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Activation of cannabinoid receptor type 1 impairs spatial and temporal aspects of episodic-like memories in rats

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The endocannabinoid system modulates many brain functions, including episodic memories, which contain memories of time and places. Most studies have focused on the involvement of the endocannabinoid system in spatial memory; however, its role in temporal memory is not well understood. Few studies have tested whether the unilateral endocannabinoid system is sufficient to modulate memory retrieval. Here, we tested whether type 1 cannabinoid receptors in the right hippocampal cornu ammonis area 1 region are enough to modulate the retrieval of episodic memories, specifically their spatial and temporal components. Because rats have innate preferences for displaced or old familiar objects, we changed the locations of "old familiar" and "recent familiar" objects in an open field and measured the rats' exploration times to evaluate spatial and temporal memory. To address the influence of the type 1 cannabinoid receptors on the retrieval of episodic-like memories, two doses of arachidonylcyclopropylamide, a selective type 1 cannabinoid receptor agonist, were infused into the cornu ammonis area 1 of rats ten minutes before the discrimination trials. We observed that rats injected with a low dose of arachidonylcyclopropylamide spent less time investigating displaced objects, suggesting spatial memory impairment, whereas those receiving a high dose explored old familiar objects less frequently, suggesting temporal memory impairment. This indicates that unilateral activation of type 1 cannabinoid receptors in the cornu ammonis area 1 impairs the spatial and temporal aspects of episodic memories. This research mimics the influence of marijuana intoxication effects in humans, such as spatial and temporal disintegration.

Keywords

Endocannabinoid system; marijuana intoxication; CA1 hippocampus; episodic memory; temporal disintegration; spatial memory

1. Introduction

The endocannabinoid system (ECS) in the brain modulates various functions, including motor (Martinez et al., 2012), sensory (Green et al., 2003), and cognitive (Green et al., 2003; Jacobus et al., 2009; Messinis et al., 2006) functions. Memory, regardless of whether it is declarative or nondeclarative, is a cognitive function that has been frequently reported to be modulated by the ECS. In human subject studies, declarative memory is usually impaired by marijuana intoxication (Ranganathan and D'Souza, 2006). In rodent models, whether ECS facilitates or impairs declarative memory is inconclusive; it seems that the administration method profoundly influences the results of behavioral tasks. To test the mechanisms of ECS-modulated memory processing, some reports used systemic injections (Deadwyler et al., 2007; Lichtman, 2000; Lichtman and Martin, 1996), while others used local infusion (Atsak et al., 2012; de Oliveira Alvares et al., 2005, 2006). Interestingly, opposite effects can be demonstrated with different administration routes. However, the route of administration does not always result in opposite effects. For example, systemic or local injections of fatty acid amide hydrolase inhibitor, which increases endogenous levels of the cannabinoid receptor agonist, into the cornu ammonis area 1 (CA1) or basolateral amygdala decreases the retrieval of fear memory (Segev et al., 2018). The abovementioned literature reveals an interesting question: which brain areas contribute to marijuana intoxication?

The hippocampus is a brain area that expresses a high density of type 1 cannabinoid (CB1) receptors (Herkenham et al., 1990; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998), which are the major receptors that bind endocannabinoids. In the hippocampus, CB1 receptors are mainly distributed in the synaptic terminals of gamma aminobutyric acidergic (GABAergic) (Chen et al., 2003; Katona et al., 1999) and glutamatergic neurons (Kawamura et al., 2006). Substantial literature indicates that the ECS in the hippocampus modulates memory processing from acquisition, consolidation, and retrieval to extinction (Marsicano and Lafenetre, 2009). In laboratory animal studies, hippocampal-dependent memory tasks could be impaired by systemically administering CB1 receptor agonists (Deadwyler et al., 2007; Lichtman and Martin, 1996), and CB1 receptor antagonists could facilitate memory processing (Deadwyler et al., 2007; Lichtman, 2000). It is nearly impossible to locally inject drugs into the human brain to study anatomically specific mechanisms of marijuana-related memory effects. However, anatomically restricted studies on cannabinoid-modulated memory in rodents are also limited (Quillfeldt and de Oliveira Alvares, 2015). Although systemic cannabinoid effects could arise from multiregional actions, the hippocampus is hypothesized to be an important mediator of marijuana-related memory effects, owing to its abundant CB1 receptors (Herkenham et

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al., 1990; Mailleux and Vanderhaeghen, 1992; Tsou et al., 1998). Also, memory is widely defined as "past experiences that persist over time" (Buzsaki, 2006). Different brain regions contribute to processing different types of memory. For example, procedural memory depends on the striatum and cannot be retrieved consciously. Therefore, this kind of memory is classified as non-declarative memory. On the other hand, declarative memories, which are believed to be hippocampus-dependent, can be retrieved consciously and thus declared. In a simplified stage model of memory, "encoding," "storage," and "retrieval" are necessary for processing memory (Melton, 1963).

This research is focused on the retrieval of declarative memory. We locally infused CB1 receptor agonists into the hippocampal CA1 in rats to show whether the memory-retrieval functions were impaired by activation of CB1 receptors in the CA1. Regarding the infusion location, Klur et al. (2009) reported that the dorsal hippocampus might show behavioral lateralization of memory retrieval. Their results suggest that inactivation of the right or both hippocampi disrupts retrieval in a spatial water maze task. Unilateral microinjections of endocannabinoid-related drugs into the central nucleus of the amygdala (right side) (Hsiao et al., 2012) and ventral hippocampus (right side) (Roohbakhsh et al., 2009) showed effects in behavioral tests. It would also be interesting to test whether activation of the CB1 receptors in the right CA1 impairs memory retrieval. Therefore, we targeted the right CA1 and tested whether the right-side ECS in the CA1 is sufficient for disrupting memory retrieval. We hypothesized that this study would show whether the right CA1-based ECS affects memory retrieval.

Although there are conflicting reports on the role of CB1 receptors in learning and memory (de Oliveira Alvares et al., 2005, 2006; Deadwyler et al., 2007; Lichtman, 2000; Lichtman and Martin, 1996), the inability to estimate time, also referred to as "temporal disintegration," is consistently found in marijuana users, mostly regarding overestimating the amount of time that has elapsed (Atakan et al., 2012). Nevertheless, temporal information is one of the critical elements of episodic memory, in addition to spatial memory and object recognition. In the brain, the hippocampus is a vital brain region that processes episodic memory, supported by the discovery of place preference (O'Keefe and Dostrovsky, 1971) and time preference (MacDonald et al., 2011; Pastalkova et al., 2008) cells in this region. Also, lesions of the hippocampus have been shown to impair time estimation in rats (Meck et al., 1984). The abovementioned findings support the idea that the ECS in the hippocampus may play a critical role in modulating episodic memory (another type of declarative memory), including temporal information. The mechanisms involved in processing spatial and temporal memory are complex.

To mimic the influences of marijuana intoxication on the aspects of spatial and temporal memory, we subdivided memory into three components: type (episodic), phase (retrieval), and brain area (hippocampal CA1). We adapted Dere et al. (2005) three-trial object exploration task, which can be used to evaluate episodic memory in terms of recognition (what), spatial (where), and temporal (when) information. We targeted the effects of memory retrieval because human studies have revealed that cannabis users are less impaired when learning new information but have difficulty recalling newly acquired information (Ilan et al., 2004; Miller et al., 1977). However, the timing of drug injection profoundly affects the outcome of behavioral performance (de Oliveira Alvares et al., 2008). For example, infusing drugs immediately after training is reported to manipulate memory consolidation, whereas administration before the test trial influences memory retrieval. Thus, we infused a CB1 receptor agonist into the CA1 before the discrimination trials. Our results partially mimic the influence of marijuana intoxication on spatial and temporal disintegration.

2. Materials and methods

2.1 Substances

A stock solution of water-soluble arachidonylcyclopropylamide, or ACPA (5 mg, Cat. No. 1781, Tocris, Bristol, UK;), was dissolved in 1 ml of pyrogen-free saline (PFS) and stored at -20 °C until administration. Previous reports have shown that the ACPA microinjection dose is effective from 1 ng to 10 ng (Moghaddam et al., 2010; Mohammadmirzaei et al., 2016; Zarrindast et al., 2008). The effects of ACPA on memory have been tested in the ventral hippocampus (Mohammadmirzaei et al., 2016). Therefore, we chose doses of 3.125 ng/1μL and 12.5 ng/1μL (prepared from a stock solution of 5 mg/mL; 5/(4×10^5) dilution for the high dose and 5/($4 \times 4 \times 10^5$) for the low dose) in the present study.

2.2 Animals

Fourteen male Sprague-Dawley rats (250-300 g; BioLASCO Co., Ltd, Taiwan) were randomly separated into two groups (low-dose group n = 7; high-dose group n = 7). Before surgery, the animals were housed in home cages that were placed in a temperature-maintained room (23 ± 1 °C) with a 12:12 h light: dark cycle. Food and water were available ad libitum. After undergoing surgery, the animals were individually housed in home cages in the same room. To avoid the influence of sleep deprivation on hippocampus-dependent memories (Hagewoud et al., 2010), all experiments were performed during the dark period.

2.3 Surgery

Animals were sedated with 5% isoflurane and subcutaneously injected with an analgesic (buprenorphine, 0.03 mg/kg) and atropine (0.04 mg/kg) to prevent the accumulation of saliva. Isoflurane (1.5 to 2.5%) was used for the maintenance of anesthesia during surgery. Five stainless steel screws were surgically anchored onto the frontal, parietal, and interparietal bones. Klur et al. (2009) have reported that inactivation of the right dorsal hippocampus disrupts memory retrieval in a spatial water maze task, which suggests lateralization of the hippocampus for memory retrieval. Therefore, a microinjection guide cannula (26 gauge, O.D. 0.46 mm, I.D. 0.24 mm; Plastics One, Roanoke, USA) was implanted above the right CA1 (AP, -3.8 mm; mL, 3 mm; DV, 2.5 mm relative to bregma; Fig. 1A). The coordinates were adopted from the Paxinos and Watson rat atlas (Paxinos and Watson , 2008). The screws and cannula were then cemented to the skull with dental acrylic (Tempron, GC Co., Tokyo, Japan). At the end of the surgery, the incision was treated topically with gentamicin. Carprofen (5 mg/kg) was given subcutaneously for postsurgery analgesia. The animals were allowed to recover for seven days before the initiation of experiments. All procedures performed in this study were approved by the National Taiwan University Animal Care and Use Committee.

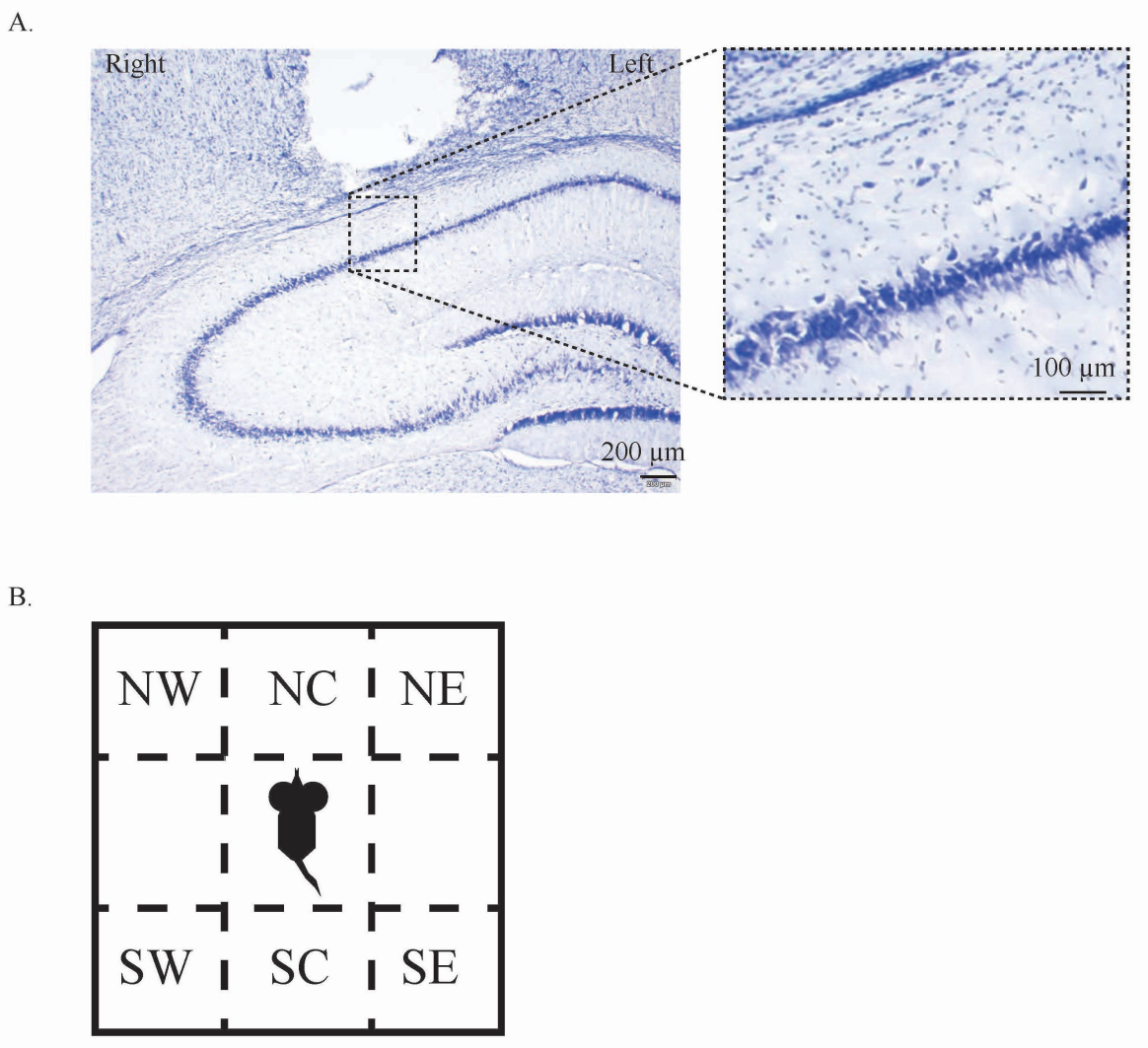

Figure 1. Photograph of a histologically processed section depicting the injection site and a schematic drawing of the coordinates for the objects. (A) The microinjection cannula was aimed at the dorsal CA1. (B) The objects were placed in the northwest (NW), north-center (NC), northeast (NE), southwest (SW), south-center (SC), and southeast (SE) sections depending on which trial was being executed. The rats always faced north at the beginning of each trial.

2.4 Histology

At the end of the experiments, the rats were anesthetized by an intraperitoneal injection of a cocktail of Zolitel (40 mg/kg; Virbac, Carros, France) and Xylazine (10 mg/kg; Sigma-Aldrich, St. Louis, Missouri, USA). Then, the animals' reflexes were checked by pinch tests. After the rats lost withdrawal reflexes, they were perfused with 4% formalin through the heart, and their brains were collected and sliced to confirm the location of the microinjection of cannula (Fig. 1A). The brains were cut into 30 μm coronal sections in a cryostat microtome and then treated with Nissl stain. The rats were excluded if cannula implantation missed CA1 or resulted in severe lesions in CA1. 14 out of 15 animals were involved.

2.5 Apparatus and objects

All experiments were executed in a temperature-(23 ± 1 °C) and illumination-(140 ± 10 lux) controlled behavioral room with a digital camera on the ceiling. The open field was surrounded by four plastic boards (60 cm × 50 cm), which were attached and to the floor, resulting in a 60 cm × 60 cm × 50 cm space. The floor was covered with a black, nonreflective material. The plastic boards were decorated with some visual cues. The plastic boards and visual cues used in the control sessions were different from those used in the ACPA sessions. Object sets (four identical objects in each set; two sets in the control sessions and two sets in the ACPA sessions; Fig. 2) were assembled from LEGO-like bricks. These objects were attached to the open field with double-sided tape during the experiments so that the rats could not move them. The positions of novel objects were randomized when executing the experiments on different rats to eliminate the potential confounding factor of area preferences. The floor, the walls of the open field, and all objects were first cleaned with water and then with 75% ethanol solution at both the beginning and the end of each trial to eliminate the odor of subjects of interest during trials. The double-sided tape was also replaced when cleaning. The investigators wore lab gowns, masks, and gloves during the whole experiment.

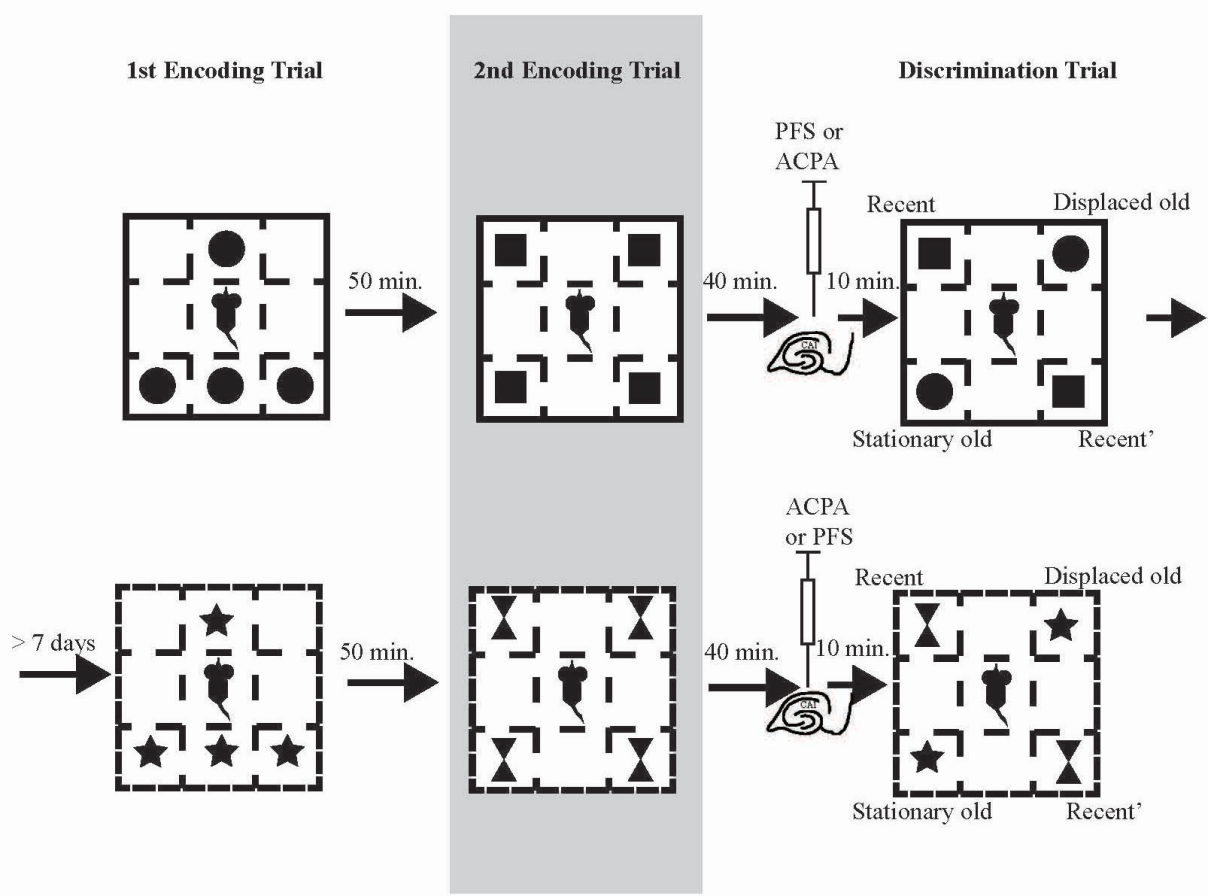

Figure 2. Schematic drawing of the protocols of the three-trial object exploration task. The subjects had two encoding trials with different objects placed in different locations. Ten minutes before the discrimination trial, a CB1 receptor agonist or PFS was infused into CA1. One week later, a similar protocol was conducted, but with different object sets. Moreover, if the subjects received PFS before, they were alternately injected with the CB1 receptor agonist and vice versa. The walls of the open field and their visual cues used in the control sessions were different from those in the ACPA sessions.

2.6 Experimental procedures

The experimental procedures were adapted from Dere et al. (2005) (Fig. 1B and 2). Briefly, an experimental session consisted of two encoding trials and one discrimination trial. One microliter of PFS or 1 μL of ACPA was chosen at random and alternatively administered into the right CA1 10 minutes before the discrimination trials. Each rat received PFS in the control session and 3.125 ng/μL or 12.5 ng/μL ACPA in the ACPA session, depending on its group. Therefore, the order for the executing control session or ACPA session was random for each rat. Sessions were carried out at least one week apart. During the experiment, the rats were allowed to freely explore the open field for 10 minutes in each trial. Between trials, the rats waited for 50 minutes in their home cages. The open-field arena was then divided into a 3-by-3 grid, and four identical copies of the object were placed into the grids during the encoding trials. The specific locations of the objects are detailed below. In the first encoding trial, the objects were arranged in a triangle-shaped spatial configuration. The original article described these four places as (Fig. 1B) the center of the northern wall (NC), the center of the southern wall (SC), the southwest corner (SW), and the southeast corner (SE). In the second encoding trial, each of the four objects in the other set was placed in the four corners (northwest (NW), northeast (NE), SW, SE). In the discrimination trial, the objects in the NE and SW corners were replaced with objects from the first trial.

2.7 Data collection and statistics

The object exploration time was defined as the amount of time the rats directed their noses toward objects at a distance of less than 2 cm (Leger et al., 2013; Lueptow, 2017). The time spent climbing or leaning on objects was not included, unless the rats also directed their noses toward the objects. The object exploration time was measured offline by well-trained investigators using stopwatches. A single-blind procedure was used for measuring the exploration time to minimize subjective bias. All values acquired from video files are presented as the means ± standard error of the means (SEMs) for the indicated sample sizes. The statistical analyses were performed with SPSS (Version: 10.0.7, IBM, New York, USA). A two-tailed paired t-test was performed to test for significant differences in within-subject data, and an unpaired t-test was used to test significant differences in between-subject data. $P < 0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1 Estimates of episodic memory for "What & When" and "What & Where" discrimination

To test whether a single infusion of ACPA disturbs memory retrieval, we planned to observe the rats' tendency of exploration for old familiar, recent familiar, stationary, and displaced objects after microinjecting ACPA or PFS into the right CA1. Two doses of ACPA (low dose, 3.125 ng/μL; high dose 12.5 ng/μL) were tested in separate groups of rats. Moreover, two control experiments, a low-dose control (PFS) and a high-dose control (PFS), were needed to test whether the baseline innate preferences of these groups were different. The order for executing the control session or ACPA session was random for each rat. According to Dere et al. (2005), the baseline innate preferences are stronger for old familiar and displaced objects. The coordinates of the novel objects are illustrated in Fig. 1B. Alternating the objects among different places across different trials created place, sequence, and object differences (Dere et al., 2005).

Fig. 2 displays the experimental protocols. Injections in the right CA1 (Fig. 1A) were performed 10 minutes before the discrimination trials (Fig. 2). The infusion of either PFS or ACPA was chosen at random, and one week later, a similar protocol was performed, but the alternate injection type was administered (i.e., drug or vehicle). Although within-subjects designs have the advantage of using fewer lab animals due to their high power and less variability in detecting significance, habituation of the experiment or prelearned effects could still be confounding factors for the subjects' exploration times. Therefore, we first measured the difference between the exploration time of the animals that received PFS first (n = 8) and that of the animals that received PFS after finishing an ACPA experiment (n = 6). We found no difference with regard to the order of administration (old familiar $T(12) = 1.25$, $P = 0.24$; recent familiar $T(12) = 1.59$, $P = 0.14$; displaced $T(12) = 1.75$, $P = 0.11$; stationary $T(12) = 0.20$, $P = 0.85$). We then inspected recency discrimination in the control experiments. By comparing exploration in the SW+NE regions to that in the NW+SE regions (Fig. 3A and 3B), top illustration, black objects), we measured the rats' interests in old familiar objects (SW+NE) and recent familiar objects (NW+SE) during the last trial, i.e., the discrimination trial. The rats that received vehicle injections spent significantly more time investigating older objects than recent objects (Fig. 3A, left 2 bars; vehicle, old: 40.53 ± 9.30 sec vs. recent: 23.65 ± 5.25 sec, $T(6) = 2.78$, $P < 0.05$; Fig. 3B, left 2 bars; vehicle, old: 15.70 ± 2.91 sec vs. recent: 10.63 ± 2.70 sec, $T(6) = 2.91$, $P < 0.05$). Then, the rats' spatial discrimination ability (Fig. 3C and 3D) was estimated by observing the duration they spent exploring objects in the NE (displaced object) or the SW (stationary object) regions. Both control groups showed significant interest in displaced objects (Fig. 3C, left 2 bars; vehicle, displaced: 55.74 ± 13.60 sec vs. stationary: 25.32 ± 7.09 sec, $T(6) = 2.71$, $P < 0.05$; Fig. 3D, left 2 bars; vehicle, displaced: 26.49 ± 5.64 sec vs. stationary: 4.91 ± 1.28 sec, $T(6) = 3.76$, $P < 0.01$).

The findings from the control experiments suggest that the injection of the vehicle into the right CA1 did not impair memory for differentiating objects' recency and location. We also noticed that the exploration time of the low-dose controls was longer than that of the high-dose controls. Although we randomized the experimental order of the PFS and ACPA tests, we did not randomize the experimental order between the low-dose group and the high-dose group. In this case, the high-dose ACPA group and its control test were finished earlier. For some unknown reasons, the high-dose group of animals showed less interest in objects (this will be explained in the Discussion section). However, the confounding factor did not affect the trend of the control results; that is, rats with vehicle injections in the low- or high- dose group all had more significant interest in old familiar objects (Fig. 3A and 3B, left 2 bars) or displaced objects (Fig. 3C, left 2 bars).

3.2 ACPA impairs temporal and spatial memory

We further tested the effects of the hippocampal CB1 receptors on memory retrieval. After infusing 3.125 ng ACPA into CA1, the rats still spent a significant amount of time exploring old familiar objects (Fig. 3A, right 2 bars; low dose, old: 46.65 ± 11.72 sec vs. recent: 24.52 ± 3.40 sec, $T(6) = 2.56$, $P < 0.05$). These results suggest that a low dose of a CB1 agonist may not disrupt the retrieval of temporally associated memories. In contrast, the retrieval of spatial memories may be influenced by a low dose of ACPA because in their control periods (1 μL of PFS), the animals spent more time exploring displaced objects (Fig. 3C, left 2 bars); however, the same group of animals spent a similar amount of time investigating stationary and displaced objects after receiving low-dose ACPA (Fig. 3C, right 2 bars; low dose, displaced: 52.66 ± 14.98 sec vs. stationary: 40.64 ± 10.97 sec, $T(6) = 1.01$, $P = 0.35$).

Inconsistent effects of marijuana intoxication on memory have been reported in open-field (Ranganathan and D'Souza, 2006) and animal studies (Atsak et al., 2012; de Oliveira Alvares et al., 2005, 2006). We hypothesized that the level of intoxication results in different percentages of CB1 receptors being activated, further leading to different influences on memory. Therefore, we tested rat memory retrieval functions after a high dose of ACPA. The data showed that during their control periods, the rats explored old familiar objects much more often than recent familiar objects (Fig. 3B, left 2 bars), but this effect was inhibited by high-dose ACPA (Fig. 3B, right 2 bars; high dose, old: 18.85 ± 2.81 sec vs. recent: 23.07 ± 4.29 sec, $T(6) = -1.49$, $P = 0.19$), which suggests that the retrieval of temporal memories may be disrupted by high doses CB1 receptor agonists in the right CA1. ACPA rats even spent more time exploring recent familiar objects than the control rats (Fig. 3B; control recent vs. ACPA recent; $T(6) = -2.52$, $P < 0.05$). However, a high dose of ACPA had little effect on spatial memories, given that we still observed a long duration of exploring the displaced objects (Fig. 3D, right 2 bars; high dose, displaced: 25.11 ± 2.62 sec vs. stationary: 12.59 ± 4.37 sec, $T(6) = 2.78$, $P < 0.05$). A summary of the results is presented in Table 1.

4. Discussion

4.1 Experimental design and the results

Our results demonstrate that a high dose of the CB1 agonist impaired the retrieval of sequentially ordered events, partially mimicking the temporal disintegration effects seen in marijuana intoxication (Atakan et al., 2012), and a low dose of ACPA disrupted the retrieval of spatial memories. Our data support previous findings showing that marijuana intoxication damages memory retrieval (Curran et al., 2002). We also demonstrated that activation of the right CA1 is sufficient for impairing memory retrieval. Al-

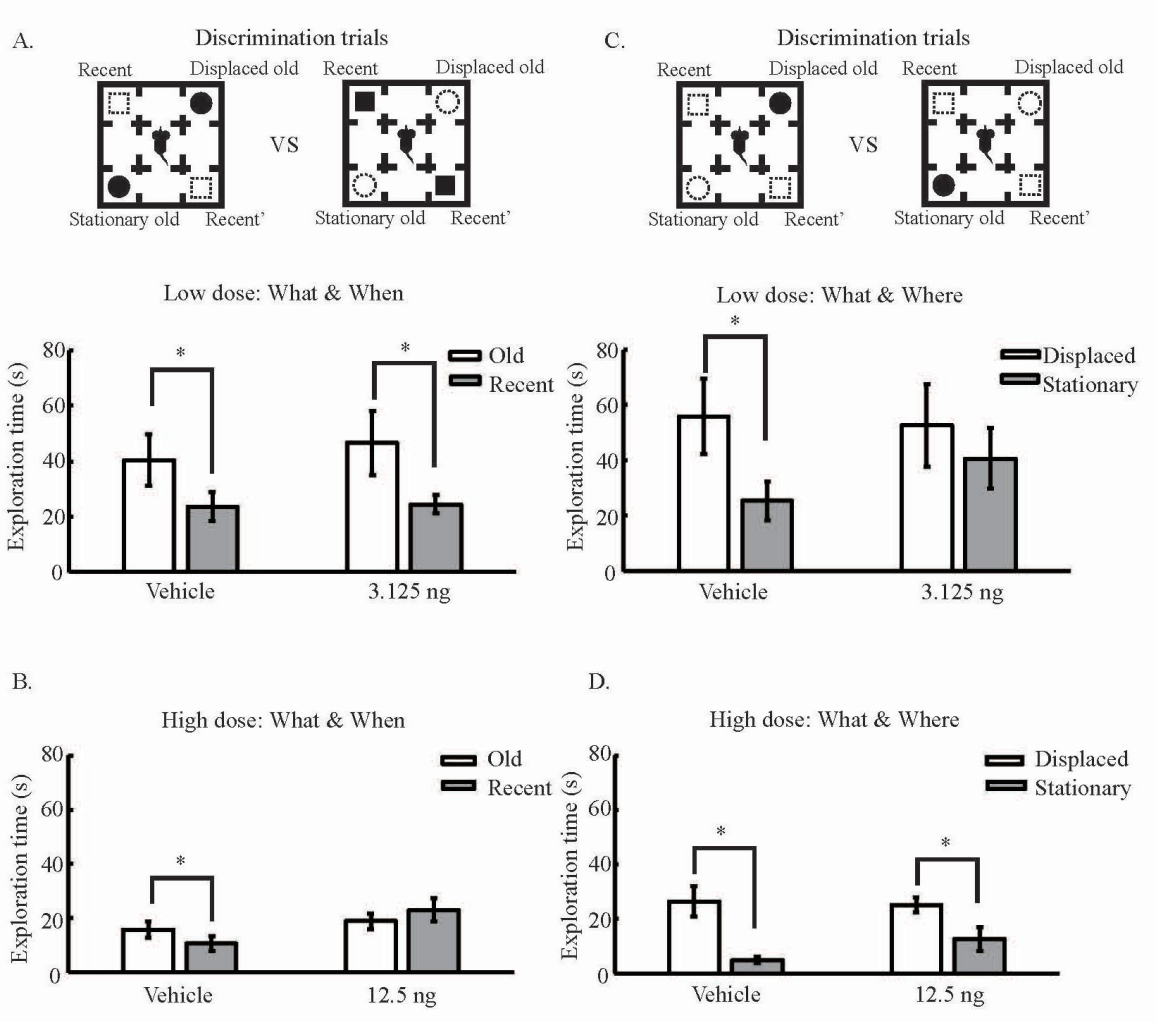

Figure 3. Exploration times during the discrimination trials. The top panel is an illustration showing which exploration times were compared (exploration times of the black objects were calculated). The time the rats spent exploring the old familiar and recent familiar objects demonstrated their ability to discriminate object sequences that require memories of "what" and "when" (A and B). Rats require memories of "what" and "where" to differentiate displaced and stationary objects (C and D). (A) and (C) depict the results for the low-dose group and (B) and (D) depict those of the high-dose group. The bars depict the means ± SEMs. * represents a significant difference, $P < 0.05$.

though the rodent hippocampus might lateralize the function of memory retrieval (Klur et al., 2009), the compensatory and remaining functions of the contralateral CA1 still cannot be examined in the present study. Nevertheless, two key points need to be discussed: 1). we reused the animals to test exploration time after administrating PFS (or ACPA), and 2). the total exploration time was less in the high-dose group than in the low-dose group. Although testing the vehicle effects in a new group could have ruled out the potential habituation or prelearned effects from the reused animals, we still chose to retest in the same animal since the design is less affected by individual variation.

To minimize the abovementioned retesting confounding factors, we randomized the PFS and ACPA administration order. Moreover, we randomized the objects, the walls of the open field, and the environmental cues. Also, we compared the object exploration times between rats infused with PFS first and those infused with PFS later; we found no significant difference between these two groups. By retesting the same animals, measuring the vehicle effects of the low-dose and high-dose groups provides the levels of baseline interest of each group. Therefore, the data represent the tendency in exploration time after ACPA administration (Table 1). We think it is critical to obtain the baseline interest because the between-subjects variability might mask the results of the experiment is not controlled perfectly. Taking our data as an example, the total exploration time was less in the high-dose group than in the low-dose group (Fig. 3A and 3B).

In our opinion, this result was caused by not randomizing the examining order between groups. We finished the experiment of the high-dose group first (including the ACPA and PFS tests) and then performed the same procedure for the low-dose group. For some unknown reasons, the high-dose group did not explore for a similar amount of time as the low-dose group. We suspect that the low-dose group had more time to adapt to the investigators and showed many natural, innate responses to the objects. However, the data still provide some evidence of the ACPA effects, because we tested the vehicle effect of the two groups and demonstrated

Table 1. Summary table. Summary of the rats' preferences for objects after each manipulation.

Manipulation \ Most frequented objects	Recent or old familiar objects	Stationary or displaced objects
PFS (low-dose group)	Old familiar objects	Displaced objects
ACPA (low-dose group)	Old familiar objects	Equal
PFS (high-dose group)	Old familiar objects	Displaced objects
ACPA (high-dose group)	Equal	Displaced objects

the same tendency of exploring objects after the same manipulation (Table 1). Our data also show that the low-dose group had impaired spatial memory and that the high-dose group had disturbed temporal memory. We suspect that different memories may engage the CB1 receptors from different neurons. Although it is difficult to test this hypothesis, some reports have proposed hypothetical mechanisms for how CB1 agonist doses influence neurons differently (Busquets-Garcia et al., 2018).

One of the causes of this phenomenon might be linked with the distributions of the CB1 receptor on different cell types. CB1 receptors are present in the synaptic terminals of GABAergic (Chen et al., 2003; Katona et al., 1999) and glutamatergic neurons (Kawamura et al., 2006) of the hippocampus. Comparing the CB1 receptor densities on the GABAergic and glutamatergic neurons in CA1, the GABAergic neurons present with a higher density (Kawamura et al., 2006). Specifically, CB1 receptors are mainly present on perisomatic interneurons that contain cholecystokinin (CCK) (Katona et al., 1999). CCK-positive interneurons serve to fine-tune glutamatergic neurons in the CA1 (Freund, 2003), so activation of CB1 receptors may disinhibit CA1 functions. However, CB1 density is not the only factor that determines which CB1receptor-expressing neurons are engaged. Steindel et al. (2013) reported that the CB1 receptors in hippocampal glutamatergic neurons have a higher efficacy of functioning than nearby GABAergic neurons (Steindel et al., 2013). Although the mechanisms of the differential recruitment of CB1 receptors in different neuron types remain unclear, different doses of CB1 receptor agonists theoretically can lead to different or even opposite functions.

4.2 CB1 receptors modulate spatial memories

Riedel and Davies (2005) reviewed several rodent studies about CB1 receptor-modulated spatial memories. The tools used to study this effect include water mazes, radial arm mazes, T and Y mazes, and delayed match-to-position tasks. Most studies systemically administered drugs and showed impairment of memories following the administration of CB1 receptor agonists (Riedel and Davies, 2005). Lichtman et al. (1995) showed that both the systemic and intrahippocampal administration of a CB1 agonist impairs spatial memories.

In this study, we used ACPA (in Tocrisolve™100) dissolved in normal saline, which has better binding potential and selectivity for CB1 receptors and rules out vehicle effects that water-insoluble CB1 agonists typically possess. In our results, the injection of 12.5 ng ACPA into CA1 still preserved the knowledge of "where". Robbe et al. (2006) finding also hints that the activation of CB1 receptor impairs sequential memory, but that spatial memory may still be intact. They reported that CB1 receptor agonists impair spike timing coordination but not the firing rate of place cells, which encode spatial information about the environment (Robbe et al., 2006). The use of Dere et al. (2005) three-trial object exploration task can also test the effects of drugs on temporal memories, which have been less often addressed by other reports. Thus, we are more interested in the aspects of CB1 receptor-modulated temporal memories.

4.3 CB1 receptors modulate temporal memories

Impairment in recalling the sequential order of events has been tested in place cell studies (Robbe and Buzsaki, 2009; Robbe et al., 2006), not only at the behavioral level, as we presented above; Robbe et al. (2006) influential results indicated that CB1 receptor agonists disrupt the coordination of CA1 cell assemblies, including the discharge time of theta sequences, and destroy their firing patterns in a theta cycle (i.e., theta precession interference). The firing time of a theta sequence corresponds to the order in a place field; therefore, the activation of CB1 receptors impairs the order of memories. Robbe et al. (2006) utilized an intraperitoneal injection approach; thus, it is still unclear whether the local ECS in the hippocampus contributed much to their findings and which phase of memory processing was affected. Hajos et al. (2000) immersed hippocampal slices in a solution containing a CB1 receptor agonist and subsequently abolished kainic acid-induced CA3 gamma oscillations. This result suggests that CB1 receptors negatively modulate the retrieval of spatial and temporal memories because CA3 gamma oscillations occur during memory retrieval (Bieri et al., 2014).

Some investigators trained animals to test the memory of sequences. Farovik et al. (2010) trained rats to recognize 10 two-odor sequences and showed that both CA1 and CA3 were essential to distinguish ordered events, but CA1 is especially crucial for differentiating two-odor sequences at longer intervals, such as 10 sec. Another report illustrated that damaged hippocampi significantly decreased the rate at which rats could correctly distinguish odors in a sequence task but not in an odor recognition task (Fortin et al., 2002). The present method uses rats' innate behaviors to test sequential order memory and saves time in training animals, but other "when-related" functions, such as timing, must be investigated using more complex tasks. According to our results, we speculate that the ECS in the hippocampus is involved in the process of processing time information in the brain.

4.4 Limitations of the three-trial object exploration task

This task has a possible confounding factor because the exploration time of old familiar objects is different from that of both the stationary and displaced objects. Therefore, Dere et al. (2005) further compared the time spent exploring stationary old familiar objects and the mean time spent exploring the two recent familiar

objects. Their results indicated that rats spent significantly more time exploring the stationary old familiar object (Dere et al., 2005), but we did not see this tendency in our studies. We suggest that if we want to see a significant difference between the time rats spent exploring the old stationary object and the mean time spent exploring recent familiar objects, the rats must spend much more time exploring the old stationary objects. However, it is not practical to expect the rats not to show more attention to old displaced objects, since rats also have innate preferences toward displaced things. Also, Dere et al. (2005) used a one-tailed t-test to measure the significance of these factors. This may imply that the importance between the old stationary objects and the recent objects is low.

5. Conclusions

Our main findings suggest that activation of the right CA1 disrupts the retrieval of episodic memories and that different doses of ACPA may result in the impairment of spatial or temporal memories.

Abbreviations

ACPA, arachidonylcyclopropylamide; CA, cornu ammonis area; CB1, type 1 cannabinoid; CCK, cholecystokinin; ECS, endocannabinoid system; GABAergic, gamma aminobutyric acidergic; NC, north-center; NE, northeast; NW, northwest; PFS, pyrogen-free saline; SC, south-center; SE, southeast; SEM, standard error of the mean; SW, southwest.

Ethics approval and consent to participate

All procedures performed in this study were approved by the National Taiwan University Animal Care and Use Committee (Approval No: NTU107-EL-00108).

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Conflict of Interest

The authors declare no conflict of interest.

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