Review Article

Activation and Deactivation of 5-HT1 Receptor of Accumbens Shell Area does not Alter ACPA-induced Aversive Memory Deficit in Male Rat

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Abstract

Background: Some studies have indicated a close relation between serotonergic and cannabinoidergic systems in several brain regions. Thus, the aim of current study is investigating the effect of 5-HT1 receptors of accumbens shell (Acb shell) on aversive memory deficit induced by ACPA (cannabinoid CB1 receptor agonist) using test-retest protocol of elevated plus-maze (EPM) in male Wistar rats.

Method: Bilateral guide cannulae were implanted to allow microinjection of ACPA, CP94253 HCL (5-HT1 receptor agonist agonist) or GR127935 HCL (5-HT1 receptor antagonist).

Results: Post-test intra-Acb shell of ACPA (0.002 µg/rat), CP94253 (0.5 and 5 ng/rat) and GR127935 (5 ng/rat) increased the percentage of open-arms time (%OAT) in the EPM task compared to the control group, indicating aversive memory deficit. Moreover, concurrent microinjection of the subthreshold dose of CP94253 and GR127935 into Acb shell did not alter open-arms exploratory behavior induced by intra-Acb shell of ACPA on the retest day.

Conclusion: Our data suggests that Acb shell 5-HT1 receptor does not affect aversive memory deficit induced by ACPA in the Acb shell.

Keywords: Accumbens shell, ACPA, aversive memory, 5-HT1 receptors agonist and antagonist

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Introduction

The plant *Cannabis sativa* is commonly known as cannabis or marijuana which comprises about 60 terpenophenolic compounds, generally known as part of the group of plants, cannabinoids. For hundreds of years, Marijuana has been used all over the world for both medical and recreational purposes. While it has been known to have both positive and negative side effects, it does entail certain alarming side effects. The positive side effects generally cause relaxation, stress relief and a sense of calmness. On the other hand, the negative side effects have been known to include nausea, sickness, vomiting, dizziness and headaches.[1] Furthermore, cannabis can also produce a sense of euphoria, lethargy, confusion, depersonalization, altered time sense, impaired motor performance, memory defects, paranoia, depression, fear, anxiety and hallucinations.[1]

The endogenous cannabinoid system has been already widely recognized, and many researchers have expanded and researched further into the field of pharmacology and therapeutics of cannabinoids.[2]

Three kinds of cannabinoid receptors have been identified in humans and other animals, namely, CB1, CB2 and CB3 receptors.[3–5] The CB1 receptor is associated with mediating and its effects are focused mainly on memory and cognitive functioning regions of the brain. The CB2 receptors are not known to be associated with any cannabinoid-related cognitive effects of the brain so far.[6] Both CB1 and CB2 cannabinoid receptors have been identified to be part of the family of G protein coupled receptors, as there are other known receptor subtypes. Both the CB1 and CB2 receptors have been identified to possess endogenous and exogenous cannabinoid compounds, which in themselves include five structurally diverse classes.[7] The cannabinoid receptors can be identified in different regions of the brain including the cortex, hippocampus, amygdala, basal ganglia, cerebellum, and the emetic centers of the brain stem which are responsible for an individual's behavior. Alternatively in other regions of the brain, lower levels are found for example in the thalamus, pons, and the rest of the brainstem.[8] CB1 receptors also occur at low to moderate levels in the nucleus accumbens. In this region, CB1 receptors have a similar pattern to that of the striatum. CB1 receptors can be found on terminals inside the glutamatergic prefrontal cortex accumbens within the pathways.[9]

Pharmacologically, the endocannabinoid system acts in such a way that it will affect the neurotransmission systems including: gamma-aminobutyric acid (GABA), glutamate, the main biogenic amines (dopamine, noradrenaline and serotonin), acetylcholine and opioids. Each CB1 brain receptor regulates the activation or deactivation of these neurotransmitter systems differently in various regions of the brain. However, the majority of the research conducted has dealt with the hippocampus and nucleus accumbens.[10]

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New information has been recently uncovered related to serotonin's effects on behavior. Throughout the past few decades, researchers have become more aware of the various effects serotonin has on behavior and further investigated into the matter. There has been vast development regarding certain drugs and their action on the serotonergic system of the brain, allowing progressive treatment for depression, anxiety, appetite regulation, and post-traumatic stress disorders. Many have investigated the level and role of serotonin in emotional states and behavior.[11] At least seven serotonin receptors have been identified which have various effects on behavior. Recent studies have created a sense of empathy from researchers in the role of serotonin's cognitive functions which directly affect the memory and learning region of the brain.[12–16] The serotonin receptor subtypes which have been identified are receptors, 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_6, and 5-HT_7.[12,15] Studies have been conducted specifically to examine the effects of serotonin agonists on the particular region of learning and development. However, research findings support the notion that no direct link exists between the agonist 5-HT_{1A} receptor subtype, and its proposed adverse effect of causing learning impairment.[11]

Through the research conducted, the results suggest the notion that behavioral adaptation to stress is accompanied by sensitization of 5-HT_{1A}-mediated neurotransmission[17] and that it causes an increase in the appearance of this receptor in the brain, which is associated with reducing anxiety-like behaviors.[18] Hence, the study's findings support the notion that activation of 5-HT_{1A} receptors protects animals against various emotional and behavioral changes, which can be triggered by stressful stimuli, possibly by putting into place mechanisms which would be involved in the ability to cope with stressful situations.

The nucleus accumbens can be divided into two main sections; the core and shell. There is a greater quantity of 5-HT synapses in the NAc shell, and there are further incidents of synaptic contacts in the NAc shell compared to the core.[19] Mutually, the core and shell sub-regions of the NAc produce a heavier serotonergic innervation than that of the raphe nucleus,[20] whereas in the 5-HT-labeled axon terminals inside the shell, there are frequently formed synaptic contacts of symmetric variety, which are comparative to the 5-HT-labeled axon terminals in the core region.[21] Inside the NAc shell, the 5-HT-containing axon terminals are usually formed symmetrically in the inhibitory form and synapses with GABAergic neurons and its targets. This indicates a neuromodulatory function (largely inhibition) for the 5-HT on GABAergic neurons produced inside the NAc shell.[20]

Based on previous data, this study addresses the serotonergic system effects and its interactions with cannabinoids and the influence they have on aversive memory.

Materials and Methods

Subjects

Male Wistar rats (Tehran University of Medical Sciences, Tehran, Iran) weighing 250 g – 300 g (2 – 3 months) upon surgery, were housed in groups of five per cage in a temperature-controlled room (22 ± 2°C) under standard laboratory conditions, with free access to food and water, with a 12-h light/12-h dark cycle (lights on at 7:00 AM). Each animal was used once only. Eight animals were used in each group of experiments. The experiments were carried out during the light phase of the cycle. Animals' treatment and maintenance were conducted in accordance with the Principles of Laboratory Animal Care (NIH publication No. 85–23, revised 1985) as well as the Animal Care and Use Guidelines of Tehran University of Medical Sciences.

Drugs

CB1 cannabinoid receptor agonist (Arachidonoyl cyclopropamide, ACPA), 5-HT_1 receptor agonist (CP94253 HCL) and 5-HT_1 receptor antagonist (GR127935 HCL) were dissolved in saline solution (0.9%). These drugs were supplied by Tocris, Biosciences, UK. Control animals received saline.

Elevated plus-maze (EPM) apparatus

An EPM was used made of Plexiglas and consisting of two opposite open-arms (50 × 10 cm) surrounded by a 1 cm high ledge, and two enclosed-arms (50 × 10 × 40 cm). The maze was set up 50 cm above the floor. The junction area of the four arms (central platform) measured 10 × 10 cm.[22–24]

Stereotactic surgery and drug infusion

Rats were intraperitoneally anesthetized using ketamine hydrochloride 10% (Alfasan, Woerden, Holland; 50 mg/kg) plus xylazine 2% (Alfasan, Woerden, Holland; 4 mg/kg) then positioned in a stereotactic frame. The upper incisor bar was set at 3.3 mm below the intermural line so that the skull aligned horizontally between bregma and lambda. Two unilateral guide-cannulae (through which an injection cannula could be inserted for drugs, saline or vehicle applications, 5 – 7 days later) were stereotaxically implanted over the left and right Acb shell. Taking bregma as the reference point, the coordinates for the Acb shell were AP = +1.7 mm, ML = ±0.8 mm and DV = 5.9 mm, according to the atlas of Paxinos and Watson.[25] The cannulae were fixed to the skull by means of acrylic resin and two stainless steel screws. By the end of surgery, a stylet was introduced inside each guide cannula to prevent possible occlusion. After surgery, the rats were placed again in their home cages in groups of four, similar to before surgery. Five to seven days post-surgery, the rats received a bilateral infusion into the Acb shell using dental needles (27-gauge) introduced through guide cannulae. The injection needles were advanced until their tips reached 1 or 2 mm below the cannulae's end. Then, 0.3 μL/side of solution was injected into Acb shell. This was done during 60 s, using a 1 μL glass Hamilton syringe. A polyethylene catheter was interposed between the upper end of dental needles and the microsyringes. The displacement of an air bubble inside the polyethylene catheter was used to monitor the drug flow. To allow proper infusion, needles were removed 60 s after the completion of injection.

General conditions and data collection

In the present study, EPM test-retest method was chosen to investigate anxiety and the acquisition learning process. Recent studies have shown that using the test-retest sessions in the EPM results in a qualitative shift in emotional state. Thus, unconditioned fear in the test session would possibly transform to learned avoidance during the retest.[26,27] In our study, animals were given a post-test intracerebral drug injection. Thus, drug effects on consolidation of memory formation with the subsequent long-term effects on memory.[28,29] In general, reduced open-arms exploratory behaviors of saline or vehicle-treated groups during retest session, indicate aversive learning associated with the initial

exploration of this potentially dangerous environment.[30]

All experiments were carried out in a minimally illuminated (40-lux) room, during the diurnal phase, between 9:00 AM and 15:00 PM. Five minute EPM sessions were recorded using a video camera while a monitor and a DVD-recording system were installed in the adjacent room. After each EPM session, the apparatus was cleaned and towel dried to avoid urine impregnation. The numbers of open- (OAE) and enclosed-arms entries (EAE, an EPM index of general exploratory activity) with the four paws, as well as the time spent in open arms (OAT) were recorded. Raw data were used to calculate the percentage of time spent in open-arms [%OAT; (time in open-arms/300) × 100], the percentage of enclosed-arms entries [%EAE; (the numbers of closed-arms entries/locomotion) × 100] and open arms entries [%OAE; (the numbers of open-arms entries/locomotion) ×100] .[22,31]

Verification of cannulae placements

Upon concluding each experiment, the rats were deeply anesthetized and 1% Methylene Blue solution was injected into Acb shell (0.3 μL/side) as described in the drug section. Each animal was then decapitated, its brain removed and placed in 10% formalin solution. After 12 – 14 days, the brains were sliced and the sites of injections were verified according to the atlas of Paxinos.[25] Data from rats with cannulae placements outside the intended sites were excluded from the statistical analyses.

Statistical analysis

The data were analyzed by repeated measure analysis of variance (ANOVA) for test and retest sessions and expressed as mean ± S.E.M. Post-hoc Tukey test was performed when significant *F*-values were obtained in the ANOVA. Values of $P < 0.05$ were considered statistically significant.

Experimental design

Experiment 1: effect of post-test microinjections of 5-HT1 receptor agents on open-arms exploratory-like behaviors

To examine whether the microinjection of drugs into the Acb shell involves in memory, the drugs were infused post EPM testing. In this experiment, 8 groups of animals received saline (0.3 μL/side), CP94253 (5-HT1 receptor agonist; 0.05, 0.5 and 5 ng/rat), GR127935 (5-HT1 receptor antagonist; 0.05, 0.5 and 5 ng/rat) immediately after testing. The treated groups were retested in the EPM 24 h later, undrugged.

Experiment 2: effect of post-test microinjections of ACPA (CB1 receptor agonist) on open-arms exploratory-like behaviors

To test the possible involvement of ACPA in memory, the drug was infused after EPM testing. In this experiment, ACPA (0.0002, 0.002, 0.02 and 0.2 μg/rat) was administered immediately after testing. The treated groups were retested in the EPM 24 h later, undrugged.

Experiment 3: effect of post-test microinjections of 5-HT1 receptor agonists/antagonists on ACPA-induced open-arms exploratory-like behaviors

In order to assess the possible interaction between 5-HT_1 Acb shell receptors upon ACPA-induced exploratory-like behaviors, drugs were administered after the EPM testing session. In this experiment, 8 groups of animals received saline (0.3 μg/side) and the subthreshold dose of CP94253 (0.05 ng/rat), GR127935 (0.05 ng/rat) as intra-Acb shell microinjection, immediately after testing session. In addition, all these animals received the subthreshold or effective doses of ACPA (0.0002, 0.002, 0.02, and 0.2 μg/rat). The treated groups were retested in the EPM 24 h later, undrugged.

Results

Results from the experiment 1

Effect of post-test intra-Acb shell microinjection of CP94253 on open-arms exploratory-like behaviors

Repeated measure and post-hoc analysis showed that intra-Acb shell injection of CP94253 at applied doses increased %OAT and %OAE, while it did not alter %EAE on retest day compared to the control group, suggesting that CP94253 at applied doses (0.5 and 5 ng/rat) decreased aversive memory acquisition (Figure 1). All experimental repeated measure results are summarized in Table 1 and Figure 1.

Effect of post-test intra-Acb shell microinjections of GR127935 open-arms exploratory-like behaviors

According to repeated measure and post-hoc analysis, only the higher dose of GR127935 treatment on testing day led to increased %OAT and %OAE, but it did not alter %EAE on retest day compared to the own control group. This finding suggests that only the higher dose GR127935 induces aversive memory acquisition deficit. All experimental repeated measure results are summarized in Table 2 and Figure 1.

Results from the experiment 2

Effect of post-test intra-Acb shell microinjections of ACPA on open-arms exploratory-like behaviors.

Repeated measure and post-hoc analysis showed that intra-Acb shell injection of ACPA (0.2 μg/rat) increased %OAT (Figure 2, panel 1A) and %OAE (Figure 2, panel 1B), but did not alter %EAE (Figure 2, panel 1C) upon retest compared to the own control group, suggesting that the higher dose of ACPA impairs aversive memory acquisition. All experimental repeated measure results are demonstrated in Table 3 and Figure 2.

Results of experiment 3

Effect of post-test intra-Acb shell microinjections of CP94253 or GR127935 on open-arms exploratory-like behaviors already induced by ACPA

Two-way ANOVA and post-hoc analysis showed that intra-Acb shell injection of the subthreshold dose CP94253 or GR127935 did not alter %OAT (Figure 2, panel 2A for CP94253 and panel 3A for GR127935), %OAE (Figure 2, panel 2B for CP94253 and panel 3B for GR127935) or %EAE (Figure 2, panel 2C for CP94253 and panel 3C for GR127935) already induced by ACPA on retest day compared to their respective groups, indicating that CP94253 and GR127935 did not alter ACPA-induced amnesia. All experimental Two-Way ANOVA results are demonstrated in Table 4 for CP94253 and Table 5 for GR127935.

Discussion

Effect of intra-Acb shell ACPA on open-arms exploratory behaviors in naive rat subjected to EPM

Recent research results suggest that the effects of intra-Acb shell infusion of selective CB1 cannabinoid receptor agonist ACPA,

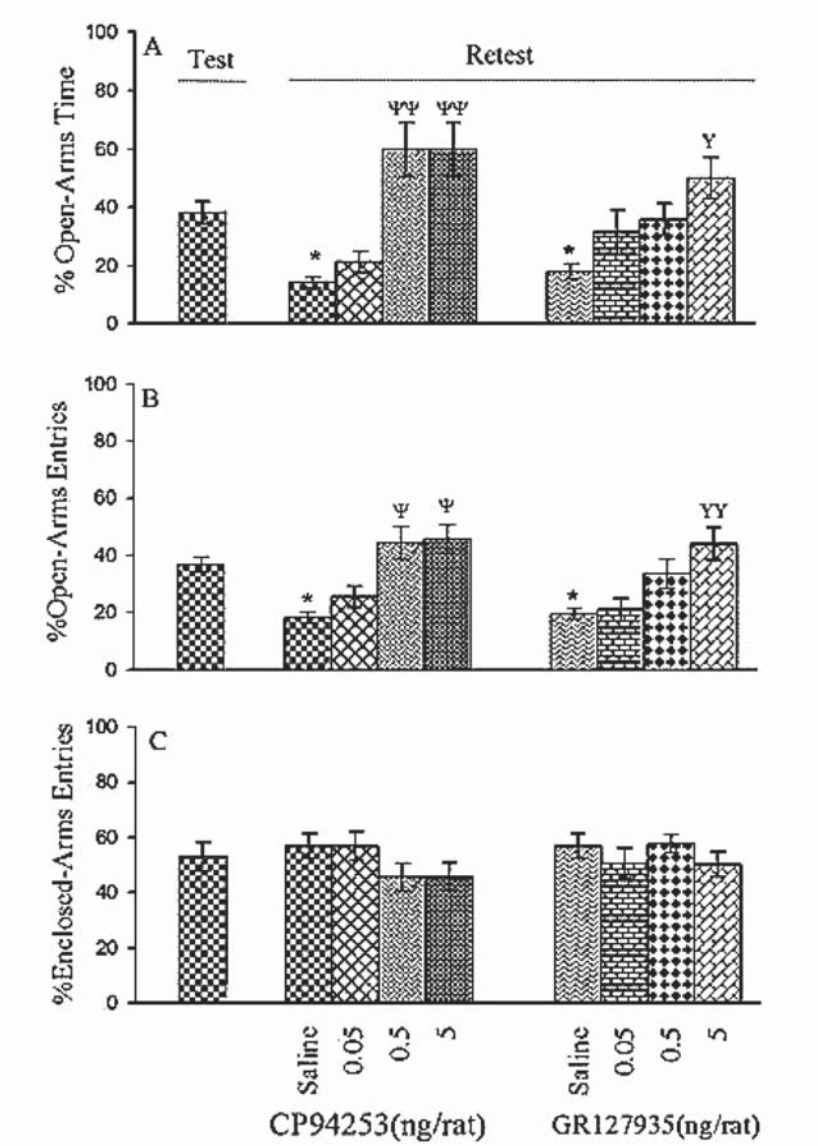

Figure 1. Open-arms exploratory behaviors following post-test intra-Acb shell microinjections of CP94253 or GR127935. After 24 h, all groups were retested in the EPM, un-drugged. %Open-Arms Time, %Open-Arms Entries and %Enclose-Arms Entries are showing in A, B and C panels respectively. Values are expressed as mean ± S.E.M (n = 8 in each group).*P < 0.05 different from saline group in the test day. Ψ < 0.05 and ΨΨP < 0.01 different from control saline group in the retest day.

Table 1. Repeated measure analysis with *P*-values for the effect of CP94253 on exploratory-like behaviors.

Experiments	Behaviors	Inter-Group		Intra-Group		Intra- Inter Group interaction		Final results conclusion for each experiment
		$F_{(1,28)}$	P	$F_{(3,28)}$	P	$F_{(3,28)}$	P	
Repeated measure analysis results for CP94253 microinjection into the Acb shell	%OAT	10.1	$P < 0.001$	6.994	$P < 0.001$	3.54	$P < 0.05$	The data showed that the higher doses of CP94253 induced amnesia
	%OAE	7.06	$P < 0.001$	3.941	$P < 0.05$	2.84	$P < 0.05$	
	%EAE	1.810	$P > 0.05$	2.999	$P > 0.05$	0.209	$P > 0.05$	

Table 2. Repeated measure analysis with *P*-values for the effect of GR127935 on exploratory-like behaviors.

Experiments	Behaviors	Inter-Group		Intra-Group		Intra- Inter Group interaction		Final results conclusion for each experiment
		$F_{(1,28)}$	P	$F_{(3,28)}$	P	$F_{(3,28)}$	P	
Repeated measure analysis results for GR127935 microinjection into the Acb shell	%OAT	4.099	$P < 0.05$	4.540	$P < 0.05$	0.487	$P > 0.05$	The data showed that the higher doses of GR127935 induced amnesia
	%OAE	3.02	$P < 0.05$	2.94	$P < 0.05$	2.132	$P > 0.05$	
	%EAE	0.009	$P > 0.05$	1.690	$P > 0.05$	1.164	$P > 0.05$	

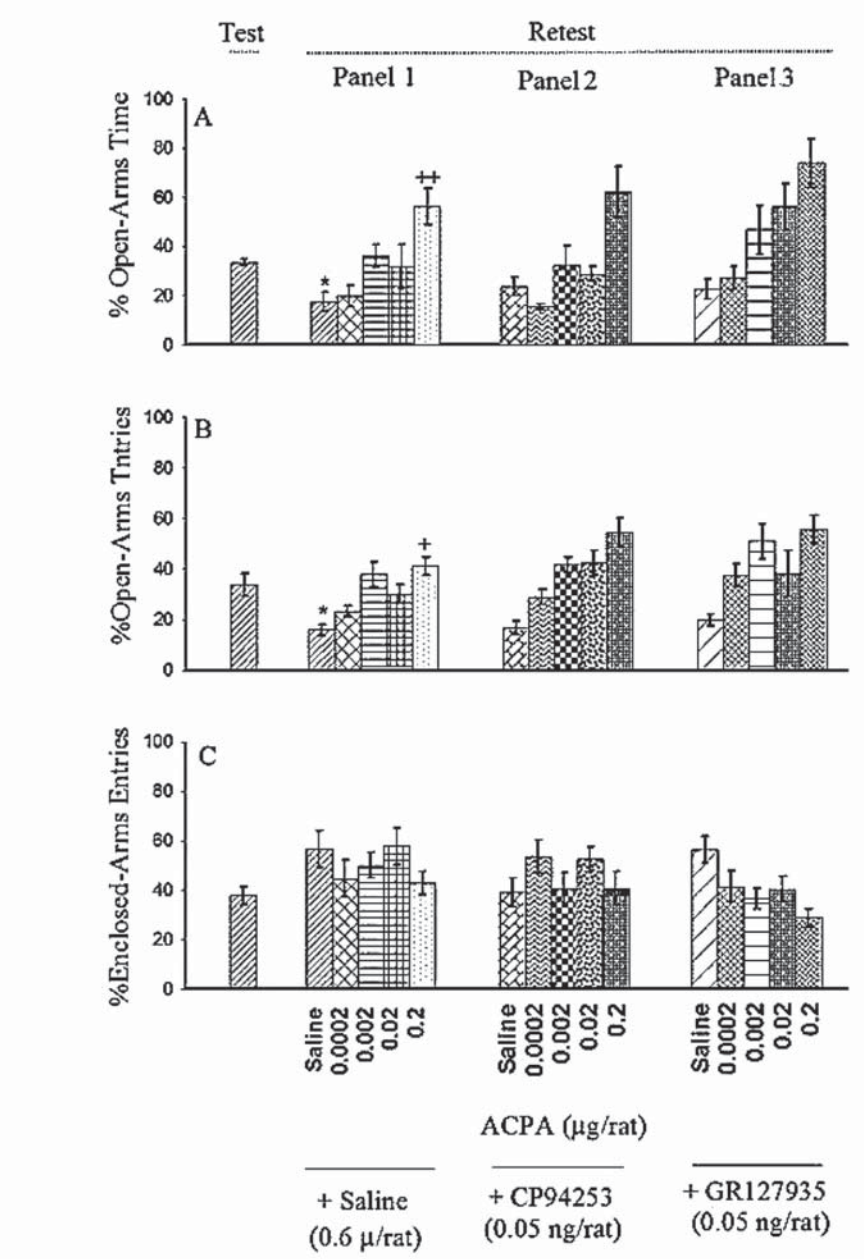

Figure 2. panel 1) shows open-arms exploratory behavior following post-test microinjections of ACPA. After 24 h, all groups were retested in the EPM, undrugged. In addition, **panel 2** for CP94253, while **panel 3** for GR127935 show the effect of intra-Acb Shell post-test injection of the subthreshold dose CP94253 and GR127935 on open-arms exploratory-like behavior induced by both the subthreshold and effective doses of ACPA. After 24 h, all groups were retested in the EPM, undrugged. Values are expressed as mean ± S.E.M (n = 8 in each group). %Open-Arms Time, %Open-Arms Entries and %Enclose-Arms Entries are showing in A, B and C panels respectively. *$P < 0.05$ different from respective saline group in **panel 1**. +$P < 0.05$ and ++$P < 0.01$ different from control saline group in panel.

Table 3. Repeated measure analysis with *P*-values for the effect of ACPA on exploratory-like behaviors.

Experiments	Behaviors	Intra-Group		Inter-Group		Intra- Inter Group interaction		Final results conclusion for each experiment
		$F_{(1,35)}$	*P*	$F_{(4,35)}$	*P*	$F_{(4,35)}$	*P*	
Repeated measure analysis results for microinjection of ACPA into AcShell	%OAT	4.945	$P < 0.05$	3.378	$P < 0.05$	3.544	$P < 0.05$	The data showed that the higher doses of ACPA induced anxiolytic-like behaviors and amnesia
	%OAE	12.508	$P < 0.001$	3.51	$P < 0.05$	3.97	$P < 0.05$	
	%EAE	2.263	$P > 0.05$	0.947	$P > 0.05$	1.746	$P > 0.05$	

Table 4. Two-way ANOVA results for the effect of CP94253 on ACPA-induced exploratory-like behaviors.

Experiments	Behaviors	Inter-Group		Intra-Group		Intra- Inter Group interaction		Final results conclusion for each experiment
		$F_{(1,70)}$	P	$F_{(4,70)}$	P	$F_{(4,70)}$	P	
Two ANOVA results between panels 1 and 2 of Figure 2	%OAT	0.028	$P > 0.05$	5.077	$P < 0.01$	1.860	$P > 0.05$	The drug of CP94253 into Acb shell did not alter amnesia like behavior induced by ACPA
	%OAE	2.872	$P > 0.05$	1.632	$P > 0.05$	1.331	$P > 0.05$	
	%CAE	2.392	$P > 0.05$	1.737	$P > 0.05$	2.117	$P > 0.05$	

Table 5. Two-way ANOVA results for the effect of GR127935 on ACPA-induced exploratory-like behaviors.

Experiments	Behaviors	Intra-Group		Inter-Group		Intra- Inter Group interaction		Final results conclusion for each experiment
		$F_{(1,70)}$	P	$F_{(4,70)}$	P	$F_{(4,70)}$	P	
Two ANOVA results between panel 1 and 3 of Figure 2	%OAT	4.864	$P < 0.01$	3.285	$P < 0.01$	1.076	$P > 0.05$	The GR127935 into Acb shell did not alter amnesia like behavior induced by ACPA
	%OAE	3.470	$P < 0.01$	5.534	$P < 0.01$	1.446	$P > 0.05$	
	%EAE	4.436	$P < 0.01$	5.528	$P < 0.01$	1.453	$P > 0.05$	

emerge into retest session. These results indicate an impairment of aversive memory acquisition in ACPA-treated animals after testing was concluded. Numerous other findings indicate that cannabinoids are anxiolytics and can adjust the behavioral and physiological response when encountering any stressful conditions.[32,33]

On the basis of scientific reports and their findings on the effects of cannabis on cognitive processes, many researchers have been motivated to investigate the effects of cannabinoids on synaptic plasticity, focusing specifically on the long-term potentiation (LTP). Cannabinoids have been known to directly affect the LTP and will often block it, making it difficult to explain the mechanisms that are involved in the process.[1] Cannabinoids have been shown to impair memory in rats, mice and monkeys, as demonstrated in various types of experimental conditions such as radial maze, instrumental discrimination tasks, Morris water maze, etc.[34] More explicit research has revealed that the CB1 receptor antagonist (SR141716A) could antagonize the effect of the CB1 receptor. Thus, this is the reason why experiments involving cannabinoid receptors and its effect are visibly more apparent and easier to distinguish.[34] Methodically, administration of cannabinoid receptor agonists (WIN 55212-2 and CP55940) increased the open arms time in the EPM in mice (i.e., an anxiolytic response); however, only at low administered doses. On the other hand, Tetrahydrocannabinol (THC) decreases the open arms time in a dose-dependent manner.[35] In addition, systemic administration of SR141716 and AM251 CB1 receptor antagonists reduced the open arms time.

Cannabinoid receptor activation has different effects on learning and memory, and these reactions all depend on the nature of the task, region of the brain and the stage in memory.[36] In a given task, Cannabinoid agonists may cause impairments to the emotional (or aversive) and rewarding memory-related processes, damaging relatable areas of the brain and sections related to memory stage-dependent manner. This result is congruent with the findings of other studies which imply exogenous acute cannabinoid treatment can be the cause of various outcomes depending on task aversion and the specific brain region involved.[37,38]

Effect of intra-Acb shell administration of 5-HT1 agents on ACPA-induced open-arms exploratory behavior

From the collective data analysis, it was discovered that intra-Acb shell infusion of CP94253 (5-HT1 receptor agonist) and GR127935 (5-HT1 receptor antagonist) given at a higher dose, could alter the impaired emotional memory of the subject. On the contrary, based on our data findings, administration of a sub-threshold dose of CP94253 and GR127935 in Acb shells did not alter the ACPA-induced did not impair aversive memory.

Other experimental studies have implied that 5-HT_{1A}R and 5-HT_{1B}R signaling play a role in acquisition and retrieval of learning; however, there is still not sufficient evidence to suggest that this receptor plays any role in the consolidation of learning. Nevertheless, studies have found that acquisition of new learning in behavioral tasks was increased when accompanied by repetitive motivation through pre-training and the administration of a 5-HT_{1A}R agonist [e.g., flesinoxan, buspirone or 8-OH-DPAT].[39]

In certain regions of the brain, such as areas which deal with cognitive processes, the 5-HT_{1B} and 5-HT_{1D} receptors are more active.[40] Regarding this, studies have confirmed, using the neurochemical and electrophysiological method, that this stimulation mediates 5-HT release in structures which are associated with cognitive processes.[41] It has been found that the presynaptic 5-HT_1B receptor stimulation decreases learning consolidation, whilst the postsynaptic 5-HT_1B receptor stimulation makes the process easier. The conclusion drawn from these findings supports the notion that the fluoxetine improved learning consolidation, through helping the interaction of 5-HT with various 5-HT postsynaptic receptors, such as the 5-$HT_{1B/1D}$ subtype .[14]

The 5-HT_{1B} heteroreceptors prevent the release of a variety of different neurotransmitters related to the kind of neurons that demonstrate them. Systemic use of 5-HT1B receptor agonists efficiently affects behavioral results obtained amid testing. The effects on behavior following this process include: increased locomotion, alterations in brain reward mechanisms and reduction in aggression. However, selective antagonists could comprise precognitive potential to some extent.[42,43]

Previously, the scientific opinion held that receptors were

activated by agonists, and they either created only one signal or several signals but with similar efficacies. On the contrary in recent studies, it has been verified that different ligands may have various ranges of efficacy in different signaling pathways. Researchers proved that when agonists with different molecular structures combined with other receptors, creating a stable, unique and apparent ligand-receptor conformation. This process allows different interactions with downstream proteins and creates distinct patterns of signaling, and finally produces cellular responses.

The 5-HT_{1B} receptor specifically targets the learning development and memory regions of the brain. The administration of agonists shows signs of reduction in performance, although the antagonists and the knockout mouse improved performance in terms of learning and memory which could be a result of mechanisms involving modulation of cholinergic neurotransmission. It can be concluded from the combined research of recent studies that presynaptic 5-HT_{1B} receptor motivation reduces learning consolidation; however, postsynaptic 5-HT_{1B} receptor motivation may make this process easier to achieve.[14]

Although direct studies regarding the interactions of cannabinoidergic and serotonergic systems exhibit the accumbens shells identified, the CB1 receptors prevents the release of serotonins in the mouse brain cortex. These receptors are possibly placed presynaptically and endogenous cannabinoids prevent their activation. The scale of inhibition is smaller than that achieved by 1) the other three presynaptic receptors on serotonergic neurons and 2) CB1 receptors on cholinergic neurons contained in the same tissue.[44]

Cannabinoids regulate serotonergic neuronal activity in the NAcc. Conducted research indicates that cannabinoids may have specific impacts, both directly and indirectly, on serotonergic neurons. The direct effects of cannabinoids are preventive, whereas the indirect effects via presynaptic circuits are excitatory. Regardless of the adverse effects, administration of WIN55 and CP55 has a moderate excitatory net result on 5-HT efflux in the NAcc of drug-naïve animals.[45] The increase of 5-HT efflux is associated with creating indirect effects in cannabinoids whilst the decrease creates a direct effect.[46]

To conclude this paper, the endocannabinoidergic system may adjust serotonergic transmissions through two potential mechanisms. The first mechanism would be by regulating the activity of afferents into serotonin-producing neurons[47] and secondly, by directly modulating the functions of a distinct subset of serotonergic neurons.[48] In relation to cannabinoidergic and serotonergic systems interactions, a number of studies have proposed the notion that cannabinoids and their receptor agonists such as anandamide and WIN55212-2, inhibit the uptake of serotonin into cortical synaptosomes, possibly through reducing the activity in the energy source, Na+/K+-ATPase.[49] Hence, if a cannabinoid receptor agonist is used, this will cause the blockage of operating transporters which eventually reflects on the flux of serotonin levels in different regions of the brain.[50]

It may be concluded from the findings of our experiments that, in the amounts administrated, the emotional memory was impaired, whilst when the interaction between agonists and antagonists serotonin and ACPA was applied, induced amnesia was not altered by the ACPA. Presumably, there may be other serotonergic receptors and/or neurotransmitters involved in this phenomenon resulting in the change in behavior which would also cause amnesia.

Author's Contribution

M. Keramati Nojedehsadat contributed to the acquisition of animal data. M. R. Zarrindast were responsible for the study concept, design and assisted with data analysis and interpretation of findings. M. Keramati Nojedehsadat and M. R. Zarrindast contributed to manuscript drafting and provided critical revision of the manuscript for important intellectual content. S. Oryan and V. Babapour contributed to edit of the manuscript. All authors critically reviewed the content and approved the final version for publication.

Conflicts of interest: *There are no conflicts of interest.*

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