdala activity. This may improve emotional regulation and decrease avoidance through memory reconsolidation and fear extinction, possibly mediated by dynamic modulation of emotional memory circuits (Feduccia and Mithoefer 2018; Johansen and Krebs 2009; Parrott 2007). Like the other 5-HT_{2A} receptor agonists that were described above, MDMA (Romano and Harvey 1994) and its derivative methylenedioxymphetamine (Romano et al. 1991) have been shown to facilitate fear conditioning in preclinical models, presumably through a 5-HT_{2A} receptor dependent mechanism. Paradoxically, MDMA also enhances the lasting extinction of conditioned freezing and fear-potentiated startle. This was mediated by 5-HT systems in general, as it was attenuated by pretreatment with an SSRI, and 5-HT_{2A} receptors in particular, since it was attenuated by pretreatment with a selective 5-HT_{2A} receptor antagonist (Young et al. 2017). Facilitated extinction of fear conditioning has also been reported with other 5-HT_{2A} receptor agonists as psilocybin facilitates extinction of fear conditioning at low doses that tended to increase neurogenesis in the hippocampus. (Catlow et al. 2013). These findings lead to another important question: How is it the case that the same receptor contributes to both fear conditioning and fear extinction?

Possible Mechanisms

This review proposes that a central role of 5-HT may be to function as a stress detection and response system. Upregulation of 5-HT_{2A} receptors during stressful situations may be a learning mechanism that tunes the brain through plastic changes that facilitate danger avoidance in the future. This facilitation of persistent learned associations between

environmental cues and stressful situations may be excessive during life-threatening situations and overly tune sensory systems to respond to stress-associated stimuli (i.e., danger signals), and these neurobiological adaptations may allow danger signals to override the protective effects of safety signals. The administration of MDMA appears to normalize brain function and decrease PTSD scale scores in a persistent and impressive manner, suggesting a decreased salience of these danger signals. Previous studies have linked PTSD to deficits in the capacity of organisms to use safety signals to conditionally inhibit the effects of danger signals (Jovanovic et al. 2005; Kazama et al. 2012; Myers and Davis 2004; Winslow et al. 2008). Indeed, the presence of a deficit in conditioned inhibition has been reported to be a reliable biomarker of PTSD (Jovanovic et al. 2012). One plausible mechanism for the efficacy of MDMA is that it facilitates learning between the current environment and safety signals through indirect stimulation of 5-HT_{2A} receptors. During psychotherapy, the PTSD patient makes contact with a variety of environmental stimuli that signal safety. MDMA would augment such therapy by facilitating long-lasting associations between the current environment and safety signals. As noted previously, MDMA has also been shown to increase BDNF levels (Edut et al. 2014), which would support the formation of new associations through the promotion of synaptic plasticity. In other words, MDMA may signal through 5-HT_{2A} receptors to induced BDNF-facilitated synaptic plasticity and associative learning of new safety signals. This possibility leads to the hypothesis that other 5-HT_{2A} receptor agonists may also be effective for PTSD, a matter that has not yet received extensive study. However, psilocybin has recently achieved notable success in the treatment of existential anxiety and depression in the terminally ill.

If persistent enhancements of 5-HT_{2A} receptor expression and function during stress contribute to the symptoms of PTSD, MDMA could also directly reverse some of these aberrant neuroadaptations. In support of this contention, it has been consistently shown that phenethylamines such as MDMA induce rapid tolerance and selective downregulation of 5-HT_{2A} receptors (Buckholtz et al. 1985; Leysen et al. 1989; McKenna et al. 1989; Smith et al. 2014; Smith et al. 1999). Persistent agonist-mediated downregulation of 5-HT_{2A} receptor could have equivalent effects to chronic antagonist administration. Persistent decreases in 5-HT_{2A} expression could reverse the selective tuning of cortical regions towards danger signals and attenuate glutamate release into the amygdala following danger signal processing. As 5-HT_{2A} receptors increase the output of the frontal cortex to subcortical areas, their increased expression could result in negative feedback to suppress activity in the frontal cortex. Persistent downregulation of 5-HT_{2A} receptor expression following MDMA treatment would then provide for both a persistent decrease of glutamate release into the amygdala as well as allow for a persistent normalization of frontal cortical function. It has been hypothesized that by reducing activation in brain regions implicated in the amygdala and other fear- and anxiety-related neural circuitry, MDMA may allow for reprocessing of traumatic memories and emotional engagement with therapeutic processes.

It is possible that the therapeutic efficacy of MDMA would be shared by other compounds that stimulate 5-HT_{2A} receptors or that it may be unique to MDMA. In support of the latter possibility, MDMA is known to have a complex pharmacology. MDMA can indirectly activate 5-HT_{2A} receptors and other 5-HT receptors through stimulated release of 5-HT. It also has effects at other monoamine receptors and can induce the release of hormones such

as oxytocin, cortisol, and prolactin (Murnane et al. 2010, 2012). It is possible that MDMA stimulates stress responses through the 5-HT_{2A} receptor and associated cortisol release, and that this allows for reprocessing of traumatic memories. It could simultaneously engage other 5-HT and hormonal systems (such as the prosocial effects of oxytocin) that support engagement with the therapeutic processes. Some of the effects of MDMA may be unique or specific to this single agent as SSRIs (Inoue et al. 1996) and the 5-HT releasers D-fenfluramine and meta-chlorophenylpiperazine (Graeff et al. 1996) do not seem to share the same effects of MDMA for PTSD. It is possible that some of the therapeutic effects of MDMA are mediated by interactions between 5-HT_{2A} receptors and other systems activated by its complex pharmacology. Regulation of activity of 5-HT_{2C} receptors is a plausible targets as it facilitates the retrieval of cued fear memory (Homberg 2012). Nevertheless, despite the complex pharmacology of MDMA, its activity at 5-HT_{2A} receptors appears likely to be a critical component of its therapeutic efficacy for PTSD.

Summary

5-HT is an evolutionary old molecule, is highly conserved across the animal kingdom, is widely used by the brain, and was one of the first discovered neurotransmitters. Despite this, ascribing a specific set of functions to brain 5-HT and its receptors has been difficult and controversial. This review proposes that a central role of 5-HT may be to function as a stress detection and response system. The 5-HT_{2A} receptor is the major excitatory 5-HT receptor in the brain and has been linked to the effects of drugs that produce profound sensory and cognitive changes. Upregulation of 5-HT_{2A} receptors during stressful situations may be a learning mechanism that tunes the brain through plastic changes that facilitate danger avoidance in the future. This facilitation of persistent learned associations between environmental cues and stressful situations may be excessive during life-threatening situations and overly tune sensory systems to respond to stress-associated stimuli (i.e., danger signals). These neurobiological adaptations may allow danger signals to override the protective effects of safety signals and contribute to the symptoms of PTSD. In support of this proposal, numerous studies have shown that this receptor is upregulated by a broad variety of stressors and have related 5-HT_{2A} receptor function to associative learning. Activation of the 5-HT_{2A} receptors appears to recapitulate many aspects of stressors, including activation of the HPA axis. It also appears to induce production of trophic factors such as BDNF that contribute to synaptic plasticity. In addition to being linked to learning, mood, sleep, and appetite, 5-HT has also been linked to functions that are critical for sustaining life such as mating, defensive mechanisms, dominance hierarchies, and the prevention of starvation. MDMA has shown notable success in the treatment of PTSD and have achieved status as breakthrough therapy from the FDA. It is further argued that MDMA may produce these therapeutics effects by acting through 5-HT_{2A} receptors to facilitate safety learning. MDMA may also engender agonist-mediated downregulation of 5-HT_{2A} receptors to reverse the neuroadaptations associated with danger learning. MDMA may additionally act through other 5-HT and hormonal systems to produce a stress response that allows for reprocessing of traumatic memories and that support engagement with the therapeutic processes. The 5-HT_{2A} receptor appears to have a complex and interesting role in responding to stress. Elucidating this role may provide important insights into the central

functions of the 5-HT system. It may also provide insights into the etiology of PTSD and the therapeutic benefits of MDMA.

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Table 1:

Previous studies examining the effects of various stressors on 5-HT_{2A} receptor expression or function.

5-HTP = 5-hydroxytryptophan; DOI = 2,5-dimethoxy-4-iodoamphetamine; 5-HT = 5-hydroxytryptamine;

HTR = head twitch response; 5-MeO-DMT = 5-methoxy-N,N'-dimethyltryptamine; LPS = lipopolysaccharide

Type of Stress	Outcome	Reference
<u>Studies in support of the hypothesis that stress increases 5-HT_{2A} receptor expression or function</u>		
<i><u>Physical Stress</u></i>		
Electroconvulsive shock	Increase in 5-HT ₂ receptor binding sites and 5-HTP HTR	(Goodwin et al. 1984)
Electroconvulsive shock	Increase in 5-MeO-DMT HTR	(Metz and Heal 1986)
Acute or repeated immobilization	Increase in 5-HT ₂ receptor binding sites	(Torda et al. 1988)
Chronic forced swimming	Increased DOI HTR and 5-HT ₂ availability	(Takao et al. 1995)
Toe pinch stress	Alleviation of symptoms by DOI	(Hawkins et al. 2002)
Chronic unpredictable stress	Increased 5-HT _{2A} expression	(McKittrick et al. 1995; Ossowska et al. 2001)
<i><u>Social Stress</u></i>		
Social isolation	Increased DOI HTR and 5-HT _{2A} expression	(Takao et al. 1995; Ueki et al. 2015)
Social isolation	Increased DOI HTR	(Brotto et al. 1998)
Mixed sex social housing	Increase in 5-HT ₂ receptor binding sites	(McKittrick et al. 1995)
Social defeat	Increased 5-HT ₂ availability	(Berton et al. 1998)
<i><u>Maternal Care Stress</u></i>		
Maternal separation	Increased DOI HTR and altered c-Fos expression	(Sood et al. 2018)
Prenatal exposure to LPS	Increased DOI HTR and 5-HT _{2A} expression	(Holloway et al. 2013; Wischhof et al. 2015)
Maternal variable stress	Increased DOI HTR and 5-HT _{2A} expression	(Holloway et al. 2013; Ueki et al. 2015)
<u>Studies not in support of the hypothesis that stress increases 5-HT_{2A} receptor expression or function</u>		
Adult exposure to LPS	Decreased HTR	(Kouhata et al. 2001)
Single footshock	Decreased HTR but unaltered 5-HT _{2A} expression	(Izumi et al. 2002)
Acute forced swimming	Decreased DOI or 5-HTP HTR	(Pericic 2003)
Chronic unpredictable stress	5-HT _{2A} knock mice show unaltered depression-like states	(Jaggar et al. 2017)

Table 2:

Previous studies examining the role of 5-HT_{2A} receptors in associative learning.

LSD = d-lysergic acid diethylamide; DOM = 2,5-dimethoxy-4-methylamphetamine; 8-OH-DPAT = 8-hydroxy-2-(dipropylamino)tetralin; 5-HT = 5-hydroxytryptamine; 5,7-DHT = 5,7-dihydroxytryptamine;

Type of Stimulation	Outcome	Reference
Intravenous LSD	Facilitation of associative learning	(Gimpl et al. 1979)
Systemic LSD	Facilitation of associative learning	(Harvey et al. 1988; Harvey et al. 1982)
Systemic LSD	Facilitation of associative learning	(Harvey et al. 1988; Romano et al. 2006)
Systemic LSD in humans	Facilitation of associative learning	(Hensman et al. 1991)
Depletion of 5-HT by intraventricular 5,7-DHT	No effect on LSD facilitation of associative learning	(Romano et al. 2006; Welsh et al. 1998a)
Ritanserin treatment prior to LSD	Blockade of LSD facilitation of associative learning	(Welsh et al. 1998a)
Systemic DOM	Facilitation of associative learning	(Harvey et al. 1982)
8-OH-DPAT and lisuride	No effect on associative learning	(Welsh et al. 1998a)
Inverse 5-HT _{2A} agonists	Impaired associative learning	(Welsh et al. 1998a)
Inverse 5-HT _{2A} agonists	Impaired associative learning	(Harvey 2003)
Systemic ritanserin in humans	Impaired associative learning	(Hensman et al. 1991)
Upregulation of 5-HT _{2A} receptors by chronic treatment with MDL11,939	Facilitation of associative learning	(Harvey et al. 2004)
Viral mediated recovery of 5-HT _{2A} expression in knock out mice	Rescue of associative learning	(Barre et al. 2016; Welsh et al. 1998a)
Systemic TCB-2	Facilitation of contextual and cued fear conditioning	(Zhang et al. 2013)
Ayahuasca	Facilitation of fear conditioning	(Barre et al. 2016; Favaro et al. 2015)