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Behavioral Profile of the Novel Cannabinoid Agonist AM4054

Evan McClure
University of Connecticut

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The Honors Scholar Thesis of
Evan D. McClure

Department of Psychology, University of Connecticut, Storrs Mansfield, CT 06268, USA

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I. Abstract

Although cannabinoid drugs have been used for thousands of years both recreationally and therapeutically, little has been known about their mechanisms of action until recently. Since the discovery of the endogenous cannabinoid CB_1 receptor in 1988, the behavioral profile of cannabinoid receptor ligands has been much more thoroughly defined. Cannabinoid CB_1 agonists have been shown to produce a variety of behavioral effects including suppression of locomotion, catalepsy, hypothermia, and analgesia. Research has also demonstrated that these behavioral effects can be inhibited by CB_1 receptor antagonists including SR 141716 and AM 251. Although behavioral indicators of anxiety including thigmotaxis have been observed in several different paradigms, there is inconclusive and often times contradictory evidence to define the role of anxiety in CB_1 receptor activation. The present study addressed the behavioral profile of AM 4054, a novel full agonist at the CB_1 receptor, as well as the ability of the CB_1 antagonist AM 251 to reverse these effects. To further identify and expand research on the suppression of locomotion and induction of thigmotaxis with the administration of a CB_1 agonist, experiment 1 was conducted in the open field. In this experiment, each rat (n=40) was randomly assigned one of the five treatments: vehicle, 0.16, 0.32, 0.64, or 1.25 mg/kg AM 4054. After a 30 minute pre-treatment, each subject was tested in the open field for 18 minutes. Results indicated that AM 4054 produced a dose-related suppression of locomotion as well as the subtle presence of thigmotaxis in two out of four doses. In experiment 2, subjects (n=40) received either vehicle or 2.0 or 4.0 mg/kg AM 251 60 minutes prior to testing. After 30 minutes, the subjects were given either a 0.3 mg/kg dose of AM 4054 or vehicle. After a total pretreatment duration of 60 minutes, the animals were tested on a battery of tasks including an 18 minute session in locomotor boxes. Experiment 2 was a continuation of a previous study conducted in

the same lab, which confirmed the effects of AM 4054 on this tetrad of tasks as being consistent with other cannabinoid agonists. In this experiment the effects of AM 4054 were reversed by the administration of the CB_1 antagonist AM 251. Past studies have shown that AM 4054 is a highly potent drug with behavioral actions similar to other cannabinoid CB_1 agonists. Furthermore, AM 4054 can be a useful drug in future studies, and has potential therapeutic value for the treatment of various conditions.

II. Introduction

Throughout history, the marijuana plant (*Cannabis sativa*) has been used both medicinally and recreationally in many different cultures around the world. Despite the often negative view of this drug as one of the most commonly abused substances in the United States, there has been heated political debate for and against the medicinal use of the Cannabis plant for the treatment of various health conditions. Cannabis has been used anecdotally for more than five thousand years to treat a variety of conditions including hysteria, delirium, insomnia, nausea, anorexia, glaucoma, and pain (Burns and Ineck, 2006). A relatively safe drug with virtually no potential for overdose, the marijuana plant can be an important treatment.

During the beginning of the 20th century, cannabis was commonly prescribed in the US and Europe to treat pain, pertussis, asthma, gastrointestinal disorders, Grave's disease, diarrhea, malaria, and as a sedative (Grotenhermen and Russo, 2002). In an attempt to suppress the abuse of the cannabis plant, the Controlled Substances Act of 1970 defined marijuana as a Schedule I drug, and classified cannabis as drug with no legitimate medicinal value (Burns and Ineck, 2006). As countless new studies continue to suggest otherwise, legislation has been passed in several states to allow the prescription of marijuana for select patients. Many practitioners are reluctant to recommend its use however, due to intense scrutiny and disapproval by the federal government (Burns and Ineck, 2006). Despite the legal and political controversy behind cannabis drugs, the history of marijuana has been well documented and noted within the medical field, and cannabis is still used medicinally all over the world for the treatment of several different medical conditions.

Research has shown that marijuana contains several active and inactive cannabinoids, the primary active ingredient being delta-9-tetrahydracannabinol (THC). Cannabidiol, another

major cannabinoid component of marijuana thought to have anti-oxidant and anti-inflammatory properties without the psychoactive effects of THC, was identified in 1934 (McPartland and Russo, 2001). With the discovery of THC in 1964, research on the cannabis plant moved to a much higher level (Mechoulam and Gaoni, 1967). For several years however, there were misconceptions about the pharmacologic properties of THC and Marijuana, and it was long thought that they disrupted cellular membranes, rather than being associated with a specific receptor (Munro et al., 1993).

For almost thirty years, the primary mechanism of action for THC was not known, and it was not until recently that endogenous cannabinoid receptors were identified in the central and peripheral nervous systems. With the discovery of these endocannabinoid receptors, new Cannabinoid agonists have been developed with high affinities for these receptors. Although many new drugs have been developed, few have been studied for their effects on human subjects. Ajulemic acid (CT-3) has been tested on humans, and has been found to produce fewer psychoactive effects while maintaining analgesic potential (Karst et al., 2003; Burstein et al., 2004). CT-3 is an analog of a THC metabolite, and some studies have shown that it may have anti-inflammatory effects (Zurier et al., 1998). In recent years there has been an effort to develop cannabinoid drugs that produce fewer psychoactive effects but still maintain their medical value.

Since the discovery of the cannabinoid CB_1 receptor in 1988 and the subsequent discovery of the cannabinoid CB_2 receptor in 1993, there has been a new focus of research conducted on the endocannabinoid system (Beardsley 2005). In 1992, the endogenous cannabinoid receptor ligand arachidonoylethanolamide (anandamide) was found to occur naturally in the brain (Devane et al., 1992). With the identification of endogenous agonists,

researchers have studied the biological role of endocannabinoid systems with specific emphasis on appetite, eating behavior, and body weight regulation (Kirkham 2005).

CB_1 receptor activation has been a major area of interest while brain expression of CB_2 receptors has been much less emphasized in research over the years (Gong et al., 2005). This is due mostly to the fact that CB_2 receptor activation has traditionally been associated with immune tissues in the peripheral nervous system. More recent literature on the other hand shows the expression of CB_2 receptor messenger RNA and protein localization on brainstem neurons as well as the presence of functional CB_2 receptors in the brainstem (Sickle et al., 2005). These results are in contrast to numerous previous studies that failed to detect the presence of CB_2 receptors in the brain (Gong et al., 2005). CB_1 receptors on the other hand, do occur in the periphery, but are more commonly associated with the tissues of the central nervous system. Several studies have been conducted over the years to localize CB_1 receptors to various parts of the brain, and few have been successful in doing so (Fusco et al., 2004). Immunohistochemical studies with light and confocal microscopy however, have been helpful in identifying various regions in the brain with dense CB_1 expression (Fusco et al. 2004). Research has indicated that endocannabinoid CB_1 receptors are found in CNS areas such as the basal ganglia, cerebellum, hippocampus, hypothalamus, nucleus accumbens, amygdala, cerebral cortex, brain stem, and spinal cord (Ferund et al., 2003).

Early cannabinoid agonists, created by pharmaceutical groups to have similar binding properties to delta-9-THC, built a foundation for early cannabinoid research. Noted for their high affinities for endogenous CB_1 receptors, several compounds have been created including CP 55,940 (Johnson and Melvin, 1986), WIN 55,212-2 (Ward et al., 1989), and AM 411 (McLaughlin et al., 2005). Within the past decade, researchers have developed new cannabinoid

agonists with variable affinities for both CB_1 and CB_2 receptors. Greatly varying in potency, these drugs bind to the endocannabinoid receptors and mimic the actions of endogenous cannabinoids. The development of CB_1 agonists provides critical research tools for studying cannabinoid receptor function as well as any therapeutic benefits these compounds may have for use in the medical field (McLaughlin et al., 2005).

Over the past several years, research on cannabinoid receptors has generated interest in the pharmaceutical industry. With the development of cannabinoid agonists, researchers have been able to study the behavioral effects of cannabinoid receptor activation and the possible medicinal benefits of cannabinoid drugs. In recent years, cannabinoid antagonists have been a major focus of research. With the identification of the first CB_1 selective antagonist SR141716 in 1994, a significant body of research has investigated the possible use of cannabinoid antagonists (Beardsley 2005). Cannabinoid receptors in the CNS have been implicated in the control of appetite, cognition, mood and drug dependence; recent findings support the hypothesis that CB_1 receptor antagonism might be associated with antidepressant and anti-stress effects (Witkin et al., 2005). In addition, studies have been conducted to evaluate the use of CB_1 antagonists as potential appetite suppressants.

CB_1 receptor antagonists are believed to have therapeutic potential as appetite suppressants because they affect food intake at doses that do not produce side effects such as ataxia or decreased locomotion (McLaughlin et al., 2005). However, the specific mechanisms underlying food intake reduction produced by CB_1 antagonists are not well understood (McLaughlin et al., 2005). Still, the question of whether or not increases in nausea are an important part of appetite suppression needs to be considered. A recent study concluded that the CB_1 antagonist AM 251 may reduce food intake by inducing nausea or malaise, but not because

of motor slowing or incoordination related to feeding (McLaughlin et al., 2005). The prospect of CB_1 antagonists and their use as appetite suppressants in the human population has generated much interest in the pharmaceutical industry. Before these drugs may be used for weight loss in humans, more research must be done.

Research on cannabinoid CB_1 antagonists and their role in the treatment of mood disorders and substance abuse is greatly expanding. In a recent study by Witkin et al. (2005), CB_1 receptor antagonists were noted for their promising effects in clinical studies, where they were found to treat comorbid symptoms of depression such as cognitive deficiencies, weight gain, impulsivity, and dependence disorders.

In the study of cannabinoid systems and their role in modulating anxiety, it is important to highlight the highly inconsistent, often contradictory effects of cannabinoid receptor manipulations on anxiety related behaviors (Rodgers et al., 2005). Despite years of research, the mechanisms behind cannabinoid modulation of anxiety remain poorly understood (Genn et al., 2004). Several studies have proposed that cannabinoid agonists are anxiolytic (Haller et al., 2002), while others have shown the anxiogenic effects of cannabinoid drugs (Arevalo et al., 2001; Genn et al., 2004).

The analgesic properties of CB_1 agonists present a possible pain relieving alternative for opioid drug use in the medical field. Opioid drugs including morphine have severe dependency properties that pose serious problems in the treatment of chronic pain. Chronic pain often requires a polypharmaceutical approach to management, and cannabinoids are a potential addition to the arsenal of treatment options (Burns and Ineck, 2006). In addition, Cannabinoids provide a potential approach to pain management with a novel therapeutic target and mechanism,

and are a particularly attractive prospect because they have a favorable safety profile (Burns and Ineck, 2006).

In contradiction to the belief that cannabinoid drugs are neurodegenerative, these drugs have actually been found to protect against neurotoxicity in several different paradigms (Zhuang et al., 2005). In particular, a recent study demonstrated that cultured rat hippocampal neurons were protected from excitotoxic degeneration by pretreatment with CB_1 agonists such as THC and WIN 55,212-2 (Zhuang et al., 2005). A major point of interest in this study was that the compounds were effective in preventing cell death even if administered prior to the neurotoxin exposure (Zhuang et al., 2005). In addition to their neuroprotective properties, cannabinoid agonists have been shown to be useful in the treatment of several diseases including Tourette's Syndrome (Muller-Vahl et al., 2002), multiple sclerosis (Baker et al., 2001), and Alzheimer's disease (Volicer et al., 1997).

Cannabinoid drugs and their role in cancer treatment has also been a growing research topic. In human breast cancer patients, it has been found that the endocannabinoid system with specific emphasis on anandamide, regulates cell proliferation in breast cancer cells (Grimaldi et al., 2005). Cancer chemo therapy often produces nausea and decreased appetite which may lead to malnourishment. As a possible solution, appetite studies have shown that CB_1 receptor agonists including delta-9-THC increase feeding in rats and humans (McLaughlin et al., 2003). Many properties of CB_1 agonists make them useful drugs in treating a variety of health conditions and disorders.

In the past, cannabinoid CB_1 receptor agonists have been shown to produce several behavioral actions including locomotor suppression, catalepsy, memory and attentional impairments, reduced reactivity to painful stimuli (analgesia), ataxia, and hyperphagia (Childers

and Breivogel, 1998). Numerous other papers have highlighted the suppression of operant responding in rats after the administration of a CB_1 agonist. In a study by Carriero et al. (1997), four cannabinoid agonists suppressed operant lever pressing at moderate to high doses.

In the 1991 paper by Martin et al., a clear behavioral profile for cannabinoid agonists was developed. According to this study, suppression of spontaneous locomotion, induction of catalepsy, hypothermia, and analgesia, comprises a tetrad of behaviors characteristic of CB_1 agonists (Martin et al., 1991). In a more recent paper, Wiley and Martin stressed the importance of classifying cannabinoids based on this tetrad of tasks and showing the reversal of these effects with the use of a cannabinoid antagonist (2003). In addition, a necessary component for the classification of a CB_1 agonist is that it displays similar potency on all four tests in the tetrad (Wiley and Martin, 2003).

The present study was conducted to show the behavioral profile of AM 4054, a novel agonist at the CB_1 receptor, and the results of its co-administration with AM 251. Because CB_1 agonists are often found to produce anxiogenic effects (Arevalo et al., 2001; Genn et al., 2004), locomotor suppression in the open field may reflect effects related to anxiety. Thigmotaxis, which refers to an animal's tendency to stay close to the outside of the open field when experiencing anxiety, was an important component of the study. Experiment 1 addressed the question of whether or not AM 4054 suppressed locomotion in the open field, and if thigmotaxis occurred in moderate to high doses of the drug. Experiment 2 studied the ability of the CB_1 antagonist AM 251 to reverse suppression of locomotion, catalepsy, hypothermia, and analgesia induced by the CB_1 agonist AM 4054. This experiment was an extension of past research that confirmed AM 4054 as a potent cannabinoid that had an effect on the tetrad of tasks.

III. Materials and Methods

Subjects

The present experiments were conducted using adult male Sprague-Dawley rats (Harlan Sprague Dawley, Indianapolis, IN) with no prior drug experience; (total n = 80). These rats each weighed 300 – 450 grams on the day of testing. All rats were housed in a colony room with a 12 hour light-dark cycle and kept at a temperature of approximately 22-25°C. Throughout the course of these experiments, animals received *ad libitum* access to water and standard laboratory chow. Animal protocols were approved by the institutional animal care and use committee, and all experimental methods were in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996).

Drugs

AM 4054 and AM 251 were synthesized in the laboratory of Alex Makriyannis, in the School of Pharmacy at Northeastern University. All injections were administered i.p. Vehicle for these compounds consisted of dimethyl-sulfoxide (DMSO), Tween-80 (both from Sigma, St. Louis, MO), and 0.9% saline in a 1:2:7 ratio. For experiment 1, AM 4054 was administered in a dose range of 0.16-1.25 mg/kg i.p. In experiment 2, AM4054 was administered at a single dose of 0.3 mg/kg i.p. To reverse the effects of AM 4054 in experiment 2, AM 251 was administered in a dose of either 2.0 or 4.0 mg/kg.   These doses were determined by extensive pilot studies.

Behavioral Procedures

Assessment of open field locomotion and relative interior activity

The open field chamber was constructed with a square wooden floor (115 cm X 115 cm) painted black with white lines every 23 cm, forming a five-by-five grid. A clear Plexiglas sheet covered the floor of the open field. The walls of the open field were 44 cm in height. Sessions were 18 minutes long and the rats were tested in a darkened room with dim red lamps above two corners. Both the experimental room and the open field apparatus were novel to the subjects, and each subject was tested only once. At the start of the 18 minute session, the rats were placed in one of the four corners of the open field and were faced toward the center. Blind to treatment, an observer separately counted inner and outer horizontal crossings, which were defined by the movement of all four paws from one block square to another. A hand counter was used to make these counts. Outer crossings were defined by movements into one of the 16 squares adjacent to the walls, either from another outer square along the wall or from an inner square. Inner crossings were defined as movements into and within the nine center blocks of the open field which did not run along the walls.

Assessment of locomotion, catalepsy, hypothermia, and analgesia

For the assessment of locomotor activity, rats were placed in small locomotor chambers (28 × 28 × 28 cm). The floor in each of the four chambers consisted of two wire mesh panels (27 × 13 cm) connected by a metal rod though the center. This metal rod was a fulcrum for the floor panels, which were able to move freely. Locomotion by the subjects from one area in the chamber to another produced a deflection of one or more floor panels, which closed one or more of four microswitches mounted on the outside of the chamber. These microswitch closures were detected and counted by an external computer that ran a custom program previously written in QBasic, by means of an interface (Med Associates). Animals were tested in 18 minute sessions.

In order to ensure a high baseline of locomotor counts, the chambers were novel to the subjects at the time of testing. Subjects were moved to an adjoining room immediately following the locomotion session, and were allowed to habituate for 5 minutes. Subjects were then tested for catalepsy. A thin metal bar fixed at 13 centimeters above a wooden stand was used by placing both of the subject's forelimbs over it with both hind limbs on the wooden stand. Subjects were timed for latency to remove one or both forelimbs, or to jump onto the bar. Two trials were taken for each subject, and latencies were summed. As a measure of analgesia, subjects were tested for latency on a tail-flick apparatus that inflicted a varying level of pain (Ugo Basile, Italy). In order to prevent spontaneous movement, the rats were wrapped lightly in a cloth towel. Each subject's tail was placed in contact with a heat source that contained a photosensor which was activated using a foot pedal. Any movement of the tail was detected by the photosensor, which then turned off the heat source and stopped a built-in timer. A maximum duration of 10 seconds was set to prevent damage to the rats. To measure differing levels of hypothermia, each subject's temperature was taken with a pliable, water-resistant thermistor that was inserted 6 cm into the animal's rectum. A digital thermometer was connected to the thermistor, and recorded the temperatures. Temperatures were allowed to stabilize for at least 5 seconds before being recorded.

Experiments

Experiment 1: Effects of AM 4054 on spontaneous locomotion and thigmotaxis in the open field

In the following experiment, the effects of AM 4054 on spontaneous locomotion and thigmotaxis were assessed. Rats (*n*=40) were handled for five days before testing. Each rat was randomly assigned one of the five treatments: vehicle, 0.16, 0.32, 0.64, or 1.25 mg/kg AM 4054.

After a 30 minute pre-treatment, each subject was tested in the open field for 18 minutes. The

number of inner and outer line crossings were counted and recorded.

Experiment 2: Reversal of AM 4054 behavioral effects with CB_1 antagonist AM 251

In experiment 2, a new group of animals was randomly assigned to one of five different

treatment groups to demonstrate the reversal of the behavioral effects of AM 4054, using the CB_1

antagonist AM 251. Subjects (*n*=40) received either vehicle or 2.0 or 4.0 mg/kg AM 251 60

minutes prior to testing. After 30 minutes, the subjects were given either a 0.3 mg/kg dose of

AM 4054 or vehicle. After a total pretreatment duration of 60 minutes, the animals were tested

on a battery of tasks including an 18 minute session in locomotor boxes.

Data Analyses

Behavioral data for experiment 1 and each part of the tetrad were analyzed separately

using a between subjects one-way ANOVA with dose as the between subjects factor. In cases

where assumptions of homogeneity of variance were violated, the nonparametric Kruskal-Wallis

test was used. Individual dose effects were analyzed using planned comparisons, which

compared the effects of each dose to the effects of vehicle. For the reversal tetrad in experiment

2, a behavioral effect of AM 4054 was considered reversed by AM 251 if planned comparisons

suggested the group that received AM 251 plus AM 4054 significantly differed from the group

receiving AM 4054 only. To perform the present data analysis, a computerized statistical

program (SPSS 10.1 for Windows) was used.

IV. Results

Experiment 1: Effects of AM 4054 on spontaneous locomotion and thigmotaxis in the open field

AM 4054 significantly suppressed locomotion on the inside (Fig. 1; ($F_{(4,35)}=24.27$, $p<0.001$) as well as locomotion on the outside portions of the open field (Fig 2; ($F_{(4,35)}=121.16$, $p<0.001$). Planned comparisons revealed significant differences between all doses of AM 4054 in comparison to vehicle.

Experiment 2: Reversal of AM 4054 behavioral effects with CB_1 antagonist AM 251

The behavioral effects of AM 4054 were reversed with the co-administration of the CB_1 selective antagonist AM 251. With catalepsy, an ANOVA revealed a significant overall group difference (Fig. 3; $F_{(4,35)}= 9.44$, $p<0.01$). AM 251 alone had no significant effect, while AM 4054 caused a significant induction of catalepsy compared to vehicle ($p<0.01$). Catalepsy induced by AM 4054 was significantly reduced by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<.0.01$). In the tail-flick analgesia test (Fig. 4), there was a significant overall effect ($F_{(4,35)}=9.5$, $p<.0.001$).   AM 251 alone had a significant effect ($p<0.05$), and AM 4054 caused an increase in tail-flick latency ($p<0.01$). AM 4054-induced analgesia was significantly reduced by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<0.01$; $p<0.05$). For assessment of body temperature (Fig. 5), temperatures were found to be significantly different from group to group ($F_{(4,35)}=28.68$, $p<0.001$). AM 251 alone had no significant effect, while AM 4054 caused a significant reduction in body temperatures compared to vehicle ($p<0.01$). AM 4054-induced hypothermia was significantly reduced by co-administration of AM 251 at both the 2.0 and 4.0 mg/kg doses ($p<0.01$). In the locomotor chambers, AM 4054 alone did have a significant effect on locomotion ($p<0.01$). The suppression of locomotion induced by AM 4054 was not significantly reversed by AM 251 at

either the 2.0 or 4.0 mg/kg dose. The Kruskal-Wallis test revealed a significant dose-related

effect across all groups (p<0.01).

V. Discussion

Previous research characterized AM 4054 as a potent CB_1 selective cannabinoid agonist with characteristic effects on the tetrad test, including locomotor suppression, catalepsy, analgesia, and hypothermia (Martin et al., 1991). In the present study, some of these effects were reversed with the co-administration of the CB_1 selective antagoinist AM 251 at doses that are behaviorally active in rats (McLaughlin et al., 2003).

Characterized by potency on all four tests of the tetrad, the behavioral effects of CB_1 agonists must be reversed by a CB_1 antagonist in order to fit the profile (Rinaldi-Carmona et al., 1994). In order to classify AM 4054 as a cannabinoid agonist, a tetrad was conducted. Having similar potency on all four levels of the tetrad (data not shown), it was hypothesized that a cannabinoid antagonist such as AM 251 would reverse these effects. Both the 2.0 mg/kg dose and 4.0 mg/kg dose of AM 251 significantly reversed the effects of AM4054 on three of the four tasks.

In the past, high doses of CB_1 antagonists including SR 141716A have been found to inhibit open field ambulation (Jarbe et al., 2002). In addition, past research has suggested that AM 251 alone produces a significant suppression of locomotion (McLaughlin et al., 2005). Subjects treated with both AM 4054 and AM 251 produced locomotor counts similar to vehicle, suggesting that the locomotor suppressant effects of both drugs offset each other.

Thigmotaxis, an animal's tendency to avoid the inner region of the open field and ambulate near the high walls of the outside region, has been demonstrated with anxiogenic drugs including benzodiazepine inverse agonists (Prut and Belzung, 2003). In the past, it has been suggested that changes in relative interior activity in the open field task can be used as a behavioral determinant of anxiolytic or anxiogenic effects of drugs (Prut and Belzung, 2003). In

the present study, thigmotaxis was observed at the 0.16 and 0.31 mg/kg doses of AM 4054. AM 4054 reduced locomotion in the open field with little indication of thigmotaxis at doses other than 0.16 and 0.31 mg/kg. This is not to say however that an anxiogenic effect did not exist. All doses of AM 4054 used in this study caused a profound decrease in locomotor counts in both the inner and outer portions of the open field. There was, however, a slightly more defined suppression of inside crossings than outside crossings, perhaps suggesting an anxiogenic effect of AM 4054 at doses that also suppress motor activity. It is also important to consider differences between doses. In the past, it has been suggested that cannabinoid agonists produce anxiolytic effects at low doses, but anxiogenic effects at higher doses (Genn et al., 2004). In the present study, the 0.3 mg/kg dose of AM4054 that suppressed locomotion in the locomotor cages also reduced inside and outside crossings in the open field. Because interior and exterior counts were analyzed separately, it was possible to compare the effects more efficiently. Since exterior activity was significantly suppressed at all doses, an overall motor effect was shown. Combined with the reduction in locomotion and induction of catalepsy in experiment 2, it is reasonable to suggest that AM 4054 inhibits locomotion by impairing motor control, but that the drug does in fact produce anxiogenic effects in the same dose range.

In the past it has been concluded that AM4054 produces behavioral effects consistent with other cannabinoid agonists that have their effects at the CB_1 receptor. In the future, AM 4054 can be a useful tool for further understanding CB_1 receptor activation as well as aiding the development of new cannabinoid agonists. AM 251 continues to be a useful tool for classifying new cannabinoid agonists and conducting behavioral tests. Future studies should further explore the anxiety-related properties of cannabinoid drugs in order to more fully understand endocannabinoid system roles in regulating anxiety.

VI. References

Arévalo C, de Miguel R. Hernández-Tristán R. Cannabinoid effects on anxiety-related behaviours and hypothalamic neurotransmitters, Pharmacol Biochem Behav 70 (2001), pp. 123–131.

Burns TL and Ineck JR. Cannabinoid analgesia as a potential new therapeutic option in the treatment of chronic pain. The Annals of Pharmacotherapy: Vol. 40, No. 2, pp. 251-260.

Burstein SH, Karst M, Schneider U, Zurier RB. Ajulemic acid: a novel cannabinoid produces analgesia without a "high." Life Sci 2004;75:1513-22.

Carriero D, Aberman J, Lin SY, Hill A, Makriyannis A, Salamone JD. A detailed characterization of the effects of four cannabinoid agonists on operant lever pressing. Psychopharmacology (1998), 137: 147-156.

Devane WA, Hanus L, Breuer A, Pertwee RG, Stevenson LA, Griffin G. Isolation and structure of a brain constituent that binds to the cannabinoid receptor, Science 258 (1992), pp. 1946–1949.

Genn RF, Tucci S, Parikh S, File SE. Effects of nicotine and a cannabinoid receptor agonist on negative contrast: distinction between anxiety and disappointment? Psychopharmacology 177 (2004), pp. 93–99.

Gong JP, Onaivi ES, Ishiguro H, Liu QR, Tagliaferro PA, Brusco A, Uhl GR. Cannabinoid CB_2 receptors: immunohistochemical localization in rat brain. Brain Res. 2006 Feb 3;1071(1):10-23. Epub 2006 Feb 9.

Grimaldi C, Pisanti S, Laezza C, Malfitano AM, Santoro A, Vitale M, Caruso MG, Notarnicola M, Iacuzzo I, Portella G, Di Marzo V, Bifulco M. Anandamide inhibits adhesion and migration of breast cancer cells. 2006 Feb 15;312(4):363-73. Epub 2005 Dec 15.

Grotenhermen F, Russo E, eds. Cannabis and cannabinoids: pharmacology, toxicology, and therapeutic potential. New York: Haworth Integrative Healing Press, 2002.

Haller J, Bakos N, Szirmay M, Ledent C, Freund TF. The effects of genetic and pharmacological blockade of the CB_1 cannabinoid receptor on anxiety. Eur J Neuroscience 2002;161395-8.

Järbe TU, DiPatrizio NV, Makriyannis A. (-)-Adamantyl-Delta8-tetrahydrocannabinol (AM-411), a selective cannabinoid CB_1 receptor agonist: effects on open-field behaviors and antagonism by SR-141716 in rats, Behav Pharmacol 15 (2004), pp. 517–521.

Johnson MR and Melvin LS. The discovery of nonclassical cannabinoid analgetics. In: R. Mechoulam, Editor, Cannabinoids as therapeutic agents, CRC Press Inc., Boca Raton, FL (1986).

Karst M, Salim K, Burstein S, Conrad I, Hoy L, Schneider U. Analgesic effect of the synthetic cannabinoid CT-3 on chronic neuropathic pain. JAMA 2003;290:1757-62.

McLaughlin PJ, Lu D, Winston KM, Thakur G, Swezey LA, Makriyannis A, Salamone JD. Behavioral effects of the novel cannabinoid full agonist AM 411. Pharmacol Biochem Behav. 2005 May;81(1):78-88. Epub 2005 Apr 21.

McLaughlin PJ, Winston K, Swezey L, Wisniecki A, Aberman J, Tardif DJ, Betz AJ, Ishiwari K, Makriyannis A, Salamone, JD. The cannabinoid CB_1 antagonists SR 141716A and AM 251 suppress food intake and food-reinforced behavior in a variety of tasks in rats, Behav Pharmacol 14 (2003), pp. 583–588.

McLaughlin PJ, Winston KM, Limebeer CL, Makriyannis A, Salamone JD. The cannabinoid CB_1 antagonist AM 251 produces food avoidance and behaviors associated with nausea but does not impair feeding efficiency in rats. Psychopharmacology [in press].

McPartland J, Russo E. Cannabis and cannabis extracts: greater than the sum of their parts? J Cannabis Ther 2001;1:103-32.

Mechoulam R, Gaoni Y. Isolation, structure and partial synthesis of an active constituent of hashish. J Am Chem Soc 1967;86:1646-7.

Muller-Vahl KR, Schneider U, Koblenz A, Jobges M, Kolbe H, Daldrup T. Treatment of Tourette's syndrome with delta 9-tetrahydrocannabinol (THC): a randomized crossover trial, Pharmacopsychiatry 35 (2002), pp. 57–61.

Munro S, Thomas KL, Abu-Shaar M. Molecular characterization of a peripheral receptor for cannabinoids. Nature 1993;365:61-5.

Prut L, Belzung C. The open field as a paradigm to measure the effects of drugs on anxiety-like behaviors: a review. Eur J of Pharmacology 2003;463: 3-33.

Rodgers RJ, Evans PM, Murphy A. Anxiogenic profile of AM251, a selective cannabinoid CB_1 receptor antagonist, in plus-maze-naïve and plus-maze-experienced mice. Behavioral Pharmacology. 2005, 16:405-413.

Sandyk R, Awerbuch G. Marijuana and Tourette's Syndrome. J Clin Psychopharmacol 1988;8: 444-445.

Van Sickle MD, Duncan M, Kingsley PJ, Mouihate A, Urbani P, Mackie K, Stella N, Makriyannis A, Piomelli D, Davison JS, Marnett LJ, Di Marzo V, Pittman QJ, Patel KD, Sharkey KA.Identification and functional characterization of brainstem cannabinoid CB_2 receptors. Science. 2005 Oct 14;310(5746):329-32.

Ward SJ, Childers SR, Pacheco M. Pravadoline and aminoalkylindole (AAI) analogues: actions which suggest a receptor interaction, Br J Pharmacol 98 (1989), p. 831.

Wiley JL and Martin BR. Cannabinoid pharmacological properties common to other centrally acting drugs, Eur J Pharmacol 471 (2003), pp. 185–193.

Zurier RB, Rossetti RG, Lane JH, Goldberg JM, Hunter SA, Burstein SH. Dimethylheptyl-THC-11-oic acid: a nonpsychoactive anti-inflammatory agent with a cannabinoid template structure. Arthritis Rheum 1998;41:163-70.

VII. Figure Captions

Figure 1. AM 4054 reduced inside crossings in the open field. Data points represent mean (± S.E.M.) number of total inside crossings in the open field. **$p<0.01$ difference between dose of AM 4054 and vehicle using planned comparisons.

Figure 2. AM 4054 reduced outside crossings in the open field. Data points represent mean (± S.E.M.) number of total outside crossings in the open field. **$p<0.01$ difference between dose of AM 4054 and vehicle using planned comparisons.

Figure 3. AM 251 reverses catalepsy induced by AM 4054. Bars represent group means (± S.E.M.). **$p<0.01$ difference between AM 4054 alone and vehicle. ‡$p<0.01$ difference between AM 251-pretreated animals and AM 4054 alone using planned comparisons.

Figure 4. AM 251 reverses analgesic effects of AM 4054. Bars represent group means (± S.E.M.). *$p<0.05$; **$p<0.01$ difference between AM 4054 alone and vehicle. †$p<0.05$; ‡$p<0.01$ difference between AM 251-pretreated animals and AM 4054 alone using planned comparisons.

Figure 5. AM 251 reverses hypothermia induced by AM 4054. Bars represent group means (± S.E.M.). **$p<0.01$ difference between AM 4054 alone and vehicle. ‡$p<0.01$ difference between AM 251-pretreated animals and AM 4054 alone using planned comparisons.

Figure 6. AM 251 reverses locomotor suppression induced by AM 4054. Bars represent group means (± S.E.M.). **$p<0.01$ difference between AM 4054 alone and vehicle.

Figure 1.

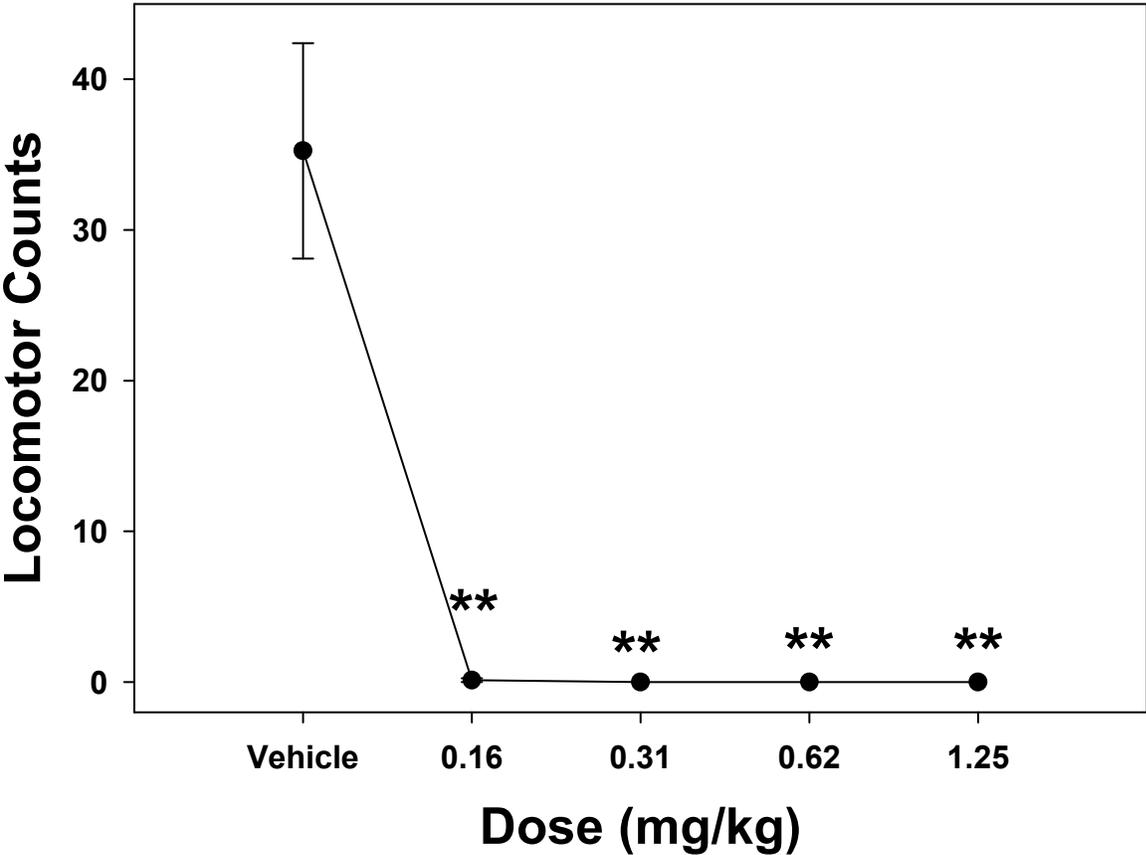# AM 4054 IMPAIRS INSIDE CROSSINGS IN THE OPEN FIELD

Figure 2.

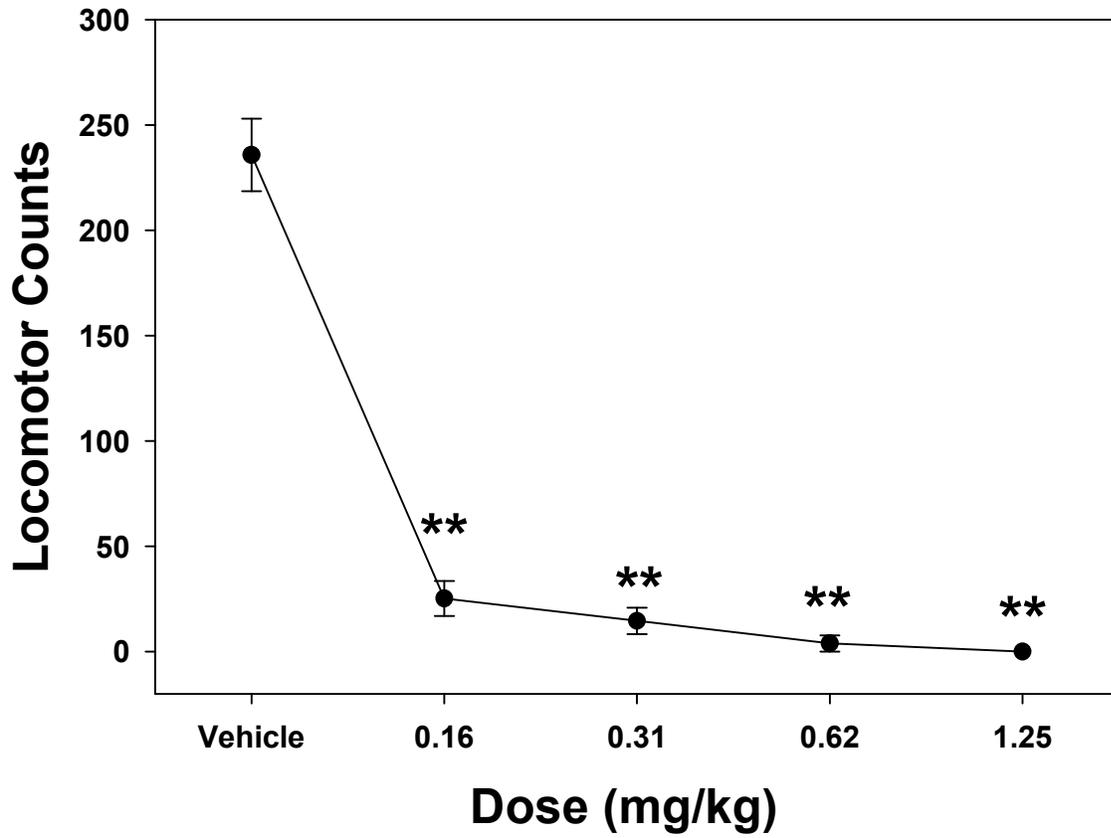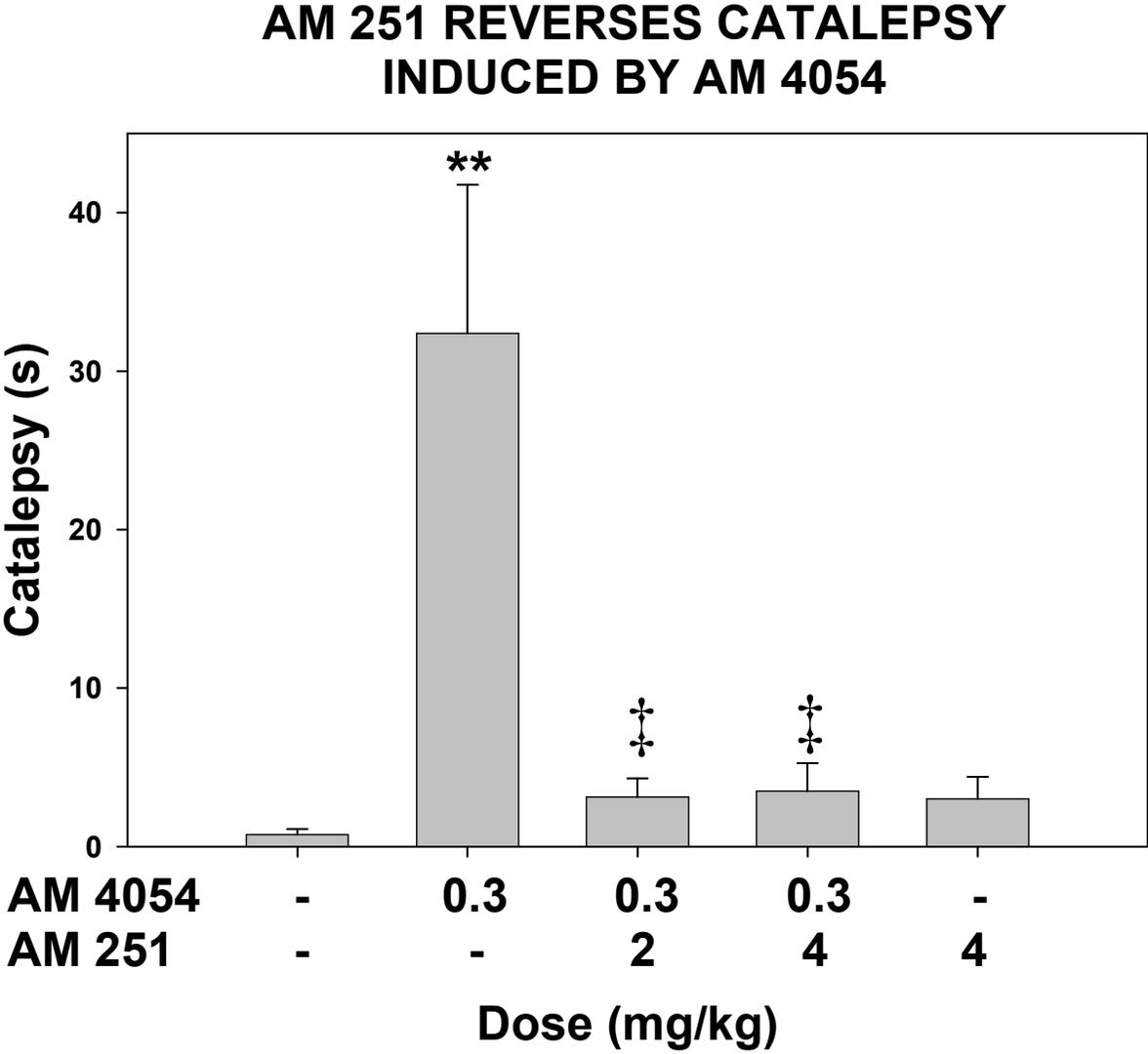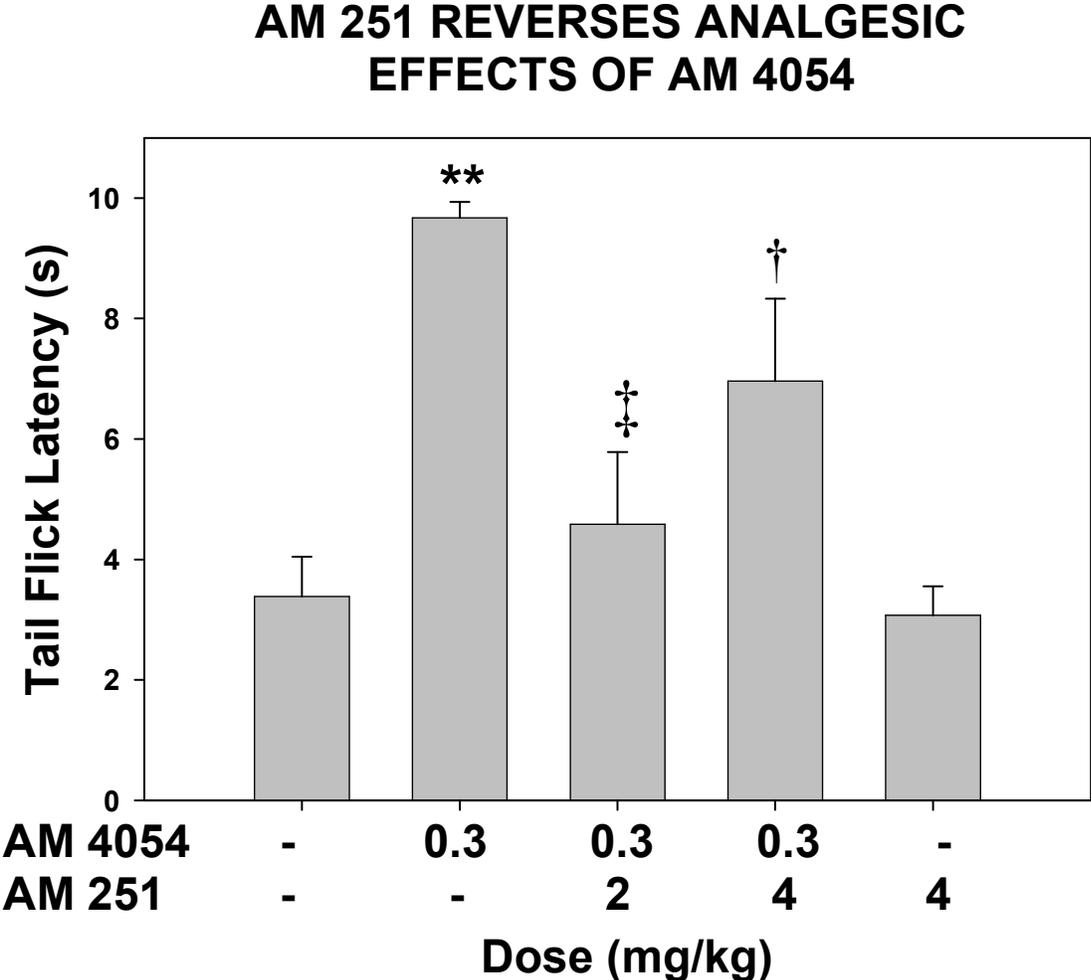AM 4054 IMPAIRS OUTSIDE CROSSINGS
IN THE OPEN FIELD

Figure 3.

AM 251 REVERSES CATALEPSY INDUCED BY AM 4054

Figure 4.

AM 251 REVERSES ANALGESIC EFFECTS OF AM 4054

Figure 5.

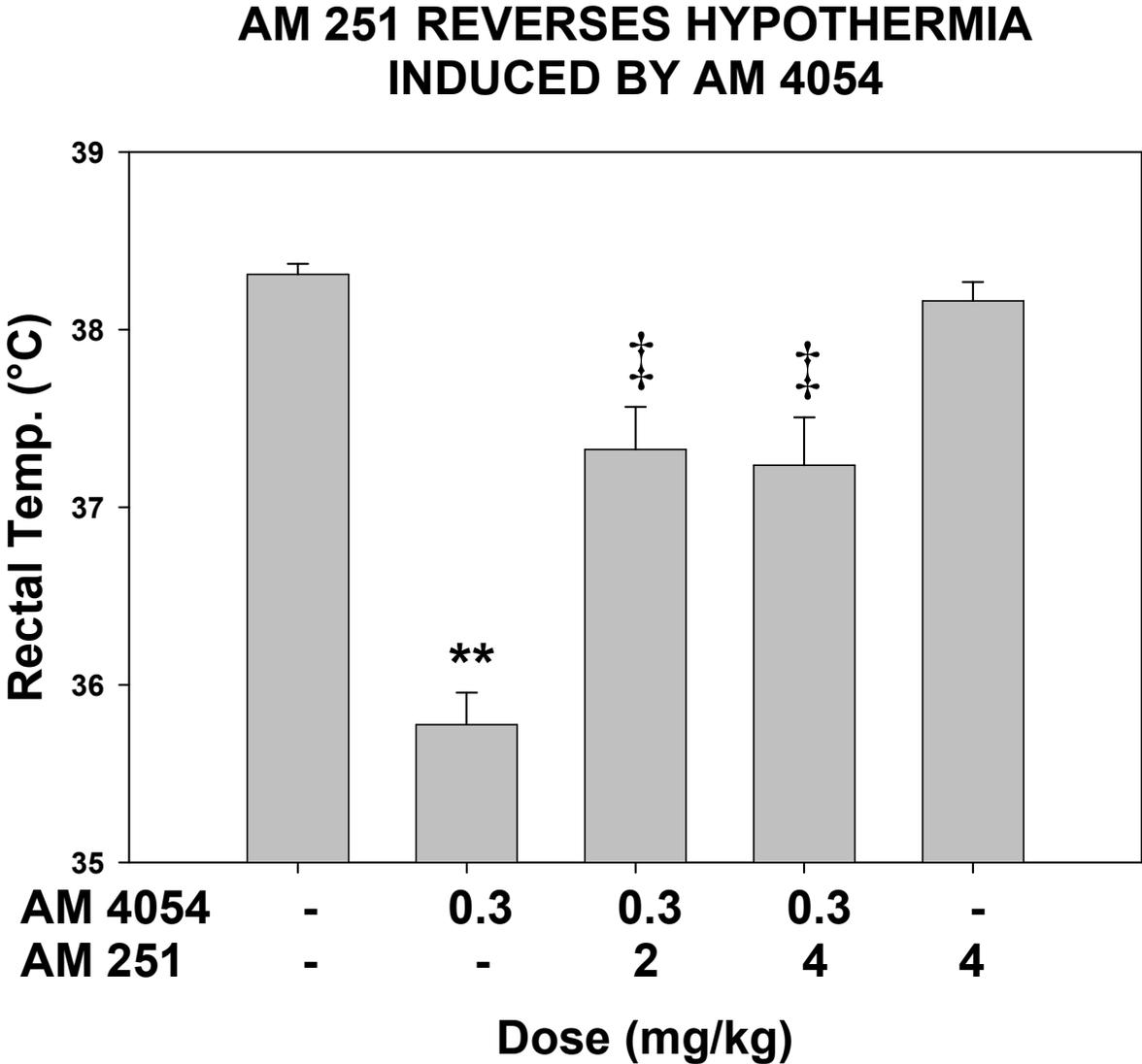**AM 251 REVERSES HYPOTHERMIA INDUCED BY AM 4054**

Figure 6.

AM 251 REVERSES LOCOMOTOR SUPPRESSION INDUCED BY AM 4054