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Effects of Cannabidiol on MK-801-Induced Locomotor Sensitization in Mice

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Abstract

Previous research has shown that cannabidiol (CBD), a non-psychoactive compound in the hemp plant *Cannabis sativa*, may be useful in treating drug craving, one of the hallmarks of drug addiction. However, the neural mechanism by which CBD attenuates craving is poorly understood. Studies from other laboratories have shown that neuroplastic changes associated with brain NMDA glutamate systems may at least partially serve as a neural mechanism for craving. In the current study, the noncompetitive NMDA receptor antagonist MK-801 maleate was used to induce locomotor sensitization, a form of NMDA glutamate-mediated neuroplasticity, in mice to test the sensitization-attenuating potential of CBD. Separate groups of mice (N=8) received either CBD (1.0 mg/kg, i.p.) or saline thirty minutes prior to an intraperitoneal injection of MK-801 (0.5 mg/kg) or saline and tested for locomotor performance in an open field (Induction Trial). Seventy-two hours later all mice, regardless of drug pretreatment, were tested for locomotor activity following a second administration (0.5 mg/kg, i.p.) of MK-801 (Sensitization Trial). Results revealed a significant difference across groups for the Induction Trial, with groups receiving SAL-MK801 and CBD-MK801 significantly more active than SAL-SAL and CBD-SAL groups. Pretreatment with CBD had no effect on the locomotor activating effects of MK-801 during the Sensitization Trial with similar levels of locomotor performance across drug groups. Possibilities for the lack of CBD effects are discussed, as well as implications and future research directions.

Introduction

Drug addiction is a significant social and economic problem in society. Every year addiction costs the United States over $122 billion in time spent on treatment and $15 billion in health insurance (Rehab International, 2012). Up until the 1960's drug addiction was thought to be a moral flaw in character (Wise, 2002). It was then that technological advances in experimental techniques discovered that non-human animals could be taught to press a lever to begin self-administering a drug and that they maintained the behavior. Thus, the idea that a substance could be inherently addictive, more or less regardless of one's moral character began to hold among medical practitioners and scientists (Julien, et al., 2011). Today, the American Psychiatric Association considers addiction a disorder and lists it in its Diagnostic and Statistical Manual, Fourth Edition (APA, 2000). When one ingests a psychoactive drug repetitively or in excess, it may lead to a neuronal and neurotransmitter dysregulation called sensitization (Vezina, 2007). However, how do neural sensitization processes in the brain result in addiction and are there effective methods for treatment?

The brain is a very plastic organ and adapts throughout one's life (Julien, et al., 2011). Therefore, it is thought that some neuroadaptation takes place in the process of addiction as a result of drug exposure. One known way neuroadaptation manifests itself is sensitization. Sensitization can be viewed as a model of this neuroplasticity because it occurs in response to chronic or high doses of a drug, leading to anticipation of receiving the drug and drug-seeking behavior, a phenomenon also known as "craving".

Behaviorally, drug craving is a central hallmark of addiction and may be used as a model for sensitization (Steketee and Kalivas, 2011).

In terms of sensitization expression, increased locomotor activity, i.e., locomotor sensitization, is one way this neuroadaptation is expressed. Locomotor sensitization means that locomotor activity will increase *despite* no change in the dosage of a drug between administrations, making it an extremely useful model for measuring sensitization.

There is evidence that alterations in glutamatergic systems in addition to dopaminergic systems might be responsible for sensitization, i.e. neuroplasticity, particularly the mesolimbic dopamine pathway, glutamatergic pathways, and NMDA glutamate receptors (Cornish and Kalivas, 2000). There is also evidence that NMDA receptor-antagonizing psychostimulants cause sensitization (or craving) due to these altered dopaminergic and glutamatergic system interactions (Kalivas and Alesdatter, 1993).

Sensitization is known to be caused by psychostimulants. A particularly useful psychostimulant drug for inducing locomotor sensitization is MK-801 maleate. MK-801 acts as an NMDA receptor antagonist, therefore altering glutamatergic and dopaminergic systems in the brain (Wong, et al., 1986). These same NMDA glutamate receptors are those partially responsible for some of the synaptic plasticity and neuroadaptation taking place in the brain, in conjunction with dopaminergic systems. Given that sensitized locomotor reactions to MK-801 is a form of neuroplasticity, locomotor activity can be used as a model for sensitization.

Surprisingly, there are not many drugs available for the treatment or attenuation of the sensation, i.e., the craving sensitization. Although its exact mechanism is unknown, cannabidiol (CBD) has been shown to be effective in attenuating sensitization in heroine-addicted rats (Ren, et al., 2009). A drug similar to cannabidiol is Rimonabant and has also been shown to attenuate drug-seeking behavior (Morgan, et al., 2010). Perhaps then, cannabidiol can attenuate MK-801-induced locomotor sensitization in mice.

Using MK-801-induced locomotor activity as a model for sensitization in the brain, this experiment aims to explore the possibility of the non-psychoactive substance cannabidiol as a potential attenuator of stimulant-induced sensitization.

Addiction

There are two levels on which to understand addiction: neurologically and behaviorally. Neurological addiction takes into account structures, neurotransmitters, and neural activity. Ideally one would observe exactly what happens in the brain during addiction development. Behavioral observation is useful diagnostically and in settings where neural activity observation is unavailable or not easily possible. It is also relevant on a psychological level: what or how the subject feels and their actions. Neural activity can be inferred or suggested through behavioral observation, e.g., locomotor activity.

Behaviorally, addiction can be thought of as a "downward spiral" in terms of development (APA, 2000; Figure 1). Beginning with the ingestion of a significant amount of a drug, during which sensitization takes place, the brain adapts to the increased amount of drug and begins to anticipate it. This period is called the

"incubation period". Then craving begins to occur, finally leading to bingeing, withdrawal, and then craving again, spiraling into a cycle resulting in addiction (APA, 2000). Sensitization is a key step in this spiral as it is the beginning of the downward spiral.

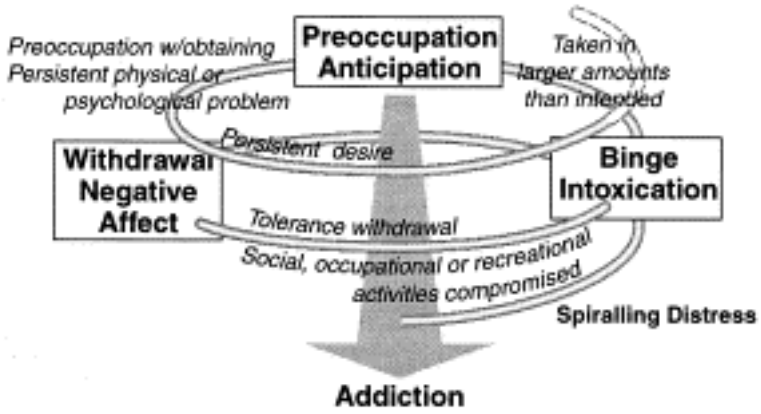

Figure 1. The addiction cycle according to the DSM-IV (APA, 2000).

Incentive-Sensitization Theory

The explanation of how drug mechanisms lead to addiction in the brain addressed in this experiment is the Incentive-Sensitization Theory developed by Terry Robinson and Kent Berridge in the 1990's. The theory essentially states that the brain experiences "sensitization" when a drug is taken in excess and begins to anticipate receiving the drug. This leads to "wanting" and "craving". "Wanting" is having the desire for a drug and "craving" is a need for the drug, influenced by motivation (Robinson & Berridge, 2001; 2008). Sensitization is thought to underlie compulsive drug-seeking behavior (Wise, 2002). This constant anticipation leads to eventual relapse, resulting in the downward spiral of addiction as described by the DSM-IV (Figure 1).

In the cycle of addiction, sensitization can be understood by being the opposite of tolerance. Instead of the receptors in the brain becoming accustomed to the drug and its effects, the brain becomes sensitive, that is, more easily activated by both the drug itself and that which is associated with it. It can be characterized as "a hypersensitivity to the incentive motivational effects of drugs and drug-associated stimuli" (Robinson and Berridge, 2008).

There are three main categories of sensitization: behavioral sensitization and neural sensitization, in addition to incentive sensitization. Behavioral sensitization in the context of drugs, is the enhancement of the motor-stimulant response to drugs. It is this enhancement that is elemental to addiction (Robinson and Berridge, 2001). Psychomotor sensitization is a complex dose-dependent mechanism occurring within the brain (2001). It is thought to be caused by neurons and synapses becoming hypersensitive to a drug's effects, as if "looking" for the drug. Psychomotor sensitization persists significantly longer than tolerance. Tolerance is becoming accustomed to a drug's effects, while sensitization is becoming more receptive to a drug's effects and drives the "want". Once one is sensitized to a particular drug, he or she remains so up to years afterward, thus explaining high relapse rates in addicts (Robinson and Berridge, 2001).

The concept of "liking" and "wanting" also must be addressed, for it is this distinction that leads to the sensitized incentive. According to Robinson and Berridge, wanting something and liking something are two different things, though usually connected (2008). Wanting something gives one incentive to attain it, while liking something simply gives that person pleasure, but not necessarily the incentive. The

dissociation between this incentive value and the reward production of drugs, and those of natural rewards allows for the liking/wanting distinction (2008). A drug is more likely to be obtained from "craving" rather than "liking". Incentive-sensitization can be manifested through this unconscious wanting (implicitly) or through a conscious craving (explicitly) (2008).

Craving is the *learned* response of drug administration and its positive reinforcing effects (the "high" sensation) (Goldstein & Volkow, 2002). According to Robinson and Berridge, there are two mechanisms for sensitization to occur: context-specific and intermittent dosages of a drug. Context-specific sensitization is induced when the drug is administered in a particular environment consistently. In a study by Volkow and colleagues (2004) experiments using rats revealed craving to be stimulated by environmental factors. Intermittent dosages of a drug also may induce sensitization, simply due to repeated exposure. Single high doses may also induce sensitization (Karler, et al., 1994).

Mesolimbic Dopamine System, Glutamate, and Reward

Returning to sensitization induction, almost all drugs of abuse activate a common path: the mesolimbic dopamine system. Whether the drug is an agonist and directly stimulates dopamine release in the brain or blocks the inhibitory messages for dopamine by affecting NMDA glutamate receptors, abused drugs increase the amount of dopamine in this system (Julien, et al., 2011). The mesolimbic dopamine system is made up of a variety of structures, such as the ventral tegmental area (VTA) and nucleus accumbens, including other limbic pathways, as well as neurotransmitters such as

acetylcholine, glutamate, and GABA. The system as a whole involves cholinergic input from the brainstem, input from cortical structures like the medial and occipital prefrontal cortex, glutamatergic signals from the amygdala, GABA from striatal structures, and glutamatergic and GABAergic inputs in the nucleus accumbens (Figure 2). The nucleus accumbens in particular is a significant component of the mesolimbic dopamine system and plays a paramount role in addiction because of its "reward" sensation and the expression of sensitization (Cornish and Kalivas, 2000; Koob and Nestler, 1997).

Originally it was thought that the ability of drugs of abuse to increase levels of dopamine in the limbic system was the only necessary reinforcing effect that led to addiction (Goldstein & Volkow, 2002). A view presented by Koob and Le Moal (2000) presents the idea that addiction is essentially a dysregulation of neurotransmission in the brain's reward system and incorporates more than dopaminergic systems, but also glutamatergic systems, stress mechanisms, environmental factors, and genetics. Nonetheless, dopamine still plays a major key part in addiction and the bulk of the responsibility may be attributed to it (Wise, 2002).

In the process of stimulant addiction, the drug can also stimulate the release of glutamate from the amygdala, which activates dopaminergic neurons in the VTA, from which dopamine is released to the other components of the mesolimbic dopamine system (Cornish and Kalivas, 2000; Figure 2).

Glutamate is a nonessential amino acid and known to be an excitatory neurotransmitter in the brain. It is one of the brain's most common neurotransmitter and plays a significant role in activating NMDA receptors, which release calcium ions

(Kalivas and Alesdatter, 1993; Uzbay, et al., 2000). Excessive release of glutamate can cause neurotoxicity because of these stimulatory effects.

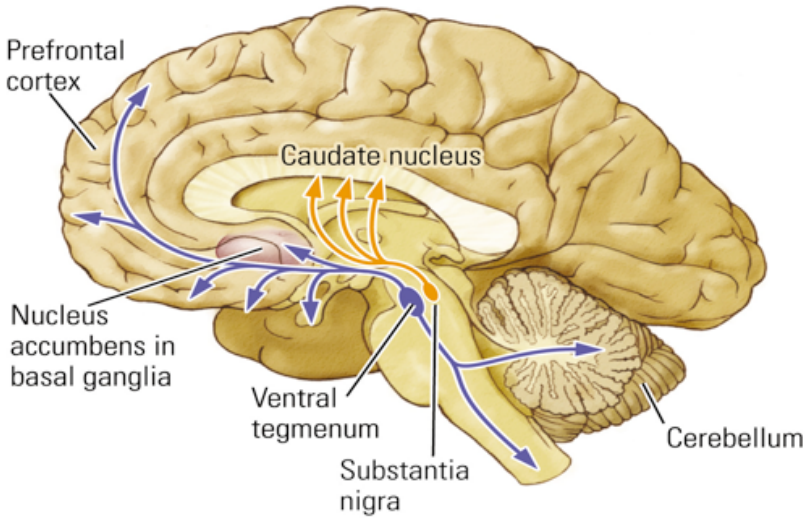

Figure 2. Mesolimbic Dopamine System and Nigro-Striatal Dopamine System (Adapted from Heimer, L., 1995).

Glutamate receptors are prevalent in the brain and are especially high in concentration in the cerebral cortex, hippocampus, basal ganglia, septum, and amygdala – structures also associated with addiction (Julien, et al., 2011). Interactions between dopaminergic and glutamatergic systems in the brain are unclear, but are significantly accountable for psychostimulant-induced locomotor activity (Burns, et al., 1994).

A study by Cornish and Kalivas (2000) testing cocaine sensitization found that "recruitment of glutamatergic afferents to the nucleus accumbens…[are] a necessary neural event" to produce the neuroadaptation known as sensitization (P.4). They also found that repeated exposure to cocaine resulted in increased recruitment.

NMDA Receptors

Due to their involvement with glutamate, NMDA receptors are key players in stimulant sensitization. N-methyl-d-aspartic acid receptors (NMDAR) are ionotropic glutamate receptors in the brain responsible largely for the regulation of synaptic plasticity in learning, memory, and cognitive ability. This synaptic plasticity is largely responsible for the sensitization response to drugs (Irifune, et al., 1995; Karler, et al., 1994; Wang and McGinty, 1999).

NMDAR are co-localized with alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors on 70% of synapses, also players in sensitization (Julien, et al., 2011) and are activated by glutamate in the presence of glycine or serine. Usually while at resting membrane potential, the NMDA receptor is blocked by a magnesium ion. Direct depolarization or activation of AMPA receptors or kainate receptors removes the magnesium block, allowing for free ion flow. Excessive signaling with this receptor can lead to neurotoxicity (Julien, et al., 2011). Neurotoxicity is caused by the influx of calcium through the NMDA glutamate receptor, potentially due to their high permeability, and prevents the sending of inhibitory signals (Stout, et al., 1998).

Antagonists

Perhaps because NMDA glutamate receptors play a critical role in neuronal plasticity, antagonists of these receptors cause sensitization. It has been shown that rats self-administer PCP, an NMDAR antagonist, into the frontal cortex, along with other NMDA antagonists (Goldstein and Volkow, 2002). This activates glutamate release in the VTA, then triggering the rest of the mesolimbic dopamine pathway.

When the NMDA receptor is blocked artificially, as with antagonists such as MK-801, PCP, and ketamine, symptoms such as hyper-locomotor activity are present. This hyper-locomotion occurs from the prefrontal cortex and nucleus accumbens releasing glutamate to NMDA receptors in the VTA, which release GABA onto dopaminergic systems. When these receptors are blocked by MK-801, GABA is not released, and dopaminergic systems are not inhibited – potentially leading to addiction (Carey, et al., 1995; Carlsson and Carlsson, 1989; Foster and Wong, 1987).

Sensitization and Stimulants

In addition to Robinson and Berridge's theory, Steketee and Kalivas (2011) describe behavioral sensitization to be "defined by the augmented motor-stimulant response that occurs with repeated, intermittent exposure to a specific drug" (P. 349).

Repeated stimulant exposure can induce sensitization in humans (Robinson and Berridge, 2001). There are many studies providing evidence of sensitization (Carey, et al., 1995; Karler, et al., 1994; Vezina, 2007; Zukin and Zukin, 1979). In a study by Leith and Kuczenski (1982) it was found that the stimulant amphetamine causes stereotypy and locomotor sensitization after chronic doses. A study by Cornish and Kalivas (2000) found that sensitization could be induced by cocaine. Xu and Domino (1994) confirmed sensitization from MK-801 exposure as well.

When animals (and humans) become sensitized to the effects of a given drug, particularly psychomotor stimulants such as MK-801, the sensitization tends to last a significantly long amount of time. This increased length of vulnerability causes the mesolimbic dopamine system to become more responsive towards the drug (Wise,

2002). The dopamine system can remain sensitized to cues and stimulation associated with the drug, leading to craving (Julien, et al., 2011). The brain becomes hypersensitive to the acquisition and expectation of receiving the drug of abuse.

Locomotor sensitization is best known to be caused by stimulants. A number of different drugs fall under the category of stimulants, such as cocaine, amphetamine, nicotine, methylphenidate, MK-801, and caffeine. Stimulants are known to cause sensitization and their effects by disrupting the neurotransmission of dopamine and glutamate in the brain (Wang and McGinty, 1999). Either by inhibiting reuptake or dopamine breakdown in the synapse, promoting excessive release of dopamine in the synapse, or by affecting glutamate receptors, it is generally thought that stimulants affect dopamine release in the mesolimbic dopamine system (Kelley and Berridge, 2002). Unsurprisingly, dopamine stimulation in this area accounts for stimulants' highly addictive properties.

Psychostimulants' effects on the central nervous system may manifest themselves in the body also, and can confirm the efficacy of a drug. In very high doses they can lead to stereotypic behavior (Leith and Kuczenski, 1982). Stereotypy is a typical behavioral effect caused by very high doses of stimulants. Stereotypy is not necessarily sensitization-related, but caused by the excessive release of DA. It is defined by repetitive movement with no obvious purpose or function, e.g. a rat sniffing the same area repeatedly and excessively. Stereotypy can also be stillness in obscure positions. In mice and rats stereotypic behavior includes excessive grooming, standing on hind legs, and repeated purposeless behavior.

Moderately high doses of psychostimulants also lead to hyper-locomotor activity. This increased motor activity can be accounted for through the amplified release of dopamine. Psychological effects of stimulants include euphoria, heightened alertness, and in high doses agitation and restlessness (Andine, et al., 1999; Carey, et al., 1995; Carlsson and Carlsson, 1989). Locomotor (behavioral) sensitization can be used as an indirect measurement of sensitization induction when it is positive, i.e., when locomotor activity is present. If there is no locomotor activity present and is the only model used to measure sensitization induction, it is no longer sufficient due to the unreliability in measuring the transition from motivated locomotor activity to stereotypy. This makes locomotor activity an excellent model for measuring sensitization behaviorally.

Mechanism and Effects of MK-801 on Sensitization

In order to induce sensitization in this experiment, the stimulant MK-801 maleate was used. MK-801 maleate ((+)-5-methyl-10, 11-dihydro-5H-dibenzo [a,d] cyclohepten-5,10-imine maleate) is an anticonvulsant, has anesthetic and anticonvulsant properties, and is considered a PCP-type drug (Koek, et al., 1993). Though less potent than phencyclidine, MK-801 induces higher locomotor activity and was found to be a selective non-competitive NMDA receptor antagonist, the effects of which are use- and dose-dependent (Irifune, et al., 1995). Endogenous glutamate and other amino acids enhance MK-801's binding to NMDA receptors. The binding mechanism has been suggested to act by blocking open channels associated with these NMDA receptors. Because of this block, GABA is then not released onto dopaminergic

neurons, meaning continued release of dopamine due to the lack of inhibitory neurotransmitter.

As a PCP-type drug, MK-801 induces PCP-like locomotor activity in rats (Everitt and Wolf, 2002). This includes Straub tail, hind leg ataxia, elongated body posture, head twitches, in addition to cognitive effects, such as increased irritability and increased sensitivity to audial and tactile stimuli. A study by Carlsson and Carlsson (1989) found that there was increased locomotion in mice when administered MK-801, behavior thought to be due to interactions between glutamatergic and dopaminergic systems, potentially including serotonin (Cornish and Kalivas, 2000; Koek, et al., 1993; Loscher & Honack, 1992). Carlsson and Carlsson also found that haloperidol, a dopamine receptor antagonist did not antagonize the MK-801-induced locomotion, suggesting that this locomotor activity is independent of dopamine and noradrenaline release and must then be attributed to NMDA antagonizing.

Sensitization studies using phencyclidine can be applied to MK-801 because of their similarity in effects and mechanism. A rat brain study by Zukin and Zukin (1979) found that phencyclidine had the highest affinity in the hippocampus, then the cervical spinal cord, hypothalamus, caudate nucleus, frontal cortex, cerebellum, and least in the medulla, pons, and amygdala (1979). These regions are part of the mesolimbic dopamine system, accounting for susceptibility to addiction and direct induction of sensitization. Phencyclidine was also found to induce locomotor sensitization through antagonizing NMDA receptors (Xu and Domino, 1993).

Many studies have shown Phencyclidine (PCP), an illicit drug similar to MK-801, to induce psychomotor sensitization (Koek, et al., 1993; Wong, et al., 1986; Xu and

Domino, 1993). However, it is suggested that it does not require multiple doses to induce sensitization, but one high dose is sufficient (Xu and Domino, 1993). In a study by Benvenga and colleagues, MK-801 was also shown to potentially share a similar mechanism with ketamine (1991). To date, the only other competitive substances to MK-801 are PCP and ketamine, further supporting the possibility of a similar mechanism to those drugs.

Endocannabinoids and Sensitization

The endocannabinoid system has two main types of receptors: CB_1 and CB_2. Both receptors are G-protein-coupled receptors and are present throughout the brain in higher prevalence than receptors for glutamate and GABA. They are most prevalent in the cortex, hippocampus, basal ganglia, cerebellum, and spinal cord (Julien, et al., 2011). CB_1 receptors are found mostly in the central nervous system and interact with GABAergic neurons. Several glutamatergic and cholinergic telencephalic and cerebellar neurons also express the CB_1 receptors (de Fonseca, et al., 2005; Piomelli, 2003). CB_2 receptors are found largely in structures associated with the immune system (de Fonseca, et al., 2005).

"CB_1 receptors are found on the presynaptic terminals and act to inhibit calcium ion flux… activation of cannabinoid receptors inhibits the release … glutamate from presynaptic terminals" (Julien, et al., 2011, P. 482). Perhaps then, they may play a role in attenuating sensitization.

There is evidence that cannabinoid receptors inhibit the reuptake of dopamine and glutamate in the mesolimbic dopamine system, however, contrary to the

psychoactive component of *Cannabis sativa* delta-9THC, cannabidiol has low potency in this respect.

Mechanism and Effects of Cannabidiol on Sensitization

Not many drugs are available for the attenuation of craving, however, cannabidiol (CBD) has been found potentially to attenuate sensitization-induction (Long, et al., 2006; Mechoulam, et al., 2007; Morgan, et al., 2010; Moreira and Guimaraes, 2005). Cannabidiol is a component of the marijuana plant *Cannabis sativa*, but does not have psychoactive effects. It was found to be an antagonist to CB_1 and CB_2 receptor agonists, including noradrenaline (Mechoulam, et al., 2007). A study by a group in Aberdeen (Pertwee, 2008) showed cannabidiol is a CB_1-agonist and noradrenaline antagonist. The group concluded that cannabidiol acts on presynaptic sites in order to antagonize the agonists and did not *directly* antagonize the receptors. Cannabidiol's effects on CB_2 may also be beneficial for the immune system in preventing hyper-inflammatory responses in the central nervous system (Sacerdote, et al., 2005).

In a recent experiment testing CB_1 and CB_2 receptor inhibitors and heroin addiction reinstatement, a drug similar in mechanism to cannabidiol, Rimonabant, prevented drug-seeking behavior (Maldonado, et al., 2006). Rimonabant essentially blocks CB_1 activity, which are also involved in reward and motivation, similarly to the mesolimbic dopamine system. Knowing this, it is possible that cannabidiol should also be capable of preventing the reinstatement of drug-seeking behavior in previously sensitized animals (Fattore, et al., 2003). However, its exact mechanism is not fully understood.

Hypothesis and Proposed Experiment

Using the model of incentive-sensitization for addiction, the stimulant MK-801 was used to investigate the possibility of cannabidiol preventing the induction of sensitization in mice. MK-801's ability to induce sensitization in conjunction with research suggesting cannabidiol's attenuating effects on sensitization were the basis for this experiment. Based on this previous research, it was posited that cannabidiol would have attenuating effects on the MK-801-induced sensitization and will be shown through a decrease in locomotor activity in the mice pretreated with cannabidiol and MK-801.

Methods and Materials

Subjects

Thirty-two female Swiss-Webster mice (Simonsen Labs, Gilroy CA), weighing approximately 22 g upon arrival from the breeding facility, were housed in groups of 4 in standard polyethylene mouse cages in a temperature-controlled vivarium ($21^{o} \pm 2^{o}$ C). The vivarium had a 12 hr. light/dark cycle (lights on: 0700 hours) and all behavioral testing was conducted during the light portion of the cycle. Food and tap water were freely available throughout the experiment. The experimental protocol including procedure, drugs and injection regimen was approved by the Institutional Animal Care and Use Committee of the Keck Science Center.

Apparatus and Equipment

Locomotor activity testing was conducted in a separate room using 4 open-top, clear, Rubbermaid plastic bins (48 x 35 x 10 cm), with the floor of each bin demarcated

into 6 equal squares (dimensions of each square = 16 x 11.33 cm) using a black permanent marker. Mice were exposed to the bins only during the locomotor activity measurement period. Test bins were thoroughly cleaned after testing each mouse with a non-toxic cleaning agent (Method Pro Chef Multi-surface) to prevent any locomotor effects resulting from olfactory cues.

Procedure

Locomotor activity testing was divided into two test sessions: the Induction Test and the Sensitization Test. For the Induction Test half the mice received an intraperitoneal (i.p.) injection of cannabidiol (1.0 mg/kg) while the remaining mice received saline. Thirty minutes later, half the cannabidiol and saline-pretreated mice received either MK-801 (0.5 mg/kg, i.p.) or saline yielding the following treatment groups: saline-saline (SAL-SAL), saline-MK-801 (SAL-MK801), cannabidiol-saline (CBD-SAL) and cannabidiol-MK-801 (CBD-MK801). Five minutes following the second drug administration mice were individually placed in the test bins and their locomotor performance was videotaped and scored over a 60 min. test session.

Locomotor activity score was measured by counting the total number of line crossings the mouse made over the test session. The mouse needed to pass over a line with both its front and back paws for it to be considered a line crossing. Locomotor testing for each mouse was randomized across drug groups.

Seventy-two hours following the Induction Test mice were retested for locomotor activity following an i.p. administration of 0.5 mg/kg of MK-801 (Sensitization Test). The procedure for the sensitization test was identical to that of the induction test with

the exception that all mice were challenged with an i.p. administration of MK-801 (see Figure 3).

	TEST DAY 1				TEST DAY 2
Group N = 32	Pre-treatment		Induction Dose	72 hour drug wash out period	Sensitization Dose
A N = 8	Cannabidiol (1.0 mg/kg)	30 Minutes	MK-801 (0.5 mg/kg)		MK-801 (0.5 mg/kg)
B	Saline (0.9% 1.0 mg/kg)		MK-801		MK-801
C	Cannabidiol		Saline		MK-801
D	Saline		Saline		MK-801

Figure 3. Experiment week set up.

Testing and injections for both experimental days were performed during the same time of day consistently; between 9am and 1pm. Testing was done in the same, standard environment with low lighting and low sound (B29, Keck Science Building).

Drug Preparation

Cannabidiol (Sigma Chemicals, St. Louis MI) was dissolved in 1.0 ml of 99.9% ethanol and brought up to volume with 0.9% sterile saline. CBD control mice received saline mixed with 1.0 ml ethanol. MK-801 maleate (Sigma Chemicals, St. Louis MI) was dissolved in 0.9% sterile saline. All drug administrations were performed using sterile injection procedures.

Statistical Analysis

Each animal's line crossings during the one-hour test session were totaled for both the Induction and Sensitization tests yielding that animal's locomotor activity score. A two-way analysis of variance (ANOVA) was conducted on the locomotor activity scores with Pretreatment Drug as the between groups factor and Test as with within groups factor. Separate one-way ANOVA's were also conducted on the Induction Test and Sensitization Test. Statistical significance was set at 0.05. A Tukey HSD test ($p = 0.05$) was used to compare means associated with significant main effects and interactions.

Results

In order to test for an interaction between test trial and pretreatment drug group to see whether cannabidiol did indeed have an effect on locomotor sensitization, a two-way 2x4 factorial ANOVA was carried out on Pretreatment Group (SAL-SAL, SAL-CBD, CBD-MK801, and SAL-MK801) and Test Trial (Induction and Sensitization), both between-subjects variables (Figure 4). There was a significant main effect for Test Trial, $F(3,56) = 4.836$, $p = 0.032$, and for Pretreatment Group, $F(3,56) = 4.197$, $p = 0.009$. Mean locomotor activity during the Sensitization Trial ($M = 1866.063$, $SE = 107.790$) was significantly higher than in the Induction Trial ($M = 1530.844$, $SE = 107.790$), as expected since all mice were treated with MK-801 during the Sensitization Trial. There was also a significant interaction between Pretreatment Group and Test Trial, $F(3, 56) = 3.010$, $p = 0.038$. The CBD-MK801 group had the highest locomotor activity ($M = 2018.688$, $SE = 152.438$) followed by SAL-MK801 ($M = 1884.500$, $SE = 152.438$), SAL-

CBD (M = 1562.125, SE = 152.438), and lastly SAL-SAL (M = 1328.500, SE = 152.438). This was also not unexpected.

The Tukey HSD test showed a significant interaction between SAL-SAL and CBD-MK801 ($p = 0.012$, SE = 215.580), however, no other significant interactions were observed (Figure 4).

The Levene's Test for Equality of Error Variances was violated ($p < 0.01$), however the factorial ANOVA is robust despite a violation of homogeneity.

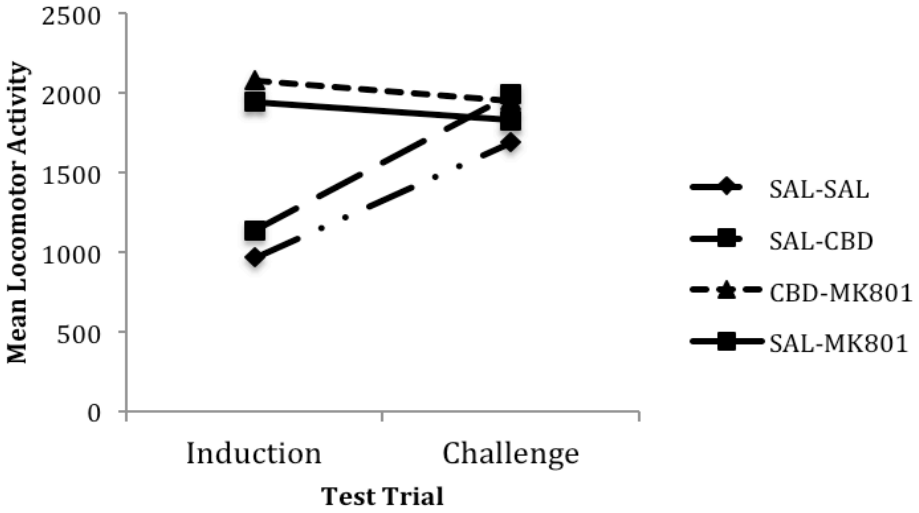

Figure 4. Two-way 2x4 factorial ANOVA interaction for Test Trial and mean locomotor activity scores of Pretreatment Groups. Data is unaltered, N = 64.

Sensitization induction appears not to have occurred, since there was no significant increase in the SAL-MK801 pretreatment group from the Induction Test to the Sensitization Test (Figure 4). Mice as a whole responded with very large ranges of locomotor activity in each group when placed in the experiment bins during the Induction Trial, which may be the reason for a lack of significance. An ideal interaction

among the groups would have been a diverging interaction between SAL-MK801 and CBD-MK801, with SAL-MK801 increasing in mean locomotor activity (Figure 5). However, this did not occur, thus suggesting the absence of sensitization.

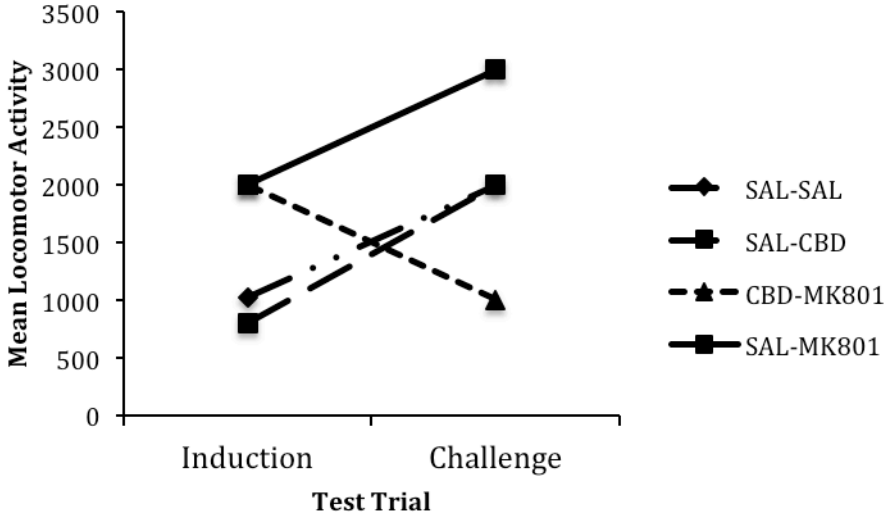

Figure 5. Ideal two-way 2x4 factorial ANOVA interaction for Test Trial and mean locomotor activity scores of Pretreatment Groups.

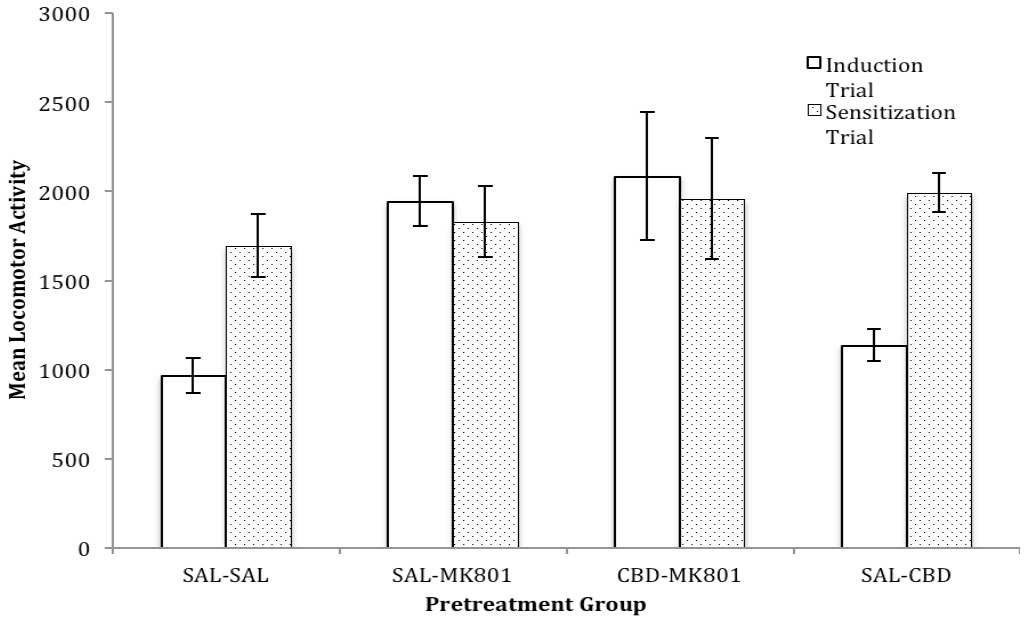

Figure 6. Comparison between Induction Trial activity and Sensitization Trial activity across Pretreatment Groups, (N = 32, SE).

To test if MK-801 induced significant differences in mean locomotor activity across pretreatment groups, a one-way ANOVA was carried out for each trial. There were significant differences across groups within the Induction Trial, $F(3,28) = 7.598$, $p = 0.001$ (Figure 5). A post-hoc Tukey HSD test was run for detailed comparisons. There were significant differences between groups. SAL-SAL mean locomotor activity ($M = 965.13$, $SE = 99.850$) was significantly lower than SAL-MK801 activity ($M = 1941.25$, $SE = 140.328$), $p = 0.011$, as expected; SAL-SAL mean locomotor activity was also lower than CBD-MK801 activity (2082.50, $SE = 359.447$), $p = 0.003$, as expected. SAL-MK801 mean locomotor activity was also significantly higher than SAL-CBD ($M = 1134.50$, $SE = 88.445$), $p = 0.043$, as was CBD-MK801, $p = 0.014$, as expected. There was no significant difference between SAL-SAL and SAL-CBD, $p = 0.701$, nor between SAL-MK801 and CBD-MK801 ($p = 0.924$, Figure 7). This confirms MK-801 effectively induced hyper-locomotor activity in MK-801-treated mice.

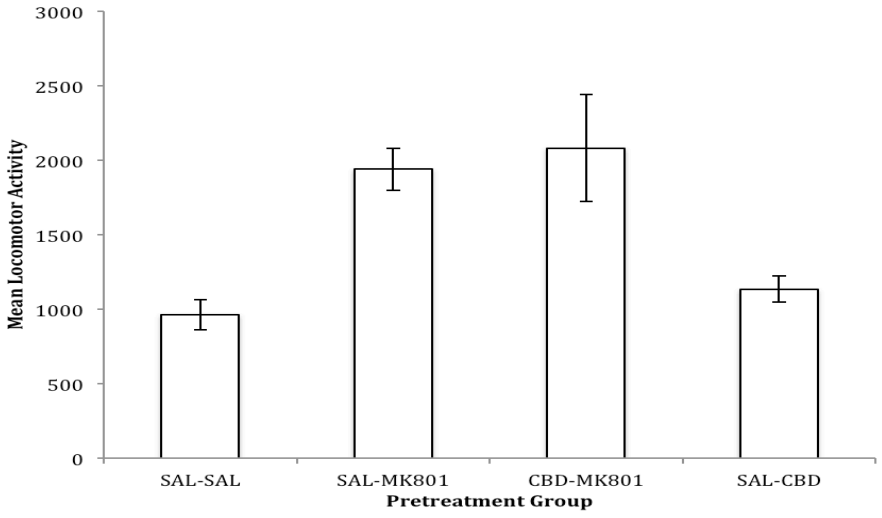

Figure 7. Bar graph of mean locomotor activity scores of Pretreatment Groups across Induction Trial, error bars are standard error. Data is unaltered, N = 32.

The Levene Statistic for Homogeneity of Variances was significant at $p < 0.000$, therefore a Welch test ($p < 0.000$) and Brown-Forsythe test ($p = 0.005$) were also conducted. The ANOVA is robust against this violation, however.

To test for sensitization induction as well as effects of cannabidiol, a one-way ANOVA was also carried out mean locomotor activity across Pretreatment Groups during the Sensitization Trial. However, there were no significant differences among groups during the Sensitization Trial, $F(3,28) = 0.357$, $p = 0.784$ (Figure 8). Statistically all mice performed equally in locomotor activity. There was a lesser violation of variance; Welch and Brown-Forsythe produced non-significant results ($p = 0.637$, $p = 0.784$ respectively).

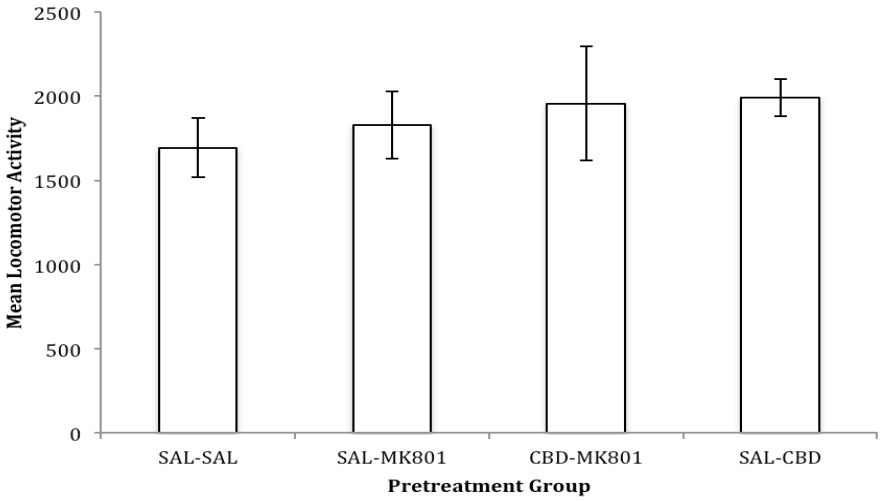

Figure 8. Mean locomotor activity scores of Pretreatment Groups across Sensitization Trial, error bars are standard error. Data is unaltered, N = 32.

Discussion and Conclusions

The results indicated that Cannabidiol did not have a significant effect in preventing sensitization induction from MK-801 maleate in mice. In fact, sensitization induction appears not to have occurred, since there was no significant increase in the SAL-MK801 group from the Induction Trial to the Sensitization Trial (Figure 6). Clearly this suggests that the two-dose method of inducing sensitization to MK-801 is insufficient and should be altered to at least 4 chronic doses of MK-801 similar to the study by Carey and colleagues (1995).

Other possibilities for a lack of significance include length of induction period, dose, stereotypy, lack of baseline activity level, individual mouse variability, novel environment, and sample size per group.

The induction period consisted of only one dose administered 72 hours before challenge dose testing. Although it was shown that a single high dose could induce sensitization, it is possible that the 0.5 mg/kg dose was not high enough (Hargreaves and Cain, 1995). However, the reason this dose was used instead of a higher dose was to prevent stereotypy, because locomotor activity was used as a measurement of drug effects. Unfortunately stereotypy was still present, which may have also affected the activity scores. However, it is also possible that 72 hours was too long a washout period. Returning to Wise (2002), it is possible that although sensitization lasts a "very long time", it had already decreased by 72 hours post pretreatment. Due to this factor, the wash out period is suggested to decrease to 48 hours.

The dose of cannabidiol also could have been much too low (1.0 mg/kg). Perhaps it should be double the dose for future experiments to guarantee effects. In a study by

Parker and colleagues (2004) they used a dose of 5 mg/kg cannabidiol. A precaution is that it would be necessary to avoid sensitization to cannabidiol as a result of the increased dosage.

It is possible that because the mice were active during weigh-ins, the animal's weight was inaccurately measured and the dose of MK-801 was too high, causing the stereotypy. In observing the animals it is determinable which animals received CBD-MK801 and which received SAL-MK801.

Another possibility for lack of significance, individual variation, became a more significant problem than expected. Equality of variance was violated for all tests; therefore a Welch test was also performed. A baseline activity score for each animal, which would then be compared to pretreatment locomotor score and then the sensitization dose score, might have provided better homogeneity. Statistically, it would also allow for mean change in score, which could be compared instead of total mean scores across groups. This would account for individual variation on a daily basis, as well as satisfy homogeneity tests.

The novel experimental environment also may have been a factor. Mice particularly in the SAL-SAL group responded with very high locomotor activity when placed in the experiment bins. The locomotor activity score range for this group was 582 – 1325 line crossings per 60 minutes. This suggests that measurement should not have begun until 15-30 minutes after drug administration and placement in the environment so animals could adjust. In addition, a study by Hargreaves and Cain (1995) stated peak activity began 30 minutes post-injection of MK-801. In a study by Carlsson and Carlsson (1989) it was found that peak locomotor activity occurred

around ninety minutes post-injection. That is to say locomotor activity measurement ended before peak activity even occurred. Either way, measurement should start later and potentially last longer.

In conclusion, if this experiment were to be repeated, a baseline activity test should be performed first to measure natural locomotor activity. The sensitization-induction period should be altered to chronic administrations of CBD/Saline/MK-801 over a number of days, then to administer the MK-801 challenge dose and measure locomotor activity. If the test were redone with significant results, cannabidiol would have potential to becoming an agent in preventing addiction, the mechanism of sensitization would be better understood, and addiction may potentially be treated effectively and permanently.

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