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Research report

Stimulation of 5-HT_{1A}, 5-HT_{1B}, 5-$HT_{2A/2C}$, 5-HT_3 and 5-HT_4 receptors or 5-HT uptake inhibition: Short- and long-term memory

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Abstract

In order to determine whether short- (STM) and long-term memory (LTM) function in serial or parallel manner, serotonin (5-hydroxtryptamine, 5-HT) receptor agonists were tested in autoshaping task. Results show that control-vehicle animals were modestly but significantly mastering the autoshaping task as illustrated by memory scores between STM and LTM. Thus, post-training administration of 8-OHDPAT (agonist for 5-$HT_{1A/7}$ receptors) only at 0.250 and 0.500 mg/kg impaired both STM and LTM. CGS12066 (agonist for 5-HT_{1B}) produced biphasic affects, at 5.0 mg/kg impaired STM but at 1.0 and 10.0 mg/kg, respectively, improved or impaired LTM. DOI (agonist for 5-$HT_{2A/2C}$ receptors) dose-dependently impaired STM and, at 10.0 mg/kg only impaired LTM. Both, STM and LTM were impaired by either mCPP (mainly agonist for 5-HT_{2C} receptors) or mesulergine (mainly antagonist for 5-HT_{2C} receptors) lower dose. The 5-HT_3 agonist mCPBG at 1.0 impaired STM and its higher dose impaired both STM and LTM. RS67333 (partial agonist for 5-HT_4 receptors), at 5.0 and 10.0 mg/kg facilitated both STM and LTM. The higher dose of fluoxetine (a 5-HT uptake inhibitor) improved both STM and LTM. Using as head-pokes during CS as an indirect measure of food-intake showed that of 30 memory changes, 21 of these were unrelated to the former. While some STM or LTM impairments can be attributed to decrements in food-intake, but not memory changes (either increase or decreases) produced by 8-OHDPAT, CGS12066, RS67333 or fluoxetine. Except for animals treated with DOI, mCPBG or fluoxetine, other groups treated with 5-HT agonists 6 h following autoshaping training showed similar LTM and unmodified CS–head-pokes scores.

Keywords: Autoshaping; 5-HT receptors; Learning rat

1. Introduction

Growing evidence from invertebrate [2,3,4,27] and mammalian species, including human shows [66,67] that serotonin (5-hydroxytryptamine, 5-HT) systems play a role in memory consolidation, short- (STM) and long-term memory (LTM) [7,21,39,44]. In mammalian species, these cognitive processes occur in brain areas such as hippocampus, amygdala, caudate nucleus, hypothalamus and cortex. Significant changes in brain 5-HT systems function and receptors appear as results of memory formation, aging and Alzheimer's disease (see [44,49], for review; also [11,17,29,32,36,39,47,49,50,57,69]). Notably, using receptor binding profiles, common secondary messenger coupling and functional activity ligands, seven families of 5-hydroxytryptamine receptors and subtypes (5-$HT_{1A/1B/1D/1E/1F}$, 5-$HT_{2A/2B/2C}$, 5-$HT_{3A/3B}$, 5-$HT_{4A/4B}$, 5-$HT_{5A/5B}$, 5-HT_6 and 5-$HT_{7A/7B/7C/7D}$) have been identified [18,19,61,62]. Timely questions are the nature of memory [14], including the pharmacological, molecular and theoretical basis of STM and LTM. It is unknown if STM is merely a step towards LTM, or both are separate entities [23,25]. Direct participation of 5-HT has been demonstrated in human and animals by decreasing 5-HT brain levels using acute 5-HT depletion, which impaired memory formation (see [12,65,67] for reviews); in contrast, enhancing brain serotonin activity by means of its precursor (i.e., tryptophan) improved memory in animals ([15], see [51], for review). This evidence is consistent with the result that post-training (but not pre-) administration of 5-HT uptake inhibitors improved memory consolidation by increasing 5-HT intersynaptic concentrations, requiring protein and mRNA synthesis of multiple 5-HT receptors ([40–42]; see also e.g., [50]). This notion is consistent with the fact that 5-HT itself displays a differential affinity for 5-HT receptors [40]. 5-HT systems might exert their effects directly and/or by modulating neurotransmitters such as

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acetylcholine and glutamate (see for e.g., [7,20,33,39]), then it is possible that by augmenting 5-HT tone of different 5-HT receptors during memory formation, this cognitive process would be affect. As STM temporarily stores information on the basis of changes in preexisting connections due to covalent modifications of preexisting proteins and LTM stores this information more permanently through the growth of new connections as a result of transcription and translation of certain genes, a process called memory consolidation [1,6,13,22,24–27,38,39,70]. Notably, 5-HT receptors are metabotropic or ionotropic [19] they depict different times and duration courses of action as well differential affinity for serotonin (see [40]), which would provide might support to STM and/or LTM. Hence, the aim in this work was to determine the effects of agonists for 5-HT_{1A} to 5-HT_4 receptors or 5-HT uptake inhibitor in STM and LTM by using an associative learning task, namely autoshaping, where memory formation is gradually and progressive [39]. Herein, STM and LTM are define in terms of their neurobiological basis, since it was previously reported [39] that the inhibition of hippocampal protein synthesis or new mRNA did not produce a significant effect on autoshaping STM performance but it did impair LTM; nonetheless their non-contingent administration of protein inhibitors at 6 or 24 h following training [39] or immediately before testing showed no effects.

It should be noticed that from invertebrates to mammalians the same animals have been used to study STM and LTM [23,27,39], in behavioral tasks requiring one trial (e.g., passive avoidance) as well as multitrial and progressive process (e.g., gill withdrawal reflex in Aplysia). Hence, in this study Pavlovian/instrumental autoshaping (P/I-A) learning task was selected because requires multitrial, which generates a gradual learning of conditioned responses (CR) [39,40,44,46]. Notably, a large number of serotonergic and physiological (e.g., aging) mechanisms have been tested in autoshaping and the present doses were selected in base to previous dose-response studies (see [39,40,56]), which produced reproducible effects in memory consolidation or STM and LTM. In an autoshaping or sign-tracking setting, a hungry animal is given a Pavlovian sequential pairing (stimulus–stimulus [S–S]) of a lighted key or a retractable-illuminated lever conditioned stimulus (CS) and unconditioned stimulus (US) (for review see [40]). Autoshaped responses or conditioned responses (CR) result from the Pavlovian S–S association and are sustained by the instrumental response–stimulus (R–S) association such as peck, lever-press or contact lever-press responses. Autoshaping is sensitive to small increases or decreases in various behavioral parameters (i.e., not measuring the same event twice), including sign tracking (i.e., autoshaping response directed toward to the localized retractable and illuminated lever) and goal tracking (i.e., heads-pokes directed to the food-magazine, the place where the US is delivered). Importantly, Pavlovian S–S and R–S associations are mediated by hippocampus and striatum, respectively [40] and these associations have allowed using important behavioral control. For instance, comparison between a trained group with P/I-A associations showed 10–15% of CR, during the second and third sessions; in contrast, truly random control group [63] displayed 3–5% of CR [51], while the operant level was 0.6% of CR [40]. Importantly, increases or decreases of the autoshaped response may be independent of food intake, since animals do not learn the CR under diverse conditions of food-deprivation levels [46] and pre- or post-training administration of drugs that increase (e.g., 8-hydroxy-2-(di-n-propylamino) tetralin, 8-OH-DPAT) food-intake in free-feeding animals, had no effect in retrained animals with food deprivation; however, 8-OH-DPAT increased the CR in free-feeding groups in a dose-dependent manner. Notably, a well-known suppresser of food-intake such as d-amphetamine facilitated memory consolidation in a P/I-A task in food-deprived rats [54] and in a recent work ([39], see also [30]) 5-HT, cholinergic or glutamatergic antagonists modulated STM and LTM regardless to head-pokes during CS (CS–head-pokes). It should be keep in mind that some 5-HT drugs could produce some unspecific motor or e.g., food-intake effects, hence it is important to have some behavioral (indirect) index of food-intake during the STM and LTM experiments. In this work, head-pokes/CS was used as an indirect index to detect food-intake. To our knowledge there is no previous evidence about the effects of 5-HT receptor agonists in STM and LTM.

2. Materials and methods

2.1. Subjects

Male Wistar rats (12-weeks-old, weight, 230–260 g) were collectively housed ($n = 8$–10) in jumbo cage (53 cm × 43 cm × 19 cm), in a temperature- and light-controlled room under a 12 h light/dark cycle (lights on at 7:00 a.m.) at a constant temperature of 23 °C, with water and food provided *ad libitum* for a week. This was eventually followed by a reduction in body weights to 85% by gradually reducing the food-intake (see below). The local institutional committee for the use of animal subjects approved the present experimental protocol (Project No. 047/02).

2.2. Autoshaping apparatus

Autoshaping test [40,53,55,57], STM and LTM [39] protocols have been previously described. In short, the autoshaping learning task apparatus (Coulbourn Instruments, Lehigh Valley, PA) included a standard attenuation system, with the following inner dimensions: 25 cm × 29 cm × 25 cm (width × length × height) and consisted of a metal frame and transparent Perspex and aluminum walls and a bars floor. Solid-state programming equipment was used for control and recording. An acrylic retractable lever was mounted 4 cm above the floor and 10 cm from the right and left walls. The lever microswitch was adjusted to provide a 10 g force for operation. A food magazine for rat pellets (Bio-Serv, Frenchtown, NJ) was located 5 cm to the right of the lever and 3 cm above the floor. A photocell was mounted with the food magazine to measure head-pokes during the presence of CS (head-pokes/CS). A house light was located in the right top corner and maintained being turned on during session period.

2.3. Behavioral protocols

Behavioral protocols include food-magazine and autoshaping training the latter is composes by STM and LTM (see below).

2.3.1. Food-magazine

Individually each rat was placed in an experimental chamber for a habituation period (≈15 min), having access to 50 food pellets (45 mg each) previously placed inside the food-magazine. The criterion was that once the animal ate all 50 food-pellets and presented 150 nose-pokes (as measured by a photocell) into the food-magazine, immediately afterward the autoshaping training program was initiated. Testing sessions were realized later at 1.5 h (for STM) and 24 h (for LTM). Importantly, 1.5 and 24 h were selected considering that hippocampal