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Blockade of cannabinoid CB_1 receptors alters contextual learning and memory

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Abstract

The endocannabinoid system appears to have an important role in specific aspects of learning and memory, yet there has been no systematic study of the role of cannabinoid receptors in contextual fear conditioning. The present study examined the effects of cannabinoid CB_1 receptor blockade on the acquisition, consolidation, and expression of contextual fear using the selective cannabinoid CB_1 receptor antagonist AM251. AM251 produced a decrease in the expression of contextual fear when administered prior to training, testing, or both. This effect was observed when footshock was signaled by an auditory cue but not in an unsignaled shock version of the task. Moreover, blocking cannabinoid CB_1 receptors had no effect on consolidation of contextual memory regardless of the conditioning paradigm. These data indicate that inhibition of cannabinoid CB_1 receptors produces specific deficits in processing contextual information and that the effects of CB_1 antagonists on contextual learning may differ from effects on other types of learning.

Keywords: Fear learning; Memory; Rat; Associative learning; Endocannabinoid

1. Introduction

Compounds such as anandamide and 2-arachidonyl-glyercol act on cannabinoid-specific receptors in the brain (e.g., cannabinoid CB_1 receptor; Devane et al., 1992; Matsuda et al., 1990; Sugiura et al., 1995; Wilson and Nicoll, 2002). These endocannabinoids are rapidly synthesized in postsynaptic cells upon depolarization and bind to cannabinoid CB_1 receptors located on presynaptic terminals, altering the subsequent release of neurotransmitters such as gamma-aminobutyric acid (GABA; Katona et al., 2001; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001). The activation of cannabinoid CB_1 receptors thus represents a novel mechanism of fast retrograde synaptic transmission, yet the normal function of this system is just starting to be uncovered.

Cannabinoid CB_1 receptors are particularly concentrated in limbic structures such as the hippocampus and amygdala (Katona et al., 2001; Tsou et al., 1998), suggesting that endocannabinoids may contribute to learning and memory. Indeed, the results of recent studies indicate that endocannabinoids may have a modulatory role in hippocampal-dependent learning and affect short-term synaptic plasticity (de Oliveira Alvares et al., 2005; Kreitzer, 2005). Cannabinoid CB_1 receptor knockout mice or mice treated with a cannabinoid receptor antagonist are able to initially learn the location of a hidden platform in the Morris water maze but exhibit impaired performance when the platform position is subsequently changed (Varvel et al., 2005; Varvel and Lichtman, 2002). In addition, the cannabinoid CB_1 receptor antagonist AM251 has been shown to impair memory consolidation in an inhibitory avoidance paradigm (de Oliveira Alvares et al., 2005, 2006). Nonetheless, there has yet to be a systematic study of the involvement of the endocannabinoid system on the acquisition or expression of contextual fear. Contextual fear conditioning, like spatial learning, is largely dependent on the hippocampus (Anagnostaras et al., 2001; Corcoran and Maren, 2001; Holland and Bouton, 1999). If the endocannabinoid system has a fundamental role in regulating hippocampal-dependent learning and memory, disruption of cannabinoid CB_1 receptors would be

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expected to affect contextual fear conditioning as well. On the other hand, endocannabinoids may contribute differently to spatial and contextual learning especially since separable brain systems are thought to underlie these forms of learning (Burwell et al., 2004; Good and Honey, 1997).

The present study thus examined the effects of AM251 (1-(2,4-dichlorophenyl)-5-(4-iodophenyl)-4-methyl-*N*-1-piperidinyl-1*H*-pyrazole-3-carboxamide), a selective cannabinoid CB_1 receptor antagonist, on the acquisition and expression of conditioned fear to a training context. Previous studies indicate that the hippocampus is differentially involved in contextual fear learning depending on whether or not the unconditioned stimulus is signaled by a discrete cue (Phillips and LeDoux, 1994). When footshock immediately follows presentation of an auditory stimulus, for example, conditioning to the context is thought to occur in the "background" since the phasic auditory cue is the stimulus that principally becomes associated with the foot shock. In the absence of a discrete cue, conditioning to the context occurs in the "foreground" since contextual cues are the only stimuli available to be associated with the foot shock. It has previously been shown that hippocampal lesions disrupt only background contextual conditioning (Phillips and LeDoux, 1994). Thus it was expected that AM251 would have different effects on signaled versus unsignaled contextual fear conditioning.

2. Materials and methods

Three experiments were included in this study. Experiment 1 examined the effects of pre-training and/or pre-testing injections of AM251 on contextual fear conditioning when a tone preceded the delivery of footshock (i.e., signaled footshock version of the task). Experiment 2 was identical except that the tone did not precede the delivery of the footshock (i.e., unsignaled footshock task). In Experiments 3A and 3B, AM251 was injected immediately after training in either the signaled or unsignaled version of the task (Experiments 3A and 3B, respectively) to assess effects cannabinoid CB_1 receptor blockade on consolidation of contextual memory.

2.1. Subjects

One hundred-eight male Long–Evans rats (~350 g) obtained from Charles River Laboratories (Montreal, Canada) were used in the three experiments included in this study. All rats were maintained on a 12-h light–dark cycle with free access to food and water and in accordance with IACUC-approved protocols, AAALAC requirements, and the European Community guidelines for the use of experimental animals.

2.2. Drug preparation and administration

AM251, a selective cannabinoid CB_1 receptor antagonist (provided by Dr. Alexandros Makriyannis, Northeastern University), was dissolved in dimethylsulfoxide (DMSO) immediately prior to the training or testing session. Rats were treated (i.p.) with either vehicle (DMSO) or AM251 15 min prior to being placed in the conditioning chamber. A dose of 3 mg/kg (volume of 3 mg/ml) was chosen for this study based on previous findings (Chambers et al., 2006; Rodgers et al., 2005) in which AM251 was evaluated in a plus-maze paradigm and conditioned taste aversion. Since potential state dependent learning effects have not been examined in previous studies, a factorial design was used in Experiments 1 and 2. The various pre-training and pre-testing administrations of vehicle and AM251 used in Experiments 1 and 2 resulted in the treatment groups outlined in Table 1.

2.3. Apparatus

Behavioral training was conducted in an operant testing environment interfaced with a personal computer and controlled by MED-PC V.4 software (Med Associates, St. Albans, VT). Experiments were conducted in 24 × 30.5 × 29 cm operant test chambers with modular component aluminum panels in the front and back, Plexiglas side panels and top, and a floor constructed of 0.48 cm rods placed 1.6 cm apart. Scrambled alternating current was delivered through the grid floor by a constant current shock source. A speaker connected to a programmable audio input generator was located at the top right corner of the front panel and used to deliver the auditory cue (a 1500 Hz, 86 dB tone) in Experiments 1 and 3A. A partially shaded houselight (28 V, 100 mA) mounted centrally at the top of the front wall illuminated the chamber during training and testing. Each conditioning chamber was placed in a sound-attenuating cubicle (SAC). A video camera was mounted on the outside of the back wall of the SAC, with the lens protruding through a hole in the back wall, which provided full view of the rat in the entire chamber.

2.4. Behavioral procedures

2.4.1. Training

In each experiment, training took place as described previously (Bucci et al., 2000, 2002; Maren et al., 1997). Rats were placed in individual chambers on the training day and after 3 min received three training trials (64-s inter-trial interval, ITI). In Experiments 1 and 3A, the auditory stimulus (tone) was presented for 10-s followed immediately by delivery of a constant current shock (1.0 mA for 1.0 s) delivered through the floor grid. In Experiments 2 and 3B, the tone was not included

Table 1
Treatment groups in Experiments 1 and 2

Group	Training session	Test session (s)	*n* (Experiment 1)	*n* (Experiment 2)
Veh–Veh	Vehicle	Vehicle	8	11
Veh–AM	Vehicle	AM251 (3.0 mg/kg)	11	8
AM–Veh	AM251 (3.0 mg/kg)	Vehicle	10	8
AM–AM	AM251 (3.0 mg/kg)	AM251 (3.0 mg/kg)	11	8

Note that in Experiment 1, the same compound was injected before the context test session as well as the cue test session.

in the trials. In all experiments, rats were removed from the chambers and returned to the home cage 64 s after the final shock was delivered.

2.4.2. Testing

Forty-eight hours after the training session, rats were returned to the original training context for 8 min to assess fear conditioning to the training context. No shocks were delivered during this session. Forty-eight hours after the context test session, rats were placed in a new context (the same training chambers were used with card board covering the floor grid, opaque white paper covering the chamber walls, and a dish of a Vicks Vapo-Rub mixed with vinegar placed in the chamber). The original tone was presented 8 times for 10 s (64 s ITI) beginning 2 min after the rat was placed in the chamber. Again, no shocks were delivered during this session. Behavioral responses during the training and testing days were recorded on videotape for subsequent analysis.

All rats received the context test session first, followed by the cue test session since this has previously been shown to be the optimal method for obtaining the most independent assessment of both auditory and contextual fear conditioning in the same rats (Maren et al., 1997). Nevertheless, a small study was conducted to determine if the order of testing would impact the observed drug effects in this study. When the cue test session was conducted prior to the context test session, identical results were obtained with rats treated with AM251 (data not shown).

2.5. Behavioral observation

Freezing served as the index of conditioned fear and was operationally defined as total motor immobility except for breathing (Blanchard and Blanchard, 1969; Fanselow, 1980). On the training day, the incidence of freezing behavior was recorded during the 64-s periods following each trial (post-shock freezing). Freezing behavior was recorded every 8 s during a 64-s epoch. During the context test session, freezing was observed every 8 s during the 8-min observation period. For the cue test session, freezing was recorded every 2 s during each 10-s presentation of the tone. The frequency of freezing behavior was converted to a percentage of total observations. A single primary observer scored all of the behavioral data, while a second observer scored a subset of the data to assess objectivity. Both observers were blind to treatment condition and their observations were highly correlated ($r^2=0.8$; $P<0.01$).

2.6. Data analysis

Analyses of freezing behavior during training and cue testing was conducted using repeated measures analysis of variance (ANOVA) with Group as the between-subjects variable and Trial as the within-subjects variable. For the context test session, a one-way ANOVA was conducted using Group as the between-subjects variable. Significant main effects were followed up with appropriate pair-wise comparisons. An alpha level of 0.05 was adopted for all analyses.

3. Results

3.1. Experiment 1

One rat in the AM251–Veh group and two rats in the Veh–Veh group were excluded from the analysis because they showed no signs of conditioned fear during the training session due to technical difficulties with shock delivery in one of the conditioning chambers. The sample sizes in each group are noted in Table 1.

The level of post-shock freezing was comparable in all groups during the training session [main effect of Group, $F(3,36)=0.8$, $P>0.5$], indicating that treatment with AM251 prior to training did not affect acquisition of the freezing response. The mean levels of freezing over the course of training were 63.2±3.9% (Veh–Veh), 58.9±3% (Veh–AM), 60±4.4% (AM–Veh), and 55.7±2.7% (AM–AM). All rats exhibited greater freezing over the course of training as indicated by a main effect of Trial [$F(3,108)=191.6$, $P<0.0001$].

The primary data of interest were freezing during the context test session, which is illustrated in Fig. 1. A one-way ANOVA revealed a main effect of Group [$F(3,36)=3$, $P<0.04$] on freezing to the context. Post hoc pair-wise comparisons confirmed that each of the groups receiving AM251 exhibited less freezing behavior compared to the Veh–Veh control group (Ps<0.0001). There were no significant differences between the groups that received AM251 (Ps>0.2), indicating that the reduction in contextual fear was comparable in each of the drug-treated groups compared to controls. In contrast to the results of the context test session, each group treated with AM251 exhibited more freezing behavior than the Veh–Veh control group during the cue test session, consistent with a previous report (Marsicano et al., 2002) of impaired extinction of the cue-specific fear response following treatment with a CB_1 antagonist (main effect of Group [$F(3,36)=3$, $P<0.04$]). The mean levels of freezing during the tone test session were 74.2±12% (Veh–Veh), 97.2±1.3% (Veh–AM), 92.5±4.5% (AM–Veh), and 93.9±2.8% (AM–AM).

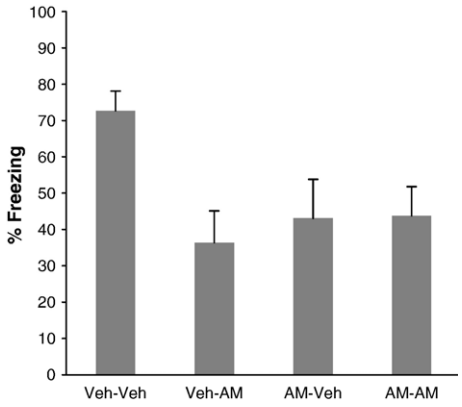

Fig. 1. Freezing behavior observed during the context test session in Experiment 1. Rats in each of the AM251-treated groups exhibited significantly less freezing to the training context compared to vehicle-treated control rats. Data are means ±S.E.M.

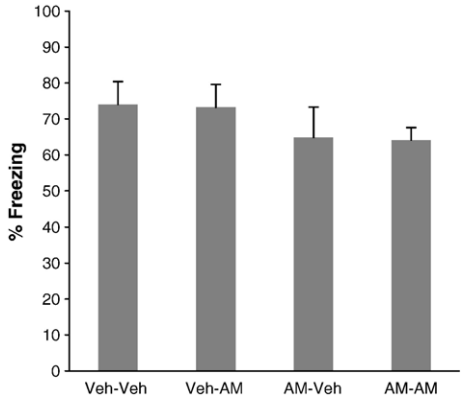

Fig. 2. Freezing behavior observed during the context test session in Experiment 2. There were no group differences in freezing during the context test session. Data are means±S.E.M.

3.2. Experiment 2

The sample sizes in each group included in Experiment 2 are noted in Table 1. The level of post-shock freezing in Experiment 2 was comparable in all treatment groups. There was no significant main effect of Group [$F(3,31)=2.5$, $P>0.1$] and mean levels of freezing across the training session were 57.4±2.9% (Veh–Veh), 59.4±3.8% (Veh–AM), 50±2.6% (AM–Veh), and 48±4.4% (AM–AM). All rats exhibited greater freezing over the course of training as indicated by a main effect of Trial [$F(3,93)=193.8$, $P<0.0001$]. During the context test session, freezing behavior was comparable in all treatment groups as shown in Fig. 2. A repeated measures ANOVA did not reveal a main effect of Group [$F(3,31)=0.7$, $P>0.6$].

3.3. Experiment 3

After the training session in Experiments 3A and 3B, rats were randomly divided into two groups and received either vehicle ($n=8$ in Expt. 3A, $n=7$ in Expt. 3B) or AM251 ($n=8$ in Expt. 3A, $n=7$ in Expt. 3B) immediately after being removed from the conditioning chambers. In Experiment 3A, there were no significant differences in post-shock freezing between the rats that were assigned to the two groups [main effect of Group, $F(1,14)=0.3$, $P>0.6$]; the mean percentages of freezing behavior observed during the training session were 59±2.9% (Veh) and 61.8±3.7% (AM). In Experiment 3B, mean percentages of freezing behavior were also comparable: 67±1.7 (Veh) and 63.4±2.1 (AM) [main effect of Group, $F(1,12)=1.8$, $P>0.2$]. Freezing during the subsequent context test session did not differ between vehicle-treated rats and AM251-treated rats in either experiment, as shown in Fig. 3; there was no significant main effect of Group in Experiment 3A (signaled footshock task; [$F(1,14)=0.3$, $P>0.6$]) or Experiment 3B (unsignaled fear conditioning task [$F(1,12)=0.2$, $P>0.7$]).

4. Discussion

The present study represents the first systematic investigation of the effects of cannabinoid CB_1 receptor blockade on contextual fear conditioning. Three experiments were designed to comprehensively assess effects on acquisition, expression, and consolidation of contextual learning, and to determine if any alterations in mnemonic function were due to state-dependent effects on learning. Rats treated with AM251 before the training session or test sessions (or both) in Experiment 1 successfully acquired a conditioned fear response as indicated by comparable levels of post-shock freezing compared to control rats. During the context test session, however, freezing was decreased in all AM251-treated rats compared to vehicle-treated rats, replicating our previous studies (Arenos, 2004; Arenos et al., 2004; Musty et al., 2005) and indicating that expression of contextual fear is impaired by blocking cannabinoid CB_1 receptors.

In contrast, freezing to the tone in Experiment 1 was increased in AM251-treated rats compared to controls during the tone test session. These data are consistent with a previous report in which blockade of cannabinoid CB_1 receptors increased freezing to an auditory cue during an extinction session (Marsicano et al., 2002). The consistent observation of elevated levels of freezing in response to a discrete stimulus (tone) following blockade of cannabinoid CB_1 receptors further supports the notion that cannabinoid CB_1 receptors may not have the same role in contextual and cue-specific fear conditioning. In addition, by blocking cannabinoid CB_1 receptors, treatment with AM251 may alter the processing of discrete cues and contextual cues and the neurochemical balance between structures that support fear conditioning (i.e., hippocampus and amygdala). This could result in the enhanced expression of fear to the auditory cue at the expense of processing the contextual stimuli.

The lack of effect of AM251 on acquisition of the freezing response during the training session in Experiment 1 is consistent with several other studies indicating that manipulation of cannabinoid CB_1 receptors does not impact initial acquisition in either spatial or fear learning paradigms (Marsicano et al., 2002; Varvel and Lichtman, 2002; Varvel et al., 2005). However, treatment with AM251 prior to training did affect the subsequent expression of contextual and cue-specific fear in Experiment 1 (i.e., group Veh–AM). In fact, administration of AM251 either

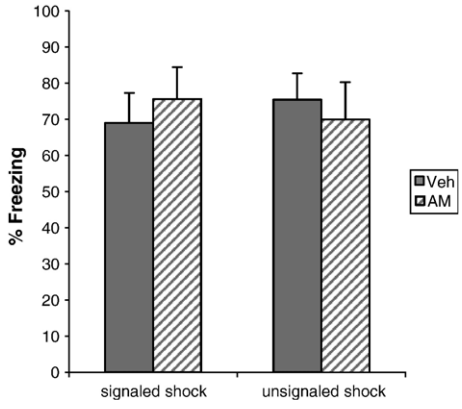

Fig. 3. Freezing behavior observed during the context test sessions in Experiment 3A (signaled shock) and Experiment 3B (unsignaled shock). Freezing during both test sessions was comparable in control rats and rats treated with AM251 immediately following training. Data are means±S.E.M.

before training and/or testing produced comparable changes in freezing during the test sessions. These data are consistent with the current notion that cannabinoid CB_1 receptors may play a modulatory role in learning (de Oliveira Alvares et al., 2005; Kreitzer, 2005).

The decrease in freezing to the training context produced by AM251 using the signaled fear conditioning task in Experiment 1 conflicts with the results of two recent reports (Finn et al., 2004; Suzuki et al., 2004). In the studies by Finn et al. (2004) and Suzuki et al. (2004), freezing to the training context was increased when the CB receptor antagonist SR141716 was administered. However, in those studies training did not involve presentation of a discrete, phasic conditioned stimulus (e.g., a tone). Similarly, when we used an unsignaled paradigm in Experiment 2, AM251 failed to decrease the expression of contextual fear as it did in Experiment 1. It is possible that an increase in freezing was not observed in AM251-treated rats in Experiment 2 because of a species difference (mice in Suzuki et al., 2004) or the use of a different conditioning procedure (fear conditioned analgesia in Finn et al., 2004). In addition, the contrasting results could be due to the use of different compounds to block cannabinoid receptors. In fact, there is evidence that both SR141716 and AM251 may be inverse agonists (Mukhopadhyay and Howlett, 2005; Shearman et al., 2003). It has also been suggested that the SR141716 compound used in the studies by Finn et al. (2004) and Suzuki et al. (2004) may affect putative CB_3 receptors in addition to cannabinoid CB_1 receptors (Breivogel et al., 2001).

In Experiment 2, the tone was not included in the training session and in that case the same treatment regimen and dose of AM251 failed to affect freezing during the context test session. The differing effects of AM251 on freezing during the context test sessions in Experiments 1 and 2 may reflect differential involvement of the hippocampus during acquisition in the two versions of the tasks. It is thought that processing contextual information may rely more on the hippocampus when context conditioning is secondary to conditioning to a discrete stimulus (a tone) that is presented immediately before footshock is delivered (Phillips and LeDoux, 1994). In contrast, the hippocampus may not be as critically involved in processing contextual information when the tone is absent and contextual cues are the only stimuli available to be associated with the foot shock. Indeed, it has been shown that damage to the hippocampus affects only background contextual conditioning (Phillips and LeDoux, 1994). The present data thus indicate that the involvement of cannabinoid CB_1 receptors in processing contextual information depends on task parameters, likely reflecting differential involvement of the hippocampus.

In the third experiment of the present study, administration of 3 mg/kg of AM251 immediately after training did not affect the consolidation of contextual fear memory. In contrast, it has recently been reported that AM251 impaired memory consolidation in an inhibitory avoidance paradigm (de Oliveira Alvares et al., 2005, 2006). In those studies, AM251 delivered after training reduced the latency to cross into the side of the chamber paired with footshock. It is difficult to compare these findings, however, since AM251 was administered systemically in the present study and directly into the hippocampus in the studies by de Oliveira Alvares et al. (2005, 2006), necessitating the use of different doses of AM251. Perhaps an effect on consolidation would have been observed in the present study if a higher dose of AM251 was used. In the context of the present study, however, it was important to use the same dose in each experiment to directly compare effects on contextual fear conditioning across preparations and treatment regimens to determine if a comparable blockade of cannabinoid CB_1 receptors uniformly affected multiple forms and stages of fear learning.

The results of the present experiments do not appear to be due simply to performance deficits. For example, it could have been that AM251 simply impaired the ability to perform the freezing response, resulting in lower levels of contextual freezing in groups treated with AM251 prior to the test sessions. This is unlikely however, since the same group of rats that exhibited decreased freezing during the context test session also exhibited elevated freezing during the tone test session. Cannabinergic compounds often alter nociception (Malan et al., 2001) which could have affected freezing following delivery of the footshock. Although this is manifest primarily as anti-nociception following administration of agonists, it is possible that AM251 could have hypernociceptive properties, resulting in increases in freezing behavior. This does not appear to have been the case since rats treated with AM251 prior to training exhibited levels of post-shock freezing that were equivalent to controls. In addition, the changes in freezing observed in the vehicle–AM251 or AM251–vehicle groups in Experiment 1 do not appear to be due merely to state-dependent effects produced by administering different compounds prior to training versus testing sessions since freezing in these groups was comparable to rats treated with AM251 both before training and before testing (AM251–AM251). Recent studies indicate that AM251 does not affect locomotor activity levels (de Oliveira Alvares et al., 2005), and so drug-induced changes in activity cannot account for the present findings. Thus it is unlikely that the results obtained in this study simply reflect a performance deficit or a state-dependent effect. Finally, an alternative explanation for the different effects of AM251 on cue-specific and contextual fear is that treatment with AM251 produced a general impairment in the ability to process contexts. However, further analysis of the cue test session data in Experiment 1 refutes that possibility since both vehicle-treated rats and AM251-treated rats exhibited little or no freezing before the first tone was presented in a new context, in contrast to the level of freezing observed when they were placed back in the original training context (data not shown).

The primary focus of this study was to examine the involvement of cannabinoid CB_1 receptors in contextual fear conditioning, a type of learning that had not been systematically examined with respect to endocannabinoid involvement. Assessing the role of cannabinoid receptors in processing contextual information is important to further establish the involvement of the endocannabinoid system in learning and memory as well as to provide additional insight into the

neurobiological mechanisms of contextual fear conditioning. Indeed, contextual fear conditioning is another well-studied form of learning and one that also has direct implications for substance abuse relapse (e.g., Bouton, 2002; Lopez-Moreno et al., 2004) as well as the development of anxiety disorders (Bouton et al., 2001). The present results provide evidence that cannabinoid CB_1 receptors may be differentially involved in mediating contextual and auditory fear conditioning and suggest that cannabinoid CB_1 receptor blockade may disrupt the processing of contextual information in situations involving both discrete and contextual stimuli.

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References

Anagnostaras, S.G., Gale, G.D., Fanselow, M.S., 2001. Hippocampus and contextual fear conditioning: recent controversies and advances. Hippocampus 11, 8–17.

Arenos, J.D., 2004. The role of the endocannabinoid system in fear learning. University of Vermont Undergraduate Honors Thesis.

Arenos, J.D., Musty, R.E., Bucci, D.J., 2004. Antagonism of the Cannabinoid-1 (CB_1) Receptor has Differential Effects on Cued and Contextual Fear Conditioning. Program No. 1006.6. 2004 Abstract Viewer/Itinerary Planner. Society for Neuroscience, Washington, DC.

Blanchard, R.J., Blanchard, D.C., 1969. Crouching as an index of conditioned fear. J. Comp. Physiol. Psychol. 67, 370–375.

Bouton, M.E., 2002. Context, ambiguity, and unlearning: sources of relapse after behavioral extinction. Biol. Psychiatry 52, 976–986.

Bouton, M.E., Barlow, D.H., Mineka, S., 2001. A modern learning theory perspective on the etiology of panic disorder. Psychol. Rev. 108, 4–32.

Breivogel, C.S., Griffin, G., Di Marzo, V., Martin, B.R., 2001. Evidence for a new G protein-coupled cannabinoid receptor in mouse brain. Mol. Pharmacol. 60, 155–163.

Bucci, D.J., Phillips, R.G., Burwell, R.D., 2000. Contributions of postrhinal and perirhinal cortex to contextual information processing. Behav. Neurosci. 114, 882–894.

Bucci, D.J., Saddoris, M.P., Burwell, R.B., 2002. Contextual fear discrimination is impaired by damage to the postrhinal or perirhinal cortex. Behav. Neurosci. 116, 479–488.

Burwell, R.B., Saddoris, M.P., Bucci, D.J., Wiig, K.J., 2004. Corticohippocampal contributions to spatial and contextual learning. J. Neurosci. 24, 3826–3836.

Chambers, A.P., Koopmans, H.S., Pittmas, Q.J., Sharkey, K.A., 2006. AM251 produces sustained reductions in food intake and body weight that are resistant to tolerance and conditioned taste aversion. Br. J. Pharmacol. 147, 109–116.

Corcoran, K.A., Maren, S., 2001. Hippocampal inactivation disrupts contextual retrieval of fear memory after extinction. J. Neurosci. 21, 1720–1726.

de Oliveira Alvares, L., de Oliveira, L.F., Camboim, C., Diehl, F., Genro, B.P., Lanziotti, V.B., Quillfeldt, J.A., 2005. Amnestic effect of intrahippocampal AM251, a CB_1-selective blocker, in the inhibitory avoidance, but not in the open field habituation task, in rats. Neurobiol. Learn. Mem. 83, 119–124.

de Oliveira Alvares, L., Genro, B.P., Van Breda, R.V., Pedroso, M.F., Da Costa, J.C., Quillfeldt, J.A., 2006. AM251, a selective antagonist of the CB1 receptor, inhibits the induction of long-term potentiation and induces retrograde amnesia. Brain Res. (Electronic publication ahead of print).

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. Science 258, 1946–1949.

Fanselow, M.S., 1980. Conditional and unconditional components of post-shock freezing. Pavlov J. Biol. Sci. 15, 177–182.

Finn, D.P., Beckett, S.R.G., Richardson, D., Kendall, D.A., Marsden, C.A., Chapman, V., 2004. Evidence for differential modulation of conditioned aversion and fear-conditioned analgesia by CB1 receptors. Eur. J. Neurosci. 20, 848–852.

Good, M., Honey, R.C., 1997. Dissociable effects of selective lesions to hippocampal subsystems on exploratory behavior, contextual learning, and spatial learning. Behav. Neurosci. 111, 487–493.

Holland, P.C., Bouton, M.E., 1999. Hippocampus and context in classical conditioning. Curr. Opin. Neurobiol. 9, 195–202.

Katona, I., Rancz, E.A., Acsady, L., Ledent, C., Mackie, K., Hájos, N., Freund, T. F., 2001. Distribution of CB1 cannabinoid receptors in the amygdala and their role in the control of GABAergic transmission. J. Neurosci. 21, 9506–9518.

Kreitzer, A.C., 2005. Neurotransmission: emerging roles of endocannabinoids. Curr. Biol. 15, R549–R551.

Lopez-Moreno, J.A., Gonzalez-Cuevas, G., de Fonseca, F.R., Navarro, M., 2004. Long-lasting increase of alcohol relapse by the cannabinoid receptor agonist WIN 55,212-2 during alcohol deprivation. J. Neurosci. 24, 8245–8252.

Malan Jr., T.P., Ibrahim, M.M., Deng, H., Liu, Q., Mata, H.P., Vanderah, T., Porreca, F., Makriyannis, A., 2001. CB2 cannabinoid receptor-mediated peripheral antinociception. Pain 93, 239–245.

Maren, S., Aharonov, G., Fanselow, M.S., 1997. Neurotoxic lesions of the dorsal hippocampus and pavlovian fear conditioning in rats. Behav. Brain Res. 88, 261–274.

Marsicano, G., Wotjak, C.T., Azad, S.C., Bisogno, T., Rammes, G., Cascio, M. G., Hermann, H., Tang, J., Hofmann, C., Zieglgansberger, W., Di Marzo, V., Lutz, B., 2002. The endogenous cannabinoid system controls extinction of aversive memories. Nature 418, 530–534.

Matsuda, L.A., Lolait, S.J., Brownstein, M.J., Young, A.C., Bonner, T.I., 1990. Structure of a cannabinoid receptor and functional expression of the cloned cDNA. Nature 346, 561–564.

Mukhopadhyay, S., Howlett, A.C., 2005. Chemically distinct ligands promote differential CB_1 cannabinoid receptor–Gi protein interactions. Mol. Pharmacol. 67, 2016–2024.

Musty, R.E., Arenos, J.D., Bucci, D.J., 2005. Blockade of the CB_1 Receptor with AM251 Alters the Processing of Contextual Information. Program No. 654.1. 2005 Abstract Viewer/Itinerary Planner. Society for Neuroscience, Washington, DC.

Ohno-Shosaku, T., Maejima, T., Kano, M., 2001. Endogenous cannabinoids mediate retrograde signals from depolarized postsynaptic neurons to presynaptic terminals. Neuron 29, 729–738.

Phillips, R.G., LeDoux, J.E., 1994. Lesions of the dorsal hippocampal formation interfere with background but not foreground contextual fear conditioning. Learn. Memory 1, 34–44.

Rodgers, R.J., Evans, P.M., Murphy, A., 2005. Anxiogenic profile of AM-251, a selective cannabinoid CB1 receptor antagonist, in plus-maze-naive and plus-maze-experienced mice. Behav. Pharmacol. 16, 405–413.

Shearman, L.P., Rosko, K.M., Fleischer, R., Wang, J., Xu, S., Tong, X.S., Rocha, B.A., 2003. Antidepressant-like and anorectic effects of the cannabinoid CB1 receptor inverse agonist AM251 in mice. Behav. Pharmacol. 14, 573–582.

Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku, K., 1995. 2-Arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. Biochem. Biophys. Res. Commun. 215, 89–97.

Suzuki, A., Josselyn, S.A., Frankland, P.W., Masushige, S., Silva, A.J., Kida, S., 2004. Memory reconsolidation and extinction have distinct temporal and biochemical signatures. J. Neurosci. 24, 4787–4795.

Tsou, K., Brown, S., Sanudo-Pena, M.C., Mackie, K., Walker, J.M., 1998. Immunohistochemical distribution of cannabinoid CB1 receptors in the rat central nervous system. Neuroscience 83, 393–411.

Varvel, S.A., Lichtman, A.H., 2002. Evaluation of CB1 receptor knockout mice in the Morris water maze. J. Pharmacol. Exp. Ther. 301, 915–924.

Varvel, S.A., Anum, E.A., Lichtman, A.H., 2005. Disruption of CB1 receptor signaling impairs extinction of spatial memory in mice. Psychopharmacology 179, 863–872.

Wilson, R.I., Nicoll, R.A., 2001. Endogenous cannabinoids mediate retrograde signaling at hippocampal synapses. Nature 410, 588–592.

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