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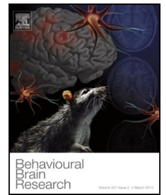

Research report

Interaction between hippocampal serotonin and cannabinoid systems in reactivity to spatial and object novelty detection

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HIGHLIGHTS

- Activation or blockade of 5-HT4 receptors impaired spatial memory.
- CB1 receptors activation impaired spatial and novelty, while their blockade facilitated novelty.
- Subthreshold dose of RS67333 restored ACPA response.
- Subthreshold dose of RS23597 differently affect ACPA signaling.
- Effective dose of AM251 blocked the effect of 5-HT4 agents on ACPA responses.

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ABSTRACT

Functional interaction between cannabinoid and serotonin neuronal systems have been reported in different tasks related to memory assessment. The present study investigated the effect of serotonin 5-HT4 agents into the dorsal hippocampus (the CA1 region) on spatial and object novelty detection deficits induced by activation of cannabinoid CB1 receptors (CB1Rs) using arachidonylcyclopropylamide (ACPA) in a non-associative behavioral task designed to forecast the ability of rodents to encode spatial and non-spatial relationships between distinct stimuli. Post-training, intra-CA1 microinjection of 5-HT4 receptor agonist RS67333 or 5-HT4 receptor antagonist RS23597 both at the dose of 0.016 μg/mouse impaired spatial memory, while cannabinoid CB1R antagonist AM251 (0.1 μg/mouse) facilitated object novelty memory. Also, post-training, intraperitoneal administration of CB1R agonist ACPA (0.005-0.05 mg/kg) impaired both memories. However, a subthreshold dose of RS67333 restored ACPA response on both memories. Moreover, a subthreshold dose of RS23597 potentiated ACPA (0.01 mg/kg) and reversed ACPA (0.05 mg/kg) responses on spatial memory, while it potentiated ACPA response at the dose of 0.005 or 0.05 mg/kg on object novelty memory. Furthermore, effective dose of AM251 restored ACPA response at the higher dose. AM251 blocked response induced by combination of RS67333 or RS23597 and the higher dose of ACPA on both memories. Our results highlight that hippocampal 5-HT4 receptors differently affect cannabinoid signaling in spatial and object novelty memories. The inactivation of CB1 receptors blocks the effect of 5-HT4 agents into the CA1 region on memory deficits induced by activation of CB1Rs via ACPA.

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1. Introduction

Among the numerous serotonin (5-HT) receptors, the 5-HT4 receptors, appear to be of particular importance in cognitive processes [1–3]. High density of 5-HT4 receptors in the limbic sys-

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tem, mainly on the pyramidal neurons of hippocampus, suggests a potential role in learning and memory [4,5]. These receptors belong to the superfamily of G-protein coupled receptors which are positively coupled to adenylate cyclase (AC) [6] leading to the intracellular accumulation of cAMP. This increase of cAMP observed after the application of 5-HT4 agonists contributes to the neuronal excitability of pyramidal cells of hippocampus by inhibiting potassium channels [7,8]. Behavioral studies have shown that 5-HT4 selective agonists improve social learning in rats [9], enhance accuracy performances in delayed matching tasks in macaques [10] and enhance place and object recognition in young adult rats [11]. Indeed, a pro-cognitive effect for 5-HT4 receptor agonists in several phases of memory has been confirmed [12].

Taken in the light of the existence of a high density of cannabinoid CB1 receptors (CB1Rs) in the hippocampal formation and their role in learning and memory, it is not surprising that hippocampus is strongly modulated by *exo*- and *endo*-cannabinoids [13–15]. The CB1R is a G-protein coupled receptor (Gi/o class G proteins) and is primarily located on presynaptic terminals [16]. CB1R agonists inhibit adenylyl cyclase and subsequently decrease intracellular cAMP levels. Also, activation of CB1Rs mediates a wide range of effects on ion channels, including voltage-dependent calcium and potassium channels [17]. Together, CB1R-mediated intracellular signaling results in reduced cellular excitability and reduced neurotransmitter release [18]. Because CB1Rs are located on both GABAergic and glutamatergic terminals, their activation leads to the suppression of both inhibitory and excitatory synaptic transmission in the brain [19–21]. A number of behavioral procedures including reward-based radial maze tasks, the water maze, contextual fear conditioning, passive avoidance and novel object recognition have shown that CB1R agonists disrupt different stages of spatial and non-spatial memory, namely acquisition, consolidation and retrieval processes [22,23]. Radial maze studies examining spatial memory indicate that administration of Δ^9-THC (the major psychoactive constituent of cannabis) and other CB1R agonists usually impair rodents' spatial working memory abilities [24,25]. Galanopoulos et al. reported that WIN55, 212-2 (0.3 mg/kg) disrupted non-associative learning, different aspects of short- and long-term recognition memory (storage and retrieval) and retention of spatial memory. They proposed that learning deficit induced by WIN55, 212-2 appeared to be CB1R dependent since pretreatment with SR141716A (0.03 mg/kg) prevented the WIN55, 212-2 response [15].

Accumulating evidence suggests that there is an interaction between CB1Rs and the 5-HT neuronal system [2,13]. We previously reported the involvement of 5-HT4 receptors in learning deficits induced by activation of CB1Rs in passive avoidance step-down task [26] and fear conditioning apparatus [27]. As we know these tasks are dependent upon the encoding and retrieval of emotionally aversive and inherently stressful training events. While these types of memories are important, they do not reflect the typical day-to-day experiences or memories most commonly affected in human disease. In addition, stress hormone release alone can modulate memory and thus obscure or artificially enhance these types of tasks. To avoid these sorts of confounds, we have utilized tasks testing animals' memory for object location and novel object recognition. These tasks involve exploiting rodents' innate preference for novelty, and are inherently not stressful [28]. Therefore, the purpose of the present study was to investigate whether hippocampal 5-HT4 receptors are involved in spatial and object novelty detection memory consolidation impairments induced by CB1R agonist arachidonylcyclopropylamide (ACPA) in a non-associative task.

2. Material and methods

2.1. Subjects

Male NMRI mice, weighing 30–35 g at the time of surgery, were used. They were kept in plastic cages, with free access to water and food, and maintained under a 12/12 regular light/dark cycle. All experiments were performed during the light phase. Mice for this study were obtained from Institute of cognitive science (ICSS, Tehran, Iran). Animals were handled in accordance with approved institutional animal care procedures and NIH guidelines. Mice were housed in an animal facility different from the room where experiments took place.

2.2. Surgery

Mice were subjected to surgery under anesthesia (pharmacological mixture: ketamine hydrochloride 50 mg/kg plus xylazine 5 mg/kg). They were implanted bilaterally with 22-gauge stainless steel cannulae, aimed at the CA1 region of the hippocampus (AP: −2 mm posterior to the bregma; ML: ± 1.6 from midline; V: −1.5 from to dura). Cannulae coordinates were derived from [29]. Stylets were used to prevent the cannulae from clogging. The tip of the cannulae was left 1 mm above the desired injection site. Mice were allowed to recover for 5–7 days following surgery.

2.3. Drugs and microinjections

ACPA (arachidonylcyclopropylamide; N-(2-cyclopropyl) 5Z, 8Z, 11Z, 14Z eicosatertraenanmide) and AM251 (N-(piperidin-1-yl)5-(4-iodophenyl) 1-(2,4-dichlorophenyl)4-methyl-1H-Pyrazole-3-carboxamide) purchased from (Tocris, Bristol, UK) were used. ACPA was obtained in Tocrisolve™ (a soya oil and water emulsion) and diluted directly into sterile 0.9% saline and at the volume of 10 ml/kg administered intraperitoneally. AM251 was dissolved in dimethylsulphoxide (DMSO; up to 10% v/v) and sterile 0.9% saline and a drop of Tween 80 and was bilaterally injected into the CA1 region in a volume of 0.5 μl/side. 5-HT4 receptor agonist RS67333 and 5-HT4 receptor antagonist RS23597 were dissolved in sterile 0.9% saline and microinjected into the CA1 region.

Microinfusions were delivered to hand restrained, conscious animals. Stylets were withdrawn and 27-gauge injectors were inserted into cannulae. These injectors were connected via polyethylene tubing to 1 μl Hamilton microsyringes. Total volume infused was 0.5 μl in 60 s, into either CA1 region. Injectors were left in place for an additional minute, to allow diffusion of the solutions into the tissue.

2.4. Description of the experimental apparatus

The apparatus used for the study was the same as in previous reports [30,31]. It was a metal circular box, 60 cm in diameter with a 20 cm-high wall. The floor was painted white and divided into 16 identical sectors by black lines. The apparatus was placed into a soundproof room surrounded by a visually uniform environment. The apparatus was illuminated by a red light (80 W) located on the ceiling. A video camera above the field was connected to a video recorder and a monitor. Five objects were simultaneously present in the open field: A cube (a metal-plated parallelepiped, measuring 7 cm × 4 cm × 4 cm with irregular holes distributed on all sides), a cone (a plastic cone on a transparent cylinder base, 8 cm in diameter and 6 cm high), a ladder (a small plastic white ladder, 5 cm wide and 16 cm high, having 10 steps connected to the two parallel arms, 3 cm thick), a cylinder (a black cylinder, 10 cm high and 4 cm in diameter, having a 2 cm in diameter hole on the top), a glass of steel

(12 cm high and 5 cm in diameter having a black handle. The initial arrangement was square-like with a central object (plastic cone). A sixth object (named corner) was used to assess the reactivity to non-spatial novelty. It consisted of two gray regularly pierced iron squares (10 × 10 cm) forming a 90° angle.

2.5. Assessment of reactivity to spatial and non-spatial novelty

The ability to react to spatial and non-spatial novelty was investigated accordingly to previously described procedure [31–33]. The general procedure consisted of three different phases: a familiarization phase, a habituation phase and a test phase. On the 1 st day mice were individually placed to a single familiarization session of 6 min, during which they were introduced in the empty arena, in order to become familiar with the apparatus and to record the baseline level of locomotor activity (session 1, S1). Subjects were then removed and placed back in the holding cage. After 5 min interval, mice were placed back to the open field for five successive sessions with 6 min period and 3 min interval in the open field containing the objects. The animals were returned to their home cages immediately after each session. The objects were placed in a square configuration with a central object (cone) in sessions 2–4 (S2–S4). The drug infusions were done immediately after S4. Next day, test phase including S5 (spatial novelty session) and S6 (non-spatial novelty session) sessions were done. In S5, the configuration was changed by displacing two objects: the cone replaced the cylinder, which was itself displaced at the periphery of the open field (between ladder and parallelepiped), so that the initial square arrangement was changed to a new spatial arrangement. In S6, one of the familiar non-displaced objects (NDOs; glass of steel) was replaced by a new object (corner) in the same location. All the objects were touched and manipulated before each session.

2.6. Data collection and statistic

Data collection was performed by a trained observer blind to drug treatment, with the use of a computer keyboard and specific software. Two behavioral parameters were considered: locomotor activity and object exploration. Locomotor activity was recorded by counting the number of sector crossed by the animals during session 1 (S1). During S2–S6, object exploration was scored as the time spent by the animal in contact with an object. A contact was defined as the subject's snout actually touching the object. To measurement the spatial memory, the mean time that mouse contacted with objects in S5 [both with displaced objects (DO) and non-displaced objects (NDO)] was subtracted from the mean time spent in contact with the same object in S4. In addition, to measure the object novelty detection, the mean time that the animal contacted with objects in S6 [both substituted object (SO) and non-substituted object (NSO)] was deducted from the mean time spent in contact with the same object in S5. Given the normality of distribution and the homogeneity of the data variance, the results were statistically evaluated by one- and two-way ANOVA for the analysis of spatial and novelty discrimination. Further analyses for individual 'between-group' comparisons were carried out through post hoc Tukey's test. Independent *t*-test analysis was done for the last three group in Fig. 2 compared to their respective groups. In all comparisons, $P < 0.05$ represented a statistical significance.

2.7. Study design

Eight animals were used in each experimental group. All infusions were done immediately after S4. In combination studies, at first ACPA was administrated. After 5 min, microinjections into the CA1 region were performed.

2.7.1. Study 1: effect of post-training, intra-CA1 microinjection of RS67333, RS23597 or AM251 on spatial and object novelty detection memory consolidation

In this experiment, mice were divided to three sets of four groups. The first four groups received different doses of RS67333 (0, 0.004, 0.008 and 0.016 μg/mouse). The second four groups were microinjected with different doses of RS23597 (0, 0.004, 0.008 and 0.016 μg/mouse). The last four groups received different doses of AM251 (0, 0.05, 0.1 and 0.2 μg/rat). All microinjections were performed into the CA1 region, immediately after S4.

2.7.2. Study 2: effect of post-training, intraperitoneal microinjection of ACPA plus saline, subthreshold dose of RS67333 or subthreshold dose of RS23597 and effect of effective dose of AM251 on ACPA, combination of RS67333 and ACPA or combination of RS23597 and ACPA response

In this experiment, 15 groups of animals were used. The first four groups received intraperitoneal microinjection of different doses of ACPA (0, 0.005, 0.01 and 0.05 mg/kg). The second four groups received RS67333 (0.004 μg/mouse) plus saline or different doses of ACPA. The third four groups received RS23597 (0.004 μg/mouse) plus saline or different doses of ACPA. The last three groups received ACPA (0.02 mg/kg) plus AM251 (0.1 μg/mouse), combination of RS67333 (0.004 μg/mouse) and AM251 (0.1 μg/mouse) or combination of RS23597 (0.004 μg/mouse) and AM251 (0.1 μg/mouse).

3. Results

3.1. Effects of all interventions on locomotor activity and time of contacts

Locomotor activity analysis showed that there is no significant difference between groups in S1 section for all intervention, thus the mean ± S.E.M. has been shown in Table 1. Moreover, similar analysis for the time of contacts (S2-S4) indicated that there is also no any significant differences as compare to respective control, thus these results were showing in the figures without analysis representation (panels A in all Figures). The time of contacts (S5 and S6) has been shown in panel A, generally. Further detail has been demonstrated in panels B and C.

3.2. Effect of post-training, intra-CA1 microinjection of RS67333, RS23597 or AM251 on spatial and object novelty detection memory consolidation

One-way ANOVA and Tukey's post hoc analysis showed that post-training, intra-CA1 microinjection of RS67333 induced a significant difference DO [$F(3, 28) = 27.90$, $P < 0.001$] and SO [$F(3,28) = 4.13$, $P < 0.05$] but not NDO [$F(3,28) = 0.51$, $P > 0.05$] and NSO [$F(3, 28) = 1.45$, $P > 0.05$] parameters. RS67333 at the dose of 0.016 μg/mouse decreased DO (Fig. 1 B and C, right panel).

One-way ANOVA and Tukey's post hoc analysis showed that post-training, intra-CA1 microinjection of RS23597 (0.016 μg/mouse) decreased DO [$F(3,28) = 56.42$, $P < 0.001$] but did not alter NDO [$F(3,28) = 0.58$, $P > 0.05$], SO [$F(3,28) = 2.64$, $P > 0.05$] and NSO [$F(3,28) = 1.42$, $P > 0.05$] parameters (Fig. 1 B and C, middle panel).

Moreover, One-way ANOVA and Tukey's post hoc analysis showed that post-training, intra-CA1 microinjection of AM251 produced a significant difference in NDO [$F(3, 28) = 14.63$, $P < 0.001$], SO [$F(3, 28) = 18.00$, $P < 0.001$] and NSO [$F(3,28) = 3.11$, $P < 0.05$] but did not alter DO [$F(3,28) = 2.60$, $P > 0.05$] parameters. AM251 at the dose of 0.1 μg/mouse increased NDO and SO (Fig. 1B and C, left panel).

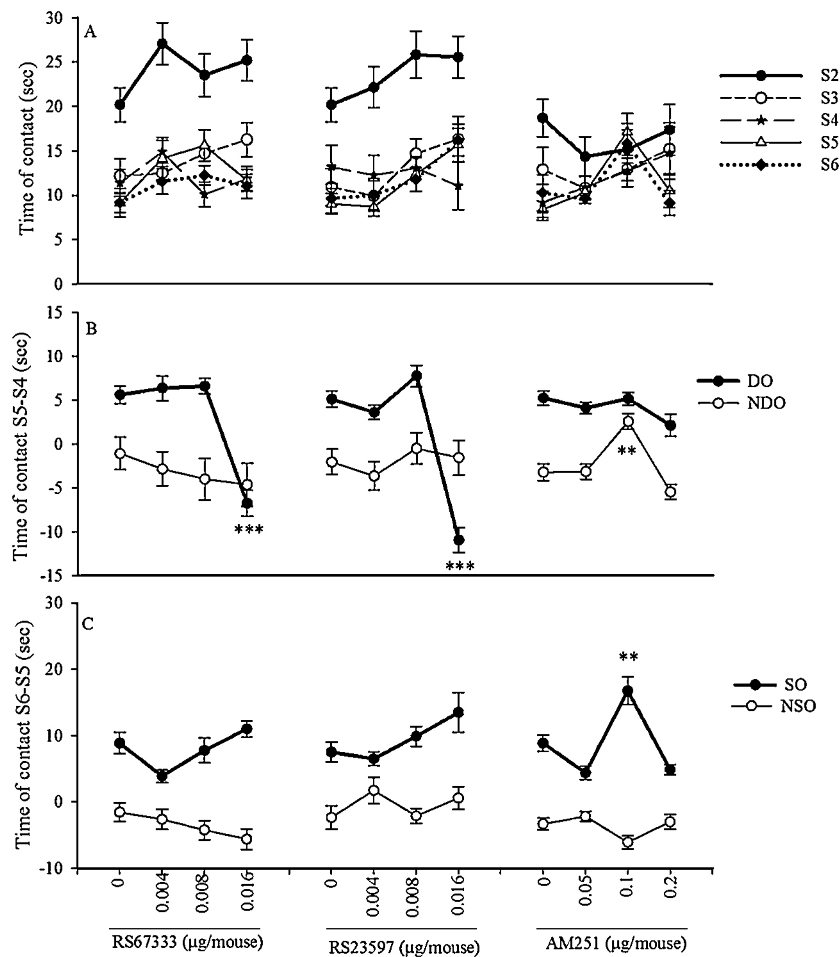

Fig. 1. Effect of post-training, intra-CA1 microinjection of RS67333, RS23597 or AM251 on spatial and object novelty detection memory consolidations. Animals were microinjected with different doses of RS67333 (0, 0.004, 0.008 and 0.016 μg/mouse), RS23597 (0, 0.004, 0.008 and 0.016 μg/mouse) or AM251 (0, 0.05, 0.1 and 0.2 μg/mouse), immediately after S4. The time spent in contact with objects in sessions 2–6 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5. Values are expressed as mean ± S.E.M (n = 8 in each group). ***P < 0.001 and **P < 0.01 different from its respective saline group.

In conclusion, both RS67333 and RS23597 impaired spatial memory consolidation, while AM251 facilitated object novelty detection memory consolidation.

3.3. Effect of post-training, intraperitoneal microinjection of ACPA plus saline, subthreshold dose of RS67333, subthreshold dose of RS23597 and effect of effective dose of AM251on ACPA, combination of subthreshold dose of RS67333 and ACPA or combination of subthreshold dose of RS23597 and ACPA

One-way ANOVA and post hoc analysis revealed that ACPA induced a significant difference in DO [F (3,28) = 26.22, P < 0.001], NDO [F (3,28) = 6.01, P < 0.01], SO [F (3,28) = 30.94, P < 0.001] and NSO [F (3,28) = 5.39, P < 0.01] parameters. All doses of ACPA decreased DO and SO, while the doses of 0.01 and 0.05 mg/kg increased NSO.

Two-way ANOVA revealed an interaction between RS67333 treatment and ACPA dose in DO [dose effect: F (3,56) = 14.16, P < 0.001; treatment effect: F (1,56) = 50.20, P < 0.001; dose-treatment interaction: F (3,56) = 13.02, P < 0.001], NDO [dose effect: F (3,56) = 2.18, P > 0.05; treatment effect: F (1,56) = 1.75, P > 0.05; dose-treatment interaction: F (3,56) = 4.43, P < 0.01], SO [dose effect: F (3,56) = 4.46, P < 0.01; treatment effect: F (1,56) = 32.61, P < 0.001; dose −treatment interaction: F (3,56) = 16.07, P < 0.001] and NSO [dose effect: F (3,56) = 1.69, P > 0.05; treatment effect: F (1,56) = 9.11, P < 0.01; dose-treatment interaction: F (3,56) = 8.43, P < 0.001]. Further analysis by post hoc showed that a subthreshold dose of RS67333 increased ACPA response at the all doses or two higher doses in DO or SO, respectively. Also, combination of RS67333 and the middle dose of ACPA decreased NSO compared to its respective group.

Two-way ANOVA revealed an interaction between RS23597 treatment and ACPA dose in DO [dose effect: F (3,56) = 31.08, P < 0.001; treatment effect: F (1,56) = 1.78, P > 0.05; dose-treatment interaction: F (3,56) = 39.69, P < 0.001], SO [dose effect: F (3,56) = 13.17, P < 0.001; treatment effect: F (1, 56) = 66.02, P < 0.001; dose-treatment interaction: F (3,56) = 26.78, P < 0.001], NSO [dose effect: F (3, 56) = 1.10, P > 0.05; treatment effect: F (1,56) = 0.19, P > 0.05; dose −treatment interaction: F (3,56) = 2.88, P < 0.05] but not NDO [dose effect: F (3,56) = 8.68, P < 0.001; treatment effect: F (1,56) = 0.86, P > 0.05; dose-treatment interaction: F (3,56) = 0.95, P > 0.05]. Further analysis by post hoc test showed that a subthreshold dose of RS23597 decreased or increased ACPA response at the dose of 0.01 or 0.05 mg/kg in DO, respectively. Also, combination of RS23597 with the lower or the middle dose of ACPA increased SO compared to their respective groups.

Independent *t*-test showed that mice received combination of AM251 (0.1 μg/rat) with ACPA (0.02 mg/kg) increased

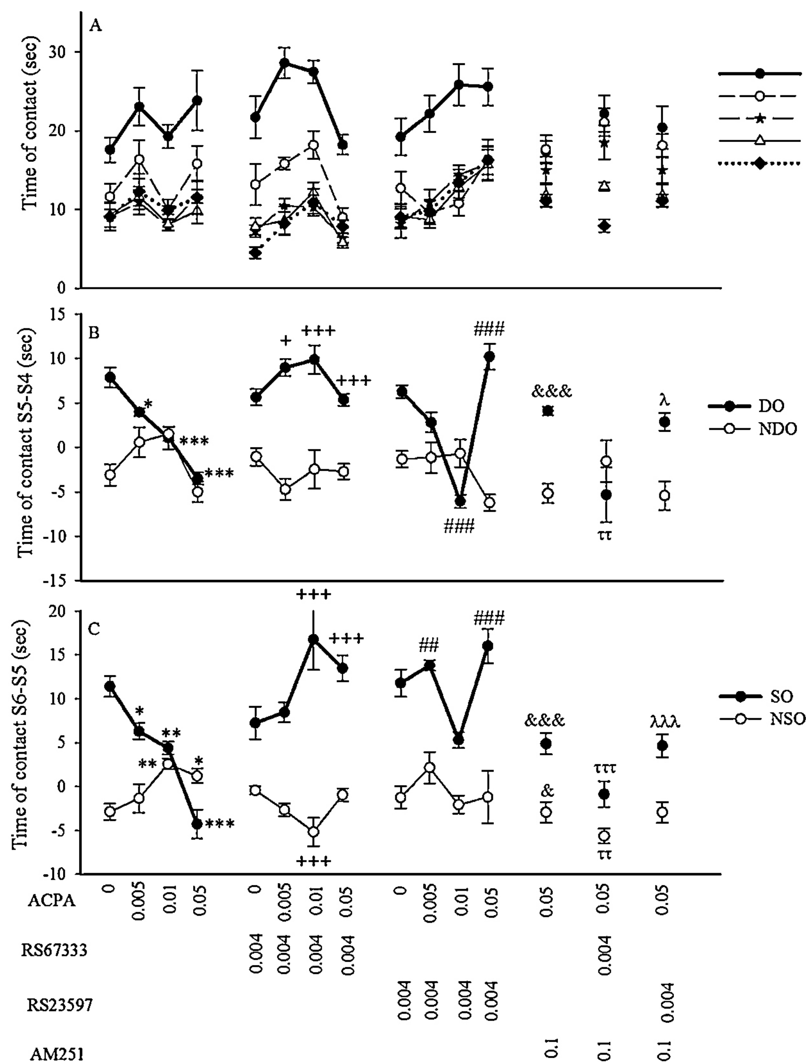

Fig. 2. Effect of post-training, intraperitoneal microinjection of ACPA plus saline, subthreshold dose of RS67333, subthreshold dose of RS23597 and effect of effective dose of AM251on ACPA, combination of subthreshold dose of RS67333 and ACPA or combination of subthreshold dose of RS23597 and ACPA. 15 groups of animals were used in this study. 12 groups of animals received post-training, intraperitoneal administration of different doses of ACPA (0, 0.005, 0.01 and 0.02 mg/kg) plus saline (1 μl/mouse), RS67333 (0.004 μg/mouse) or RS23597 (0.004 μg/mouse). Three groups of animals received ACPA (0.02 mg/kg) plus AM251 (0.1 μg/mouse), combination of AM251 (0.1 μg/mouse) and RS67333 (0.004 μg/mouse) or combination of AM251 (0.1 μg/mouse) and RS23597 (0.004 μg/mouse). The time spent in contact with objects in sessions 2–6 (A). Reaction to object displacement is measured as time spent in contact with either displaced (DO) or non-displaced (NDO) objects in session 5 minus the same in session 4 (B). Reaction to object substitution is measured as time spent in contact with either substituted (SO) or non-substituted (NSO) objects in session 6 minus the same in session 5. Values are expressed as mean ± S.E.M (n = 8 in each group). $^{***}P<0.001$, $^{**}P<0.01$ and $^{*}P<0.001$ different from saline group; $^{+++}P<0.001$ and $^{+}P<0.001$ different from respective ACPA group; $^{\#\#\#}P<0.001$ and $^{\#\#}P<0.01$ different from respective ACPA group; $^{\&\&\&}P<0.001$ and $^{\&}P<0.001$ different from ACPA (0.02 mg/kg) group; $^{\tau\tau\tau}P<0.001$ and $^{\tau\tau}P<0.01$ different from RS67333/ACPA (0.02 mg/kg) group; $^{\lambda\lambda\lambda}P<0.001$ and $^{\lambda}P<0.001$ different from RS23597/ACPA (0.02 mg/kg) group.

DO [t (14) = 1.95, P < 0.001] and SO [t (14) = 0.002, P < 0.001], while decreased NSO [t (14) = 0.78, P < 0.05] compared to ACPA (0.02 mg/kg)-treated group. Moreover, it was found no significant difference in NDO [t (14) = 0.11, P > 0.05] between two groups.

Independent *t*-test showed that mice received combination of AM251 (0.1 μg/mouse) plus RS67333 (0.004 μg/mouse) and ACPA (0.02 mg/kg) decreased DO [t (14) = 11.59, P < 0.01], SO [t (14) = 0.08, P < 0.001], NSO [t (14) = 0.28, P < 0.01] but did not alter NDO [t (14) = 7.65, P > 0.05] compared to RS67333 (0.004 μg/mouse) and ACPA (0.02 mg/kg)-treated group.

Independent *t*-test showed that mice received combination of AM251 (0.1 μg/mouse) plus RS23597 (0.004 μg/rat) and ACPA (0.02 mg/kg) decreased DO [t (14) = 4.92, P < 0.05] and SO [t (14) = 0.24, P < 0.001] but did not alter NDO [t (14) = 0.86, P > 0.05] and NSO [t (14) = 10.30, P > 0.05] compared to RS23597 (0.004 μg/mouse) and ACPA (0.02 mg/kg)-treated group.

In conclusion, ACPA impaired both spatial and non-spatial novelty memory consolidations. A subthreshold dose of RS67333 restored both ACPA-induced deficits. Moreover, a subthreshold dose of RS23597 potentiated or restored ACPA response at the middle or the higher dose on spatial memory, respectively. Meanwhile, RS23597 potentiated ACPA-induced non-spatial novelty deficit at the lower and the higher doses. Furthermore, effective dose AM251 reversed both ACPA-induced memory deficits, while blocked responses induced by ACPA (0.02 mg/kg) plus RS67333 (0.004 μg/mouse) or RS23597 (0.004 μg/mouse) on both memories.

4. Discussion

The main goal of the present work was to investigate the potential role of the hippocampal 5-HT4 receptors in spatial and object novelty detection memory deficits induced by activation of CB1 receptors via ACPA. This study examined interaction between 5-HT4 and cannabinoid CB1 systems in consolidation of memories assessed in a non-associative learning task.

Table 1
The animals' locomotor activity represented by the number of crossings during S1 in different treatment groups.

Treatment	S1
Saline (1 μl/kg)	141.25 ± 11.06
RS67333 (0.004 mg/kg)	189.75 ± 15.38
RS67333 (0.008 mg/kg)	189.25 ± 8.43
RS67333 (0.016 mg/kg)	180.12 ± 15.19
Saline (1 μl/mouse)	148.25 ± 12.81
RS23597 (0.004 μg/mouse)	180.87 ± 14.25
RS23597 (0.008 μg/mouse)	190.62 ± 17.09
RS23597 (0.016 μg/mouse)	186.25 ± 11.53
Vehicle (1 μl/muse)	136.75 ± 11.99
AM251 (0.05 μg/mouse)	155.00 ± 17.59
AM251 (0.1 μg/mouse)	159.62 ± 15.60
AM251 (0.2 μg/mouse)	151.00 ± 14.49
Saline (1 ml/kg)	159.25 ± 16.07
ACPA (0.005 mg/kg)	185.62 ± 19.73
ACPA (0.01 mg/kg)	173.12 ± 13.26
APA (0.05 mg/kg)	159.87 ± 27.24
RS67333 (0.004 μg/mouse) + Saline (1 ml/kg)	196.12 ± 26.24
RS67333 (0.004 μg/mouse) + ACPA (0.005 mg/kg)	136.50 ± 18.96
RS67333 (0.004 μg/mouse) + ACPA (0.01 mg/kg)	145.50 ± 18.32
RS67333 (0.004 μg/mouse) + ACPA (0.05 mg/kg)	113.62 ± 10.97
RS2597 (0.004 μg/mouse) + Saline (1 ml/kg)	171.12 ± 13.57
RS23597 (0.004 μg/mouse) + ACPA (0.005 mg/kg)	180.87 ± 14.25
RS23597 (0.004 μg/mouse) + ACPA (0.01 mg/kg)	215.62 ± 12.48
RS23597 (0.004 μg/mouse) + ACPA (0.05 mg/kg)	186.25 ± 11.53
AM251 (0.1 μg/mouse) + ACPA (0.05 mg/kg)	185.62 ± 19.73
AM251 (0.1 μg/mouse) + RS67333 (0.004 μg/mouse) + ACPA (0.05 mg/kg)	173.12 ± 13.26
AM251 (0.1 μg/mouse) + RS23597 (0.004 μg/mouse) + ACPA (0.05 mg/kg)	159.87 ± 27.24

Our findings with post-training, intraperitoneal administration of cannabinoid CB1 receptor agonist ACPA are consistent with previous findings showing that ACPA impaired both spatial and object novelty detection memory consolidations in the same line of mice [31]. In addition, our data indicate that microinjection of cannabinoid CB1 antagonist AM251 into the CA1 region of hippocampus facilitated object novelty memory consolidation. Also, effective dose of AM251 prevented both ACPA-induced memory impairments. Regarding to these results and the crucial role of hippocampus in memory formation, we can suggest that ACPA via activation of CB1 receptors in the hippocampus impairs memory consolidation.

The obtained data show that microinjection of 5-HT4 receptor agonist RS67333 or 5-HT4 receptor antagonist RS23597 into the CA1 region impaired spatial memory consolidation. The memory impairment was observed at the higher dose of these drugs. Moreover, a subthreshold dose of RS67333 reversed both spatial and object novelty detection memory deficits induced by ACPA. Interestingly, a subthreshold dose of RS23597 produced a modulatory effect on ACPA response in spatial memory, so that it potentiated or restored ACPA response at the middle or higher dose, respectively. In addition, subthreshold dose of RS23597 restored ACPA response at the lower or higher dose in object novelty memory consolidation. Recently, Hgena and Manahan-Vaughan reviewed the effect of 5-HT4 agonists and antagonists on hippocampus-dependent learning tasks. Although the most of studies show that activation of these receptors supports enhancing effects in cognitive functions, opposing results have been also reported. In general, pre-training activation of 5-HT4 receptors results in an enhanced ability to learn, whereas post-training injection of 5-HT4 agonists impairs memory consolidation. Behavioral experiments suggest that the time-point of activation or inactivation of 5-HT4 receptors is critical for positive or negative effects on memory performance and highlight the significance of these receptors for the targeted modulation of different components of memory [5]. This discrepancy in 5-HT4 receptor function on cognitive performance may be correlated with its capacity to activate different signaling pathways or receptor heterogenity. It has been well known that the activation of 5-HT4 receptors increases intracellular cAMP levels, which is a key player in memory events and associated synaptic plasticity events, such as long-term potentiation (LTP) [6,34]. In spite of this known pathway for 5-HT4 receptor agonist, there is a report indicating an increase in cAMP phosphodiesterase (PDE) activity in the prefrontal cortex and hippocampus following 5-HT4 receptor stimulation. This may be part of a negative feedback regulation mediated by cAMP [35]. In addition to classical cAMP/PKA signaling pathways, 5-HT4 receptors may use ERK pathways to control memory processes. The 5-HT4 receptor-mediated ERK activation seems to be dependent on Src tyrosine kinase but not G-protein or β-arrestin signaling [36]. On the other hand, two alternative splice variants for short (5-HT4S) and long (5-HT4L) isoforms of the 5-HT4 receptor have been cloned, which could be the structural basis for functional diversity [37]. Also, it has been suggested that 5-HT4 receptors behave as dimers or oligomers. Depending on the occupation of one or two binding sites in a 5-HT4 receptor dimer, a graduated response can be generated, which is an adaptive advantage in the brain [38]. Meanwhile, 5-HT4 receptors present constitutive (ligand independent) activity, although they contribute to the function of the receptor only to a small extent. This activity explains the differences between expected and observed effects of agonists and antagonists of the 5-HT4 receptors [6]. In line with our results, plethora of evidence reporting that activation of 5-HT4 receptors ameliorates cognitive impairments [5]. In addition, a modulatory effect for RS23597 on ACPA response in auditory fear conditioning has been reported in our laboratory [27]. A highly specialized neuromodulatory role for 5-HT4 receptors in synaptic plasticity and hippocampus-dependent cognition has been proposed [5]. 5-HT4 receptors seem to be located somatodendritically and presynaptically. A presynaptic potentiating effect of 5-HT4 receptor on glutamate release has been described in hippocampal neurons [39]. Since neurotransmitter release of various transmitters including 5-HT is modulated by 5-HT4 agonists, a presynaptic localization of 5-HT4 receptors on 5-HT neurons seems possible [40]. In view of this fact that there are various mechanisms for 5-HT4 receptor function, it is difficult to discuss results. Consideration of signaling pathway molecules can at least partly help to results interpretation.

Finally, our results indicate that post-training, intra-CA1 microinjection of effective dose of AM251 blocked improvement effect of subthreshold dose of RS67333 or RS23597 on ACPA response at the higher dose in both memories. Therefore, we can suggest that there is a close interaction between 5-HT4 and cannabinoid systems in the CA1 region of dorsal hippocampus in spatial and object novelty detection memory consolidations.

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