Brazilian Journal of health Review

Is there a relationship between hippocampus-dependent memory and 5-ht2a receptors? Insights from a systematic review

Há uma relação entre a memória hipocampo-dependente e receptores 5-HT2a? Insights de uma revisão sistemática

DOI:10.34119/bjhrv3n2-086
Recebimento dos originais: 09/02/2020
Aceitação para publicação: 26 /03/2020

Rafael Danyllo da Silva Miguel
Mestre em Neuropsiquiatria e Ciências do Comportamento
Instituição: Universidade Federal de Alagoas
Endereço: Av. Manoel Severino Barbosa, s/n, Bom Sucesso, Arapiraca-AL
E-mail: rafael.miguel@arapiraca.ufal.br

José Elias Bezerra Barros
Acadêmico de Medicina
Instituição: Universidade Federal de Alagoas – Campus Arapiraca
Endereço: Av. Manoel Severino Barbosa, s/n, Bom Sucesso, Arapiraca-AL
E-mail: jose.bezerra@arapiraca.ufal.br

Gilmar Franca Nobre Junior
Acadêmico de Medicina
Instituição: Universidade Federal de Alagoas – Campus Arapiraca
Endereço: Av. Manoel Severino Barbosa, s/n, Bom Sucesso, Arapiraca-AL
E-mail: gilmar_fnjr@hotmail.com

Isabella Cristinna da Silva Costa
Acadêmico de Medicina
Instituição: Universidade Federal de Alagoas – Campus Arapiraca
Endereço: Av. Manoel Severino Barbosa, s/n, Bom Sucesso, Arapiraca-AL
E-mail: isabellacristinna0895@gmail.com

José Emerson Xavier
Mestre em Morfotecnologia
Instituição: Faculdade Regional da Bahia
Endereço: Rodovia AL 220, 3630 Senador Arnon De Melo - Planalto, Arapiraca - AL
E-mail: joseemersonxavier@gmail.com

Lisiane dos Santos Oliveira
Doutora em Nutrição
Instituição: Universidade Federal de Pernambuco
Endereço: Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE
E-mail: lisiane.nutricao@yahoo.com

Brazilian Journal of health Review

Bruna Del Vechio Koike
Doutora em Psicobiologia, com 2 pós-doutorados.
Instituição: Universidade Federal do Vale do São Francisco
Endereço: Av. José de Sá Maniçoba, S/N - Centro, Petrolina - PE
E-mail:brunadvk@gmail.com

Sandra Lopes de Souza
Doutora em Ciências Morfofuncionais
Instituição: Universidade Federal de Pernambuco
Endereço: Av. Prof. Moraes Rego, 1235 - Cidade Universitária, Recife - PE
E-mail:sanlopesufpe@gmail.com

ABSTRACT

This is a systematic review with the aim of analyzing the role of 5-HT2A receptors in hippocampal-dependent memory. In order to do this, we searched the PubMed, Science Direct, and Neuron databases between October 23 and 29, 2018, using the following descriptor combinations: memory, 5-HT2A, and hippocampus, present in the title, abstract, or keywords, with no restrictions on study date or language. Following search and selection, we analyzed risk of bias, and the results were subsequently synthesized according to the experimental model. Out of 40 articles, four were included in qualitative analysis. The data indicate that the 5-HT2A receptors in the hippocampus play an important role in the memory consolidation process, although they do not interfere in the encoding or retrieval processes of these memories. Additionally, chronic use of receptor agonists in models of Alzheimer's disease also demonstrates better performance in the object recognition tests. The action of 5-HT2A receptors has also been shown to be important to aversive memory formation, thus attributing a prominent role to these receptors in hippocampal-dependent memory processes.

Keywords: Memory, Hippocampus, Serotonin, 5-HT2A receptor

RESUMO

Esta é uma revisão sistemática com o objetivo de analisar o papel dos receptores 5-HT2A na memória dependente do hipocampo. Para fazer isso, pesquisamos os bancos de dados PubMed, Science Direct e Neuron entre 23 e 29 de outubro de 2018, usando as seguintes combinações de descritores: memória, 5-HT2A e hipocampo, presentes no título, resumo ou palavras-chave, sem restrições na data ou idioma do estudo. Após a busca e seleção, analisamos o risco de viés e os resultados foram sintetizados posteriormente de acordo com o modelo experimental. Dos 40 artigos, quatro foram incluídos na análise qualitativa. Os dados indicam que os receptores 5-HT2A no hipocampo desempenham um papel importante no processo de consolidação da memória, embora não interfiram nos processos de codificação ou recuperação dessas memórias. Além disso, o uso crônico de agonistas de receptores em modelos da doença de Alzheimer também demonstra melhor desempenho nos testes de reconhecimento de objetos. A ação dos receptores 5-HT2A também demonstrou ser importante para a formação aversiva da memória, atribuindo assim um papel proeminente a esses receptores nos processos de memória dependentes do hipocampo.

Palavras-chave: Memória, Hipocampo, Serotonina, Receptor 5-HT2A

Brazilian Journal of health Review

1 INTRODUCTION

For more than 40 years, researchers around the world have studied the brain in order to understand mechanisms related to memory (Kandel 2009). From the beginning, it has been possible to observer the existence of two distinct forms of memory, one which depends primarily on the cerebral cortex (explicit memory) and one which depends on subcortical structures (implicit memory) (see KANDEL; DUDAI; MAYFORD, 2014). The hippocampus is one of the main cortical regions related to mechanisms of learning and memory (Cohen et al. 2013). Among the diverse modalities of memory, the ones that most depend on functional integrity of the hippocampus are spatial memory (Bui et al. 2018; Teixeira et al. 2018) and object recognition (Cohen et al. 2013; Hammond, Tull, and Stackman 2004; Zhang et al. 2013). In order to perform these functions, the hippocampus requires interaction of diverse neurotransmitter systems (for review, see PALACIOS-FILARDO; MELLOR, 2019), which include the serotonergic system (Teixeira et al. 2018).

The role of serotonin in associative memory and behavioral sensitization of invertebrates has been firmly established in the literature (Bailey et al. 2000). The means by which this neurotransmitter acts on declarative memory systems in mammals, however, continues to be the focus of diverse studies. Dense serotonergic projections from the raphe nuclei reach the hippocampus (Mokler et al. 1998; Nichols 2012) and inhibit long-term potentiation (LTP), responsible for memory consolidation. Nonetheless, reduced tryptophan or serotonin are associated with cognitive deficiencies observed in patients affected by major depression, and patients recover from these deficiencies following administration of drugs that increase serotonergic signaling (Micheli et al. 2018; Yohn, Gergues, and Samuels 2017). Furthermore, recent studies have shown that optogenetic stimulation of serotonergic terminals in region 1 of Ammon's horn (CA1) promotes improvements in spatial memory and potentiates transmission at CA3-to-CA1 synapses (Teixeira et al. 2018). These findings indicate that the serotonergic system plays an important role in hippocampus-dependent memory, thus making it necessary to identify the main serotonergic neuroreceptors present in the hippocampus in order to comprehend the correlation between their functions and memory.

To date, 14 subtypes of serotonergic receptors, all of which are present in the hippocampus, have been described. One of these receptors is 5-HT2A, a member of the G protein-coupled receptor family, which is distributed mainly in the frontal cortex and the hippocampus (Meneses 2007; Nichols 2012). This receptor is associated with cognition,

decision making, and task performance, and it is involved in psychiatric disorders (ZHANG and STACKMAN JR 2015). The distribution of 5-HT2A receptors in the hippocampus suggests that this receptor may contribute to memory, acting on both pre- and post-synaptic terminals (ZHANG and STACKMAN JR 2015). The true role of 5-HT2A receptors on hippocampus-dependent memory, however, has yet to be analyzed in a systematic review, which is the objective of this study.

2 METHODS

To perform the systematic review, the authors jointly developed a search and selection protocol for articles and chose methods for assessing risk of bias in the included articles. In all of the steps described below, analyses were independently carried out by two researchers (RDSM and JEBB), and the degree of agreement between these researchers was subsequently calculated by kappa coefficient, using IBM SPSS Statistic 21 software. The analyses and elaboration of this study were based on the guidelines provided by The Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA), and the protocol utilized was registered in PROSPERO under nº122117.

2.1 ARTICLE SEARCH AND SELECTION METHODS

Bibliographic searches were carried out between 23 and 29 October 2018 on PubMed, Science Direct, and Neuron, using the following MeSH descriptors: *5-HT2A*, *memory*, and *hippocampus*, present in the title, abstract, or keywords of articles available on the investigated databases. No restrictions were applied regarding study date or language. Following the collection of articles found on the databases and exclusion of duplicate articles, the titles and abstracts were read, in order to select studies that correlated 5-HT2A receptors' function in the hippocampus with their role in memory. To this end, the authors developed a sequence of inclusion and exclusion criteria for analysis.

Firstly, only the following were included: 1- original articles; 2- articles on rodents (rats or mice), given that the distribution and density of receptors vary according to species; 3- articles with a control group, in order to infer better or worse performance due to pharmacological manipulation of the animal model studied; 4- articles that used manipulation of 5-HT2A hippocampal with receptor agonists or antagonists, seeing that the objective of our study was to analyze the function of these receptors in memory. We

excluded articles in which the control group did not receive placebo (saline solution or vehicle) at the moments when the test group received the drug of interest, thus avoiding dubious results owing to potential manipulation of the animals. Finally, we excluded articles in which no memory tests were performed before, during, or after the proposed analyses.

No restrictions were applied regarding the breed of the rats or mice utilized in the articles selected for analysis, provided that they adhered to the aforementioned criteria. Studies that used systematic application of an agonist or antagonist were included only when they performed memory tests and, following euthanasia, conducted some form of analysis of the hippocampus that might correlate performance on the test in question with the functions of 5-HT2A receptors in the hippocampus. Purely morphological studies that did not evaluate the functions of 5-HT2A receptors specifically in the hippocampus were also excluded.

2.2 DATA ANALYZED IN SELECTED ARTICLES

For each article included in this systematic review, the authors investigated the animal species utilized, age at the moment of the experiment, the number of animals per cage, temperature and dietary conditions, type of drug used, drug dose, means of drug administration, and the moment the drug was administered. Furthermore, the authors analyzed the type of memory test applied, as well as each study's objectives, results, and conclusions. For studies that used data referring to investigations related to functions of 5-HT2A receptors in the hippocampus. Additionally, experimental groups whose analyses were not directly focused on functions of 5-HT2A receptors or whose analyses did not relate to the control group were also excluded from the description of results.

2.3 RISK OF BIAS ASSESSMENT FOR SELECTED ARTICLES

To assess risk of bias, the SYstematic Review Center for Laboratory animal Experimentation (SYRCLE) was utilized. This analyzes 6 different types of bias, namely: 1- selection bias; 2- performance bias; 3- detection bias; 4- attrition bias; 5- reporting bias, and; 6- other biases. For each of the risks analyzed, symbols were attributed to indicate high risk of bias (+), moderate risk of bias (±), or low risk of bias (−). As high risk of bias is associated with significant tendencies, the results of these studies cannot be considered definitive.

Brazilian Journal of health Review

2.4 SYNTHESIS STRATEGIES

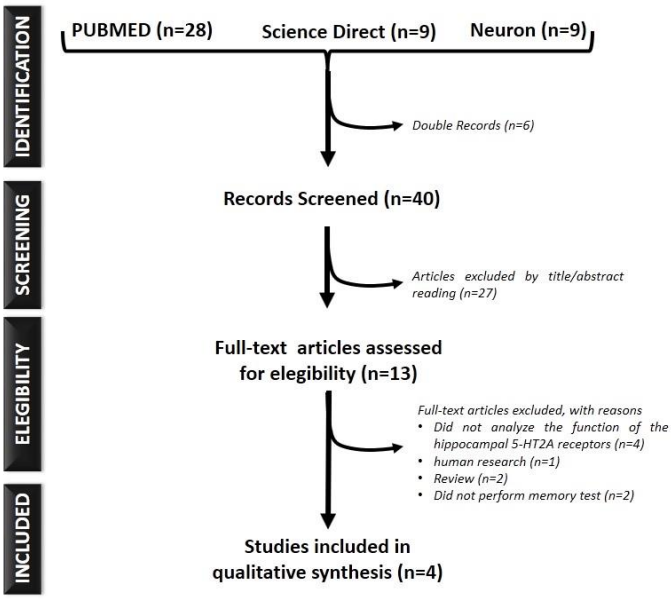
The synthesis of the results obtained was carried according to the homogeneity of the included studies, taking into consideration the animal species, animal age, animal living conditions, drug type, drug dose, means of pharmacological administration, and memory test applied.

3 RESULTS

3.1 ARTICLE SEARCH AND SELECTION

Following the bibliographic search and exclusion of duplicates, 40 articles were found in the sources utilized. However, following selection and analysis of complete texts for eligibility, only four articles were included for qualitative synthesis. A flowchart illustrating each step of the search and inclusion process is represented in **Figure 1**.

Figure 1: Flowchart showing the steps to the article search and inclusion process.



3.2 RISK OF BIAS ASSESSMENT

Risk of bias was assessed, maintaining a significantly relevant ($p > 0.001$) level of agreement between investigators, with a kappa index of 0.686. Risk of bias classification for each article included is summarized in **Table 1**.

Brazilian Journal of health Review

Table 1: Risk of bias assessment

	NASEHI et al., 2014	*ZHANG et al., 2016*	*NASEHI et al., 2017*	*AFSHAR; SHAHIDI, 2018*
Selection bias	±	±	-	±
Performance Bias	±	±	±	±
Detection Bias	±	±	±	±
Attrition Bias	±	+	±	±
Reporting Bias	-	-	-	±
Other Bias	-	-	-	±

(+ High risk of bias; ± Unclear risk of bias; − Low risk of bias)

3.3 SYNTHESIS OF INCLUDED ARTICLES

Three of the four articles included for qualitative analysis worked with mice; two used the NMRI breed, and one used the C57BL/6J breed. One study worked with Wistar rats. In the studies with mice models, pharmacological microinfusion was administered directly in CA1, whereas in the rat model the drug was administered via intracerebroventricular (ICV) injection. The study that utilized a Wistar rat model, on the other hand, performed morphological analyses of the hippocampus. As these analyses were correlated with the behavioral results obtained, however, these results are considered in the discussions. The general characteristics of the animal models utilized and information regarding the drugs administered are summarized in **Table 2**.

Brazilian Journal of health Review

Table 2: Main descriptions of animal model and experimental study design

CITATION	SPECIES	AGE	SUBSTANCE/DOSE	PHARMACOLOGICAL MANIPULATION
ZHANG, 2016	Mice C57BL/6J	8-12 weeks	TCB-2[1] (1.0 mg/kg, i.p. or 1.0µg/0.5µl in CA1) MDL 11.939[2] (0.5mg/kg, i.p)	1. TCB-2 or vehicle was administered i.p. among the groups: - 20 min before the sample session - right after the sample session - 20 min before the test session The test was done 24h after the sample session. This design yielded the following groups: **C1:** Vehicle/Vehicle/Vehicle **T1:** TCB-2/Vehicle/Vehicle **T2:** Vehicle/TCB-2/Vehicle **T3:** Vehicle/Vehicle/TCB-2 2. MDL administration produced a new group: **T4:** MDL + TCB-2 right after the sample, 10 minutes before TCB-2 administration 3. In order to evaluate the TCB-2 effect directly in CA1: **T5**: TCB-2 in CA1. **C2:** Cerebrospinal fluid in CA1.
NASEHI ET AL., 2017	Mice NMRI	5-8 weeks	Agonist (α-methyl 5-HT [5-HT2A, B e C]) or Antagonist (Cinancerine [5-HT2]) in CA1: 0.5, 5.0 e 50 ng/ mice.	The agonist or antagonist administration was performed before the inhibitory avoidance test session (first day). T1-T3 groups received increasing doses of the agonist, while T4-T6 groups received the antagonist increasing doses. The control groups C1 (agonist's control) and C2 (antagonist's control) received saline solution.
NASEHI ET AL., 2014	Mice NMRI	5-8 weeks	Agonist (α-methyl 5-HT) or Antagonist (Cinancerine) CA1 microinfusion: 0.005, 0.05 e 5ng/mice.	The agonist or antagonist administration was performed before the inhibitory avoidance test session (first day). T1-T3 groups received increasing doses of the agonist, while T4-T6 groups received the antagonist increasing doses. The control groups C1 (agonist's control) and C2 (antagonist's control) received saline solution.
AFSHAR ET AL., 2018	Wistar rats	Non informed	C1: Streptozotocin[3] (3.0 mg/kg, 10µl, i.c.v.) T1: TCB-2 (5µg/1 µl, i.c.v.)	In this study, the researchers analyzed the role of TCB-2 agonist in the memory of animals models for Alzheimer's disease (AD) induced by streptozotocin. In C1 group, AD was induced, and in T1 group, in addition to streptozotocin, TCB-2 was administered to test memory and the to evaluate the number of intact hippocampal neurons.

[1] TCB-2 ((7R)-3-bromo-2,5-dimethoxy-bicyclo[4.2.0]octa-1,3,5-trien-7yl)
[2] MDL (α-Phenyl-1-(2-phenylethyl)-4-piperidinemethanol)
[3]Streptozotocin

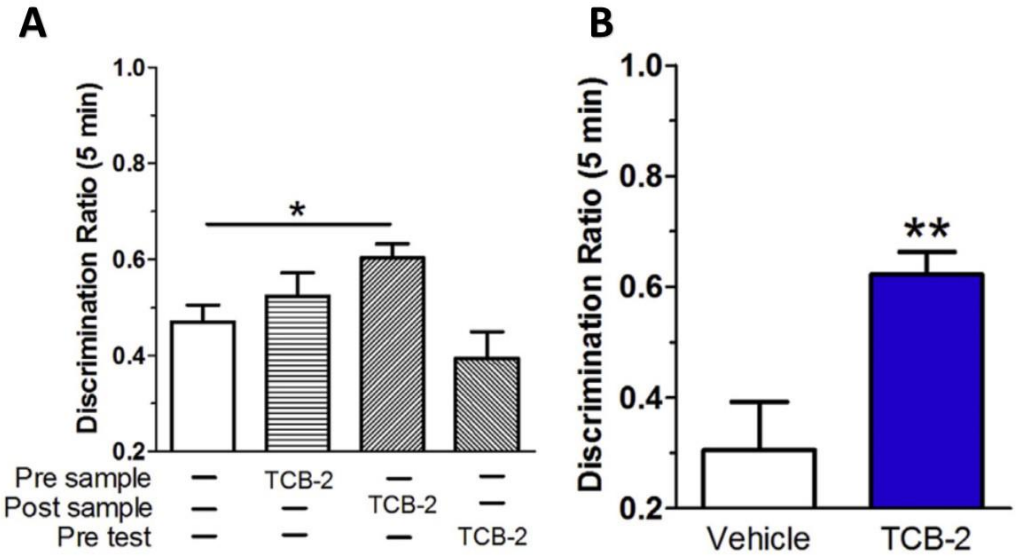
3.4 THE ROLE OF 5-HT2A RECEPTORS IN THE ENCODING, CONSOLIDATION, AND RETRIEVAL PHASES OF OBJECT MEMORY

To identify the roll of 5-HT2A receptor activation in encoding, consolidation, and retrieval processes of object memory, Zhang and collaborators (2016) organized four experimental groups (Zhang, Cinalli, Cohen, Knapp, Rios, Martínez-Hernández, et al. 2016). These groups received three intraperitoneal injections of either a 5-HT2A receptor agonist (TCB-2) or a vehicle; the injections were administered before a novel object recognition (NOR) training session, immediately after the training session, and before the test session, forming the following four groups: I. control group (vehicle/vehicle/vehicle); II. experimental group 1 (TCB-2/vehicle/vehicle); III. experimental group 2 (vehicle/TCB-2/), and; IV. experimental group 3 (vehicle/vehicle/TCB-2), in order to analyze the processes of encoding, consolidation, and retrieval, respectively.

The results of this study indicated that only administration of the agonist immediately after the training session improved performance on the object recognition memory test, in comparison with the control, which received only the vehicle (**Figure 2A**). Given that administration of the drug was systemic, however, it would not be possible to affirm that the results obtained were truly due to activation of 5-HT2A receptors in the hippocampus. For this reason, another experiment was conducted, wherein a cannula was inserted into the CA1 field of the hippocampus, infusing the vehicle (0.5 µl, n = 7) or TCB-2 (1.0 µg/0.5 µl, n = 8), immediately after the training session. This experiment verified that activation of 5-HT2A receptors in the hippocampus was, in fact, related to better performance on the NOR test (**Figure 2B**). These data suggest that 5-HT2A receptor activation in the CA1 field of the hippocampus plays an important role in the process of memory consolidation, although it did not alter encoding and retrieval processes (Zhang, Cinalli, Cohen, Knapp, Rios, Martínez-Hernández, et al. 2016).

Brazilian Journal of health Review

Figure 2: Systemic or local intrahippocampal administration of TCB-2 enhances consolidation of novel object memory. (A) Mice received TCB-2 before the sample session (TCB-2 + vehicle + vehicle, n = 10), immediately after the sample session (vehicle + TCB-2 + vehicle, n = 12), or before the test session (vehicle + vehicle + TCB-2, n = 10), which occurred 24 h later, to test object encoding, consolidation, and retrieval, respectively. Mice treated with TCB-2 after the sample session demonstrated stronger preference in new object recognition during the test session than the vehicle-treated mice (vehicle + vehicle + vehicle, n = 10), as measured by the discrimination ratio scores ($p < 0.05$). (B) Mice that received bilateral microinfusion of TCB-2 (1.0 µg/0.5 µl, n = 8) into the CA1 region exhibited enhanced performance during the test session, in comparison with mice that received intra-CA1 artificial cerebrospinal fluid (0.5 µl, n = 7). Adapted from ZHANG et al., 2016.



A longer protocol, in which ICV administration of TCB-2 (5 µg/1 µl) was carried out for 30 days preceding the memory test, also verified the benefits of 5-HT2A receptor activation for improving performance on object recognition tests (Afshar et al. 2018). This study, however, utilized Wistar rat models with Alzheimer's disease (AD) (induced by streptozotocin 3.0 mg/kg, 10 µl, ICV). In this experiment, the discrimination index in the NOR test was shown to improve in animals with AD that received TCB-2, in comparison with animals in the control group (AD + vehicle). Although this study used ICV injection, it verified the effects that this means of administration promoted with regards to survival of hippocampal neurons. They verified that ICV administration of TCB-2 promotes better neuron survival in the CA1 field of the hippocampus in rats, suggesting that 5-HT2A receptor activation may improve memory via its neuroprotective action.

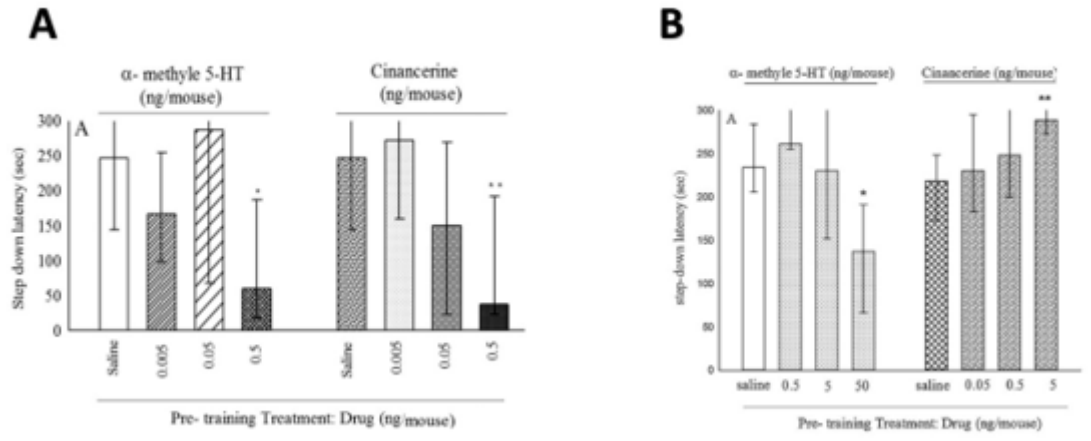
Brazilian Journal of health Review

3.5 THE ROLE OF 5-HT2A IN AVERSIVE MEMORY

Two of the four articles included performed an inhibitory avoidance test (IAT). In both articles, intrahippocampal administration of agonists, antagonists, or saline solution occurred five minutes before the training session (Nasehi et al. 2014, 2017). In the study performed in 2014, the agonist utilized was α-methyl 5-HT in concentrations of 0.005, 0.05, and 0.5 ng/mouse). This drug is a 5-HT2A/2B/2C receptor agonist. Cinancerine, which acts as a 5-HT2 receptor antagonist, was used in increasing concentrations of 0.005, 0.05, and 0.5 ng/mouse (Nasehi et al. 2014). In the 2017 article, the authors carried out an analysis using higher doses of the same agonists and antagonists administered in the previous study. The agonist concentrations were 0.5, 5.0, and 50 ng/mouse, and the antagonist concentrations were 0.05, 0.5, and 5.0 ng/mouse (Nasehi et al. 2017).

Both studies found shorter latency periods, following the highest dose of α-methyl 5-HT, suggesting that activation of 5-HT2 receptors in the hippocampus before an aversive event impairs the aversive memory encoding process (**Figure 3**) (NASEHI et al., 2014, 2017). With cinancerine, however, they found that doses of 0.5 ng/mouse showed detrimental effects on aversive memory encoding (Nasehi et al. 2014) (**Figure 3A**). Doses of 5ng/mouse, however, showed beneficial effects on the retention of this type of memory (Nasehi et al. 2017) (**Figure 3B**).

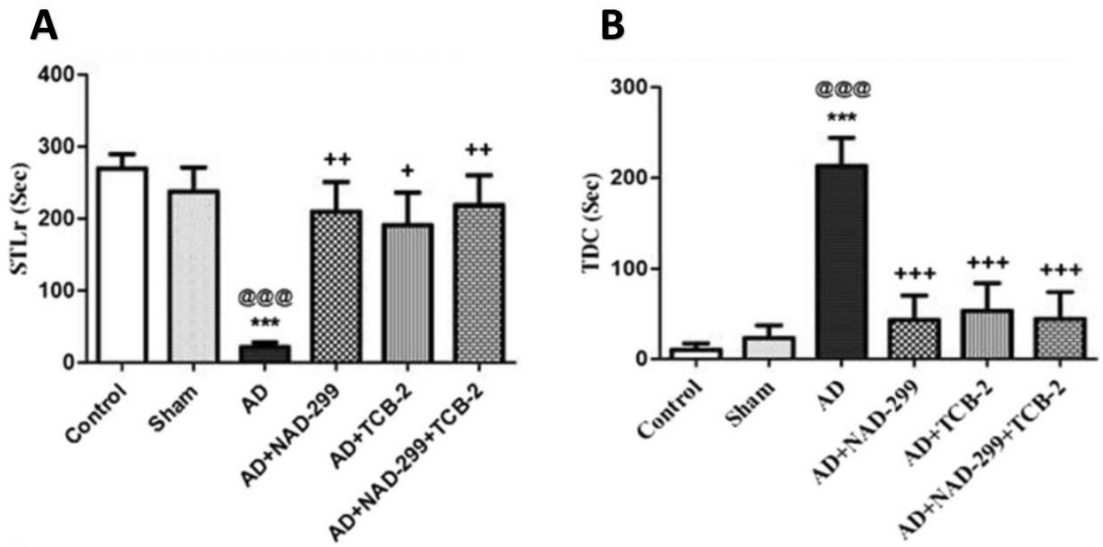
Figure 3: The latency period of the step-down test. (A) The graphs exhibit the effects of pre-training administration of α-methyl 5-HT (left, 0.5, 5 and 50 ng/mouse) and cinancerine (right, 0.05, 0.5, and 5 ng/mouse) on memory acquisition. (B) The effects of pre-training administration of α-methyl 5-HT (left, 0.005, 0.05, and 0.5 ng/mouse) and cinancerine (right, 0.005, 0.05, and 0.5 ng/mouse) on memory acquisition. Each bar is the mean ± standard error of the mean *p < 0.05 and **p < 0.01 when compared to saline/saline group (Adapted from NASEHI et al., 2014, 2017).



Brazilian Journal of health Review

In the passive avoidance learning (PAL) task, it was found that animals with AD that were treated with TCB-2 had longer latency times to enter the dark compartment during the test session (**Figure 4A**). Moreover, these animals remained less time in the dark compartment, in comparison with animals with AD alone (**Figure 4B**) (Afshar et al. 2018). These data indicate that, in addition to participating in mechanisms of object recognition memory, 5-HT2A receptors are also related to aversive memory. However, given that, in this study, TCB-2 was administered via ICV injection, the correlations between receptor action and memory can only be inferred when correlated to hippocampal neuron survival.

Figure 4: Activity of serotonergic receptors on aversive memory in mice. (A) Step-through latency during the retention trial (STLr). (B) Time spent in the dark compartment during the retention trial (TDC). Data are expressed as means ± standard error of the mean (n = 9 per group). Comparisons were made with a one-way ANOVA, which was followed by a post hoc Tukey test. ***p < 0.001 as compared with the control group. @@@p< 0.001 when compared with the sham group. +p < 0.05; ++p < 0.01; +++p < 0.001 compared to AD group.



4 DISCUSSIONS

The 5-HT2A receptors are G protein-coupled receptors, which, when activated, promote increased phospholipase C action, thus promoting greater calcium concentrations in the cytoplasmic compartment (Hagberg et al. 1998; Parrish et al. 2005). They are widely distributed throughout the central nervous system, showing an increased density in the frontal cortex and the hippocampus (ZHANG and STACKMAN JR 2015). Although they are strongly associated with psychiatric alterations, evidence has shown that these receptors

may be related to memory processes (see ZHANG and STACKMAN, 2015), given that gene polymorphisms in these receptors (HTR2A) are associated with cognitive deficiencies (Sigmund et al. 2008; Zhu et al. 2013). In CA1, it is possible to identify the presence of 5-HT2A receptors in the plasma membrane, dendrites, and dendritic spines (ZHANG et al., 2016), and they are colocalized with NMDA glutamatergic receptors (Peddie et al. 2008). Furthermore, immunoparticles for the 5-HT2A receptors have also been observed in pre-synaptic terminals of excitatory synapses with dendrites of CA1 pyramidal cells (ZHANG et al., 2016).

To analyze the effects of these receptors, it is possible to utilize one of their agonists. There are several agonists for 5-HT2A receptors, all of which have different physical-chemical properties and specificities (Nichols 2012; Parrish et al. 2005). Of these agonists, ([4-Bromo-3,6-dimethoxybenzocyclobuten-1-yl] methylamine hydrobromide), known as TCB-2, exhibits a high affinity with 5-HT2A receptors (Zhang et al. 2013). In the analyzed studies that utilized TCB-2, this agonist was observed to promote benefits for the object memory consolidation processes, both in normal mice (Zhang, Cinalli, Cohen, Knapp, Rios, and Stackman 2016) and in rat models of AD (Afshar et al. 2018). Both studies correlated the object recognition test (Bui et al. 2018; Cohen et al. 2013) with the action of 5-HT2A receptors, using TCB-2.

In CA1, serotonin promotes increased cellular excitability, given that it inhibits potassium channels and promotes activation of N-methyl-D-aspartate (NMDA) receptors, facilitating LTP (Cai et al. 2013; Celada, Puig, and Artigas 2013; Teixeira et al. 2018). On the other hand, the serotonin released in CA3 inhibits LTP or long-term depression (LTD), promoted by the mossy fibers of the dentate gyrus (Twarkowski, Hagena, and Manahan-vaughan 2016). In the study by Zhang and collaborators (2016), the agonist was administered with the assistance of a cannula directly inserted into CA1 (Zhang, Cinalli, Cohen, Knapp, Rios, and Stackman 2016).

Optogenetic analyses have shown the presence of serotonergic fibers in the stratum radiatum and the stratum lacunosum-moleculare of the hippocampus, and they indicate that the photorelease of serotonin in CA1 increases excitability of pyramidal cells to stimuli from the Schaffer collaterals, although it did not increase the response to stimuli coming from the entorhinal cortex via the perforant path (Teixeira et al. 2018). In this manner, knowing that object memory depends on the hippocampus (Bui et al. 2018; Cohen et al. 2013) and that serotonin increases neural excitability to stimuli coming from the Schaffer collaterals

(Teixeira et al. 2018), it is probable that the benefits in memory consolidation observed in the study by Zhang and collaborators (2016) is due to the increase in this excitability promoted by serotonin.

In addition to the increased excitability of pyramidal cells in CA1, the activation of 5-HT2A receptors further promotes a transitory increase in dendritic spine genesis (Yoshida et al. 2011), as well as greater expression of brain derived neurotrophic factor (BNDF) (Vaidya et al. 1997). BDNF is a neurostimulating substance that promotes neurogenesis in the hippocampus (Cameron, Hazel, and Mckay 1998). Due to this, chronic use of the agonist TCB-2 increased release of BDNF in the AD model, which both improved discrimination index and increased hippocampal neuron survival (Afshar et al. 2018). These findings indicate that the stimulation of 5-HT2A receptors in CA1 is important for object recognition memory. However, with respect to aversive memory, these receptors play another role.

Three studies analyzed aversive memory using different methods. In the studies by NASEHI (2014 and 2017), the effects of 5-HT2A/2B/2C receptor activation were analyzed via administration of the agonist α-methyl 5-HT and the antagonist cinancerine (5-HT2). As stated in the results, they verified that the administration of 0.5 ng/mouse of the agonist induced detriments to memory, showing a shorter latency time on the IAT (NASEHI et al., 2014). This result, however, was not repeated with a similar dose in the same animal model in subsequent studies, and it was only expressive with a dose of 50 ng/mouse (NASEHI et al., 2017).

Another point of divergence between these two studies was observed in the administration of the antagonist cinancerine, regarding latency time on the IAT. Whereas, in the first study, the dose of 0.5 ng/mouse induced shortened latency time (NASEHI et al., 2014), it did not promote any differences in the subsequent study when applied at the same dose (NASEHI et al., 2017). On the other hand, when cinancerine was administered at a dose of 5 ng/mouse, there was an increase in latency time, indicating an improvement in the aversive memory encoding process following previous use of this drug (NASEHI et al., 2017). This result is in opposition to the one obtained by the administration of the same drug at a dose of 0.5 ng/mouse in the prior study (NASEHI 2014). Notwithstanding these points of divergence, these two studies featured no differences regarding experimental model applied, age, living conditions, number of animals per group, memory tests, or other experimental procedures; the only difference was the concentrations of the drugs

administered. In this manner, there remains only one noncontroversial study that verified the function of 5-HT2A receptors in aversive memory.

Afshar and collaborators (2018) verified that chronic administration of TCB-2 (5µg/1 µl, ICV) promoted benefits to aversive memory on the PAL test in animals with AD (Afshar et al. 2018). This benefit may be observed during the retention test, and in the experimental session carried out 24 hours later, where the animals exhibited increased time spent in the dark compartment. It is, however, necessary for studies utilizing intrahippocampal injections of TCB-2 to be carried out in order to infer whether this observation is due to specific activation of 5-HT2A receptors present in the hippocampus.

Another point that deserves to be highlighted is that, in all the articles listed, there was a high level of unclear bias, especially with respect to performance, detection, and attrition. In animal studies, cages are not routinely stored in the animal room or vivarium in a random fashion (Kilkenny et al. 2009). Random allocation of animals is necessary in order to avoid situations where, for reasons of convenience, a given experimental group may be allocated to positions where environmental conditions are different than those provided in other positions. This is very relevant with respect to the serotonergic system, given that differences in environmental temperature and light may lead to alterations in the release of this neurotransmitter (Novotná and Janský 1976). Furthermore, in their texts, few articles using animal models specify whether the animal caregivers and the researchers carried out the analysis blindly with respect to the interventions applied to each experimental group, which implies a detection bias.

For this reason, this study highlights that even though 5-HT2A receptors in the hippocampus are strongly associated with benefits to object recognition memory and aversive memory in PAL, it is necessary that further studies are conducted on this theme, thus minimizing risks of bias.

5 CONCLUSIONS

Activation of 5-HT2A receptors in the hippocampus promotes improved performance in object recognition tests and improves aversive memory encoding processes in PAL. However, concerning analysis of aversive memory using the IAT, data were not conclusive. For this reason, it is not possible to infer whether the activation of these receptors promotes better or worse performance on this test.

Brazilian Journal of health Review

CONFLICT OF INTEREST

The authors have no conflict of interest to declare.

REFERENCES

Afshar, Simin et al. 2018. "The Effect of NAD-299 and TCB-2 on Learning and Memory , Hippocampal BDNF Levels and Amyloid Plaques in Streptozotocin-Induced Memory Deficits in Male Rats." *Psychopharmacology* 235: 2809–22.

Bailey, Craig H et al. 2000. "Is heterosynaptic modulation essential for stabilizing hebbian plasticity and memory ?" 1(October): 1–10.

Bui, Anh D et al. 2018. "Dentate Gyrus Mossy Cells Control Spontaneous Convulsive Seizures and Spacial Memory." *Neuroscience* 359(February): 787–90.

Cai, Xiang et al. 2013. "Local Potentiation of Excitatory Synapses by Serotonin and Its Alteration in Rodent Models of Depression." *Nature Publishing Group* 16(4): 464–72. http://dx.doi.org/10.1038/nn.3355.

Cameron, HA, TG Hazel, and RD Mckay. 1998. "Regulation of Neurogenesis by Growth Factors and Neurotransmitters." *Journal of Neurobiology* 36(2): 287–306.

Celada, Pau, M Victoria Puig, and Francesc Artigas. 2013. "Serotonin Modulation of Cortical Neurons and Networks." 7(April): 1–20.

Cohen, Sarah J et al. 2013. "The Rodent Hippocampus Is Essential for Nonspatial Object Memory." *Current Biology* 23(September): 1685–90.

Hagberg, Gul-Britt et al. 1998. "Stimulation of 5-HT 2A Receptors on Astrocytes in Primary Culture Opens Voltage-Independent Ca+2 Channels." *Neurochemistry international* 32: 153–62.

Hammond, Rebecca S, Laura E Tull, and Robert W Stackman. 2004. "On the Delay-Dependent Involvement of the Hippocampus in Object Recognition Memory." *Neurobiology of Learning and Memory* 82(April): 26–34.

Kandel, Eric R. 2009. "The Biology of Memory : A Forty-Year Perspective." *The journal of neuroscience* 29(41): 12748–56.

Kandel, Eric R, Yadin Dudai, and Mark R Mayford. 2014. "Review The Molecular and Systems Biology of Memory." *Cell* 157(1): 163–86. http://dx.doi.org/10.1016/j.cell.2014.03.001.

Kilkenny, Carol et al. 2009. “Survey of the Quality of Experimental Design , Statistical Analysis and Reporting of Research Using Animals.” *Plos onde* 4(11).

Meneses, Alfredo. 2007. “Stimulation of 5-HT1A, 5-HT1B, 5-HT2A/2C, 5-HT3 and 5-HT4 Receptors or 5-HT Uptake Inhibition: Short- and Long-Term Memory.” *Behavioural Brain Research* 184(june): 81–90.

Micheli, Laura, Manuela Ceccarelli, Giorgio D Andrea, and Felice Tirone. 2018. “Depression and Adult Neurogenesis : Positive Effects of the Antidepressant Fluoxetine and of Physical Exercise.” *Brain Research Bulletin* 143(September): 181–93. https://doi.org/10.1016/j.brainresbull.2018.09.002.

Mokler, David J et al. 1998. “Serotonin Neuronal Release From Dorsal Hippocampus Following Electrical Stimulation of the Dorsal and Median Raphe ´ Nuclei in Conscious Rats.” *Hippocampus* 273(8): 262–73.

Nasehi, Mohammad, Fatemeh Ghadimi, Fatemeh Khakpai, and Mohammad-reza Zarrindast. 2017. “Interaction between Harmane, a Class of β-Carboline Alkaloids, and the CA1 Serotonergic System in Modulation of Memory Acquisition.” *Neuroscience Research*. http://dx.doi.org/10.1016/j.neures.2017.03.011.

Nasehi, Mohammad, Mehdi Jamshidi-mehr, Fatemeh Khakpai, and Mohammad-reza Zarrindast. 2014. “Possible Involvement of CA1 5-HT1B/1D and 5-HT2A/2B/2C Receptors in Harmaline-Induced Amnesia.” *Pharmacology, Biochemistry and Behavior* (2013): 1–9. http://dx.doi.org/10.1016/j.pbb.2014.08.007.

Nichols, David E. 2012. “Structure – Activity Relationships of Serotonin 5-HT 2A Agonists.” *Advanced Review* 1(October): 559–79.

Novotná, R, and L Janský. 1976. “Effect of Different Environmental Temperatures on the Serotonin Concentration and Turnover in the Brain Stem of a Hibernator.” *Physiol Bohemoslov.* 25(1).

Palacios-filardo, Jon, and Jack R Mellor. 2019. “Neuromodulation of Hippocampal Long-Term Synaptic Plasticity.” *Current Opinion in Neurobiology* 54: 37–43. https://doi.org/10.1016/j.conb.2018.08.009.

Parrish, Jason C, Michael R Braden, Emily Gundy, and David E Nichols. 2005. “Differential Phospholipase C Activation by Phenylalkylamine Serotonin 5-HT 2A Receptor Agonists.” *Journal of Neurochemistry* 95(july): 1575–84.

Peddie, C J et al. 2008. “Colocalisation of Serotonin 2A Receptors with the Glutamate Receptor Subunits NR1 and GluR2 in the Dentate Gyrus : An Ultrastructural Study of a

Modulatory Role ☆.” 211: 561–73.

Sigmund, Jessica C et al. 2008. “Fine-Mapping at the HTR2A Locus Reveals Multiple Episodic Memory-Related Variants.” *Biological psychology* 79: 239–42.

Teixeira, Catia M et al. 2018. “Hippocampal 5-HT Input Regulates Memory Formation and Schaffer Collateral Excitation.” *Neuron* 98(june): 1–13. https://doi.org/10.1016/j.neuron.2018.04.030.

Twarkowski, Hannah, Hardy Hagena, and Denise Manahan-vaughan. 2016. “The 5-Hydroxytryptamine 4 Receptor Enables Differentiation of Informational Content and Encoding in the Hippocampus.” 891(September 2015): 875–91.

Vaidya, Vidita A, Gerard J Marek, George K Aghajanian, and Ronald S Duman. 1997. “5-HT 2A Receptor-Mediated Regulation of Brain-Derived Neurotrophic Factor mRNA in the Hippocampus and the Neocortex.” 17(8): 2785–95.

Yohn, Christine N., Mark M. Gergues, and Benjamin Adam Samuels. 2017. “The Role of 5-HT Receptors in Depression Tim Bliss.” *Molecular Brain* 10(1): 1–12.

Yoshida, Hiroyuki et al. 2011. “Subtype Specific Roles of Serotonin Receptors in the Spine Formation of Cortical Neurons in Vitro.” *Neuroscience Research* 71(3): 311–14. http://dx.doi.org/10.1016/j.neures.2011.07.1824.

Zhang, Gongliang et al. 2013. “Stimulation of Serotonin 2A Receptors Facilitates Consolidation and Extinction of Fear Memory in C57BL / 6J Mice.” *Neuropharmacology* 64: 403–13. http://dx.doi.org/10.1016/j.neuropharm.2012.06.007.

Zhang, Gongliang, David Cinalli, Sarah J. Cohen, Kristina D. Knapp, Lisa M. Rios, José Martínez-Hernández, et al. 2016. “Examination of the Hippocampal Contribution to Serotonin 5-HT2Areceptor-Mediated Facilitation of Object Memory in C57BL/6J Mice.” *Neuropharmacology* 109: 332–40. http://dx.doi.org/10.1016/j.neuropharm.2016.04.033.

Zhang, Gongliang, David Cinalli, Sarah J Cohen, Kristina D Knapp, Lisa M Rios, and Robert W Stackman. 2016. “Examination of the Hippocampal Contribution to Serotonin 5-HT 2A Receptor-Mediated Facilitation of Object Memory in C57BL / 6J Mice.” *Neuropharmacology* 109: 332–40.

ZHANG, GONGLIANG, and ROBERT W. STACKMAN JR. 2015. “The Role of Serotonin 5-HT 2A Receptors in Memory and Cognition.” *Frontiers in Pharmacology* 6(October): 1–17.

Zhu, Bi et al. 2013. “Neurobiology of Learning and Memory True but Not False Memories Are Associated with the HTR2A Gene.” *Neurobiology of Learning and Memory* 106: 204–

9. http://dx.doi.org/10.1016/j.nlm.2013.09.004.