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Dual Fatty Acid Amide Hydrolase and Monoacylglycerol Lipase Blockade Produces THC-Like Morris Water Maze Deficits in Mice

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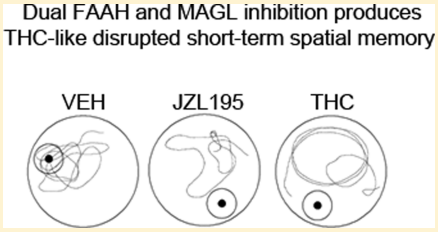
Supporting Information

ABSTRACT: Acute administration of Δ^9-tetrahydrocannabinol (THC) or exposure to marijuana smoke impairs short-term spatial memory in water maze tasks through a CB_1 receptor mechanism of action. *N*-Arachidonoylethanolamine (anandamide; AEA) and 2-arachidonoylglycerol (2-AG) are endogenous cannabinoids that are predominantly metabolized by the respective enzymes fatty acid amide hydrolase (FAAH) and monoacylglycerol lipase (MAGL). Although the MAGL inhibitor JZL184 enhances short-term synaptic plasticity, it has yet to be evaluated in the Morris water maze. Previous research demonstrated that simultaneous, complete blockade of FAAH and MAGL produces full blown THC-like effects. Thus, in the following studies we tested whether dual blockade of FAAH and MAGL would impair learning in a repeated acquisition Morris water maze task. Mice treated with the dual FAAH/MAGL inhibitor JZL195 (20 mg/kg) as well as JZL184-treated FAAH −/− mice displayed robust deficits in Morris water maze performance that were similar in magnitude to THC-treated mice. While 20 or 40 mg/kg impaired water maze performance in FAAH −/− mice, only the high dose of JZL184 disrupted performance in FAAH +/+ mice. The memory impairing effects of JZL184 were blocked by the CB_1 receptor antagonist rimonabant. Neither JZL184 nor JZL195 impaired performance in a cued version of the water maze task, arguing against the notion that sensorimotor or motivational deficits accounted for the impaired acquisition performance. JZL184 increased 2-AG levels in the hippocampus, prefrontal cortex, and cerebellum to a similar degree in FAAH −/− and +/+ mice. FAAH −/− mice, regardless of drug treatment, possessed elevated AEA levels in each brain region assessed. The results of this study reveal that concomitant increases in AEA and 2-AG disrupt short-term spatial memory performance in a manner similar to that of THC.



KEYWORDS: *Cannabinoid, memory, N-arachidonoylethanolamine (anandamide), 2-arachidonoylglycerol (2-AG), fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MAGL)*

The endogenous cannabinoid signaling system has been widely studied to understand its role in physiological and pathological processes. To date, two major endogenous cannabinoids, *N*-arachidonoylethanolamine (anandamide, AEA) and 2- arachidonoylglycerol (2-AG), and their distinct biosynthetic and degradative pathways have been extensively investigated. Both AEA and 2-AG are synthesized and released on demand, in response to diverse physiological stimuli, and are rapidly inactivated by hydrolysis after cellular reuptake. AEA[1] is metabolized by fatty acid amide hydrolase (FAAH), whereas monoacylglycerol lipase (MAGL) is the major catabolic enzyme of 2-AG.[2] These endogenous cannabinoids as well as exogenously administered cannabinoids activate two cannabinoid receptors, CB_1 and CB_2.[3,4] CB_1 receptors are heterogeneously distributed in high concentrations throughout the central nervous system and periphery and are the predominant target for the psychomimetic effects of Δ^9-tetrahydrocannabinol (THC), the primary psychoactive constituent of marijuana. CB_2 receptors are predominantly located on cells of the immune system,[4] including microglial cells and some neurons in the brain.[5,6] MAGL is located on presynaptic neurons containing CB_1 receptors[7] and, consequently, can have a significant role in controlling stimulation of these receptors. Genetic or pharmacological inhibition of FAAH or MAGL increases endogenous levels of AEA and 2-AG, respectively. Thus, the development of selective and potent inhibitors of endocannabinoid catabolic enzymes as well as the creation of FAAH −/− and MAGL −/− mice represent important tools to investigate the role of the endogenous cannabinoid signaling system in physiological and behavioral processes.

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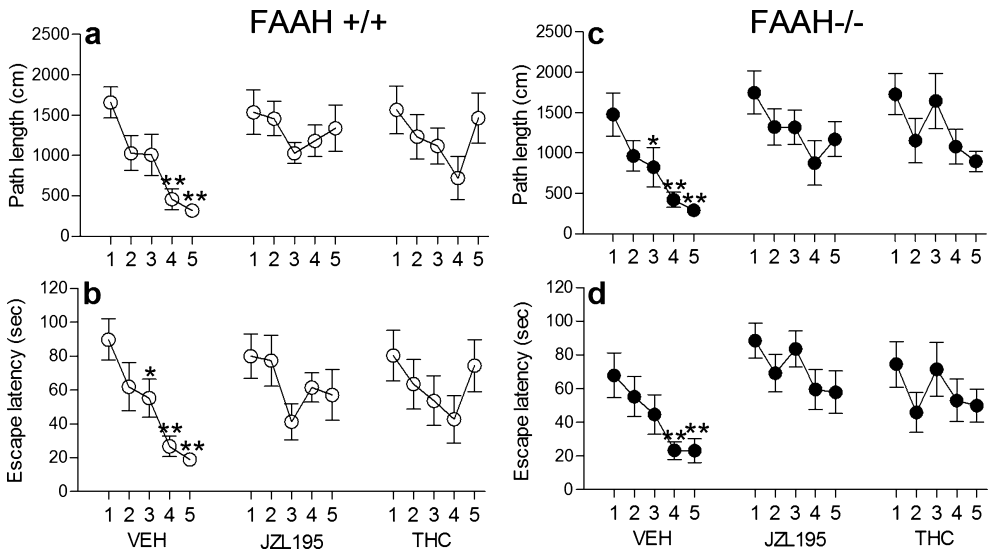

Figure 1. Dual MAGL/FAAH inhibitor JZL195 (20 mg/kg) and THC (10 mg/kg) produced similar performance deficits during the five water maze acquisition trials in FAAH +/+ and −/− mice. FAAH +/+ mice treated with vehicle, but not JZL195 or THC, significantly decreased their path lengths (a) and escape latencies (b) to the platform location in later trials as compared to trial 1. FAAH −/− mice treated with vehicle, but not JZL195 or THC, significantly decreased their path lengths (c) and escape latencies (d) to the platform location in later trials as compared to trial 1. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences from trial 1. All data are reported as the mean ± SEM, $N = 8–9$ mice/treatment group.

Marijuana, THC, and other cannabinoid receptor agonists are well known to produce disturbances in animal models of learning and memory. Specifically, cannabinoid receptor agonists selectively disrupt tasks heavily dependent on short-term (i.e., working memory), but not long-term (i.e., reference), memory in operant and spatial paradigms.[8−12] These effects are blocked by CB_1 receptor antagonists and do not occur in CB_1 −/− mice[8,10,13] indicating that they are mediated via CB_1 receptors. Additionally, FAAH −/− mice, but not wild-type mice, treated with exogenous anandamide display short-term memory impairment in the water maze that is blocked by the CB_1 receptor antagonist rimonabant.[14] In contrast, pharmacological or genetic disruption of FAAH does not affect short-term memory but significantly accelerates acquisition in the water maze paradigm.[15]

JZL184 is a highly selective and potent MAGL inhibitor that increases 2-AG brain levels up to 10-fold and produces CB_1 receptor-mediated antihyperalgesic, anxiolytic-like, hypothermic, and locomotor-suppressant effects but not cataleptic or THC subjective effects in the THC drug discrimination paradigm in mice.[16−20] Inhibition of FAAH produces antinociceptive, anti-inflammatory, anxiolytic-like, and antidepressant-like effects but does not produce the wide range of THC-like effects in mice such as catalepsy, hypothermia, hypomotility, memory-impairment, and generalization to THC in the drug discrimination paradigm.[1,21−23] In contrast, the dual FAAH/MAGL inhibitor JZL195- or JZL184-treated FAAH −/− mice produces a broad range of THC-like effects that are reversed by the CB_1 receptor antagonist rimonabant.[19] However, the consequences of simultaneous inhibition of FAAH and MAGL have yet to be evaluated in learning and memory models.

In particular, there are presently no published reports evaluating whether inhibiting MAGL or dual inhibition of FAAH and MAGL impairs short-term memory function in the water maze. Thus, the primary objective of this study was to evaluate the consequences of simultaneously inhibiting both of these endocannabinoid catabolic enzymes on mnemonic function. To this end, we tested the effects of JZL195 as well as JZL184 in FAAH +/+ and FAAH −/− mice in a spatial short-term memory water maze task. Rimonabant was used to evaluate the involvement of CB_1 receptors. In addition, FAAH +/+ or −/− mice treated with JZL184 (40 mg/kg) or JZL195-treated wild-type mice were assessed in a cued version of the water maze task in which the platform is made visible to distinguish between sensorimotor/motivational deficits and impaired memory. Finally, we quantified the impact of JZL184 administered to both genotypes on AEA and 2-AG levels in the hippocampus, prefrontal cortex (PFC), and cerebellum, brain areas known to be involved in learning and memory.

RESULTS AND DISCUSSION

Dual FAAH/MAGL Inhibitor JZL195 or THC Produces Similar Water Maze Performance Deficits in FAAH −/− and +/+ Mice. In an initial experiment, we investigated the effects of the dual FAAH and MAGL inhibitor JZL195 (20 mg/kg) on the performance of FAAH −/− and +/+ mice in a repeated acquisition task in the water maze. THC (10 mg/kg) was also evaluated in these mice as a positive control. Acquisition of FAAH +/+ mice treated with vehicle, JZL195, or THC is shown in Figure 1a and b. The one-way ANOVA revealed that vehicle [$F(4,28) = 9.1$; $p < 0.001$], but not JZL195 ($p = 0.49$) or THC ($p = 0.16$), significantly decreased path length to target across the five acquisition trials indicating that they learned the location of the hidden platform. As can be seen in Figure 1b, FAAH +/+ mice also displayed significantly reduced escape latencies across trials when treated with vehicle [$F(4,28) = 13.2$; $p < 0.001$] but failed to significantly decrease their escape latencies after 20 mg/kg JZL195 ($p = 0.16$) or 10 mg/kg THC ($p = 0.21$). These treatments elicited similar acquisition performance in FAAH −/− mice (Figure 1c and 1d). Vehicle-treated FAAH −/− mice learned the platform location during the acquisition trials [path length: $F(4, 32) = 7.9$; $p < 0.001$; escape latency: $F(4,32) = 4.3$; $p < 0.01$], whereas JZL195 (path length, $p = 0.17$; escape latency, $p = 0.42$) or THC (path length, $p = 0.08$; escape latency, $p = 0.25$) disrupted

acquisition learning. Swim speed data for FAAH +/+ and −/− mice are presented in Supporting Information, Figure 1.

Results from the probe trials revealed that FAAH +/+ mice [Figure 2a; $F(2,23) = 12.4$; $p < 0.001$] or FAAH −/− mice

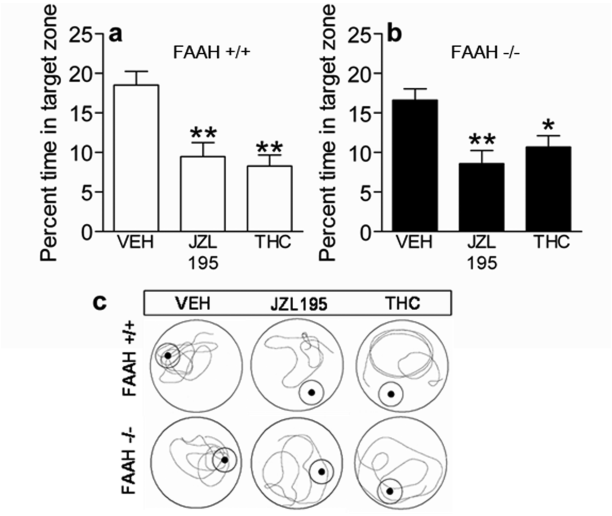

Figure 2. (a) In the probe trial, wild-type mice treated with JZL195 or THC spent significantly less time in the target zone than vehicle-treated mice. (b) FAAH −/− mice treated with JZL195 or THC spent significantly less time in the target zone than vehicle-treated mice in the probe trial. (c) Representative swim traces of FAAH +/+ and −/− mice treated with vehicle, JAL195, or THC. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences from vehicle-treated mice. All data are reported as the mean ± SEM, $N = 8–9$ mice/treatment group.

[Figure 2b; $F(2,23) = 9.5$; $p < 0.01$] spent significantly more time in the target zone when treated with the vehicle than when treated with JZL195 ($p < 0.05$) or THC ($p < 0.05$). These data also show that FAAH +/+ and FAAH −/− mice treated with JZL195 display disrupted probe trial performance that is similar to THC. Representative swim traces of both genotypes treated with vehicle, JZL195, or THC are shown in Figure 2c.

These data demonstrate that FAAH −/− and +/+ mice showed similar deficits in Morris water maze acquisition and performance during the probe trial in response to THC or the dual FAAH and MAGL inhibitor JZL195. Additionally, the disrupted probe trial performance in mice treated with JZL195 or THC further indicates that they had not acquired the task. The poor memory performance found in mice treated with THC supports previous reports indicating that acute injections of THC disrupt spatial working or short-term memory in the Morris water maze.[11−13,24]

FAAH −/− Mice Show Enhanced Sensitivity to JZL184-Induced Water Maze Performance Deficits Compared to FAAH +/+ Mice. In the first experiment, the dual FAAH and MAGL inhibitor JZL195 or THC elicited similar magnitudes of acquisition performance in both genotypes. Accordingly, we then tested whether FAAH −/− mice would be more sensitive than FAAH +/+ mice to the memory impairing effects of the selective MAGL inhibitor JZL184 on water maze acquisition.

Path length data for FAAH −/− mice are shown in Figure 3a. Vehicle-treated FAAH −/− mice displayed significant decreases in path lengths across the five trials [$F(4,44) = 7.0$; $p < 0.001$], indicating that they learned the location of the hidden platform. However, 20 mg/kg ($p = 0.68$) or 40 mg/kg ($p = 0.20$) JZL184 impaired acquisition performance in these mice. We next evaluated whether impaired performance in JZL184-treated FAAH −/− mice was CB_1 receptor mediated. In an initial study (data not shown), 3 mg/kg rimonabant did not block the memory performance deficits in FAAH−/− mice treated with 40 mg/kg JZL184. However, 5.6 mg/kg rimonabant significantly prevented the disruptive effects of 40 mg/kg JZL184 on path lengths in FAAH−/− mice [$F(4,20) = 4.5$; $p < 0.01$]. Additionally, FAAH−/− mice treated with 5.6 mg/kg rimonabant alone [$F(4,20) = 4.3$; $p < 0.05$] learned the platform location displaying significant decreases in path length during acquisition.

Escape latency data revealed a similar pattern of results in FAAH −/− mice (Figure 3b). Vehicle-treated FAAH −/− mice showed significant decreases in escape latency across the five acquisition trials [$F(4,44) = 7.0$; $p < 0.001$]. However treatment with either 20 mg/kg ($p = 0.76$) or 40 mg/kg ($p = 0.69$] JZL184 impaired performance. FAAH −/− mice administered rimonabant alone showed significantly enhanced acquisition [$F(4,20) = 3.9$; $p < 0.05$]. Rimonabant treatment also prevented the impaired acquisition caused by 40 mg/kg JZL184 [$F(4,20) = 6.5$; p, < 0.01]. Swim speed data for FAAH −/− mice are found in Supporting Information.

It should be noted that a higher dose of rimonabant than typically used was required to block the effects of dual inhibition on memory performance. FAAH−/− mice treated with JZL184 possess increased levels of both AEA and 2-AG, which may account for the higher dose of rimonabant required to block the memory impairing effects of JZL184 in FAAH−/− mice as compared to FAAH +/+ mice (discussed below). However, the increased dose of rimonabant required to block the impaired performance produced by the combination of FAAH and MAGL blockade raises the possibility of off target actions. In particular, rimonabant has been shown to act as a TRPV1 receptor antagonist in preventing capsaicin-induced impairment of LTD in GABAergic hippocampal interneurons.[25] The possible involvement of TRPV1 is bolstered by the fact that anandamide can act as an agonist at this receptor.[26,27] Delineating the receptor systems mediating the cognitive disruptive effects as well as other actions caused by dual FAAH and MAGL blockade will be a focus of future studies. Other water maze studies show that rimonabant given alone produces differential effects on spatial memory tasks that include enhanced performance,[28−31] no effect,[11,12,14] and disrupted learning in extinction[32] or new reference memory learning[33] assays. Although beyond the scope of the present study, the determination of pA2 values could provide insight into the differential sensitivity to rimonabant displayed between the FAAH −/− and +/+ mice. Nevertheless, our findings support the notion that concomitant elevation of the endocannabinoids AEA and 2-AG produces THC-like disruptions of short-term memory performance through a CB_1 receptor site of action.

The percentage of time FAAH −/− mice spent in the target zone during the probe trial is depicted in Figure 3c, and representative swim traces are found in Figure 3d. Both 20 mg/kg ($p < 0.05$) and 40 mg/kg of JZL184 ($p < 0.01$) decreased time spent in the target zone compared with vehicle treatment. However, FAAH −/− mice treated with rimonabant alone or rimonabant plus 40 mg/kg JZL spent a percentage of time in the target zone similar to that of vehicle-treated mice, indicating that they remembered the platform location.

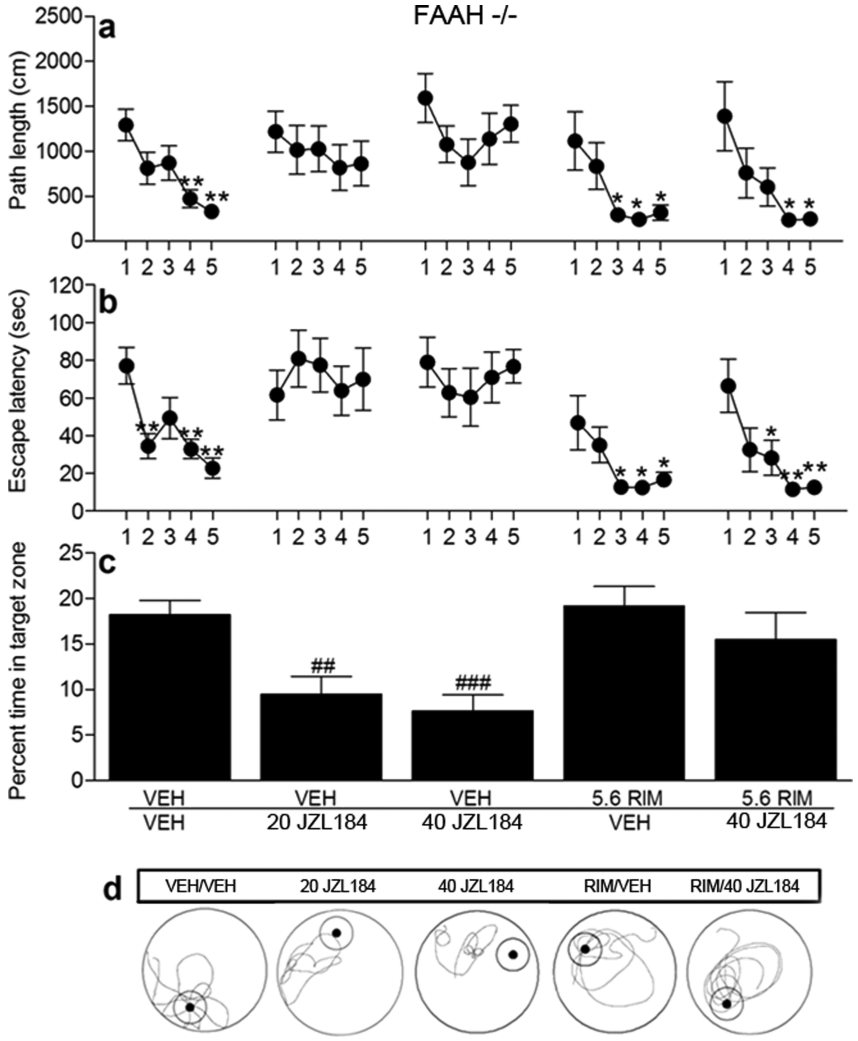

Figure 3. FAAH −/− mice are particularly sensitive to water maze acquisition deficits caused by JZL184. FAAH −/− mice treated with 20 or 40 mg/kg JZL184 did not demonstrate a significant decrease in path length (a) or escape latency (b) to the platform location. FAAH −/− mice treated with the CB_1 receptor antagonist rimonabant (RIM) and 40 mg/kg JZL184 significantly decreased their path lengths and escape latencies to the platform location in later trials indicating a CB_1 receptor mechanism of action. (c) Twenty or 40 mg/kg JZL184 led to decreases in the percentage of time spent in the target zone as compared to vehicle treatment. Rimonabant blocked the disruptive effects of 40 mg/kg JZL184 during the probe trial indicating CB_1 receptor involvement. (d) Representative swim paths of FAAH −/− mice. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences from trial 1; ## $p < 0.01$ and ### $p < 0.001$ indicate significant differences from vehicle-treated mice. All data are reported as the mean ± SEM, N = 6−12 mice/treatment group.

The effects of JZL184 in FAAH +/+ mice are shown in Figure 4. As reflected in the path length data (Figure 4a), FAAH +/+ displayed improved performance across the five acquisition trials following treatment with vehicle [$F(4,36) = 8.8$; $p < 0.001$], 20 mg/kg JZL184 [$F(4,36) = 4.4$; $p < 0.01$], or 40 mg/kg JZL184 [$F(4,36) = 3.0$; $p < 0.05$]. These mice also showed reduced path length latencies to the target following the administration of 3 mg/kg rimonabant [$F(4,32) = 12.1$; $p < 0.001$] or rimonabant (3 mg/kg) plus 40 mg/kg JZL184 [$F(4,36) = 9.2$; $p < 0.001$].

The escape latency data in FAAH+/+ mice are shown in Figure 4b. Significant decreases occurred across the five acquisition trials following vehicle [$F(4,36) = 8.8$; $p < 0.001$], 20 mg/kg JZL184 [$F(4,36) = 4.4$; $p < 0.01$], 40 mg/kg JZL184 [$F(4,36) = 3.0$; $p < 0.05$], 3 mg/kg rimonabant alone [$F(4,32) = 12.1$; $p < 0.001$], or 3 mg/kg rimonabant plus 40 mg/kg JZL184 [$F(4,36) = 9.2$; $p < 0.001$] treatment. Dunnett's post-hoc test revealed significant decreases in escape latencies from the first trial in all treatment groups, except following 40 mg/kg JZL184. Swim speed data for FAAH +/+ are found in Supporting Information.

The time FAAH +/+ mice spent in the target zone during the probe trial is shown in Figure 4c, and representative swim traces are found in Figure 4d. In FAAH +/+ mice, 40 mg/kg JZL184 disrupted performance in the probe trial [$F(4,42) = 6.2$; $p < 0.001$]. Specifically, FAAH +/+ mice spent significantly less time in the target zone when treated with 40 mg/kg JZL184 ($p < 0.05$) than when treated with the vehicle. Rimonabant (3.0 mg/kg) blocked the memory disrupting effects of 40 mg/kg JZL184 in FAAH +/+ mice indicating a CB_1 receptor mechanism of action.

FAAH +/+ mice treated with the high dose of JZL184 (40 mg/kg) showed evidence of learning the platform location (i.e., path lengths significantly decreased through the five acquisition trials); however, their escape latencies did not significantly decrease across the five acquisition trials, and they spent

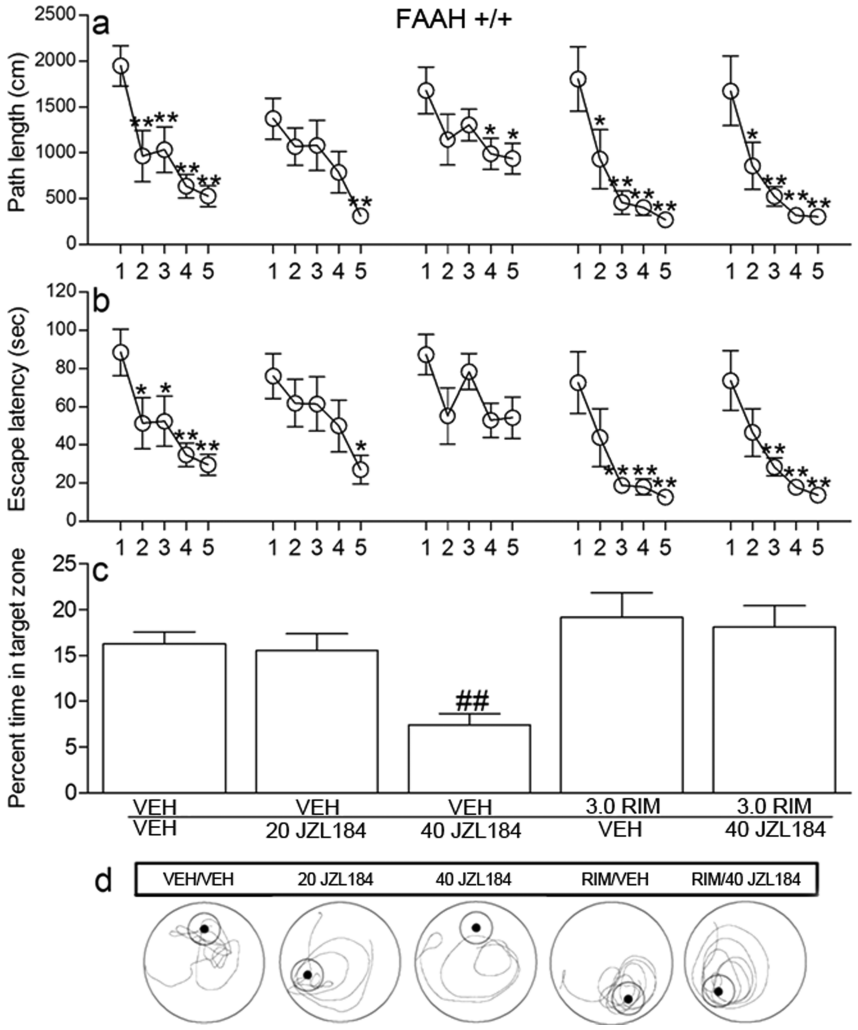

Figure 4. (a) FAAH +/+ mice are only sensitive to the disruptive effects of high dose JZL184 on water maze acquisition. Rimonabant blocked the disruptive effects of 40 mg/kg JZL184 on path length to the platform location in later trials indicating a CB_1 receptor mechanism of action. (b) FAAH +/+ mice significantly decreased their escape latencies to the platform location in later trials as compared to trial 1 in all treatment conditions. (c) During the probe test, FAAH +/+ mice treated with 40 mg/kg JZL184, but not 20 mg/kg JZL184, displayed decreases in time spent in the target zone as compared to vehicle treatment. Rimonabant prevented the disruptive effects of 40 mg/kg JZL184 during the probe trial, showing CB_1 receptor involvement. (d) Representative swim paths of FAAH +/+ mice. * $p < 0.05$ and ** $p < 0.01$ indicate significant differences from trial 1; ## $p < 0.01$ indicates significant differences from vehicle-treated mice. All data are reported as the mean ± SEM, $N = 6–12$ mice/treatment group.

significantly less time in the target zone as compared to vehicle-treated mice during the probe trial, suggesting impaired memory of the platform location. These finding are consistent with other results showing that full MAGL inhibition produces a more extensive subset of THC-like effects than that produced by full FAAH inhibition.[18] In contrast to these findings, performance in the object recognition task and acquisition of reference memory in the Morris water maze is enhanced in MAGL −/− mice.[34] We have also found here that 20 mg/kg JZL184 does not disrupt short-term spatial memory performance in FAAH +/+ mice. Busquets-Garcia et al.[35] similarly report that a low dose of JZL184 (8 mg/kg) had no effect on memory consolidation in contextual fear and object recognition tasks. Acute MAGL inhibition potentiates DSI in neurons of the hippocampus, cerebellum, and cingulate cortex,[36,37] brain areas involved in learning and memory processing, suggesting that 2-AG and MAGL may have significant roles in modulating cognitive function in other memory paradigms. Full MAGL inhibition has also been shown to elicit cannabinoid receptor independent neuroprotective effects[38] that result from decreases in arachidonic acid and proinflammatory prostaglandins. While we report that the memory impairing effects of JZL184 are blocked by rimonabant, the contribution of mechanisms independent of cannabinoid receptors on memory function as well as other physiological processes involving proinflammatory cascades should be further evaluated.

Taken together, these results reveal that FAAH −/− mice are more sensitive than FAAH +/+ mice to the disruptive effects of JZL184 on acquisition and memory performance during a probe trial. These findings are also consistent with the previous experiment showing that dual inhibition of FAAH and MAGL by JZL195 disrupts short-term spatial memory. Thus, simultaneous blockade of the primary catabolic enzymes of AEA and 2-AG elicits a THC-like disruption of spatial learning and memory in the Morris water maze. Similarly, Long et al.,[19] demonstrated that JZL195 or JZL184 treatment in FAAH −/− mice produced a broad range of THC-like effects, including catalepsy, full analgesia, and full generalization to THC in the drug discrimination paradigm. Importantly, the memory disruptive, analgesic, cataleptic, and THC-like subjective effects

caused by dual blockade of FAAH and MAGL were antagonized by rimonabant, indicating a CB_1 receptor mechanism of action.

JZL184 Does Not Impair Performance of FAAH +/+ and −/− Mice in the Cued Task. In the next set of experiments, we evaluated both genotypes in a cued task (Figure 5), in which the platform was made visible, to assess the possibility that sensorimotor or motivational deficits accounted for disrupted memory performance. In the cued task, FAAH +/+ mice treated with 40 mg/kg JZL 184 or 20 mg/kg JZL195 had swim path lengths (Figure 5a; $p = 0.93$), escape latencies (Figure 5b; $p = 0.78$), and swim speeds (Figure 5c; $p = 0.61$) that did not differ from vehicle-treated mice. Similarly, FAAH (−/−) mice treated with JZL184 or JZL195 displayed swim path lengths (Figure 5a; $p < 0.92$), escape latencies (Figure 5b; $p = 0.29$), and swim speeds (Figure 5c; $p = 0.08$) that did not differ from vehicle-treated mice. FAAH −/− and +/+ mice treated with JZL184 or JZL195 exhibited near perfect performance in the cued task, suggesting that sensorimotor or motivational deficits are unlikely to account for poor performance during the repeated acquisition trials and the probe trial. Both drug treatments and trials affected the swim speed of FAAH −/− and +/+ mice in the repeated acquisition task. In contrast, swim speeds were unaffected by treatment with JZL184 or JZL195 in the cued task. The findings in the cued task suggest that the disruptions in memory performance when both FAAH and MAGL are inhibited or with full MAGL inhibition occur independently of their effects on motor behavior.

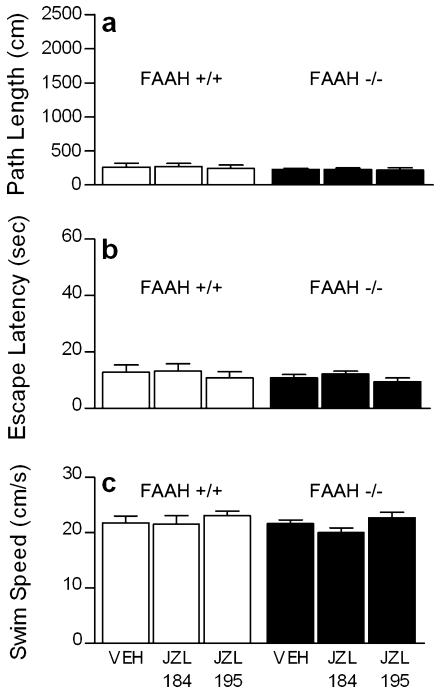

Figure 5. In the cued water maze task in which the platform is made visible, FAAH +/+ (open bars) and FAAH−/− (black bars) mice treated with 40 mg/kg JZL184 or 20 mg/kg JZL195 had path lengths to the platform location (a), escape latencies to the platform location (b), and swim speeds (c) similar to those of vehicle-treated mice. All data are reported as the mean ± SEM, $N = 11–13$ mice/treatment group.

Quantification of Endocannabinoid Levels in the Hippocampus, Prefrontal Cortex, and Cerebellum. FAAH +/+ and −/− mice were injected with vehicle or JZL184 (20 mg/kg), and 2 h later, their brains were dissected to quantify AEA and 2-AG levels in the hippocampus, prefrontal cortex, and cerebellum. Levels of 2-AG were significantly increased in the hippocampus [Figure 6a; $F(3,20) = 32.8$; $p < 0.0001$], prefrontal cortex [Figure 6b; $F(3,20) = 19.4$; $p < 0.05$], and cerebellum [Figure 6c; $F(3,20) = 42.2$; $p < 0.0001$] in both FAAH +/+ and −/− mice treated with JZL184 as compared to the vehicle. AEA levels in FAAH −/− mice were significantly greater than in FAAH +/+ mice, regardless of treatment with JZL184 or vehicle, in the hippocampus [Figure 6d; $F(3,20) = 32.6$; $p < 0.0001$], prefrontal cortex [Figure 6e; $F(3,20) = 35.7$; $p < 0.05$], and cerebellum [Figure 6f; $F(3,20) = 161$; $p < 0.0001$].

The hippocampus, PFC, and cerebellum are brain regions associated with memory and cognitive function and contain high concentrations of CB_1 receptors.[3,39] The hippocampus[40−43] and prefrontal cortex[44−46] play important roles in the effects of cannabinoids on short-term spatial memory as well as on synaptic plasticity. Studies also indicate that the cerebellum plays a role in spatial learning and memory tasks.[47−49] Here, we report that JZL184 (20 mg/kg) increased 2-AG levels approximately 4−5-fold in the hippocampus, prefrontal cortex, and cerebellum in FAAH (+/+) mice and approximately 4-, 3-, and 6-fold in these respective brain regions in FAAH −/− mice. FAAH −/− mice displayed significantly elevated brain AEA levels compared to that in FAAH +/+ mice, while JZL184 did not alter AEA levels in either genotype. These findings indicate that simultaneous increases in AEA and 2-AG brain levels result in THC-like disrupted memory performance. Previous studies have found that genetic deletion of FAAH leads to 10-fold increases in whole brain AEA levels[1] and that treatment with 16 or 40 mg/kg JZL184 produces approximately 7−8-fold increases in levels of 2-AG in whole brains.[18,19,37] The present results extend these findings by revealing increases in AEA and 2-AG levels in specific brain regions.

CB_1 receptor activation by exogenously applied cannabinoids, such as THC, lacks the regional selectivity and rapid metabolism of endocannabinoids. Consequently, the actions of endocannabinoid catabolic enzyme inhibitors do not mimic those of exogenously administered cannabinoids. While we have shown that a high dose of the MAGL inhibitor JZL184 can impair short-term memory performance, the results of the present study also reveal that a low dose of JZL184 does not impair short-term spatial memory in FAAH +/+ mice (i.e., wild-type mice). A low dose of JZL184 (8 mg/kg) was also found to have no effect on memory consolidation when evaluated in contextual fear and object recognition tasks.[35] In contrast to these findings, performance in the object recognition task and acquisition of reference memory in the Morris water maze is enhanced in MAGL −/− mice.[34] Previous research has shown that FAAH inhibition has no effect on short-term memory performance in the water maze.[14] However, the FAAH inhibitor URB597 disrupts short-term memory processing in the encoding phase of the delayed-nonmatch-to-sample task[50] and produces working memory deficits in a delayed-alternation task in the T-maze.[51] In contrast, FAAH inhibition enhances acquisition and extinction learning in spatial memory tasks,[15,52] acquisition in a passive avoidance task,[53] and consolidation in contextual fear and

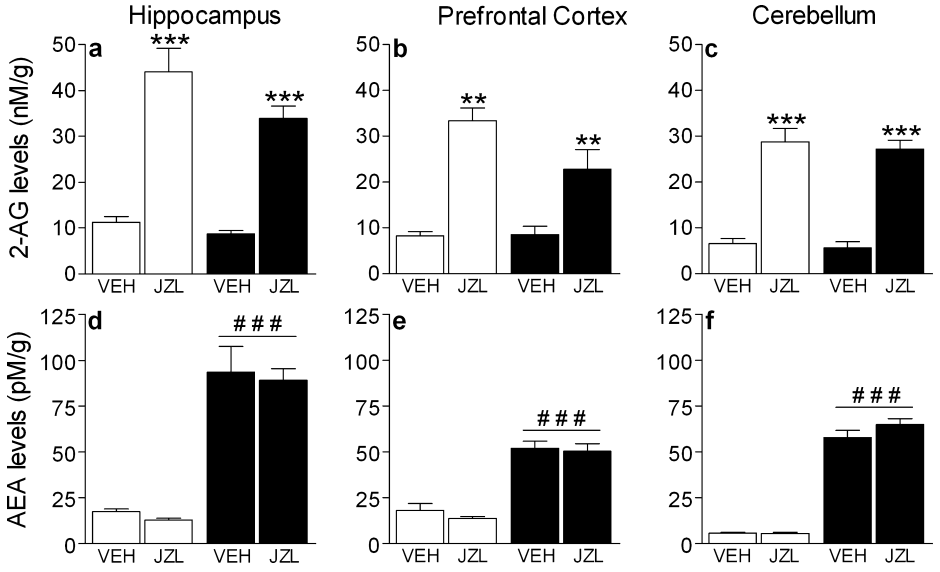

Figure 6. Twenty mg/kg JZL184 (JZL) significantly increased 2-AG levels in the hippocampus (a), prefrontal cortex (PFC; b) and cerebellum (c) of FAAH +/+ mice (open bars) and FAAH −/− mice (black bars) as compared to vehicle (VEH) treatment. ** $p < 0.01$ and *** $p < 0.001$ indicate significant differences from FAAH +/+ and −/− mice treated with vehicle; Tukey-Kramer post hoc tests. Anandamide (AEA) levels were increased in the hippocampus (d), prefrontal cortex (PFC; e) and cerebellum (f) of FAAH −/− mice (black bars), but not FAAH +/+ mice (open bars), treated with either 20 mg/kg JZL184 or vehicle. ###, $p < 0.001$, indicates significant differences from FAAH +/+ mice treated with vehicle or 20 mg/kg JZL184. All data are reported as the mean ± SEM, $N = 6$ mice/treatment group.

object recognition tasks.[35] AM404, an inhibitor of endocannabinoid uptake that also inhibits FAAH activity, impairs water maze performance[54] but has also been shown to facilitate extinction learning in conditioned freezing tasks.[55−57] These divergent findings indicate that the effects of elevating AEA or 2-AG on memory function are task specific. Moreover, taken together, these findings indicate that while endocannabinoids activate the same receptor, they can produce divergent effects on memory processing.

In summary, the present results demonstrate that the dual FAAH/MAGL inhibitor JZL195 or the administration of the MAGL inhibitor JZL184 to FAAH−/− mice impairs short-term memory performance in a manner similar to that of THC. In particular, FAAH −/− mice were more sensitive than FAAH +/+ mice to the disruptive effects of JZL184 but not JZL195 or THC. The finding that rimonabant prevented these performance deficits indicates that CB_1 receptors play a necessary role in these effects. Consistent with the notion that sensorimotor or motivational factors do not account for the disrupted memory performance, dual inhibition of FAAH and MAGL, as well as full MAGL inhibition (i.e., administration of 40 mg/kg), did not disrupt performance in the cued task. The present findings, that dual inhibition of FAAH and MAGL produces THC-like impairment of short-term spatial memory performance, supports previous research showing enhanced analgesia, catalepsy, and THC-like subjective effects in the drug discrimination paradigm[19] following concomitant increases in AEA and 2-AG. Thus, combined activation of AEA and 2-AG signaling pathways can interact in the nervous system to elicit full cannabimimetic effects.

METHODS

Subjects. FAAH −/− and +/+ mice, born in the Virginia Commonwealth University knockout colony and derived from breeders backcrossed onto a C57Bl/6 J background for at least 13 generations, served as subjects.[23] Mice were housed 4−6 per cage in an AAALAC-approved facility with a temperature (20−22 °C) and humidity controlled environment that was maintained on a 12-h light/dark cycle. Food and water were available *ad libitum* in the home cages. All experiments were approved by the Institutional Animal Care and Use Committee at Virginia Commonwealth University.

Drugs and Chemicals. JZL184 and JZL195 were synthesized as described previously.[18,19] THC and rimonabant (RIM) were provided by the National Institute on Drug Abuse (Bethesda, MD). All drugs were dissolved in a 1:1 mixture of absolute ethanol and alkamuls-620 (Rhone-Poulenc, Princeton, NJ) and diluted with saline to a final ratio of 1:1:18 (ethanol, alkamuls, and saline, respectively). JZL184, JZL195, and rimonabant were injected via the intraperitoneal (i.p.) route of administration, and THC was administered via the subcutaneous (s.c.) route of administration. All drugs were injected in a volume of 10 μL/g body mass. Doses of JZL184 (20 and 40 mg/kg) and JZL195 (20 mg/kg) were based on their efficacy in elevating brain levels of endocannabinoids, while the doses of THC and rimonabant were selected based on their respective ability to impair spatial memory and antagonize cannabinoid-induced memory deficits, respectively.[11,18,19,23]

Procedures. *Morris Water Maze.* The water maze consisted of a large circular galvanized steel pool (1.8 m diameter, 0.6 m height). A white platform (10 cm diameter) was placed inside, and the tank was filled with water (22 °C) until the top of the platform was submerged 1 cm below the water surface. A sufficient amount of white paint (Proline-Latex Flat) was added to make the water opaque and render the platform virtually invisible.

The mice were trained to perform a repeated acquisition version of a spatial memory Morris water maze task in which they were required to find a hidden platform that was placed in a new location each day as previously described.[11,58] Subjects first received a single 5 min acclimation session in which no platform was present, followed by 10 days of training in a fixed-platform task (four trials per day, 10 min between each trial). Repeated acquisition training consisted of five consecutive 2-min trials, in which a new platform location was determined each day but remained constant for that day. Once the mice located the platform, they were allowed to remain on it for 30 s. Training continued until the subject reached the platform in less than 30 s on two of the last three trials on the last three of four days.

Once training criteria were met, we evaluated the effects of the dual FAAH and MAGL inhibitor JZL195 (20 mg/kg) or THC (10 mg/kg)

in the repeated acquisition task in the water maze in FAAH −/− and +/+ mice using a counterbalanced design. We then tested whether the MAGL inhibitor JZL184 disrupted performance via a CB_1 mechanism of action. The following treatment conditions were tested in both genotypes in a counterbalanced design: vehicle + vehicle, vehicle + 20 mg/kg JZL184, vehicle + 40 mg/kg JZL184, rimonabant + vehicle, or rimonabant + 40 mg/kg JZL184. In FAAH +/+ mice 3 mg/kg rimonabant was evaluated. After finding that 3 mg/kg rimonabant did not block memory performance deficits in FAAH−/− mice treated with 40 mg/kg JZL184 (data not shown), we assessed 5.6 mg/kg rimonabant in FAAH −/− mice. Vehicle, JZL184, or JZL195 was injected 2 h before testing in the water maze. Vehicle or RIM was injected 10 min before vehicle, JZL184, or JZL195. THC was injected 30 min before testing in the water maze.

On test days, mice were evaluated in the 5 trial repeated acquisition task. Ten minutes after the fifth trial, the mice were tested in a 60 s probe trial in which the platform was removed from the tank. To qualify for testing, mice were required to achieve a criterion of swimming to the platform in less than 30 s on two of the last three trials on three of the previous four training sessions and to maintain this level of performance throughout the study. Between each test session, mice were given a washout period of at least five days. The escape latency (s), the distance the mice swam (i.e., path length; cm) to the platform, and swim speed (cm/s) were evaluated in the five trial repeated acquisition task. The time the mice spent in the target zone, defined as the area immediately surrounding the target location that comprised 8% of the total surface area, was evaluated in the probe trial. An automated tracking system (Columbus Instruments, Columbus, OH) connected to a video camera was used to quantify these dependent variables. Three different cohorts of mice were used to yield sample sizes of 6−12 FAAH +/+ and −/− mice per group.

Finally, in order to assess whether sensorimotor or motivational impairments could account for impaired memory performance, mice were trained to perform in a cued version of the task in which the platform location was made visible by placing a black rubber stopper (height, 3 cm; radius, 1.5 cm) on top of the submerged platform, which extended approximately 2 cm above the surface of the water. Training in the cued task consisted of two consecutive trials for three days, during which time mice learned to find the cued platform in less than 30 s. Once these training criteria were met, vehicle, 40 mg/kg JZL184, and 20 mg/kg JZL195 were tested in a counterbalanced fashion in FAAH −/− and +/+ mice. The mean escape latency (s), the distance the mice swam (i.e., path length; cm) to the platform, and swim speed (cm/s) of the two trials were evaluated.

Extraction and Quantification of 2-AG and AEA Levels by Liquid Chromatography−Tandem Mass Spectrometry. Immediately following decapitation, brains were removed, and the hippocampus, prefrontal cortex, and cerebellum were dissected. These regions were frozen in dry ice and stored at −80 °C until the time of processing. On the day of processing, tissues were weighed and homogenized with internal standards for each sample (2 pmol of AEA-d8 and 1 nmol of 2-AG-d8; Cayman Chemical) and 1.4 mL of chloroform/methanol (2:1 v/v containing 0.0348 g of phenylmethylsulfonyl fluoride/mL). Homogenates were mixed with 0.3 mL of 0.73% w/v NaCl, vortexed, and centrifuged for 10 min at 3220*g* (4 °C). The aqueous phase and debris were collected and extracted two additional times with 0.8 mL of chloroform. The organic phases from the three extractions were pooled, and the organic solvents were evaporated under nitrogen gas. Dried samples were reconstituted with 0.1 mL of chloroform and mixed with 1 mL of ice-cold acetone. To precipitate the proteins, the mixtures were centrifuged for 5 min at 1811*g* (4 °C). The upper layer of each sample was then collected and evaporated under nitrogen. Dried samples were reconstituted with 0.1 mL of methanol and placed in autosample vials for analysis.

Levels of 2-AG and AEA were quantified using liquid chromatography−tandem mass spectrometry. The mobile phase consisted of water/methanol (10:90) with 0.1% ammonium acetate and 0.1% formic acid. The column used was a Discovery HS C18, 4.6 × 15 cm, 3 μm (Supelco, Bellefonte, PA). The mass spectrometer was run with electrospray ionization in positive mode. Ions were analyzed in a multiple-reaction-monitoring mode. The following transitions were monitored: (348 > 62) and (348 > 91) for AEA; (356 > 62) for AEA-d8; (379 > 287) and (279 > 269) for 2-AG; and (387 > 96) for 2AG-d8. A calibration curve was constructed for each assay based on linear regression with the use of the peak area ratios of the calibrators. The extracted standard curves ranged from 0.03 to 40 pmol for AEA and from 0.05 to 64 nmol for 2-AG.

Data Analyses. All data are reported as the mean ± SEM. The acquisition data were analyzed using separate one-way repeated measures analysis of variance (ANOVA) in FAAH −/− and +/+ mice for each drug treatment in each genotype. Dunnett's test was used for post-hoc comparisons in which performance in trials 2−4 were compared to trial 1 for each treatment condition. The time spent in the target quadrant in the probe trials were analyzed with one-way ANOVAs. Dunnett's post-hoc test was then used to compare each treatment condition to vehicle in the probe trial. For the cued task, the data were analyzed with one-way ANOVAs followed by the Dunnett's post-hoc test in which each treatment condition was compared to vehicle. AEA and 2-AG levels in the hippocampus, PFC, and cerebellum were analyzed using separate one-way ANOVAs. Tukey-Kramer post-hoc tests were used to determine differences between treatment conditions in each brain area. Differences were considered statistically significant at $p < 0.05$.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Swim speed data for FAAH +/+ and −/− mice treated with vehicle, JZL195, or THC and swim speed data for FAAH +/+ and −/− mice treated with vehicle, 20 mg/kg JZL1984, 40 mg/kg JZL184, rimonabant, or rimonabant plus 40 mg/kg JZL184. This material is available free of charge via the Internet at http://pubs.acs.org.

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Author Contributions

K.A.L. and R.A.A. performed the experiments and collected the data. L.E.W., J.Z.L., B.F.C., and A.H.L. conceived the experiments. L.E.W. and A.H.L. designed the studies, analyzed the data, and wrote the manuscript.

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Notes

The authors declare no competing financial interest.

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■ ABBREVIATIONS

CB_1, cannabinoid 1; AEA, *N*-arachidonoylethanolamine (anandamide); 2-AG, 2-arachidonoylglycerol; FAAH, fatty acid amide hydrolase; MAGL, monoacylglycerol lipase; THC, Δ9-tetrahydrocannabinol; ABHD6, $\alpha\beta$-hydrolase domain 6; PFC, prefrontal cortex; ANOVA, analysis of variance; RIM, rimonabant

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