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Amnestic effect of intrahippocampal AM251, a CB1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats☆

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Abstract

CB1 is the most abundant metabotropic receptor of the brain, being found in areas classically involved in learning and memory and present at higher density at presynaptic terminals. Different sets of evidence support the idea that endogenous ligands (endocannabinoids) to the CB1 receptors act as modulators of neurotransmission. In hippocampus, endocannabinoids seem to act as retrograde messengers mediating down-regulation of GABA release. Previous reports have described a cognitive impairment effect of cannabinoid agonists, or facilitation by antagonists. The scope of the present study is to investigate the effect of intrahippocampal administration of the CB1-selective antagonist, AM251, in two behavioral tasks. One hundred and twelve male Wistar rats with bilateral cannulae implanted in the CA1 region of the dorsal hippocampus were trained in a step-down inhibitory avoidance task (IA, footshock, 0.5 mA) or an open field habituation task (OF). Immediately, after training, animals received an infusion of 0.55, 5.5, and 55.5 ng/side of AM251 (Tocris), or its vehicle (DMSO/saline), via these cannulae. Our results show that AM251 disrupted memory consolidation of the IA task, but not the OF task, an effect that seems to be purely mnemonic since the drug showed no motor performance effects. Only the intermediate dose (5.5 ng/side) of AM251 was effective in IA and the absence of effect with the larger dose may be the consequence of non-specific binding. The fact that OF was not affected raises the possibility that this endogenous system requires some degree of aversiveness to be recruited. We propose that increased levels of endogenous cannabinoids in the hippocampus, following a training session, contribute to *facilitate* memory consolidation, a process that may have been disrupted with AM251.

Keywords: Memory; AM251; CB1 receptors; Dorsal hippocampus; Step-down inhibitory avoidance; Rat

1. Introduction

CB1 is the most abundant metabotropic receptor of the brain, being found in areas classically involved in learning and memory, such as the hippocampus, cortex, basal ganglia, and cerebellum (Davies, Pertwee, & Riedel, 2002; Wilson & Nicoll, 2002). Extensive evidence shows cannabinoid receptors to be negatively coupled to

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adenylate cyclase through $G_{i/o}$ proteins, leading to a reduction in cAMP production (Ameri, 1999). CB1 has been shown to act via this G protein upon the usually presynaptic N-type voltage-dependent calcium channels (Ameri, 1999; Mackie, Lai, Westenbroek, & Mitchell, 1995; Wilson & Nicoll, 2002), suggesting a role for cannabinoids in the modulation of CNS neurotransmission. Furthermore, in hippocampal preparations, cannabinoids acting via CB1 receptors were shown to inhibit the release of glutamate, acetylcholine, and noradrenaline (Davies et al., 2002). In studying DSI (depolarization-induced suppression of inhibition), endocannabinoids, such as *N*-arachidonylethanolamine (anandamide) and 2-arachidonylglycerol (2-AG) (Pertwee & Ross, 2002) are proposed as retrograde messengers mediating down-regulation of GABA release, both in the hippocampus and in the cerebellum (Kreitzer & Regehr, 2001; Wilson & Nicoll, 2002). LTP, a phenomenon itself reinforced by DSI, was shown to be indirectly modulated by endocannabinoids that reduce presynaptic neurotransmitter release, suppressing the postsynaptic membrane depolarization necessary to activate NMDA receptors (Carlson, Wang, & Alger, 2002; Wilson & Nicoll, 2002).

This presynaptic inhibition of neurotransmitter release by cannabinoids is reinforced by the fact that CB1 receptors are present at higher density at the presynaptic terminal relative to the soma (Wilson & Nicoll, 2002) and that they can amplify the inhibition of voltage-dependent calcium channels by reducing the duration of the action potential by the activation of two different types of potassium channels (Ameri, 1999; Mackie et al., 1995).

In the hippocampus, CB1 receptors seem to be located basically in the presynaptic portions of the GABAergic axon terminals, mostly on CCK-releasing basket cells, explaining the inhibition of GABA release by CB1 agonists (Katona et al., 1999; Wilson & Nicoll, 2002). Most behavioral studies of the CB1 employing pharmacological manipulations in animal models, however, involve systemic, not intracerebral treatments; for example, systemic *pretraining* administration of natural agonists Δ^9-THC or anandamide, or synthetic agonists like WIN55,212-2, CP55940 or HU-210, are known for their learning impairment effect, particularly in hippocampus-dependent tasks such as the 8-arm radial maze, spatial alternation in a T-maze, or a delayed matching/non-matching to position task with lever presentation (Davies et al., 2002; Lichtman, Dimen, & Martin, 1995; Wilson & Nicoll, 2002); by the other side, systemic *pretraining* administration of the antagonist SR141716A in the inhibitory avoidance task in mice have not shown any per se effect upon memory (Mazzola, Micale, & Drago, 2003). In this last task, i.c.v., but not i.p. post-training administration of Δ^9-THC have impaired memory consolidation (Mishima et al., 2001).

Except for a late spatial memory improvement in rats (Lichtman, 2000) and a facilitatory action in an olfactory social recognition task (Wilson & Nicoll, 2002), both under systemic treatments, the antagonist SR141716A is usually reported as without effect by itself (Da Silva & Takahashi, 2002; Davies et al., 2002). Interesting results in the water maze task show the absence of effect of cannabinoids once mice have learned the position of the platform, i.e., the endocannabinoid system seems not to be involved in memory retrieval (Da Silva & Takahashi, 2002; Varvel, Hamm, Martin, & Lichtman, 2001). Recently, CB1 receptors were shown to be required for memory extinction, but not for memory acquisition or consolidation, in an auditory fear-conditioning test (Marsicano et al., 2002).

This scenario suggests that the endogenous cannabinoid system could be activated in a learning situation to modulate the synaptic plasticity underlying a cognitive process; if this is true, the administration of a selective antagonist should interfere negatively with any endogenous role of this system. Considering the importance of CB1 system in the hippocampus (Herkenham et al., 1991), and the fact that this brain structure is also deeply involved in the memory processing of different behavioral tasks (Izquierdo & Medina, 1995; Squire, 1992), we decided to study the effect of post-training intrahippocampal administration of AM251, a CB1-selective antagonist, in rats trained in inhibitory avoidance and the open field habituation tasks, both known to be mediated by different brain structures, including the hippocampus (Izquierdo et al., 1993).

2. Materials and methods

One hundred and twelve (112) male Wistar rats (age 2–3 months, weight 210–300 g) from our breeding colony were used in this experiment. Animals were housed in plastic cages, 4–5 to a cage, under a 12 h light/dark cycle and at a constant temperature of 24 ± 1 °C, with water and food ad libitum. All animals were anesthetized by a mixture of ketamine and xilazine (i.p., 75 and 10 mg/kg, respectively) and bilaterally implanted with a 27-gauge guide cannulae aimed at AP −4.2 mm (from bregma), LL ± 3.0 mm, DV 1.8 mm, just 1.0 mm above area CA1 of the dorsal hippocampus (according to Paxinos & Watson, 1998).

Once recovered from surgery (48 h), the animals were submitted to a training session in the step-down inhibitory avoidance (IA) or the open field habituation (OF) task; 24 h later they were tested for the corresponding task (Izquierdo et al., 1992). This task was carried out in an automatically operated, brightly illuminated box, in which the left extreme of the grid (42.0×25.0 cm grid of parallel 0.1 cm caliber stainless steel bars spaced 1.0 cm apart) was covered by a 7.0 cm wide, 5.0 cm high

formica-covered platform. Animals were placed on the platform and their latency to step-down placing their four paws on the grid was measured. In the training session, immediately upon stepping down, the animals received a 0.5 mA, 3.0 s scrambled footshock. In the test session no footshock was given, and a ceiling of 180 s was imposed on the step-down latency. At the time of infusion, right after training, 30-gauge cannulae were fitted into the guide cannulae. The tip of the infusion cannulae protruded 1.0 mm beyond that of the guide cannulae and was, therefore, aimed at the pyramidal cell layer of CA1 in the dorsal hippocampus (Fig. 1), with the 0.5 μl volume being administered at a 20 μl/h rate. The animals were divided into groups receiving bilateral infusions of 0.5 μl, either of AM251 (Tocris: 2, 20, and 200 μM solutions, resulting in concentrations of 0.55, 5.5, and 55.5 ng per side/hemistructure injected), or of its vehicle (phosphate buffered saline with 8% dimethylsulfoxide) administered immediately after training. The selected doses cover a range consistent with the final concentrations in ex vivo/cell culture studies (Wilson & Nicoll, 2001).

Open field habituation was studied using a 50 cm high, 60 × 40 cm plywood box with a frontal glass wall and a linoleum floor divided in 12 equal rectangles. Animals were left there for 3 min both in the training and the test session, and the number of rearings and crossings between sectors were counted each time. The difference in the number of rearings, or of crossings between rectangles, between the two sessions was considered a measure of retention of habituation to the open field: if the animals had habituated to the field during the first session, they should recognize it as familiar, and, in consequence, the number of rearings and crossings should be smaller in the second session (Rosat, Da-Silva, Zanatta, Medina, & Izquierdo, 1992). The number of crossings in the test session may also be used as a control for the possible motor and general performance effects of the drug administered 24 h before.

Statistical analysis of the behavioral data (latencies to step-down in IA and number of rearings and crossings in OF) was limited to the animals with correct cannula placements (Fig. 1)—97 out of 112 operated, as described in Izquierdo et al. (1992). Since all the studied variables, including the step-down latencies, passed a normality test (Kolmogorov–Smirnov test with Lilliefors' correction), differences among groups were evaluated by a one-way ANOVA, and the differences compared by Tukey's pairwise HSD post hoc test.

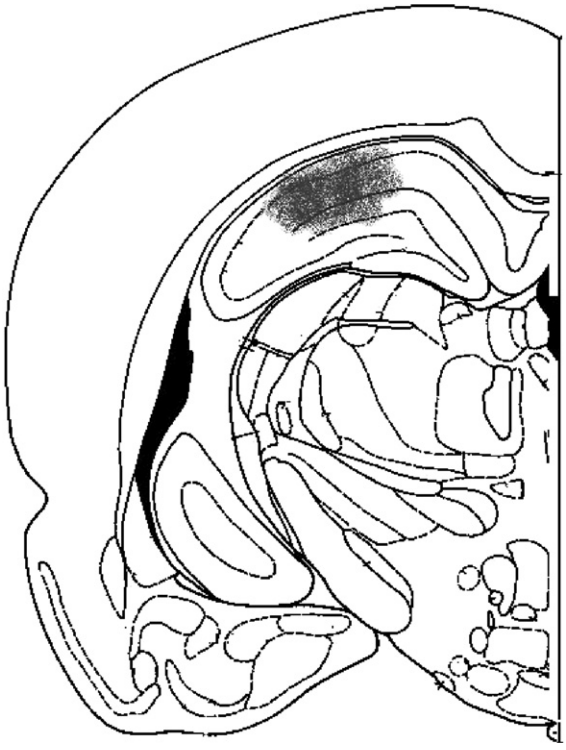

Fig. 1. Drawing representing AP plane −4.3 mm adapted from the atlas of Paxinos and Watson (1998) showing the extent of the area reached by our infusions in the rat dorsal hippocampus (stippled areas represent typical regions of accepted animals, as dyed by 2% methylene blue/0.5 μl saline infused through the same cannulae).

3. Results and discussion

Results are shown in Figs. 2 and 3A and B. In the inhibitory avoidance, comparisons among test latencies were possible since there was no statistically significant

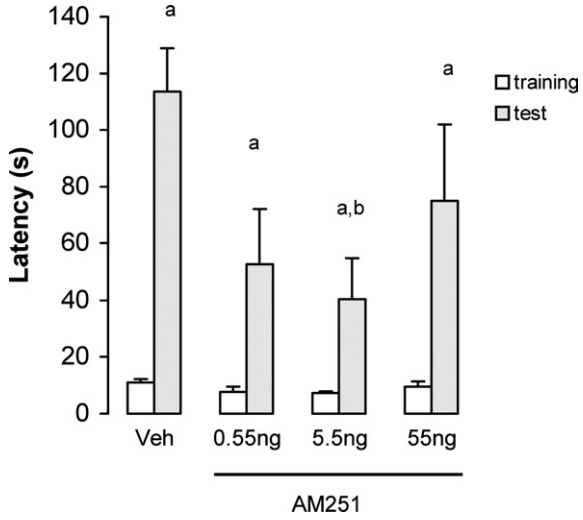

Fig. 2. Effect of AM251 in the open field habituation task. Data expressed as means ± SEM of the number of rearings and crossings. Data expressed as means ± SEM of the number of rearings and crossings. One-way ANOVA shows no statistically significant differences between the groups' means, either for the training session (rearings, $P = .300$, crossings, $P = .520$), or for the test session (rearings, $P = .629$, crossings, $P = .941$). (A) Each of the three experimental groups, respectively, vehicle, 0.55 and 5.5 ng/side treated groups, show a significant difference between training and test session rearings ($P < .001$ in all groups, Paired samples t test) and crossings ($P < .001$ in all groups, Paired samples t test).

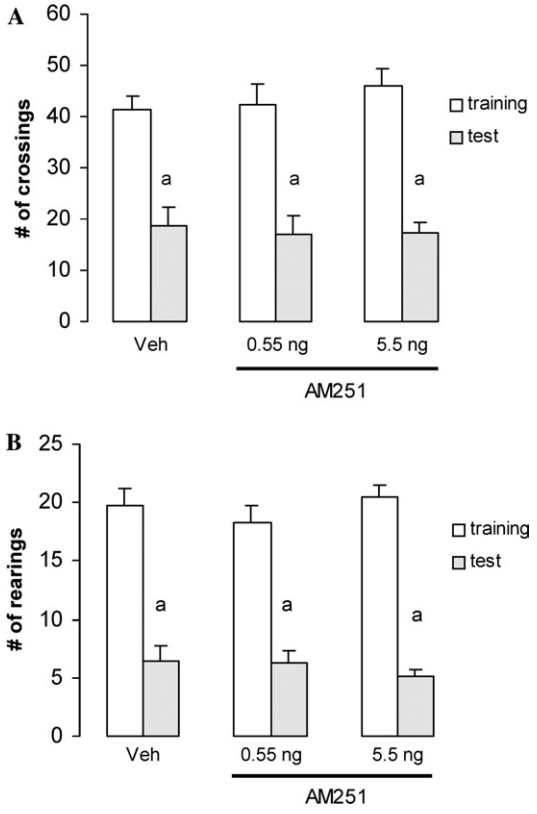

Fig. 3. Effect of AM251 in the step-down inhibitory avoidance task. Data expressed as means ± SEM of step-down latencies. One-way ANOVA shows no significant difference among training session latencies ($P = .155$). (A) Each of the four experimental groups have shown a significant difference between training and test session latencies ($P = .000$, .046, .039, and .034, Paired samples t test); (B) Only the 5.5 ng/side group of AM251 displayed a significant difference in the test session latency compared to the control group ($P = .020$, Tukey HSD test).

difference among the training session latencies (one-way ANOVA, $P = .155$); test session latencies, however, exhibited a significant difference ($P = .017$). A post hoc all pairwise multiple comparison procedure demonstrated that only the group treated with 5.5 ng/side of AM251 was significantly different from the control (vehicle-injected) group ($P = .020$, Tukey HSD test); groups treated with the other doses were not different from the control ($P > .050$). Each of the four experimental groups, respectively, vehicle, 0.55, 5.5, and 55.5 ng/side treated groups, presented a significant difference between training and test session latencies ($P = .000$, .046, .039, and .034, Paired samples t test), i.e., all groups learnt the task.

In the open field habituation task (Figs. 3A and B), the one-way ANOVA test showed no statistically significant differences between the groups' means, either for the training session (rearings, $P = .300$, crossings, $P = .520$), or for the test session (rearings, $P = .629$, crossings, $P = .941$). Each of the three experimental groups, respectively, vehicle, 0.55 and 5.5 ng/side treated groups, demonstrated significant differences between training and test session rearings ($P < .001$ in all groups, Paired samples t test) and crossings ($P < .001$ in all groups, Paired samples t test), i.e., all groups learnt the task.

Since there was no significant statistical differences among the mean values of the test session crossings, motor performance effects of both studied doses of AM251, including the IA effective one (5.5 ng/side), injected 24 h before (Fig. 3B), may be discarded.

Our results show that the antagonist, AM251, when administered into the rat hippocampus immediately after training, disrupts memory consolidation of the IA task (Fig. 2), but not the OF task (Figs. 3A and B). The effect seems to be purely mnemonic since: (a) the drug shows no motor performance effects in response to the drug (Fig. 3A), which could favor a false positive for the intermediate dose in the IA test session, and (b) AM251 was administered immediately after training, when acquisition had already finished and its effects could be only attributed to an interference with the consolidation process (McGaugh, 1966). In support of a specific effect of AM251 upon hippocampal CB1 receptors, it should be remembered that the amnestic effect took place with the lower, more selective dose (5.5 ng/side), not with the higher one (that may bind to non-specific targets into the hippocampus).

The different response observed for the two behavioral tasks requires explanation, particularly if we consider that the OF habituation task conforms better to the well-known role of the hippocampus in spatial-related behavioral situations (Izquierdo & Medina, 1995; Squire, 1992). However, there is no reason to suppose that every single modulatory system in a brain structure must conform to a "general pattern" of functioning, particularly a neuromodulatory system such as the endocannabinoid system. In particular, our results suggest that hippocampal endocannabinoids are not acting upon the *consolidation* of the OF habituation task. Conversely, the fact that IA is sensitive to AM251 administered immediately after training raises the possibility that this system requires some degree of aversiveness or alertness in order to be recruited. The release of endocanabinoids in response to a tone presentation, previously paired with a shock, has been demonstrated in the amygdala (Marsicano et al., 2002).

The amnestic effect of this CB1 antagonist appears to contrast with some reports in the literature, where cannabinoids are more frequently described to cause disruptive effects on memory and cognition (Ameri, 1999). Some of these effects were attributed to the high density of CB1 receptors in the hippocampus (Herkenham et al., 1991), but most of these studies investigated only systemic effects, usually i.p. (Davies et al., 2002; Mazzola et al., 2003; Wilson & Nicoll, 2002), and the observed effects cannot be attributed a priori to any specific brain target structure. An exception to this was a study by Lichtman et al. (1995) who reported memory deficits in the 8-arm radial maze following an *intrahippocampal*

injection of the *agonist*, CP55,940, but not of anandamide. This finding is important for comparison with ours, however, the scope of the investigation cited was not to study declarative memory, as we have done, but working memory, a different process (Bianchin, Mello e Souza, Medina, & Izquierdo, 1999). Egashira, Mishima, Iwasaki, and Fujiwara (2002) studying the injection of Δ^9-THC into the hippocampus, found an impairment of the spatial memory of this same behavioral task, a finding that contrasted with ours.

In contrast, SR141716A, a frequently used CB1 antagonist, is almost always administered along with other drugs, usually to check for the selectivity of agonist-induced deficits (see Ameri, 1999; Davies et al., 2002; Wilson & Nicoll, 2002). Injected alone, only when systemically, it was found to cause memory improvement in rats (Lichtman, 2000; Terranova et al., 1996; Wolff & Leander, 2003) or no effect at all (Da Silva & Takahashi, 2002; Davies et al., 2002). Marsicano et al. (2002), however, reported that systemic SR141716A disrupted extinction of aversive memories in mice.

We propose that increased levels of endogenous cannabinoids in the hippocampus, occurring immediately after training, contribute to *facilite* memory consolidation: the CB1 endogenous modulation acts to decrease the activity of local (basically GABAergic) inhibitory networks within the dorsal hippocampus, leading to some decisive disinhibition of the output of the (for instance) glutamatergic pyramidal neurons. CB1 antagonists, such as AM251, may disrupt this endogenous modulatory system leading to the observed amnestic effect, at least in the step-down inhibitory avoidance task. This is fully consistent with the observation that endocannabinoids might facilitate hippocampal long-term potentiation through the suggested retrograde inhibition of presynaptic GABA release (Carlson et al., 2002). In this respect, it is important to consider the possibility that AM251 could be acting as an inverse agonist, with its own metabolic effects, a possibility already demonstrated for SR141716A (Landsman, Burkey, Consroe, Roeske, & Yamamura, 1997): the similarity of actions for these drugs is suggested by the fact that: (a) the chemical structure of AM251 is almost identical to that of SR141716A (Gatley, Gifford, Volkow, Lan, & Makriyannis, 1996), and that (b) under certain circumstances both AM251 and SR 141716A inhibit G-protein activity (Savinainen, Saario, Niemi, Jarvinen, & Laitinen, 2003). This possibility, in principle, does not conflict with our proposal that endogenous cannabinoids in the hippocampus support memory consolidation by counterbalancing inhibitory actions within that structure, but adds to the consequences of activating CB1 receptors in the inverse direction, both suggesting the involvement of the endocannabinoid system in cognitive processes.

This anatomical/functional hypothesis may be complicated by the observation that many GABAergic synapses seem to be insensitive to cannabinoids (Wilson & Nicoll, 2002; Wilson, Kunos, & Nicoll, 2001), furthermore endogenous cannabinoids interfere with other known modulatory hippocampal systems, such as the cholinergic system (Christopoulos & Wilson, 2001), and even with calcium homeostasis via NMDA (Hampson et al., 1998) and/or VGCCs (Mackie et al., 1995; Wilson & Nicoll, 2002). Finally, AM251 may be itself binding to different targets, such as the putative "CB3" receptor, postulated to explain the persistence of the effect of SR141716 or the agonists anandamide and WIN55212-2 (but not Δ^9-THC) in CB1-knockout mice (Breivogel, Griffin, Di Marzo, & Martin, 2001), or the cannabinoid modulatory site in the NMDA-glutamatergic receptor (Hampson et al., 1998).

As related by Wilson and Nicoll (2002), it is premature to conclude that endocannabinoids promote learning, since studies with CB1-deficient, genetically modified mice report both impairment and facilitation of memory, as well as locomotor deficits. Investigations with the CB1/"CB3" antagonist, SR141716, have demonstrated no effect, or a facilitatory effect on memory with evidence once more of hyperlocomotive effects of the same drug (Breivogel et al., 2001). Considering our amnestic effect with the antagonist AM251 into the hippocampus we may suppose (despite the fact that this was not directly addressed here) that the key to the well-known amnestic (systemic) effect of cannabinoid CB1 agonists—including the effect observed with i.p. Δ^9-THC in the same aversive task here used (Miller, Drew, & Joyce, 1973; Mishima et al., 2001)—may not reside in the hippocampus, as usually suggested by different authors (see review in Davies et al., 2002), but, perhaps, in some sort of articulated action of different brain structures still to be identified.

References

Ameri, A. (1999). The effects of cannabinoids on the brain. *Progress in Neurobiology, 58*, 315–348.

Bianchin, M., Mello e Souza, T., Medina, J. H., & Izquierdo, I. (1999). The amygdala is involved in the modulation of long-term memory, but not in working or short-term memory. *Neurobiology of Learning and Memory, 71*, 127–131.

Breivogel, C. S., Griffin, G., Di Marzo, V., & Martin, B. R. (2001). Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. *Molecular Pharmacology, 60*, 155–163.

Carlson, G., Wang, Y., & Alger, B. E. (2002). Endocannabinoids facilitate the induction of LTP in the hippocampus. *Nature Neuroscience, 5*(8), 723–724.

Christopoulos, A., & Wilson, R. I. (2001). Interaction of anandamide with the M1 and M4 muscarinic acetylcholine receptors. *Brain Research, 915*, 70–78.

Da Silva, G. E., & Takahashi, R. N. (2002). SR 141716A prevents delta 9-tetrahydrocannabinol-induced spatial learning deficit in a Morris-type water maze in mice. *Progress in Neuropsychopharmacology and Biological Psychiatry, 26*(2).

Davies, S. N., Pertwee, R. G., & Riedel, G. (2002). Functions of cannabinoid receptors in the hippocampus. *Neuropharmacology, 42*, 993–1007.

Egashira, N., Mishima, K., Iwasaki, K., & Fujiwara, M. (2002). Intracerebral microinjections of delta 9-tetrahydrocannabinol: Search for the impairment of spatial memory in the eight-arm radial maze in rats. *Brain Research, 952*(2).

Gatley, S. J., Gifford, A. N., Volkow, N. D., Lan, R., & Makriyannis, A. (1996). ^{123}I-labeled AM251: A radioiodinated ligand which binds in vivo to mouse brain cannabinoid CB1 receptors. *European Journal of Pharmacology, 307*, 331–338.

Hampson, A. J., Bornheim, L. M., Scanziani, M., Yost, C. S., Gray, A. T., Hansen, B. M., et al. (1998). Dual effect of anandamide on NMDA receptors-mediated responses and neurotransmission. *Journal of Neurochemistry, 70*, 671–676.

Herkenham, M., Lynn, A. B., Johnson, M. R., Melvin, L. S., de Costa, B. R., & Rice, K. C. (1991). Characterization and localization of cannabinoid receptors in the rat brain: A quantitative in vitro autoradiographic study. *Journal of Neuroscience, 11*, 563–583.

Izquierdo, I., Da Cunha, C., Rosat, R., Jerusalinsky, D., Ferreira, M. B. C., & Medina, J. H. (1992). Neurotransmitter receptors involved in post-training memory processing by the amygdala, medial septum and hippocampus of the rat. *Behavioural and Neural Biology, 58*, 16–26.

Izquierdo, I., & Medina, J. H. (1995). Correlation between the pharmacology of long-term potentiation and the pharmacology of memory. *Neurobiology of Learning and Memory, 63*, 19–32.

Izquierdo, I., Medina, J. H., Bianchin, M., Walz, R., Zanatta, M. S., Da Silva, R. C., et al. (1993). Memory processing by the limbic system: Role of specific neurotransmitter systems. *Behavioural Brain Research, 58*, 91–98.

Katona, I., Sperlagh, B., Sik, A., Kafalvi, A., Vizi, E. S., Mackie, K., et al. (1999). Presynaptically located CB1 cannabinoid receptors regulate GABA release from axonal terminals of specific hippocampal interneurons. *Journal of Neuroscience, 19*, 4544–4558.

Kreitzer, A. C., & Regehr, W. G. (2001). Cerebellar depolarization-induced suppression of inhibition is mediated by endogenous cannabinoids. *Journal of Neuroscience, 21*(20), RC174.

Landsman, R. S., Burkey, T. H., Consroe, P., Roeske, W. R., & Yamamura, H. I. (1997). SR141716A is na inverse agonist at the human cannabinoid CB1 receptor. *European Journal of Pharmacology, 334*, R1–R2.

Lichtman, A. H. (2000). SR141716A enhances spatial memory as assessed in a radial-arm maze task in rats. *European Journal of Pharmacology, 404*(1–2), 175–179.

Lichtman, A. H., Dimen, K. R., & Martin, B. R. (1995). Systemic or intrahippocampal cannabinoid administration impairs spatial memory in rats. *Psychopharmacology, 119*, 282–290.

Mackie, K., Lai, Y., Westenbroek, R., & Mitchell, R. (1995). Cannabinoids activate an inwardly rectifying potassium conductance and inhibit Q-type calcium currents in AtT20 cells transfected with rat brain cannabinoid receptor. *Journal of Neuroscience, 15*, 6552–6561.

Marsicano, G., Wotjak, C. T., Azad, S. C., Bisogno, T., Rammes, G., Cascio, M. G., et al. (2002). The endogenous cannabinoid system controls extinction of aversive memory. *Nature, 418*, 530–534.

Mazzola, C., Micale, V., & Drago, F. (2003). Amnesia induced by B-amyloid fragments is counteracted by cannabinoid CB_1 receptor blockade. *European Journal of Pharmacology, 477*(3), 219–225.

McGaugh, J. L. (1966). Time-dependent processes in memory storage. *Science, 153*, 1351–1358.

Miller, L. L., Drew, W. G., & Joyce, P. (1973). Delta 9-THC: Effect on acquisition and retention of a one-trial passive avoidance response. *Behavioural Biology, 8*, 421–426.

Mishima, et al. (2001). Characteristics of learning and memory impairment induced by delta 9-tetrahydrocannabinol in rats. *Japanese Journal of Pharmacology, 87*, 297–308.

Paxinos, G., & Watson, C. (1998). *The rat brain in stereotaxic coordinates* (4th ed.). San Diego: Academic Press.

Pertwee, R. G., & Ross, R. A. (2002). Cannabinoid receptors and their ligands. *Prostaglandins, Leukotrienes, and Essential Fatty Acids, 66*(2–3), 101–121.

Rosat, R., Da-Silva, R. C., Zanatta, M. S., Medina, J. H., & Izquierdo, I. (1992). Memory consolidation of a habituation task: Role of *N*-methyl-D-aspartate, cholinergic muscarinic and GABA-A receptors in different brain regions. *Brazilian Journal of Medical and Biological Research, 25*(3).

Savinainen, J. R., Saario, S. M., Niemi, R., Jarvinen, T., & Laitinen, J. T. (2003). An optimized approach to study endocannabinoid signaling: Evidence against constitutive activity of rat brain adenosine A1 and cannabinoid CB1 receptors. *British Journal of Pharmacology, 140*(8).

Squire, L. R. (1992). Memory and the hippocampus: A synthesis from findings with rats, monkeys and humans. *Psychological Review, 99*, 195–231.

Terranova, J. P., Storme, J. J., Lafon, N., Perio, A., Rinaldi-Carmona, M., Le Fur, G., et al. (1996). Improvement of memory in rodents by the selective CB1 cannabinoid receptor antagonist, SR 141716. *Psychopharmacology (Berlin), 126*(2), 165–172.

Varvel, S. A., Hamm, R. J., Martin, B. R., & Lichtman, A. H. (2001). Differential effects of Δ^9-THC on spatial reference and working memory in mice. *Psychopharmacology, 157*, 142–150.

Wilson, R. I., & Nicoll, R. A. (2002). Endocannabinoid signaling in the brain. *Science, 296*, 678–682.

Wilson, R. I., & Nicoll, R. A. (2001). Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. *Nature, 410*, 558–592.

Wilson, R. I., Kunos, G., & Nicoll, R. A. (2001). Presynaptic specificity of endocannabinoid signaling in the hippocampus. *Neuron, 31*, 453–462.

Wolff, M. C., & Leander, J. D. (2003). SR141716A, a cannabinoid CB1 receptor antagonist, improves memory in a delayed radial maze task. *European Journal of Pharmacology, 477*(3), 213–217.