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DIET-RELATED CHANGES IN SENSITIVITY TO THE PHARMACOLOGICAL EFFECTS OF DELTA-9-TETRAHYDROCANNABINOL

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A dissertation submitted in partial fulfillment of the requirements for the degree of Doctor of Philosophy at Virginia Commonwealth University.

by

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Abstract

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Recent evidence suggests that sustained consumption of a high-fat diet is associated with reduced CB_1 receptor expression in some brain areas. Many of the neuromodulatory functions of endogenous cannabinoids are mediated by the CB_1 receptor. The CB_1 receptor also mediates the behavioral and physiological effects of Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive constituent of marijuana. While high-fat diets are associated with region-specific changes in CB_1 receptor expression, it is not clear whether such changes are behaviorally relevant. To that end, separate groups of male and female rats were placed on either a high-fat diet or a standard

diet. Cannabinoid function was determined in a triad of measures (e.g., hypothermia, gross locomotion, time on bar apparatus) at postnatal day 30 (PD30), PD44, PD68 and PD114. These age points respectively correspond to rodent models of early adolescence, late adolescence, early adulthood and full maturity in humans. Male rats were also tested at PD37 and PD61. Subsequently, the antinociceptive properties of Δ^9-THC and the effect of Δ^9-THC on food intake were also measured. After 38 days, female rats maintained on a high-fat diet were significantly less sensitive to the psychomotor effects of Δ^9-THC than were the female rats maintained on the control diet. These diet-related differences persisted into full maturity. Female rats maintained on a high-fat diet were also less sensitive to changes in food intake caused by Δ^9-THC than were female rats maintained on the control diet. In contrast, the hypothermic effects of Δ^9-THC were not differentially affected by the type of diet consumed. Likewise, female rats maintained on a high-fat diet exhibited tail-flick latencies that were indistinguishable from those of female rats maintained on the control diet. With two minor exceptions, and in sharp contrast to female rats, sensitivity to the pharmacological effects of Δ^9-THC was not differentially affected by the type of diet in male rats. In short, female rats maintained on a high-fat diet appeared to be cross-tolerant to the psychomotor and hyperphagic effects of Δ^9-THC while male rats maintained on a high-fat diet exhibited responses to Δ^9-THC that were virtually indistinguishable from control animals.

Introduction

The Endogenous Cannabinoid System

The endogenous cannabinoid, or endocannabinoid, system is a lipid-based signaling system that is composed of two known ligands, several putative ligands, at least two types of G-protein-coupled receptors, a pair of hydrolytic enzymes, and may also include a dedicated reuptake transporter. Endocannabinoids act at the CB_1 receptor to modulate neural impulses in the central nervous system, to modulate activity in the reproductive system and the gastrointestinal and also play an important role in regulating lipogenesis (Di Marzo *et al*., 1998; Cota *et al*., 2003). The CB_2 receptor is found primarily in immune cells (Munro *et al*., 1993; Galiegue *et al*., 1995) where it modulates immune function (Schatz *et al*., 1997; Cabral, 2001). Recent reports suggest that CB_2 receptors are also detectable in neurons (Brusco *et al*., 2008), but these data remain controversial and the function of these receptors, if present, is unclear.

Endocannabinoids are most commonly synthesized in the postsynaptic density of GABAergic and glutamatergic neurons (Rodriguez de Fonseca *et al*., 2005). Once synthesized, endocannabinoids can traverse backward across the synaptic cleft to inhibit presynaptic neural impulses (Wilson and Nicoll, 2001). Endocannabinoids may attenuate inhibitory signals in some brain areas by limiting the release of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA; Maneuf *et al*, 1996; Wilson *et al*., 2001) through a process known as depolarization-induced suppression of inhibition (DSI;

Pitler and Alger, 1994; Kreitzer and Regehr, 2001[A]). During DSI, endocannabinoids act to reduce calcium-ion uptake at the axon terminals of the presynaptic neuron, which suppresses the binding of vesicles to the cell membrane and the subsequent release of GABA into the synapse. Similarly, endocannabinoids may produce depolarization-induced suppression of excitation (DSE) by inhibiting the release of the excitatory neurotransmitter glutamate (Kreitzer and Regehr, 2001[B]). Endocannabinoids also appear to inhibit the action of dopaminergic (Giuffrida et al, 1999) and serotoninergic neurons (Best and Regehr, 2008). In keeping with their neuromodulatory role, endocannabinoids are only synthesized when and where they are needed. The interval during which endocannabinoids exert their physiological influence is brief because they are degraded rapidly.

Though CB_1 receptors are distributed widely throughout the brain, they are densely packed in the cerebral cortex, striatum, globus pallidus, substantia nigra, nucleus accumbens, hippocampus, and the cerebellum (Herkenham *et al*., 1990; Bliss and Collingridge, 1993; Collins *et al*., 1994; Miller and Walker, 1995; Herkenham *et al*., 1991; Herkenham *et al*., 1990; Rodriguez de Fonseca; 1998). The list of functions associated with these brain areas (e.g., cognition, locomotor function, learning and memory, reward, coordination and balance) highlight the physiological significance of the endocannabinoid system. Endocannabinoids are also known to play an important role in energy balance and metabolism. Although CB_1 receptor densities are relatively low in the hypothalamus (Fitton and Pertwee, 1982; Mattes *et al*., 1994), activation of the G-

proteins that are coupled to CB_1 receptors in the hypothalamus is highly efficient (Breivogel *et al.,* 1997).

Elucidation of the endocannabinoid system began in earnest with the isolation of (-)Δ^9-tetrahydrocannabinol (Δ^9-THC), the primary psychoactive constituent of *Cannabis sativa* (Gaoni and Mechoulam, 1964). For two decades, researchers worked to understand the behavioral pharmacology of cannabinoids, all while lacking a well-understood mechanism of action. Some thought that cannabinoids, which are highly lipophilic, could exert their affects by passive diffusion into neurons or by altering the fluidity of the cell membrane (Leuschner *et al*., 1986).

The array of physiological effects produced by cannabinoids and the observation that at least some cannabinoids displayed high-affinity for a binding site in the brain, however, led many to believe that cannabinoid pharmacology must be receptor-mediated (Harris *et al*., 1978; Nye *et al*., 1985; Mechoulam, 2006). For instance, the (-)isomer of the cannabinoid agonist 11-hydroxy-delta 8-tetrahydrocannabinol-dimethylheptyl (HU210) is at least several hundred times more potent than the (+)-isomer (HU211; Little *et al*., 1989). This disparity in potency between optical isomers is indicative of stereospecific binding at a receptor (Soudjin, *et al*., 2003).

Shortly after cannabinoid receptors were discovered in the brain, (Devane *et al*., 1988), the receptor itself was cloned (Matsuda *et al*., 1990). Initially identified as SKR6, the newly cloned receptor interacted cannabinoid ligands (i.e., Δ^9-THC, CP55, 940) but failed to interact with other ligands (i.e., bradykinin, angiotensin II, cholecystokinin) in radioligand binding assays. This newly discovered receptor also produced dose-

dependent changes in forskolin-stimulated accumulation of cAMP when stimulated with Δ^9-THC and CP55, 940. These drug-induced changes in cAMP concentration occurred in a stereoselective manner; the (+)isomers of Δ^9-THC and CP55, 940 were much less potent than the (-)isomers. Importantly, SKR6 was found to be distributed in the same brain regions as the previously identified cannabinoid receptor.

Cannabinoid receptors belong to a class of receptors called G-protein-coupled receptors (GPCRs). GCPRs are metabotropic receptors and, as such, exert their physiological influence through a series of second-messengers (i.e., cAMP, PIP_3; Rodbell, 1997). These receptors and their downstream effectors regulate a complex series of downstream reactions in a process collectively referred to as "signal transduction" (Rodbell, 1995).

In an inactive state, the G-protein complex consists of a receptor with 7-transmembrane domains, a complex of 3-subunits (α, β, and γ) that is bound to the cytosolic face of the cell membrane and a molecule of guanosine diphosphate (GDP; Rodbell, 1995; Rodbell, 1997). When a ligand binds to the receptor, the complex is activated and undergoes a conformational change that decreases the affinity of the Gα subunit for GDP and increases the affinity of the Gα subunit for GTP. When GTP binds to the Gα subunit, another conformational change reduces the affinity of the Gα subunit for the G$\beta\gamma$ subunits. The Gα-GTP complex then dissociates from the rest of the GPCR complex. Once dissociated, the Gα-GTP complex activates the appropriate second messenger. In the process of activating the second messenger, the Gα-GTP complex is

dephosphorylated to form a Gα-GDP complex. Once bound to GDP, the Gα subunit again joins the Gβγ subunits and the GPCR returns to an inactive state.

The newly cloned CB_1 receptor was useful in the effort to isolate an endogenous cannabinoid ligand. The first known endocannabinoid was identified from porcine tissue samples (Devane *et al*., 1992) and became known as "anandamide". Anandamide is a contraction of the Sanskrit word for "bliss" (ananda) and the term for the central chemical moiety of the associated structure (an amide). Anandamide is a derivative of arachidonic acid and is formally known as *N*-arachidonyl ethanolamine (AEA). The phospholipid precursor to anandamide, *N*-phosphatidyl ethanolamine (NAPE), is produced when an enzyme known as *N*-acyltransferase transfers an arachidonic acid molecule to a molecule of phosphatidylcholine (Cadas *et al*., 1996; Piomelli, 2003). The production of NAPE is calcium-dependent and is regulated by cAMP. The enzymes that cleave anandamide from NAPE include *N*-phosphatidyl ethanolamine phospholipase D (NAPE-PLD), but other phospholipase enzymes likely play a role in anandamide synthesis (Leung *et al*., 2006).

Anandamide is an agonist at CB_1 receptors (Mechoulam and Fride, 1993) and has a pharmacological profile similar to that of Δ^9-THC (Crawley et al., 1993; Weidenfeld et al., 1994; Williams and Kirkham, 1999). The CB_1 receptor is typically coupled to G_i/G_o proteins. As such, CB_1 receptor stimulation by an agonist like anandamide inhibits the formation of cAMP and, consequently, reduces the activity of phosphokinase A (PKA). The downstream effects of reduced PKA activity include diminished phosphorylation of a variety of target enzymes. Recent evidence suggests that the highly lipophilic nature of endocannabinoids allows them to bind to and activate cannabinoid receptors from within

the lipid bilayer unlike hydrophilic ligands that must bind while being stabilized in the polar extracellular space (Nebane *et al*., 2008).

Several years after the identification of anandamide, a second endocannabinoid, 2-arachidonoylglycerol (2-AG), was identified (Mechoulam *et al*., 1995). While anandamide is now thought of as a partial agonist at the CB_1 receptor, 2-AG is generally regarded as a full-agonist and is found in much higher concentrations in the brain than anandamide (Gonsiorek *et al*., 2000; Sugiura *et al*., 1995; Stella *et al*., 1997). Despite the similarity of the pharmacological profile of anandamide and 2-AG, they are produced through discrete synthetic pathways.

The pathways that produce 2-AG are robust because of the variety and redundancy of the precursors and enzymes that can be employed. The primary precursors of 2-AG are phosphatidylinositol bisphosphate (PIP2), phosphatidylinositol (PI) or phosphatidylcholine (PC), all of which are phospholipids that are commonly found in the cell membrane (Sugiura *et al*., 2006). These precursors can be converted to 2-AG by the coordinated action of phospholipase A_1, phospholipase C and/or diacylglycerol lipase (Sugiura *et al*., 1995). In an alternate pathway, phosphatase enzymes can convert the conjugate of arachidonic acid and lysophosphatidic acid (2-arachidonyl LPA) into 2-AG (Nakane *et al*., 2002).

Early on, it became apparent that the duration of action of endocannabinoids is limited because the compounds are rapidly hydrolyzed (Deutsch and Chin, 1993). The primary enzyme that degrades anandamide is called fatty-acid amidohydrolase (FAAH; Cravatt *et al*., 1996). FAAH, which belongs to a class of enzymes called serine

hydrolases, is promiscuous and can hydrolyze whole classes of long-chain acylethanolamines and primary amides. FAAH is primarily found on the membranes of cytoplasmic organelles (i.e., mitochondria, smooth endoplasmic reticulum) and the intracellular face of the plasma membrane in postsynaptic neurons (Gulyas *et al*., 2004). It has been hypothesized that FAAH held the capacity to degrade 2-AG as well as anandamide, but recent work suggests that FAAH is not an important contributor to 2-AG hydrolysis in the brain (Blankman *et al*., 2007).

The enzyme that is primarily responsible for 2-AG hydrolysis is called monoacylglycerol lipase (MAGL; Dinh *et al*., 2002). MAGL has more substrate specificity that FAAH and can only hydrolyze a subset of monoacylglycerols (Matias *et al*., 2006). MAGL also seems to function presynaptically (Gulyas *et al*., 2004), while FAAH seems to function as a postsynaptic enzyme. Because the pathways that produce endocannabinoids are not under unified control and because the enzymes that degrade endocannabinoids are found in distinct subcellular regions, some have suggested that anandamide and 2-AG play unique roles in the endocannabinoid system (Chevaleyre *et al*., 2003).

Pharmacological Effects of Cannabinoids

A cluster of four tests conducted in mice, commonly referred to as the "Martin Tetrad", are used to probe for the signature effects of cannabinoid drugs. The tetrad includes measures of transient hypothermia, antinociception, locomotor inhibition and catalepsy-like behavior (Loewe, 1946; Hardman et al., 1971; Smith et al., 1994; Chaperon and Theibot, 1999).

In rodents, both anandamide and Δ^9-THC can reduce rectal temperature by in excess of 5^{o}C (Smith *et al*., 1994; Hosko *et al*., 1981). Other drugs with cannabinoid activity (i.e., CP55,940, WIN 55212-2) have also been reported to produce similar hypothermic responses (Compton *et al*., 1992; Rawls *et al*., 2007). The amount cannabinoid-induced hypothermia is dose-dependent and is affected by the route of administration. Recent evidence suggests that inhibitors of endocannabinoid hydrolysis can also reduce body temperature significantly (Burston *et al*., 2008; Long *et al*., 2009).

The mechanism by which cannabinoids reduce body temperature is unclear, but the amount and duration of hypothermia may be regulated in the anterior hypothalamus and the caudal portion of the brainstem (Schmeling and Hosko, 1976; Hosko *et al*., 1981). While cannabinoids induce hypothermia, this transient reduction in body temperatures may involve receptors other than CB_1. For example, AM404, a drug that elevates endocannabinoid levels in the synapse, produces a hypothermic response in mice that can be blocked by a vanilloid (TRPV1) receptor antagonists (Rawls *et al*., 2006). Similarly, hypothermia produced by cannabinoid agonists can be attenuated by nociceptin/orphanin FQ (N/OFQ) receptor antagonists (Rawls, *et al*., 2007).

Cannabinoids also produce catalepsy-like behavior in mice and rats (Grunfeld and Edery, 1969). Catalepsy is characterized by muscle rigidity and the maintenance of a fixed posture, even if the posture might appear to be uncomfortable (Sanberg *et al*., 1988). Catalepsy induced by Δ^9-THC may involve diminished 5-HT(2A) and 5-HT(1A) signaling in the nucleus accumbens (Sano *et al*., 2008; Egashira *et al*., 2007; Egashira *et*

al., 2006). This reduction in serotonergic signaling is likely related to the inhibitory activity of Δ^9-THC at glutamatergic neurons.

In addition to inducing catalepsy-like behavior, cannabinoids also inhibit locomotion at moderate and high doses. Varying degrees of immobility are observed after treatment with a variety of cannabinoids (i.e., anandamide, Δ^9-THC; Rodreiguez de Fonseca et al., 1998). Evidence derived from experiments with non-human primates suggests that cannabinoid-induced locomotor inhibition may be related to decreased dopaminergic signaling at dopamine D_2 neurons (Meschler *et al*., 2000). In these studies, locomotor activity was significantly inhibited when a sub-threshold dose of the CB_1 agonist levontradol was combined with sub-threshold doses of quinelorane or pergolide, both of which are dopamine D_2 agonists. The dopamine D_1 agonist SKF81297, however, did not alter the relationship between levontradol and locomotor activity.

Evidence from rats corroborate the putative relationship between dopamine D_2 receptors, cannabinoids and locomotor activity (Marcelllino *et al*., 2008). The cannabinoid agonist CP55, 940 dose-dependently reduced the affinity of dopamine D_2 receptor binding sites in membrane preparations derived from the dorsal and ventral striatum of rat brains. Sub-threshold doses of CP55, 940 attenuated the locomotor stimulation produced by quinpirole, a dopamine D_2 agonist, in these same studies.

Cannabinoids are thought to produce antinociception by synergistically interacting with opioid receptors and α_2-adrenoreceptors (Cichewicz and McCarthy, 2003; Tham *et al*., 2005; Cox *et al*., 2007). Evidence from mice suggests that cannabinoid receptors and kappa opioid receptors are co-localized in the dorsal horn of

the spinal cord and interact to modulate the transmission of noxious stimuli from sensory neurons (Smith *et al*., 1994; Reche *et al*., 1996). Blockade of CB_1 receptors by AM281 has been shown to produce a compensatory elevation of kappa opioid receptors in the spinal cord of mice (Saez-Cassanelli *et al*., 2007). In those same experiments, AM281-induced antinociception was reversed by nor-binaltorphimine, a kappa opiod antagonist. Additionally, cannabinoid receptors are thought to interact with mu opioid receptors in the brain are thought to reduce the perception of noxious stimuli (Reche *et al*., 1996). In tail-flick and hot plate tests with mice, sub-threshold doses of morphine augmented the antinociceptive properties of Δ^9-THC in a synergistic fashion. This effect was blocked by the mu-opiod receptor antagonist β-funaltrexamine as well as a kappa-opioid receptor antagonist and a CB_1 receptor antagonist.

Animal Models of Adolescence and the Development of the Endocannabinoid System

Adolescence and puberty are temporally-related events, though each has a distinct definition. Puberty refers to the attainment of sexual maturity (Graber and Brooks-Gunn, 1998) while adolescence is the gradual period of transition from childhood to adulthood (Pickles et al., 1998). Because the onset of adolescence cannot be defined by any single biochemical, endocrine or neuroanatomical milestone, it is difficult to characterize the precise timing and duration of adolescence in physiological terms (Rosenblum, 1990).

Instead, adolescence can be characterized by age-specific behaviors such as increased risk-taking and social interaction. The behavior of adolescent animals is also highly peer-directed and is characterized by novelty-seeking. In rats, adolescent-typical

behavior is commonly observed between postnatal day 28 and postnatal day 42 (Spear, 2000).

The behavioral changes associated with adolescence do not seem to be significantly related to changes in gonadal hormonal levels (Brooks-Gunn et al., 1994). Instead the maturational changes in the brain that occur around postnatal day 28-42 contribute to the age-specific behavioral characteristics of adolescence (Spear, 2000). During adolescence many brain regions (i.e., prefontal cortex) and brain systems (i.e., dopaminergic system) are undergoing important changes (Van Eden *et al*., 1990). Another system undergoing such change is the endocannabinoid system.

Endocannabinoids play an important role in neurological development (Fernandez-Ruiz *et al*., 1999) and, in rodents, the endogenous cannabinoid system continues to mature well after birth (Rodriguez de Fonseca *et al*., 1993; Belue *et al*., 1995). There are subtle sex-differences in the timing of CB_1 receptor development, but both sexes express maximum CB_1 receptor densities between postnatal day 30 and postnatal day 40. As adolescence draws to a close (postnatal day 40), male rats express greater CB_1 receptor density in the midbrain than females and that trend continues into early adulthood, although CB_1 receptor density is similar in other brain areas (Rodriguez de Fonseca *et al*., 1993). By adulthood (postnatal day 90), CB_1 receptor densities stabilize in all brain areas (Belue *et al*., 1995). Some data suggest CB_1 receptor density declines in a region specific manner (e.g., cerebellum, hypothalamus, hippocampus; Berrendero *et al*., 1998) as rats age beyond two years, but there is also evidence that whole brain receptor densities do not change significantly up to thirty-two months of age

(Belue *et al*., 1995). While the CB_1 receptor densities change during maturation, receptor affinity does not appear differ dramatically during development (McLaughlin *et al*., 1994).

While CB_1 receptors mature slowly during the postnatal period, behavioral data suggest that the endocannabinoid system reaches functional maturity during adolescence. There is some evidence that very young rodents (postnatal day 6 though postnatal day 23) exhibit little locomotor inhibition or antinociception after treatment with anandamide or Δ^9-THC (Fride and Mechoulam, 1996[A]; Fride and Mechoulam, 1996[B]). This is relevant because changes in locomotion and antinociception, along with hypothermia and catalepsy-like behavior, are hallmarks of cannabinoid pharmacology (Smith *et al*., 1994). By the onset of adolescence (postnatal day 28), however, this pattern of response changes (Schramm-Sapyta *et al*., 2007; Wiley *et al*., 2008). In adult rodents, low doses of Δ^9-THC can stimulate locomotion (Wiley *et al*., 2008), while larger doses of cannabinoid agonists typically inhibit locomotion and induce antinociception (Fride and Mechoulam, 1996[B]). It is not yet clear, however, whether these age-related differences in cannabinoid pharmacology are linked to changes in CB_1 receptor expression or sensitivity, cell signaling, cannabinoid pharmacokinetics or some other mechanism.

Perhaps related to the slow maturation of the endocannabinoid system, there seems to be a vulnerable developmental period in rodents during which cannabinoid exposure produces long-lasting behavioral alterations, especially in behavior involving cognitive tasks (Stiglick and Kalant, 1985). Other work in rodents shows that chronic pubertal treatment with cannabinoids produces long-lasting reductions in the motivation

to work for a food reward, but similar treatment during adulthood does not produce the same effect (Schneider and Koch, 2003). There is complementary evidence from humans that long-lasting attentional deficits may be related to cannabis abuse that began before age sixteen (Ehrenreich *et al*., 1999).

Sex Differences in Cannabinoid Effects

There is evidence that males and females metabolize Δ^9-THC differently. Experiments with liver cultures suggest that female rats overwhelmingly metabolize Δ^9-THC to 11-hydroxy-Δ^9-THC (Narimatsu *et al*., 1991), a potent psychoactive compound (Lemberger *et* al., 1973). In contrast, male rats metabolize Δ^9-THC to a variety of compounds (including 11-hydroxy-Δ^9-THC). This is significant because many of the other metabolites of Δ^9-THC produced in male rats are less psychoactive than 11-hydroxy-Δ^9-THC. These observed *ex vivo* differences in the metabolism of Δ^9-THC are corroborated by the observation that female rats have higher peak brain levels of 11-hydroxy-Δ^9-THC than do male rats after intraperitoneal administration of Δ^9-THC (Tseng *et al*., 2004). Importantly, blocking the metabolism of Δ^9-THC eliminates sex-differences in antinociception in rats.

Human females have been shown to achieve higher maximal blood concentrations of Δ^9-THC and do so more rapidly than males after oral consumption of cannabis (Nadulski et al., 2005). However, Δ^9-THC can accumulate in fatty tissues (Nahas et al., 1981), so it is possible that sex-related differences in body composition play a role in the bioavailability of Δ^9-THC. In contrast, other studies with humans indicate few differences

in the degree to which men and women metabolize Δ^9-THC to 11-hydroxy-Δ^9-THC, regardless of whether the drug was delivered intravenously or by mouth (Wall *et al*., 1983).

While sex-related differences in cannabinoid pharmacokinetics have been documented, there are also important sex-related differences in cannabinoid pharmacodynamic. For example, there may be sex-related differences in the degree to which cannabinoids alter neuronal signaling in the arcuate nucleus, a brain area that controls homeostatic function like food intake. There is some evidence that rapidly-inactivating, voltage-gated potassium channels (A-type K+ channels) in the hypothalamus are inhibited by cannabinoids to a greater degree in female guinea pigs than in male guinea pigs (Tang *et al*., 2005). The CB_1 receptor agonists WIN55,212 and ACEA induced a rightward shift in the inactivation curve for these currents in females, but not in males. A rightward shift in this curve suggest that when treated with WIN55,212 or ACEA, more voltage-gated potassium channels are inhibited in females than in males. Consequently, a greater number of neuronal impulses are inhibited. The inhibitory effect of cannabinoids on these potassium channels were reversed after the application of a CB_1 antagonist.

There are also important sex-related behavioral differences in cannabinoid pharmacology. When these sex differences are detected, females tend to be more sensitive to the pharmacological effects of cannabinoids than males (Cohn et al., 1972). In an experiment with humans, females treated with Δ^9-THC exhibited a greater drop in cerebral blood velocity and blood pressure after standing (orthostatic hypotension) and

reported being more dizzy than males, even though the plasma levels of Δ^9-THC were indistinguishable (Mathew *et al*., 2003). Cannabinoids may also induce antinociception and inhibit locomotion at lower doses in female rats than male rats (Tseng and Craft, 2001).

It is also interesting to note that chronic exposure to Δ^9-THC during adolescence exaggerates the relationship between nutritional status and motivation to work for a food reward as indexed by progressive-ratio breakpoints (Wright and Wiley, unpublished data). Unlike males, female rats continued to exhibit elevated progressive-ratio breakpoints even when they were tested at 100% of their pre-testing bodyweight. This observation suggests that the food reward used in these experiments may be a more efficacious reinforcer in female rats than in male rats

High-Fat Diets and the Endogenous Cannabinoid System

The endocannabinoid system is a lipid-based signaling system and endocannabinoid levels appear to be influenced by the lipid content of the diet. More specifically, the type and quantity of polyunsaturated fatty acids (PUFAs) found in the diet can directly influence the levels of endocannabinoid ligands in the body (Berger *et al*., 2001; Watanabe *et al*., 2003). When mice and piglets are fed diets enriched with omega-6 polyunsaturated fatty acids, brain levels of anandamide and 2-AG increase significantly. High levels of omega-6 polyunsaturated fatty acids are commonly found in corn oil, soybean oil and canola oil (Rodriguez-Cruz *et al*., 2005).

Diets rich in omega-6 polyunsaturated fatty acids may increase endocannabinoid levels by providing additional substrate for FAAH, an enzyme that degrades anandamide

(Matias *et al*., 2006). As previously mentioned, FAAH lacks the kind of substrate specificity that is characteristic of MAGL (Dinh *et al*., 2002). As such, FAAH has been shown to hydrolyze the amide bond of not only anandamide, but also several other common amines formed from long-chain fatty acids (Schmid *et al*., 1985; Ueda *et al*., 1995). Interestingly, chocolate contains two such fatty-acid amides (*N*-oleoythanolamine and *N*-linoleoylethanolmine) that have been shown to inhibit the hydrolysis of anandamide in brain cells, possible by acting as a substrate for FAAH (Di Tomaso *et al*.,1996).

Even short-term consumption (e.g., 1 week) of diets rich in unsaturated fatty acids can elevate brain levels of long-chain fatty acids (*N*-acylethanolamines) as well as endocannabinoids (Artmann *et al*., 2008). The rats used in this study were fed diets rich in either monounsaturated fats (i.e., olive oil) or polyunsaturated fats (i.e., safflower oil). A diet rich in safflower oil may produce elevated levels of *N*-oleoythanolamine (OEA), *N*-linoleoylethanolamine (LEA) and 2-AG, while a diet rich in olive oil may also produce elevated brain-levels of anandamide as well.

There is some evidence that the density of CB_1 receptors in some brain regions increases soon after mice are placed on a high-fat diet, but then CB_1 density declines after sustained consumption of that diet (South and Huang, 2008). After 3 weeks, animals on the high-fat diet displayed elevated CB_1 receptor density in the medial and ventral anterior olfactory nucleus, the agranular insular cortex and the hypothalamus when compared to animals maintained on a low-fat, high carbohydrate diet.

The increase CB_1 receptor density among the mice fed a high-fat diet was correlated with an increase in plasma leptin in the hypothalamus. Leptin is a circulating hormone released primarily by white adipose tissue (Zhang *et al*., 1994) that acts in rodents via hypothalamic receptors to inhibit feeding and increase energy-expenditure (Jequier, 2002). There is also an inverse relationship in the hypothalamus between exogenously administered leptin and both anandamide and 2-AG (DiMarzo *et al*., 2001).

When mice were maintained on the high-fat diet for 20 weeks, they became obese, hyperphagic and displayed decreased CB_1 receptor density in several reward-related brain areas (i.e., substantia nigra, ventral tegmental area). Though the brain endocannabinoid levels were not measured in these experiments, these results are certainly consistent with increased endocannabinoid signaling after sustained consumption of a high-fat diet.

Other evidence suggests that mice maintained on a high-fat diet have increased hepatic anandamide levels, perhaps because of the competitive inhibition of FAAH by fatty-acid amides (Osei-Hyiaman *et al*., 2005). Animals maintained on a high-fat diet also had dramatically diminished levels of FAAH activity when compared to controls, even though all the animals appeared to be synthesizing the same level of enzyme.

While endocannabinoid levels appear influenced by the types and quantities of polyunsaturated fats in the diet, high-fat diets may also be related to changes in CB_1 receptor density. When mice were maintained on a high-fat diet long enough to induce obesity, CB_1 receptor densities in the hippocampus, nucleus accumbens and entopeduncular nucleus declined (Harrold *et al*., 2002). The researchers involved in this

experiment produced their high-fat diet by supplementing normal rodent chow with Nestle condensed milk (PUFA content ranging 6%-36%; Harrold *et al*., 2002). While the fat composition of the milk used in these experiments varied significantly, two-thirds of the polyunsaturated fatty acids in condensed milk are usually of the omega-6 variety (USDA National Nutrient Database for Standard Reference, 2005).

In mice, sustained consumption of a high-fat diet that also contains elevated levels of cholesterol has been shown to reduce CB_1 receptor density in the striatum and the hypothalamus (Hayakawa *et al*., 2007). Curiously, chronic food restriction had the opposite effect. In these experiments, mice were divided into 3 groups: those receiving a normal diet, those receiving a restricted portion of a normal diet and those receiving a high-fat, cholesterol-enriched diet. After 6 weeks, both serum and brain cholesterol levels were determined and Western blots were used to determine the expression level of CB_1 receptors in the cortex, the striatum and the hypothalamus.

As expected, serum cholesterol levels significantly increased in the group fed the high-fat diet. Conversely, striatal cholesterol levels significantly decreased and there was also a non-significant trend for cholesterol levels in the hypothalamus to decrease in that same group. Importantly, the level of CB_1 receptor expression in the striatum also decreased significantly in the group that was fed the high-fat diet.

There is evidence that altering dietary fat intake can affect the composition of the cell membrane in general and, more specifically, change the composition of lipid rafts (Siddiqui *et al*., 2007). Lipid rafts are small segments of the cell membrane that contain high-levels of cholesterol and sphingolipids (Barnett-Norris *et al*., 2005). These small

pools of densely-packed saturated lipids move freely among the more loosely-packed unsaturated lipids that compose the cell membrane. Lipid rafts may permit some types of receptor proteins to coalesce (Melkonian *et al*., 1999) and increase signal transduction efficiency in the process (Moffett *et al*., 2000). It appears that lipid rafts influence binding and signaling at CB_1 receptors (Barnett-Norris *et al*., 2005) and that the cholesterol content of the lipid raft may modulate the degree to which the receptor is activated by AEA (Bari *et al*., 2005).

Interestingly, while high-fat diets decrease CB_1 receptor density in some areas of the brain, it may also increase CB_1 receptor densities in the liver (Osei-Hyiaman, *et al*, 2005). While it is not yet clear why these differences CB_1 receptor expression occur, they may be reflective of the peripheral role of cannabinoids as modulators of lipogenesis (Osei-Hyiaman *et al*., 2005, Loftus *et al*., 2001, Kim *et al*., 2002).

While a mechanism for these changes in leptin, anandamide and CB_1 receptors has not yet been demonstrated, it may be related to the various and sometimes disparate actions of endocannabinoids. Taken together, these results suggest that diet-induced obesity results in elevated levels of circulating leptin. In response to increased leptin levels, endocannabinoid synthesis declines in the hypothalamus. This diminished endocannabinoid tone results in a short-term increase in CB_1 receptor expression. If the high-fat diet is maintained for an extended period of time, however, anandamide levels rise because of the competitive inhibition of FAAH, or for some other yet unknown process. In response to the now elevated endocannabinoid tone, CB_1 receptor expression is downregulated.

The Development of Cannabinoid Tolerance

In vitro studies show that chronic treatment with cannabinoid agonists produces CB_1 downregulation (i.e., loss of binding sites) and desensitization (i.e., loss of G-protein effector activity; Sim-Selley, 2003). The endocannabinoid system has a great deal of adaptive capacity and significant cannabinoid tolerance can develop, even with intermittent exposure to cannabinoids (Wiley *et al*., 1993). The changes in CB_1 receptor density that occur after repeated exposure to exogenous cannabinoids like WIN 55,212-2 or Δ^9-tetrahydrocannabinol (Sim-Selley and Martin, 2002) are similar to the changes in CB_1 receptor density that are associated with the sustained consumption of a high-fat diet (Harrold et al, 2002).

Cannabinoid tolerance can also be measured *in vivo*. Cannabinoids transiently reduce body temperature (hypothermia), suppress spontaneous activity, induce catalepsy (Wiley and Martin, 2003) and increase food consumption (hyperphagia; Wiley et al., 2005). When animals are repeatedly treated with exogenous cannabinoids like Δ^9-tetrahydrocannabinol, hypothermia diminishes, levels of spontaneous activity return toward the baseline, catalepsy wanes (Fan *et al*., 1994), and hyperphagia dissipates (Jarbe and Di Patrizio, 2005).

Like most psychoactive drugs, cannabinoids can produce context-specific tolerance through Pavlovian conditioning (Hill *et al*., 2004). The pharmacological effects of cannabinoids serve as an unconditioned stimulus and the environment in which those effects are experienced can serve as a conditioned stimulus. When the pharmacological effects of a drug are repeatedly and exclusively paired with a particular environment (i.e.,

a behavioral pharmacology lab), context-specific (or behavioral) tolerance can develop. In this phenomenon, the conditioned stimulus acquires the ability to elicit compensatory responses that diminish the effects of the drug in question. Though some of the most striking examples of context-specific tolerance involve opioids (Siegel, 1976), similar results can be observed with repeated exposure to any psychoactive drug under the correct conditions.

A nascent body of evidence suggests that sustained consumption of a high-fat diet is related to region-specific reductions of CB_1 receptor expression in the brain. This reduction in CB_1 receptor density is similar to changes that are observed in rodents made tolerant to cannabinoids. It is not clear, however, whether the receptor changes observed after sustained consumption of a high-fat diet are behaviorally or physiologically relevant. Because of the nature of these CB_1 receptor changes, it is hypothesized that rats maintained on a high-fat diet will be less sensitive to some or all of the pharmacological effects of Δ^9-THC. Because of sex-related differences in cannabinoid responses, it is also hypothesized that diet-related behavioral and physiological in cannabinoid pharmacology will be more evident in female rats than in male rats.

Method

Animals

All of the research in this proposal was conducted on male and female Long-Evans rats (Harlan Labs, Dublin, Virginia). All rats were single-housed in 11" X 17" X 8" Makrolon cages in a temperature-controlled (20°C–22°C) environment with 12-hour light and dark cycle (lights on at 6:00 am). Water and the appropriate type of rodent chow were freely available in the home cages. Food consumption and bodyweight were recorded twice each week and average daily food intake and weight change was calculated. Each of these studies was carried out in accordance with the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996) and was approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee. Virginia Commonwealth University is an AAALAC accredited institution.

Diet

The high-fat diet used in these experiments was made by enriching standard rodent chow (Harlan Teklad Diet LM-485) with corn oil. The standard rodent chow was soaked in room-temperature corn oil (20°C-22°C) for 24 hours before being drained and dried on clean paper towels. Once prepared, the diet was sealed in plastic bags immediately and stored at 4°C for a duration of no more than 7 days. Probe trials have determined that when soaked for 24 hours in room-temperature corn oil, the resulting diet contained 4.63 kcal/g with 33.3% of those calories coming from fat (Fig. 1). This diet was appropriate for these experiments because it was similar to diets used in related experiments that were performed in other labs and because it contained levels of fat that

are similar to the dietary intake of fat of the U.S. population (Kennedy *et al*., 1999).

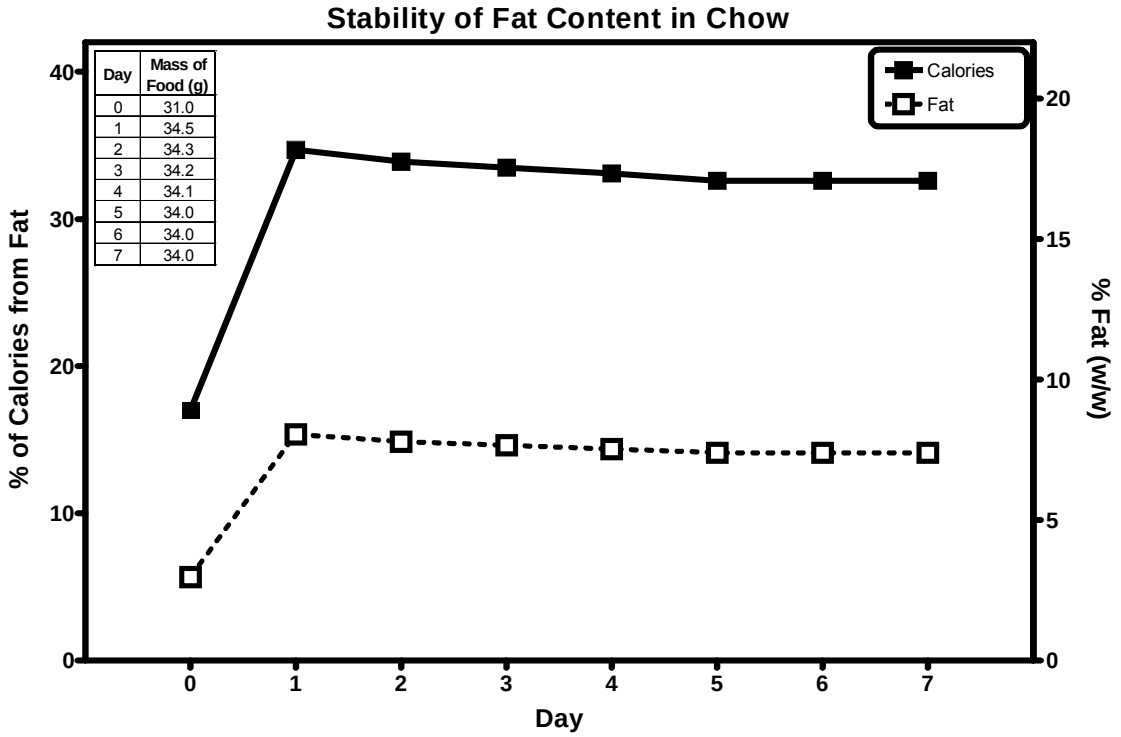

Figure 1. Fat content of high-fat diet. Unfortified chow derives 17% of digestible calories from fat. After soaking in corn oil at room temperature (20°C-22°C) for 24 hours, fat content increases to 34.7% of digestible calories. Over the course of the following 4 days, 13% of the absorbed fat leeches from the chow. By the 5th day after soaking, fat content stabilizes. The average percentage of calories derived from fat over the shelf life of the food (7 days) is 33.3%.

In contrast, rats fed the control diet (Harlan Teklad Diet LM-485; 3.75 kcal/g) derived 17% of their caloric intake from fat. The food for the control animals was kept in a sealed container that was maintained at room temperature (20°C-22°C) until dispensed into the feeding hopper in the home cage. In order to control for inter-group differences in the freshness of the food, the standard chow was replenished at the same rate as the high-fat chow (e.g., once every 7 days).

Drugs

Delta-9-THC was obtained from the Drug Supply Program of the National Institute on Drug Abuse and dissolved in a solution of Tween 80 (7.8%) and physiological saline (92.2%). All injections of Δ^9-THC were administered intraperitoneally at a volume of 1 ml/kg, with the exception the 70 mg/kg dose. The 70 mg/kg dose was derived from a 35 mg/ml solution and was administered at a volume 2 ml/kg.

Apparatus

Clear plastic cages (44 cm in length X 22.5 cm in width X 20 cm in height) covered with ventilated lids and housed in sound-attenuating cabinets were used as locomotor chambers. The chambers did not contain bedding and were cleaned with an alcohol solution between sessions. The sessions were conducted in near dark conditions as the cabinet doors were closed. A cage rack system with photocell beams along the x-axis (8 beams) and y-axis (4 beams; Lafayette Instrument, Lafayette, IN) were placed around each chamber and positioned 4.5 cm above the chamber floor. Locomotor activity was measured as total number of beams broken during the 5-minute session.

A Traceable® digital thermometer (Control Company, Friendsville, TX) equipped with a flexible, plastic-coated probe was used to measure rectal temperature. Probes measuring 1.5 mm in diameter were used for determining temperatures in adolescent rats (e.g., PD30 and PD44) and probes 3.5 mm in diameter were used for adult rats (e.g., PD68 and PD 114).

The bar apparatus that was used to measure catalepsy-like behavior consisted of a 280-mm long bolt (12.5 mm in diameter) that was attached to a vertical frame by eyebolts. The height of the bar was adjusted based upon the age of the rat (80 mm high at PD30, 100 mm high at PD44, and 130 mm at PD68 and PD114). To reduce the influence of extraneous stimuli, each bar apparatus was enclosed on the back, top and sides. The cardboard enclosure was open in the front to permit access for the experimenter. Unlike the locomotion sessions, which were conducted in near dark conditions, rectal temperature and performance on the bar apparatus will be measured under normal indoor fluorescent lighting conditions.

Procedure

Females. A group of female Long-Evans rats (n = 9), was fed a high-fat diet (4.63 kcal/g; 33% of calories from fat) beginning at postnatal day 30 (PD30). A separate group of female Long-Evans rats (n = 9) was fed a control diet (3.75 kcal/g; 17% of calories from fat) for the duration of these experiments. For female rats, cannabinoid activity was assessed in a triad of measures (hypothermia, catalepsy and spontaneous activity) on PD30, PD44, PD68, and PD114. These test points correspond to animal models of early adolescence, late adolescence, early adulthood and full maturity, respectively.

In order to obtain a full dose-effect curve in each rat, Δ^9-THC was administered in a cumulative dosing procedure (0, 3, 10, 30 and 100 mg/kg) on each of these days (Table 1). The cumulative dosing sessions began with the determination of body weight and baseline body temperature followed by an intraperitoneal injection of vehicle. Temperature was measured by inserting a rectal thermometer probe 40 mm (adolescents)

or 45 mm (adults) into the rectum. The probe was dipped in mineral oil maintained at 37^{o}C for lubrication before insertion into the rectum. The rats were then returned to their home cages for 20 minutes. When 20 minutes had elapsed, the rats were placed in a locomotion chamber for 5 minutes.

Table 1

Cumulative dosing matrix

Time (mins)	Procedure
-5	Baseline Body Temperature
0	Vehicle Injection
20	Locomotion
25	Body Temperature
30	Time on Bar Apparatus
35	3 mg/kg THC Injection
55	Locomotion
60	Body Temperature
65	Time on Bar Apparatus
70	7 mg/kg THC Injection
90	Locomotion
95	Body Temperature
100	Time on Bar Apparatus
105	20 mg/kg THC Injection
125	Locomotion
130	Body Temperature
135	Time on Bar Apparatus
140	70 mg/kg THC Injection
160	Locomotion
165	Body Temperature
170	Time on Bar Apparatus

During the locomotion session, measures of fine movement (i.e., breaking a single beam that lies adjacent to the rat's position) and ambulatory movement (i.e., breaking two or more beams as the rat moves across the chamber floor) were recorded. The software

that recorded movement in the chamber automatically discounted rapid, successive interruptions of the same beam because these types of data are indicative of stereotypic behavior and were not included in the measure of gross locomotion. The system also quantified movement along the periphery of the chamber as well as movement near the center of the chamber.

At the end of the locomotion session, body temperature was measured again and then the rats were immediately placed on the bar apparatus for up to 5 minutes. During the 5-minute bar apparatus session, the total amount of time (in seconds) that the front paws of a rat remained in contact with the bar was recorded. If both of the paws dropped from the bar, the animal was repositioned as before. The session timer was stopped briefly while the animal was repositioned. If the rat voluntarily removed both paws from the bar 10 times during the session, the session was be stopped, and amount of time spent with both paws on the bar was recorded as "zero". Each rat was tested in all three of these procedures. The total amount of time required for these measures was no more than 15 minutes (i.e., 5 minutes in the locomotion chamber plus 5 minutes on the bar apparatus plus time required for measurement of body temperature).

Immediately after the end of the bar apparatus session, the rats were injected intraperitoneally with a 3 mg/kg dose of Δ^9-THC and returned to their home cage. When 20 minutes had elapsed, the entire triad of measures was repeated. These measures were taken 3 more times after the rats have received successive intraperitoneal injections of 7 mg/kg, 20 mg/kg and 70 mg/kg Δ^9-THC. By using this method, a full dose-effect curve (0-100 mg/kg Δ^9-THC) was generated for all of the rats at each age point.

On postnatal day 128, Δ^9-THC-induced antinociception was measured with a radiant-heat, tail-flick analgesia meter (Model #1430, Columbus Instruments, Columbus, OH). The animals were weighed and injected intraperitoneally with vehicle (92.2% saline, 7.8% Tween 80) and returned to their home cage. After 30 minutes, latency to flick their tail away from the heat source was measured. Immediately after this baseline response was recorded, the animals were injected intraperitoneally with Δ^9-THC (30 mg/kg) and again returned to their home cage. After another 30 minutes had elapsed, a second tail-flick measurement was taken.

Subsequently, experiments were conducted to examine how Δ^9-THC affected food intake. In order to avoid carry-over effects, the first of the feeding trials was conducted fourteen days after the antinociception session (postnatal day 142). The rats were allowed *ad libitum* access to their normal food and water until they were weighed and injected intraperitoneally with Δ^9-THC. These studies will be conducted under free-feeding conditions because the high feeding rates that are commonly seen in food-deprived rats can obscure drug effects (Williams and Kirkham, 2002).

Once initiated, feeding trials were conducted every 7 days until all doses of Δ^9-THC (0, 0.3, 1, 3, 10 and 30 mg/kg) were tested. The various doses of Δ^9-THC were delivered a randomized order (modified Latin squares) so that drug dose did not vary systematically with age. After treatment with Δ^9-THC, the rats were returned to their home cage for 30 minutes. All of the food was removed from the home cage at this time. After the 30-minute pretreatment time had elapsed, a pre-weighed portion of the normal food (i.e., standard chow for rats on the control diet and high-fat chow for rats on the

high-fat diet) was placed in the home cage. After 1 hour, the food portion was removed, weighed and immediately returned to the cage. This procedure was repeated after another hour had elapsed. The food portion was removed and weighed again after 3 hours had elapsed and the total mass of food consumed during the feeding trial was recorded.

Males. A group of male Long-Evans rats (n = 9), was fed a high-fat diet (4.63 kcal/g; 33% of calories from fat) beginning at postnatal day 30 (PD30). A separate group of male Long-Evans rats (n = 9) was fed a control diet (3.75 kcal/g; 17% of calories from fat) for the duration of these experiments. For male rats, cannabinoid activity was assessed in a triad of measures (hypothermia, catalepsy and spontaneous activity) on PD30, PD37, PD44, PD61, PD68, and PD114. The procedure utilized at each of these age points was identical to the procedure for the described for the female rats. By using this method, a full dose-effect curve (0-100 mg/kg Δ^9-THC) was generated for all of the rats at each age point.

Subsequently, experiments were conducted to examine how Δ^9-THC affected food intake. In order to avoid carry-over effects, the first of the feeding trials was conducted fourteen days after the last cumulative dosing session (postnatal day 128). These feeding trials were conducted under both free-fed conditions and after 24 hours of food deprivation. Once initiated, feeding trials were conducted every 7 days until all doses of Δ^9-THC (0, 0.3, 1, 3, and 10 mg/kg) were tested under each condition. The nutritional status of the rats (food-deprived *vs.* free-fed) and the various doses of Δ^9-THC were delivered a randomized order so that neither condition nor drug dose varied systematically with age.

After treatment with Δ^9-THC, the rats were returned to their home cage for 30 minutes. All of the food was removed from the home cage at this time. After the 30-minute pretreatment time had elapsed, a pre-weighed portion of the normal food (i.e., standard chow for rats on the control diet and high-fat chow for rats on the high-fat diet) was placed in the home cage. After 1 hour, the pre-weighed portion of food was removed, weighed and the total mass of food consumed during the feeding trial was recorded.

When the male rats were aged 19 months, Δ^9-THC-induced antinociception was measured with a radiant-heat, tail-flick analgesia meter (Model #1430, Columbus Instruments, Columbus, OH). The procedure for measuring the antinociceptive properties of Δ^9-THC in male rats was the same as the above-described procedure that was used with the female rats.

Two separate groups of male rats (n = 4) were used to explore the possibility that context-specific (or behavioral) tolerance affected the repeated measures that are intrinsic to this experimental design (Table 1). For each group, Δ^9-THC-induced hypothermia was measured in a cumulative dosing procedure (0-100 mg/kg) on postnatal day 30 and again on postnatal day 37. For the first group, both of these experiments was conducted in an identical environment (e.g., the same behavioral pharmacology lab). For the second group of rats, the two experiments were conducted in two disparate environments (e.g., a behavioral pharmacology lab and in the vivarium).

Data Analysis

Sample size for each experiment was determined with SigmaStat (ver. 3.5; Systat Software Corporation, Point Richmond, CA). Parameters entered in this program

included effect size, standard deviation, number of groups, desired power, and alpha level. Values for the proposed studies, a small-to-medium effect size (0.15; Cohen *et al*.,

Table 2

Methodology for Examining Context-specific Tolerance

	Location of 1st Test	**Location of 2nd test**
Group 1	Behavioral Pharmacology Lab	Behavioral Pharmacology Lab
Group 2	Behavioral Pharmacology Lab	Vivarium

2003) was chosen for the power analysis. Based on standard deviations derived from probe trials, the standard deviation for these calculations will be estimated at 0.125. By convention, the desired power level will be set at 0.80 and the alpha level will be set at 0.05 (Howell, 1997).

SigmaStat calculated that a sample size of 9 per group was necessary in order to detect differences based on these parameters. This sample size was consistent with sizes used by this lab previously in similar studies and was used for all studies in this protocol. In total, 44 animals were required to complete these studies. These studies commenced on postnatal day 30 for both male and female rats and and concluded on postnatal day 190 for female rats and on or about postnatal day 578 for male rats.

All statistical analyses, except ED_{50} calculations, were conducted using SigmaStat statistical software. GraphPad Prism was used to determine ED_{50} values and 95%

confidence limits as necessary. Growth rates were compared by linear regression analysis. Bodyweights, average daily food consumption, hypothermia, locomotor activity, time with paws on bar apparatus, tail-flick latencies and food-intake were analyzed with a split-plot ANOVA. The development of context-specific tolerance was analyzed with a two-factor (dose, location of second test), repeated measures ANOVA. Changes in tail-flick latency were be analyzed with a one-factor (diet) ANOVA. Tukey post hoc tests ($\alpha = 0.05$) will be used to specify differences revealed by significant ANOVAs. The diet-related changes in potency of Δ^9-THC (ED_{50} values and 95% confidence limits) were determined by fitting a non-linear regression equation against the corresponding dose-effect curve. Each measurement was plotted against the common logarithm of the dose. In order to determine the ED_{50} values for Δ^9-THC, the amount of time with paws on bar apparatus was expressed as a percentage of the total session length (300 seconds). Similarly, locomotor activity at various doses of Δ^9-THC was expressed as a percentage of baseline locomotor activity.

Results

Effects of a High-fat Diet on Bodyweight

Females. As expected, female rats gained a significant amount of weight over the course of development [main effect of postnatal day, $F(26, 416) = 301.204$, $p < 0.001$]. Further, female rats fed a high fat diet overall weighed significantly more than those fed a control diet [main effect of diet, $F(1, 16) = 37.483$, $p < 0.001$], (Fig. 2). A significant interaction between diet and postnatal day on bodyweights was not detected, $F(26, 416) = 0.9136$, $p < 0.590$.

Males. Male rats also exhibited a significant main effect of diet on bodyweight, $F(1, 15) = 126.804$, $p < 0.001$, with those fed a high fat diet being heavier, as well as a significant main effect of postnatal day on bodyweight, $F(26, 390) = 671.428$, $p < 0.001$ (Fig. 3). No significant interaction between diet and postnatal day on bodyweight was detected, $F(26, 390) = 0.7885$, $p = 0.7631$.

Psychomotor Effects – Time on Bar Apparatus

Females. Baseline measurements of the amount of time female rats spent on the bar apparatus were conducted on postnatal day 30. At baseline, there was a significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus, $F(4, 64) = 85.164$, $p < 0.001$ (Fig. 4). When tested on postnatal day 30, both groups of rats spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg /kg and

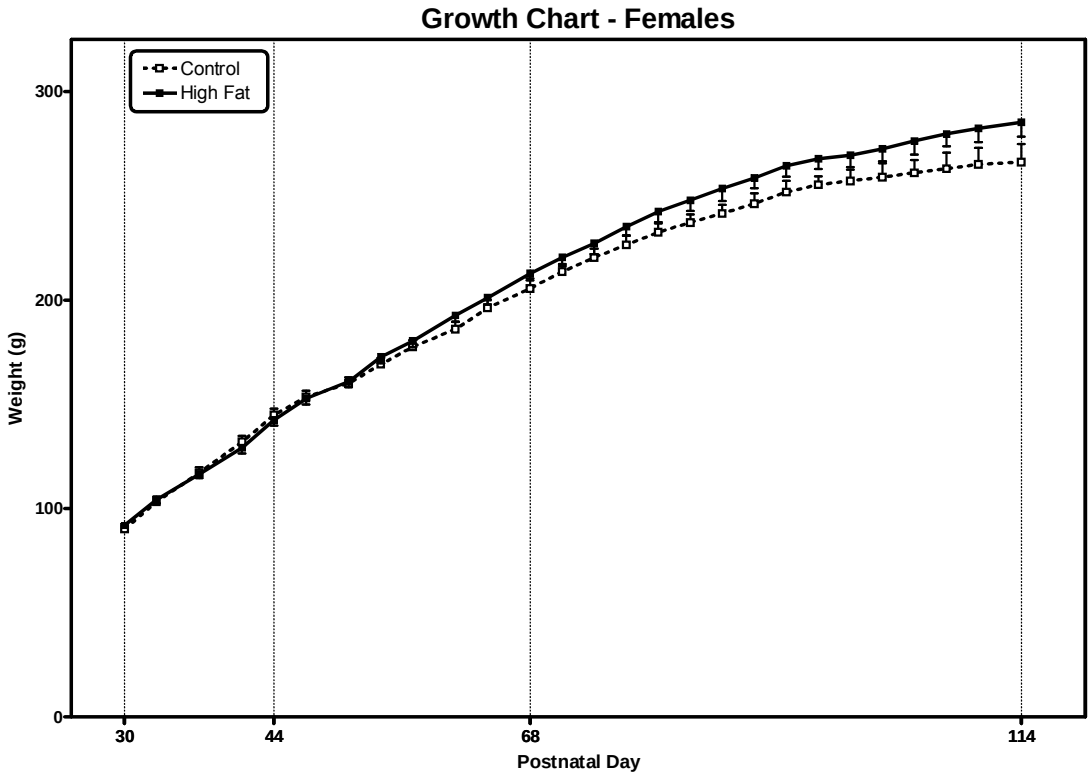

Figure 2. Growth chart for female rats. Despite the presence of significant main effects of diet and postnatal day on bodyweight, no significant interactions of diet and postnatal day on bodyweight were detected.

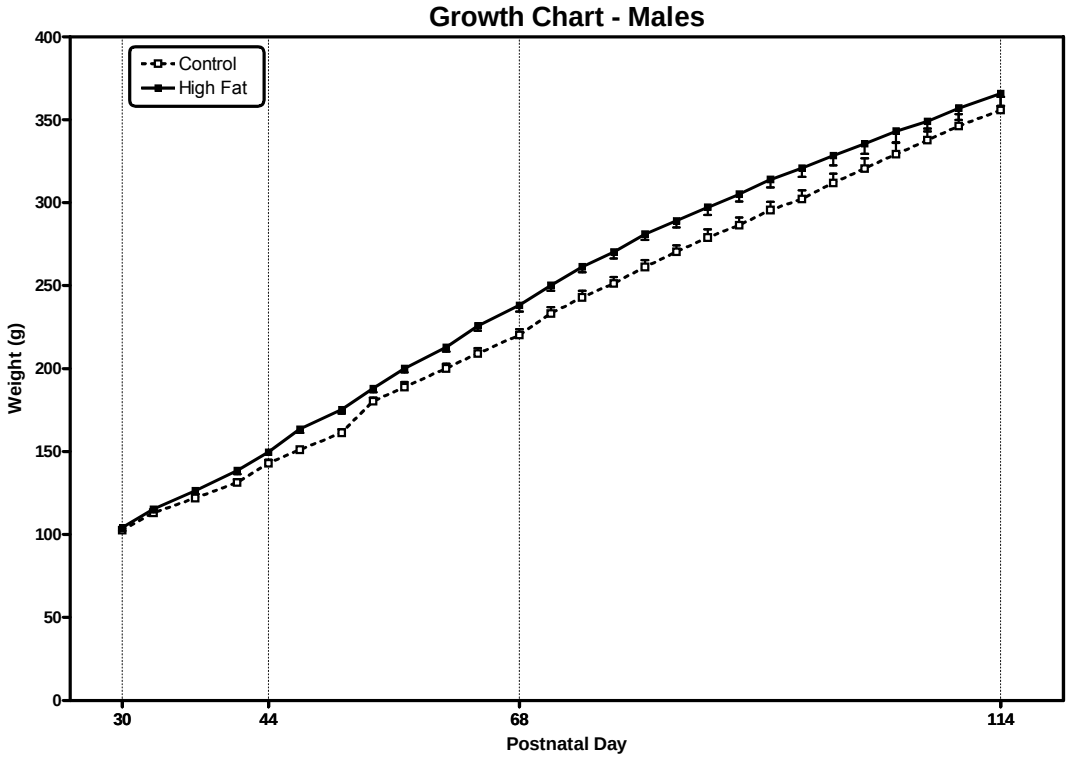

Figure 3. Growth chart for male rats. Despite the presence of significant main effects of diet and postnatal day on bodyweight, no significant interactions of diet and postnatal day on bodyweight were detected.

100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). In contrast, a 3 mg/kg dose of Δ^9-THC did not significantly increase the amount of time the rats spent on the bar apparatus. However, there was no significant main effect of diet on the amount of time spent on bar apparatus, $F(1, 16) = 0.593$, $p = 0.452$ and no significant interaction between diet and dose of Δ^9-THC was detected, $F(4, 64) = 0.606$, $p = 0.659$.

The results for the amount of time female rats spent on the bar apparatus at postnatal day 44 were similar to the results observed on postnatal day 30. At postnatal day 44, there was again a significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus, $F(4, 64) = 123.167$, $p < 0.001$ (Fig. 5). Both groups of rats spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). Again, a 3 mg/kg dose of Δ^9-THC did not significantly increase the amount of time the rats spent on the bar apparatus when tested at postnatal day 44. There was no main effect of diet on the amount of time spent on bar apparatus, $F(1,16) = 2.806$, $p = 0.113$) and no significant interaction between diet and dose of Δ^9-THC was detected, $F(4, 64) = 1.384$, ($p = 0.250$).

By postnatal day 68, however, significant diet-related differences in the amount of time spent on the bar apparatus became apparent (Fig. 6). Analyses revealed a significant interaction between the type of diet consumed and the dose of Δ^9-THC administered, $F(4, 64) = 2.685$, $p = 0.039$. At this age point, the amount of time that female rats spent with their paws on the bar apparatus after treatment with Δ^9-THC was dependent upon which

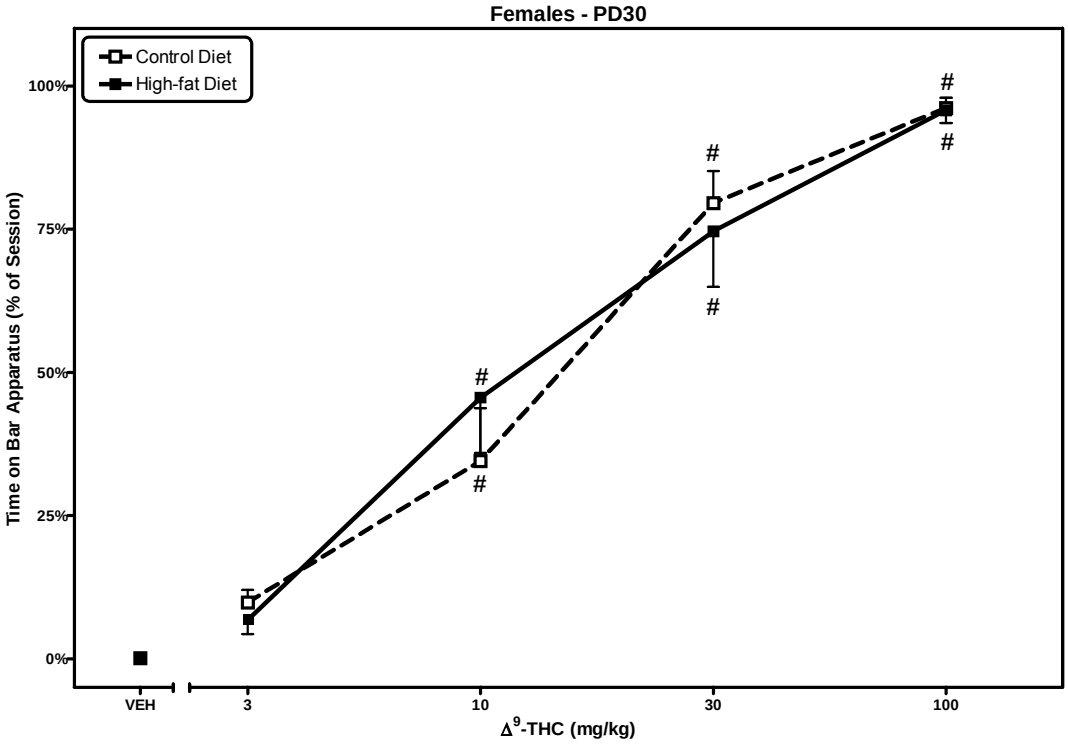

Figure 4. Time on bar apparatus for female rats at PD30. Expressed as percentage of the total session time (mean +/- SEM) that female rats remained on the bar apparatus at postnatal day 30. While Δ^9-THC produced a significant, dose-dependent increase in time on the bar apparatus, the groups exhibited indistinguishable drug responses. Data points highlighted with an "#" are significantly different from the respective vehicle-treated conditions.

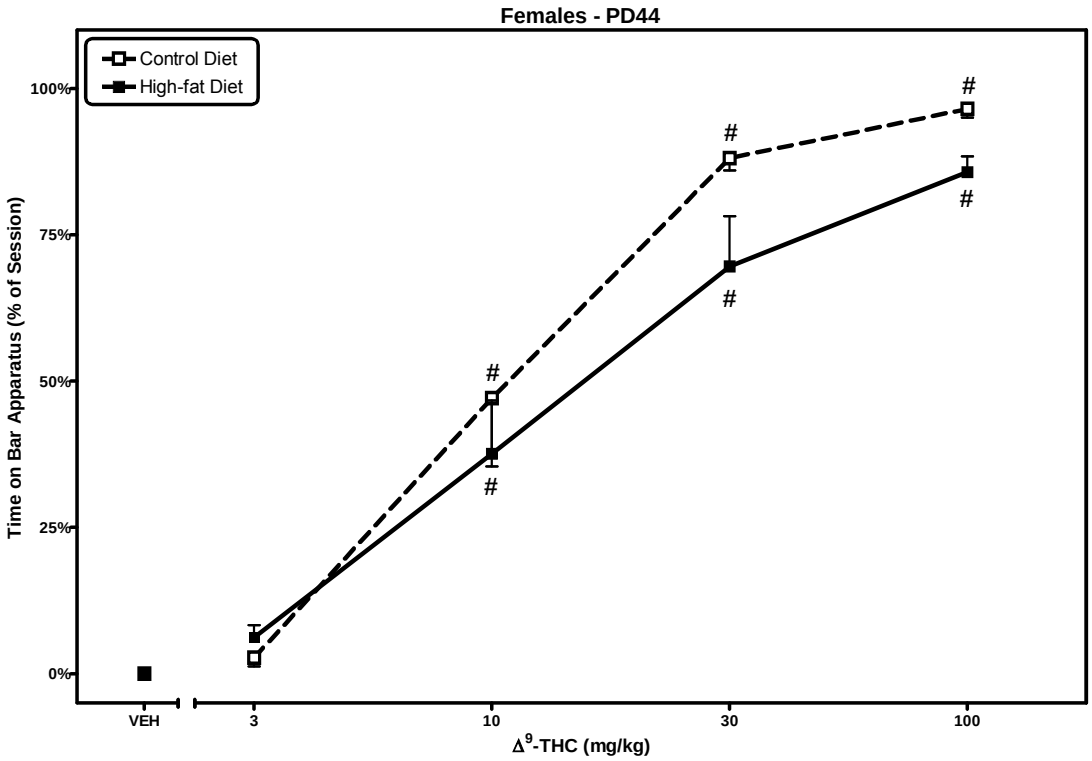

Figure 5. Time on bar apparatus for female rats at PD44. Expressed as percentage of the total session time (mean +/- SEM) that female rats remained on the bar apparatus at postnatal day 44. While Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus, no between group differences were noted. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

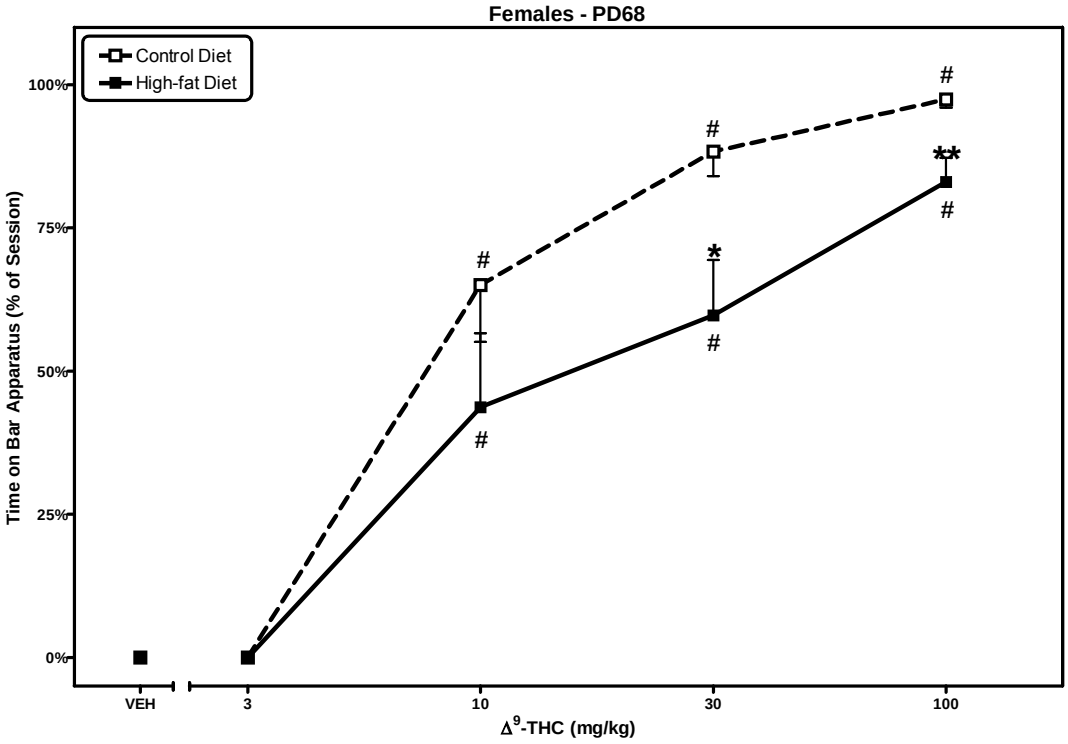

Figure 6. Time on bar apparatus for female rats at PD68. Expressed as a percentage of the total session time (mean +/- SEM) that female rats remained on the bar apparatus at postnatal day 68. A significant interaction between Δ^9-THC and the type of diet consumed was evident. Though Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus for both groups, the female rats maintained on a high-fat diet spent significantly less time on the bar apparatus than the female rats maintained on the control diet when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC. Data points highlighted with "#" are significantly different from the respective vehicle-treated conditions. Data points highlighted with asterisk are significantly different from each other. ("*****" indicates $p < 0.05$; "******" indicates $p < 0.01$.)

diet they consumed. Although Δ^9-THC produced a dose-dependent increase in the amount of time spent on the bar apparatus in both groups, the rats that were maintained on a high-fat diet spent significantly less time on the bar apparatus than did the rats maintained on the control diet when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC ($p < 0.05$ for both measures). Despite these differences in the amount of time spent on the bar apparatus at postnatal day 68, the 95% confidence intervals for the ED_{50} values of Δ^9-THC overlapped (Table 3), indicating the absence of diet-related differences in the potency of Δ^9-THC.

Table 3

Potency of Δ^9-THC in female rats.

Females		**PD30**		**PD44**	
		HFD	**Control**	**HFD**	**Control**
Bar Apparatus	**ED_{50} (mg/kg)**	12	15.7	12.9	10.3
	95% CI (mg/kg)	10.7-13.4	13.4-18.4	11.5-14.4	8.1-13.0
Locomotor Activity	**ED_{50} (mg/kg)**	147.8	46.3	n/a	2499
	95% CI (mg/kg)	19.5-1121	17.0-126.6	n/a	n/a
		PD68		**PD114**	
		HFD	**Control**	**HFD**	**Control**
Bar Apparatus	**ED_{50} (mg/kg)**	11.5	6.9	25.1	5.2
	95% CI (mg/kg)	8.8-14.9	4.9-9.7	20.6-30.6	3.5-7.7
Locomotor Activity	**ED_{50} (mg/kg)**	38	29.4	9.4	9.4
	95% CI (mg/kg)	27.1-53.2	1.9-466.2	5.1-17.2	5.1-17.2

When tested on postnatal day 114, the diet-related differences in the amount of time spent on the bar apparatus were again obvious (Fig. 7). Analyses revealed a significant interaction between the type of diet consumed and the dose of Δ^9-THC on the amount of time spent with forepaws on the bar apparatus, $F(4, 64) = 6.875$, $p < 0.001$. Again, Δ^9-THC produced a dose-dependent increase in the amount of time spent on the bar apparatus in both groups. Rats maintained on a high-fat diet spent significantly less time on the bar apparatus than did the rats maintained on the control diet when treated with 10 mg/kg Δ^9-THC and 30 mg/kg Δ^9-THC ($p < 0.05$ for both measures). On postnatal 114, the 95% confidence intervals for the ED_{50} values of Δ^9-THC did not overlap (Table 3). At this age point, Δ^9-THC was significantly less potent in the rats maintained on a high-fat diet than in the rats maintained on a control diet.

Even though there were significant inter-group differences in the amount of time spent on the bar apparatus after postnatal day 68, other non-systematic observations suggest that the measurements used in these experiments may underestimate the differences between the groups (Fig. 8). At doses of Δ^9-THC above 10 mg/kg, the female rats fed the control diet typically moved their heads and forepaws very little and appeared quite sedated while on the bar apparatus. At those same doses, the female rats fed a high-fat diet typically moved their heads, usually changed their posture at least once during the course of the 5-minute session on the bar apparatus and frequently re-positioned their paws or moved along the bar apparatus. At doses of Δ^9-THC above 30 mg/kg, female rats fed a high-fat diet commonly engaged in a series of repetitive head

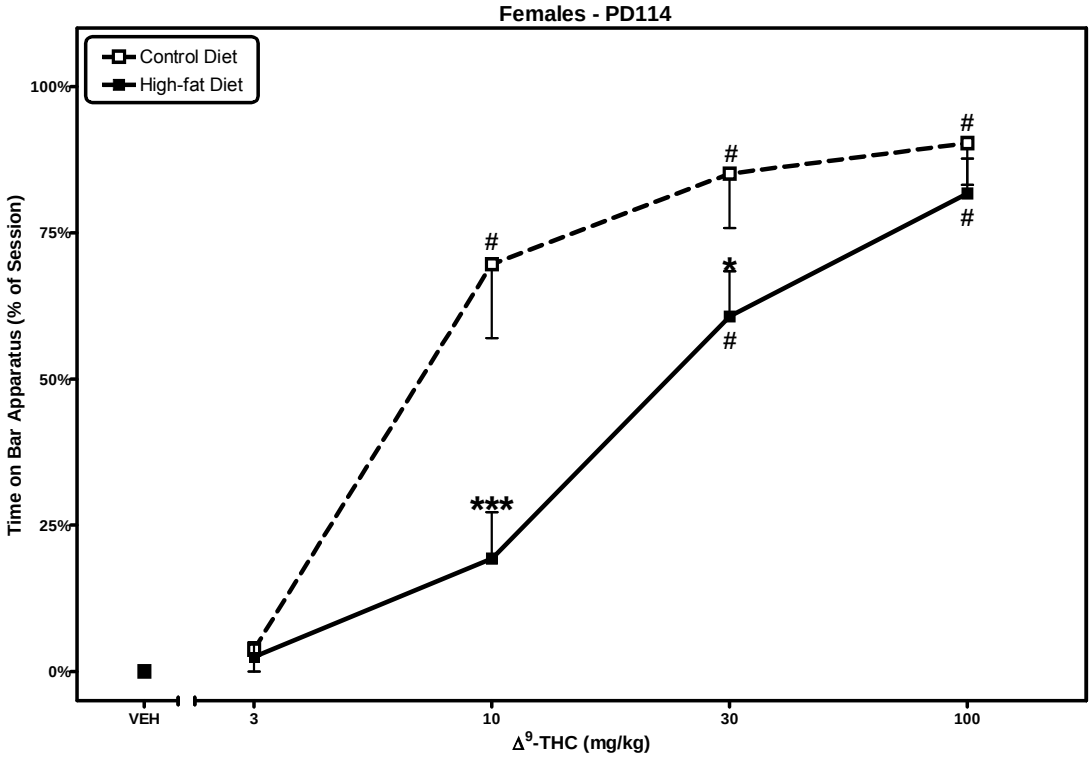

Figure 7. Time on bar apparatus for female rats at PD114. Percentage of the total session time (mean +/- SEM) that female rats remained on the bar apparatus at postnatal day 114. A significant interaction between Δ^9-THC and the type of diet consumed was evident. Though Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus for both groups, the female rats maintained on a high-fat diet spent significantly less time on the bar apparatus than the female rats maintained on the control diet when treated with 10 mg/kg and 30 mg/kg doses of Δ^9-THC. Data points highlighted with "#" are significantly different from the respective vehicle-treated conditions. Data points highlighted with asterisk are significantly different from each other. ("*****" indicates $p < 0.05$; "*******" indicates $p < 0.001$.)

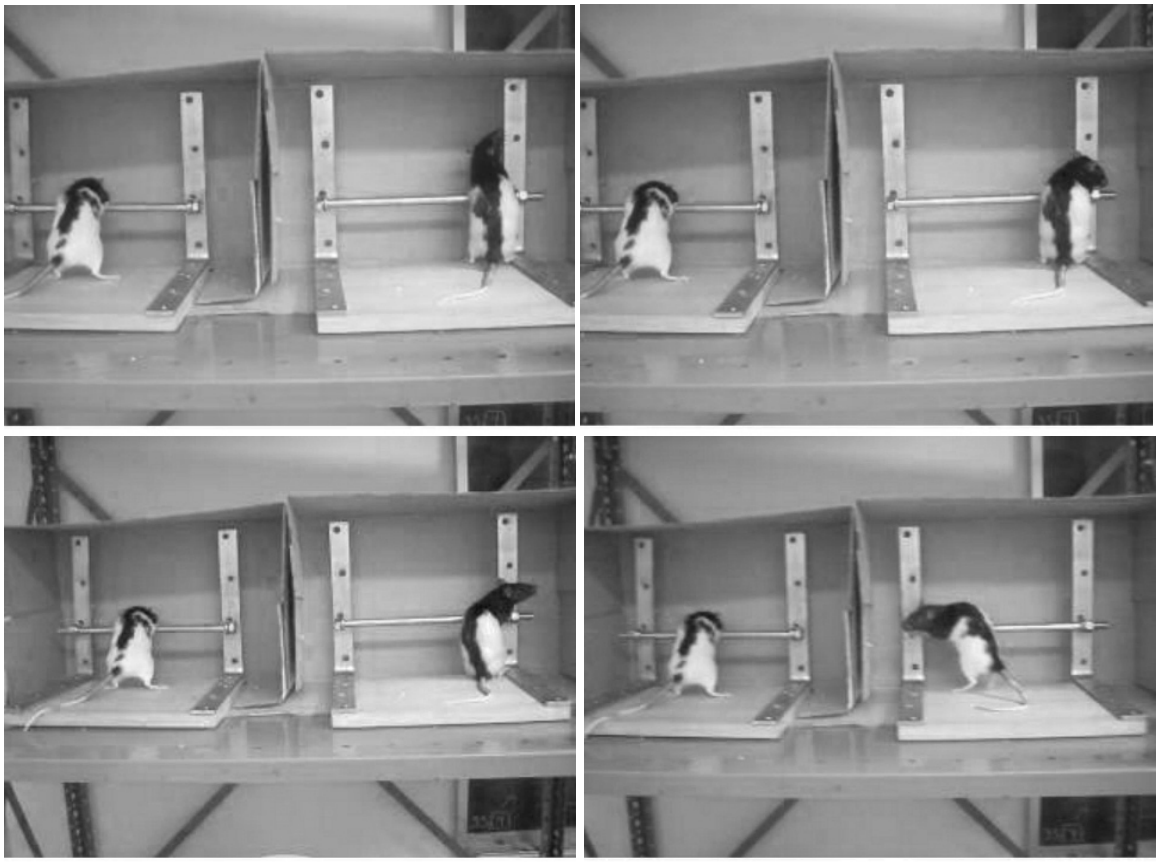

Figure 8. Differences in activity during bar apparatus sessions. Non-systematic observations suggest that the female rats maintained on a high-fat diet were significantly less affected by moderate and high doses of Δ^9-THC than were female rats maintained on the control diet. These photos were taken after the rats had been treated with a 10 mg/kg dose of Δ^9-THC. The female rat maintained on a high-fat diet (right) frequently exhibited overt head movements, shifts in posture and lateral movements along the bar apparatus. The female rat maintained on the control diet (left) was virtually immobile. These differences in activity were not captured by the operational definition the dependent variable because the front paws rarely the left bar apparatus while the rat maintained on a high-fat diet executed these movements.

movements. These movements were typically from side-to-side and were occasionally accompanied by a slight shift in posture or body position.

In general, there were no differences between the groups in the amount of time spent on the bar apparatus until the female rats reached early adulthood (postnatal day 68). After postnatal day 68, the female rats maintained on the high-fat diet were significantly less sensitive to Δ^9-THC-induced increases in the amount of time spent on the bar apparatus.

Males. Like the female rats, baseline measures of time spent on the bar apparatus for the male rats were taken on postnatal day 30. At baseline, there was a significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus, $F(4, 64) = 115.781$, $p < 0.001$ (Fig. 9). Both groups of male rats spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). A 3 mg/kg dose of Δ^9-THC, however, did not significantly increase the amount of time the male rats spent on the bar apparatus. There was no main effect of diet on the amount of time spent on bar apparatus, $F(1, 16) = 0.0534$, $p = 0.820$ and no significant interaction between diet and dose was detected, $F(4, 64) = 0.0137$, $p = 1.000$.

When the male rats were tested at postnatal day 37, the significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus persisted, $F(4, 60) = 50.829$, $p < 0.001$ (Fig. 10). Male rats maintained on the control diet spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). Male rats maintained on

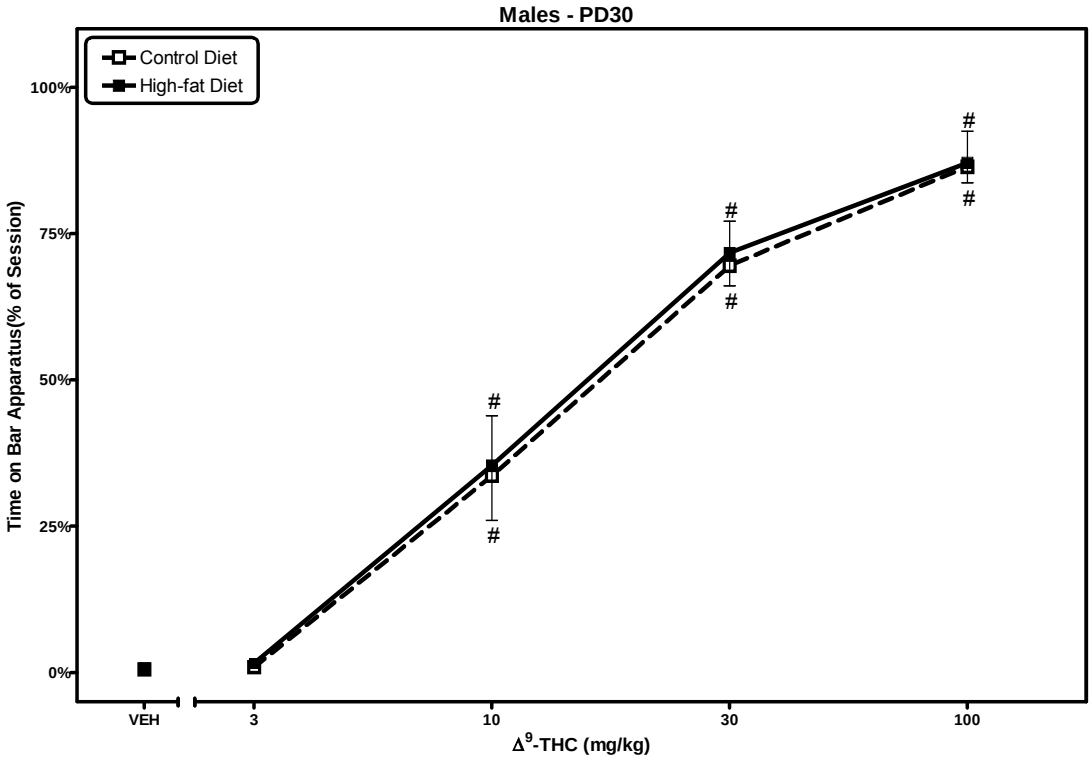

Figure 9. Time on bar apparatus for male rats at PD30. Percent of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 30. Though Δ^9-THC to produce a significant, dose-dependent increase in time on the bar apparatus, the groups were again indistinguishable in terms of their responses. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

a high-fat diet spent significantly more time on the bar apparatus when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for both measures). A 3 mg/kg dose of Δ^9-THC did not significantly increase the amount of time the rats spent on the bar apparatus for either group. At postnatal day 37, like at postnatal day 30, there was no main effect of diet on the amount of time spent on bar apparatus $F(1, 15) = 0.203$, $p = 0.659$) and no significant interaction between diet and dose was detected, $F(4, 60) = 0.419$, $p = 0.795$.

When the male rats were tested at postnatal day 44, there was again a significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus, $F(4, 60) = 30.387$, $p < 0.001$ (Fig. 12). Male rats maintained on the control diet spent significantly more time on the bar apparatus when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for both measures). Like previous tests, a 3 mg/kg dose of Δ^9-THC failed to significantly increase the amount of time the rats spent on the bar apparatus. Also like previous tests with male rats, there was no main effect of diet on the amount of time spent on bar apparatus $F(1, 15) = 0.00198$, $p = 0.965$) and no significant interaction between diet and dose was detected, $F(4, 60) = 1.316$, $p = 0.275$.

When the male rats were tested at postnatal day 61, the significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus was still apparent, $F(4, 60) = 36.510$, $p < 0.001$ (Fig. 13). Both groups of male rats diet spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). A 3 mg/kg dose of Δ^9-THC did not significantly increase the amount of time the rats spent on the bar apparatus

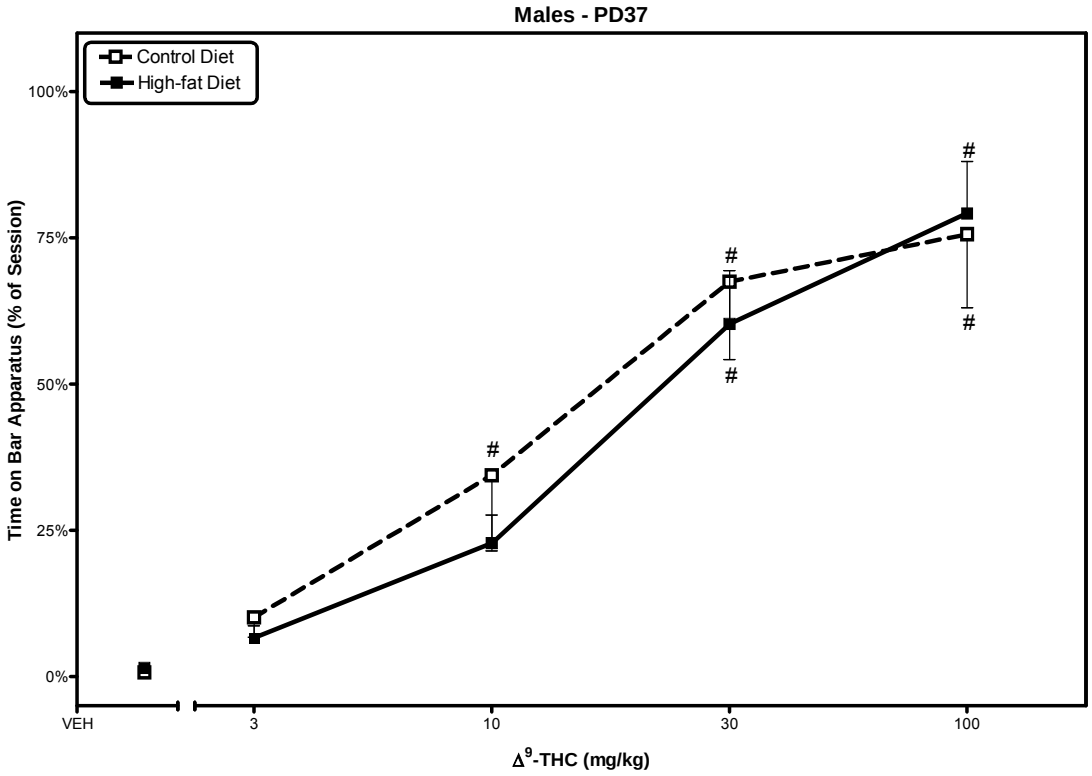

Figure 10. Time on bar apparatus for male rats at PD37. Percent of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 37. Just as at PD30, Δ^9-THC produced a significant, dose-dependent increase in time on the bar apparatus, but the responses between the groups were again indistinguishable. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

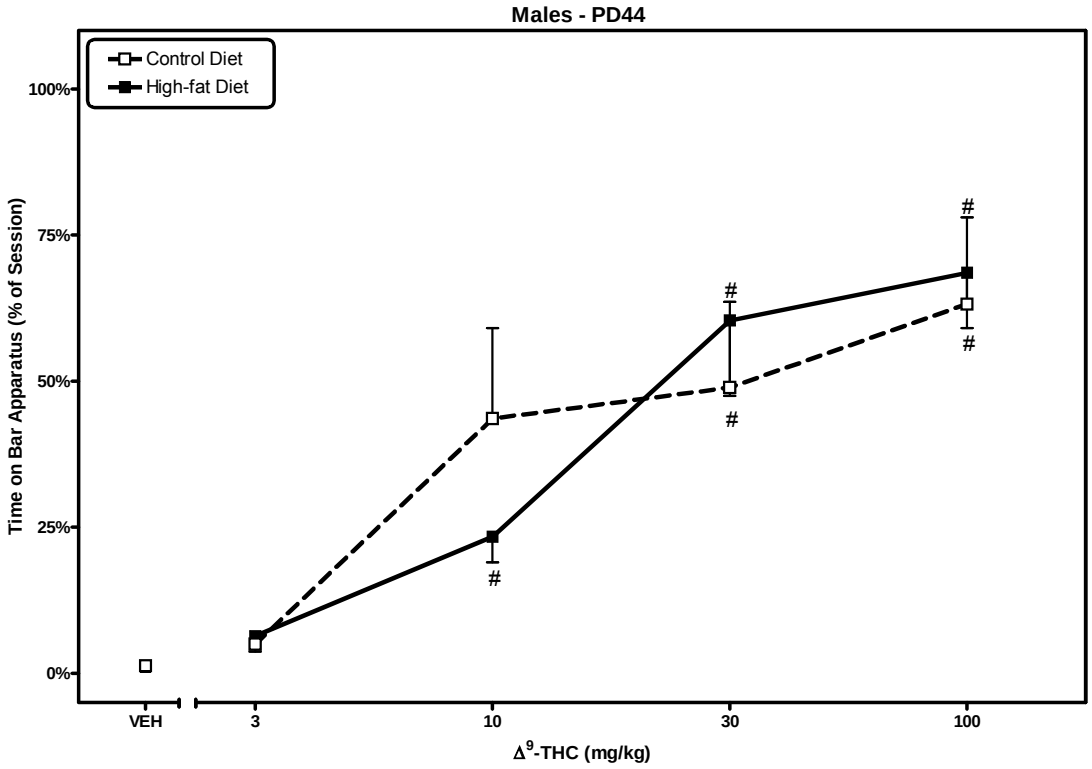

Figure 11. Time on bar apparatus for male rats at PD44. Percent of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 44. While Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus, the responses between the groups were again indistinguishable. Data points highlighted with an "#" are significantly different from the respective vehicle-treated conditions.

for either group. At postnatal day 61, there was no main effect of diet on the amount of time spent on bar apparatus $F(1, 15) = 0.599$, $p = 0.451$) and no significant interaction between diet and dose was detected, $F(4, 60) = 0.555$, $p = 0.696$.

For the male rats, the previously noted trends in the amount of time spent on the bar apparatus continued on postnatal day 68 (Fig. 13). At this age point, male rats displayed a significant main effect of Δ^9-THC on the amount of time spent on the bar apparatus, $F(4, 60) = 39.519$, $p < 0.001$. Like the previous tests, both groups of male rats spent significantly more time on the bar apparatus when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures) and a 3 mg/kg dose of Δ^9-THC did not increase the amount of time the rats spent on the bar apparatus significantly. There were again no main effect of diet on the amount of time spent on bar apparatus $F(1, 15) = 0.0176$, $p = 0.896$ and no significant interaction between diet and dose was detected, $F(4, 60) = 0.578$, $p = 0.680$.

When the male rats were tested on postnatal day 114, however, significant diet-related differences in the amount of time spent on the bar apparatus were evident (Fig. 14). At this age point, there was a significant interaction between the type of diet consumed and the dose of Δ^9-THC administered, $F(4, 60) = 8.230$, $p < 0.001$, with Δ^9-THC producing a significant dose-dependent increase in the amount of time spent on the bar apparatus in both groups. Male rats maintained on a high-fat diet spent significantly less time on the bar apparatus than did the rats maintained on the control diet when treated with 10 mg/kg Δ^9-THC ($p < 0.05$). On postnatal 114, Δ^9-THC also appeared to be significantly less potent in the male rats maintained on a high-fat diet than in the male

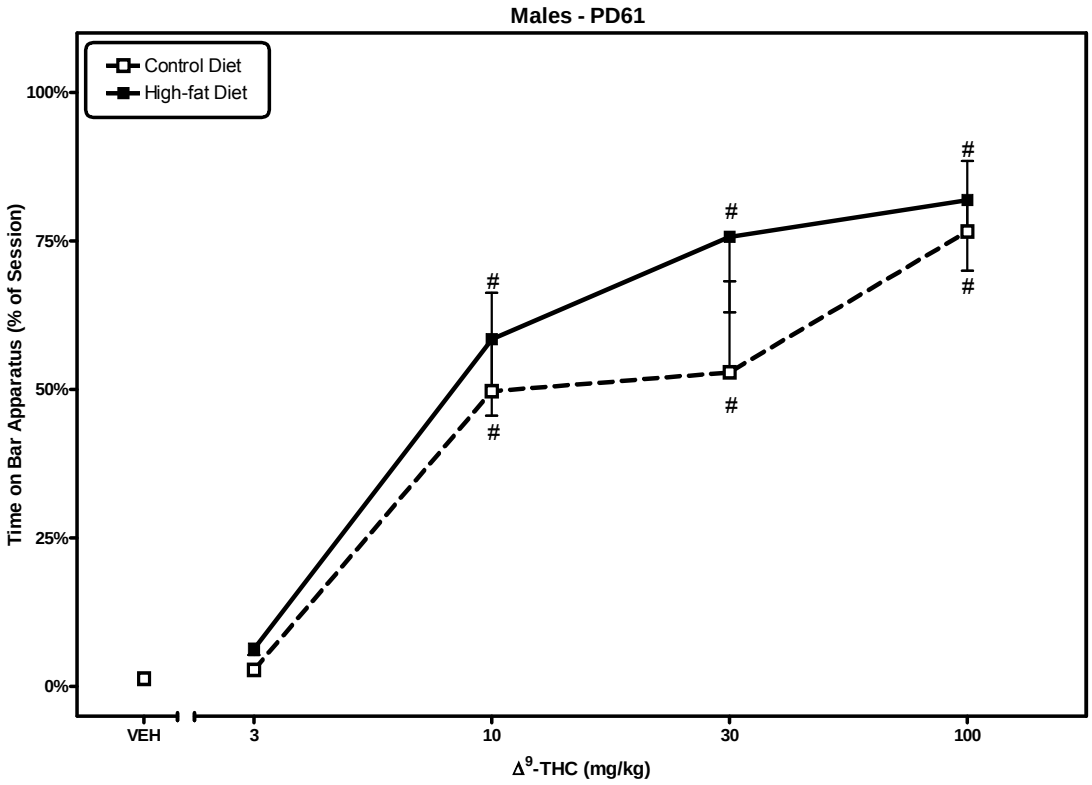

Figure 12. Time on bar apparatus for male rats at PD61. Percent of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 61. While Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus, the responses between the groups were again indistinguishable. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

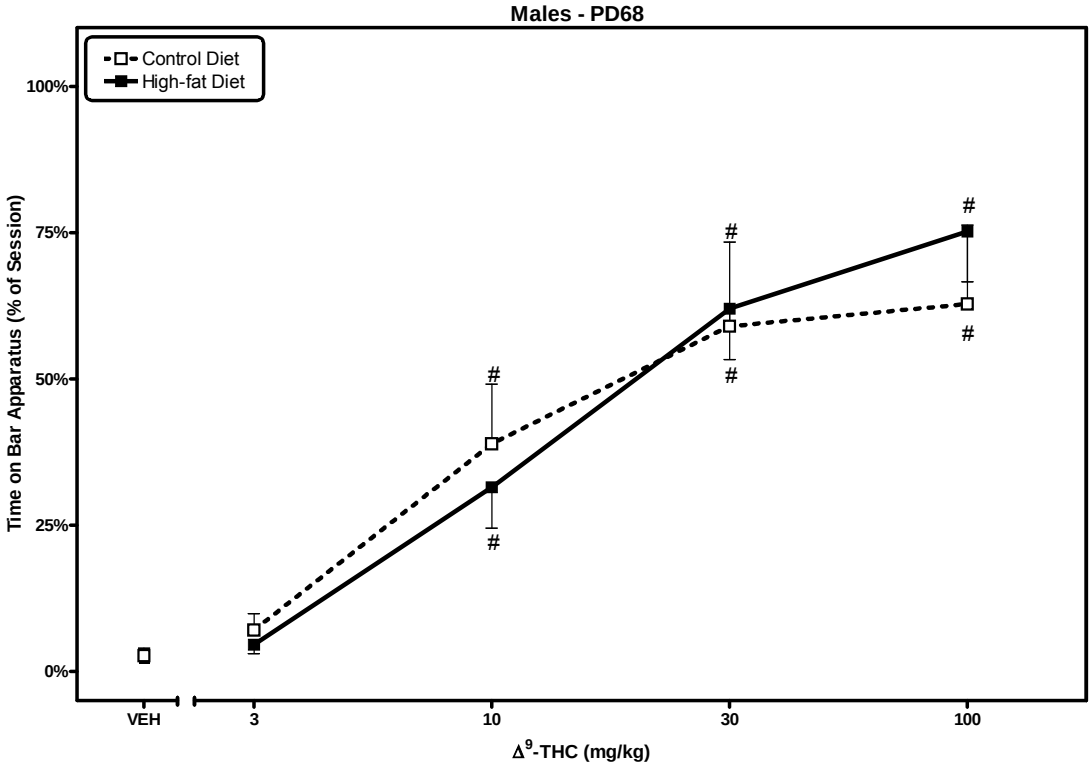

Figure 13. Time on bar apparatus for male rats at PD68. Percent of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 68. While Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus, the responses between the groups were again indistinguishable. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

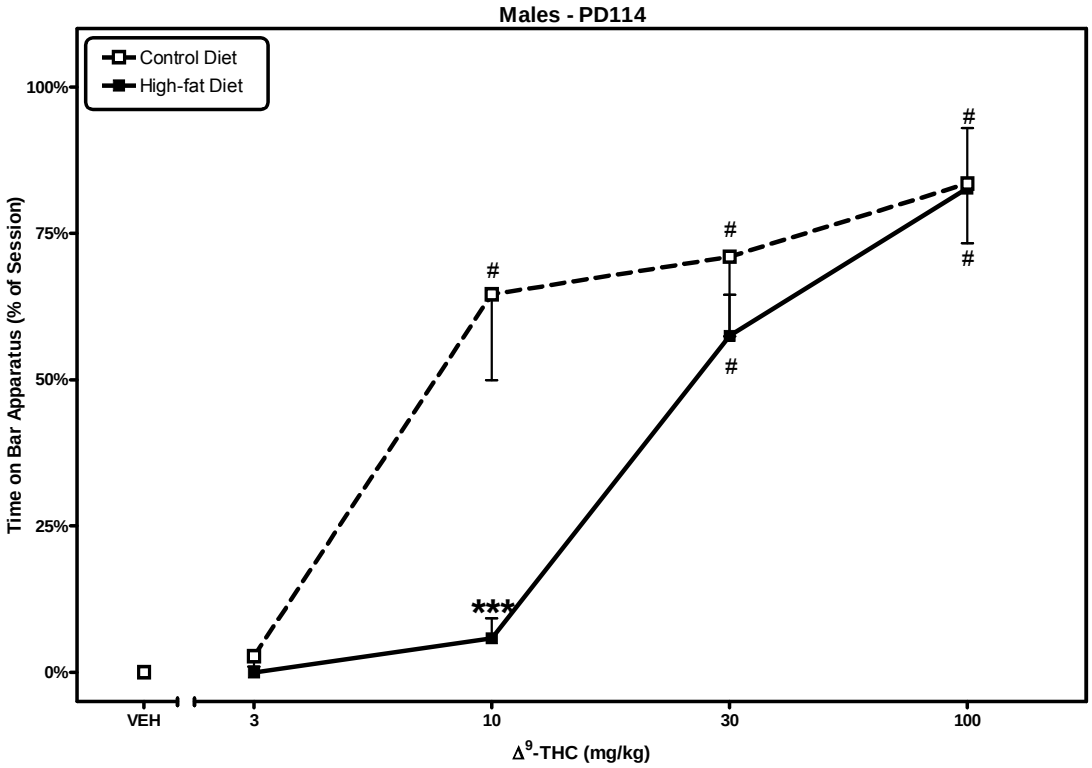

Figure 14. Time on bar apparatus for male rats at PD114. Percentage of the total session time (mean +/- SEM) that male rats remained on the bar apparatus at postnatal day 114. A significant interaction between Δ^9-THC and the type of diet consumed was evident. Though Δ^9-THC continued to produce a significant, dose-dependent increase in time on the bar apparatus for both groups, the male rats maintained on a high-fat diet spent significantly less time on the bar apparatus than the male rats maintained on the control diet when treated with a 10 mg/kg dose of Δ^9-THC. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions. Data points highlighted with asterisk are significantly different from each other. ("*******" indicates $p < 0.001$.)

rats maintained on a control diet. The 95% confidence intervals for the ED_{50} values of Δ^9-THC did not overlap (Table 4).

Table 4

Potency of Δ^9-THC in male rats.

Males		PD30		PD37		PD44	
		HFD	Control	HFD	Control	HFD	Control
Bar Apparatus	ED50 (mg/kg)	14.3	15.3	22.5	11.0	15.6	6.4
	95% CL (mg/kg)	11.8-17.3	12.8-18.4	19.1-26.5	9.3-13.1	12.3-19.9	4.5-9.2
Locomotor Activity	ED50 (mg/kg)	6.2	12.5	n/a	n/a	n/a	n/a
	95% CL (mg/kg)	4.3-9.1	8.6-18.3	n/a	n/a	n/a	n/a
		PD61		PD68		PD114	
		HFD	Control	HFD	Control	HFD	Control
Bar Apparatus	ED50 (mg/kg)	5.8	8.0	14.3	7.3	39.3	5.3
	95% CL (mg/kg)	4.2-8.0	5.2-12.2	12.2-16.9	5.7-9.4	27.2-56.8	3.4-8.3
Locomotor Activity	ED50 (mg/kg)	6.6	22.0	n/a	38.0	n/a	28.9
	95% CL (mg/kg)	5.3-8.2	13.9-34.8	n/a	27.1-53.2	n/a	22.5-37.1

Psychomotor Effects – Gross Locomotor Activity

Females. Baseline measures for gross locomotor activity in female rats were taken on postnatal day 30. At baseline, there was a significant main effect of Δ^9-THC on the

number of beams broken during locomotor testing session, $F(4, 64) = 23.764$, $p < 0.001$ (Fig. 15). Both groups of female rats broke fewer beams when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for both measures). A 3 mg/kg dose of Δ^9-THC did not significantly affect the number of beams broken during the session. There was no main effect of diet on the number of beams broken $F(1, 16) = 0.0110$, $p = 0.918$, and no significant interaction between the type of diet and the dose of Δ^9-THC on the number of beams broken was detected $F(4, 64) = 0.434$, $p = 0.783$.

At postnatal day 44, the significant main effect of Δ^9-THC on the number of beams broken persisted, $F(4, 64) = 14.058$, $p < 0.001$ (Fig. 16). Female rats broke significantly fewer beams when treated with a 100 mg/kg dose of Δ^9-THC. There was no main effect of diet on the number of beams broken $F(1, 16) = 1.910$, $p = 0.186$ and no significant interaction between the type of diet and the dose of Δ^9-THC on the number of beams broken was detected $F(4, 64) = 1.043$, $p = 0.392$.

By postnatal day 68, a significant interaction between the type of diet consumed and the dose of Δ^9-THC on the number of beams broken was evident, $F(4, 64) = 4.188$, $p = 0.004$ (Fig. 17). At this age, female rats maintained on a high-fat diet broke a significantly greater number of beams than did female rats maintained on a control diet when each group was treated with a 30 mg/kg and a 100 mg /kg dose of Δ^9-THC ($p < 0.05$ for both measures). For the females maintained on the control diet, the 95% confidence intervals for ED_{50} values of Δ^9-THC were 27.1 to 53.2 mg/kg (Table 3). In contrast, it was not possible to calculate ED_{50} values under this dosing regimen for the

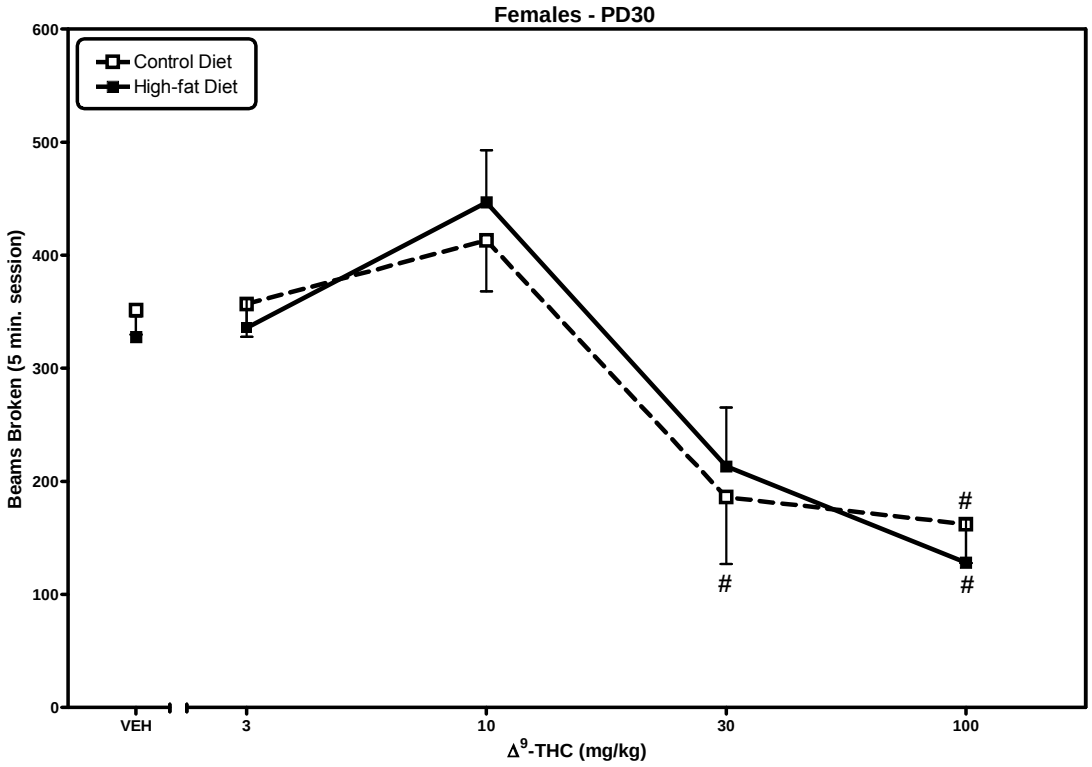

Figure 15. Locomotor activity for female rats at PD30. Number of beams broken (mean +/- SEM) by female rats after treatment with Δ^9-THC on postnatal day 30. Delta-9-THC dose-dependently decreased locomotor activity in both groups. No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

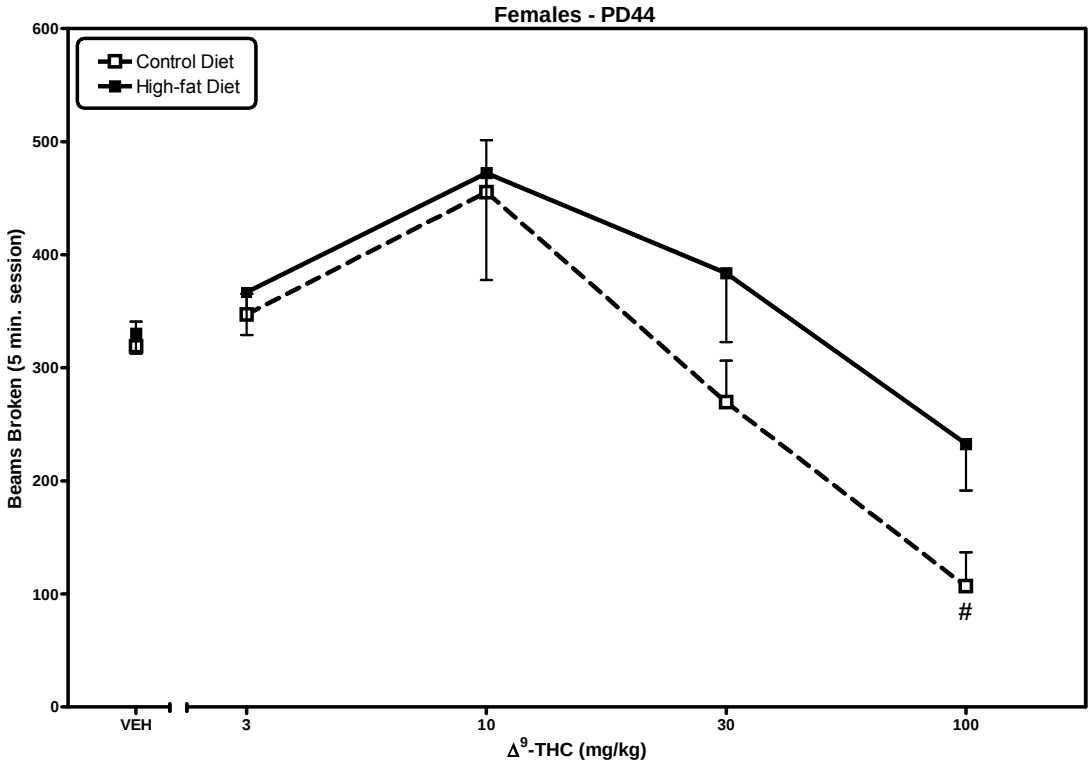

Figure 16. Locomotor activity for female rats at PD44. Number of beams broken (mean +/- SEM) by female rats after treatment with Δ^9-THC on postnatal day 44. Again, Δ^9-THC dose-dependently decreased locomotor activity in both groups. No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

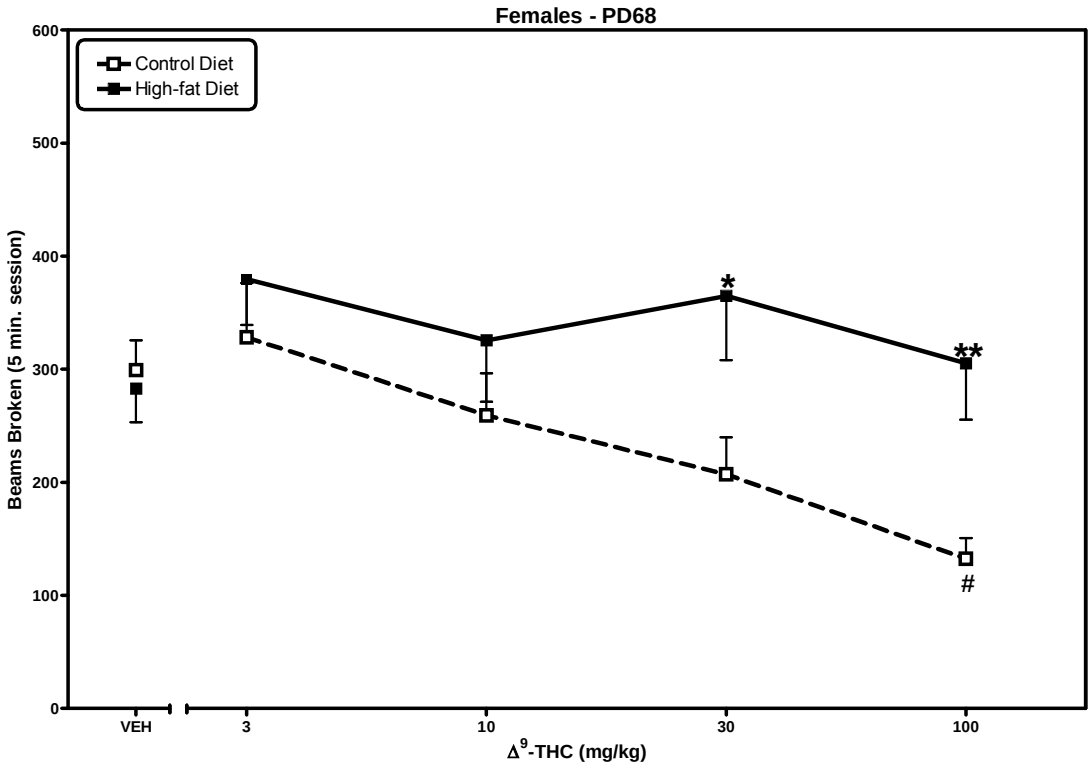

Figure 17. Locomotor activity for female rats at PD68. Number of beams broken (mean +/- SEM) by female rats after treatment with Δ^9-THC at postnatal day 68. A significant interaction between Δ^9-THC and diet on the number of beams broken was evident. Female rats maintained on the control diet broke significantly fewer beams than female rats maintained on a high-fat diet when treated with 30 mg/kg and 100 mg/kg doses of Δ^9-THC. Additionally, female rats maintained on the control diet broke significantly fewer beams when treated with a 100 mg/kg dose of Δ^9-THC than when treated with vehicle. In contrast, the number of beams broken by female rats maintained on a high-fat diet never varied significantly from the number broken under vehicle conditions, regardless of the dose of Δ^9-THC. Data points highlighted with "#" are significantly different from the respective vehicle-treated conditions. Data points highlighted with asterisk are significantly different from each other. ("*****" indicates $p < 0.05$; "******" indicates $p < 0.01$.)

female rats maintained on a high-fat diet because the relationship between the dose of Δ^9-THC and locomotor activity was non-linear.

The significant interaction between the type of diet consumed and the dose of Δ^9-THC on the number of beams broken continued at postnatal day 114, $F(4, 64) = 3.292$, $p = 0.016$ (Fig. 18). At doses of 3 mg/kg, 10 mg/kg and a 100 mg /kg of Δ^9-THC, female rats maintained on a high-fat diet broke a significantly greater number of beams than did female rats maintained on a control diet ($p < 0.05$ for all measures). Despite this difference, the 95% confidence intervals for ED_{50} values of Δ^9-THC overlapped (Table 3)

Males. Like females, baseline measures for gross locomotor activity in male rats were taken on postnatal day 30. At baseline, there was a significant main effect of Δ^9-THC on the number of beams broken during a five-minute testing session, $F(4, 64) = 1.894$, $p < 0.001$ (Fig. 19). Both groups of male rats broke fewer beams when treated with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle ($p < 0.05$ for all measures). A dose of 3 mg/kg Δ^9-THC did not significantly affect the number of beams broken during the session. At this age point, there was no main effect of diet on the number of beams broken, $F(1, 16) = 0.000484$, $p = 0.983$, and no significant interaction between the type of diet and the dose of Δ^9-THC was detected, $F(4, 64) = 1.894$, $p = 0.122$.

At postnatal day 37, changes in gross locomotor activity in male rats after treatment with Δ^9-THC were similar to what was seen on postnatal day 30. There was a significant main effect of Δ^9-THC the number of beams broken, $F(4, 60) = 8.089$, $p <$

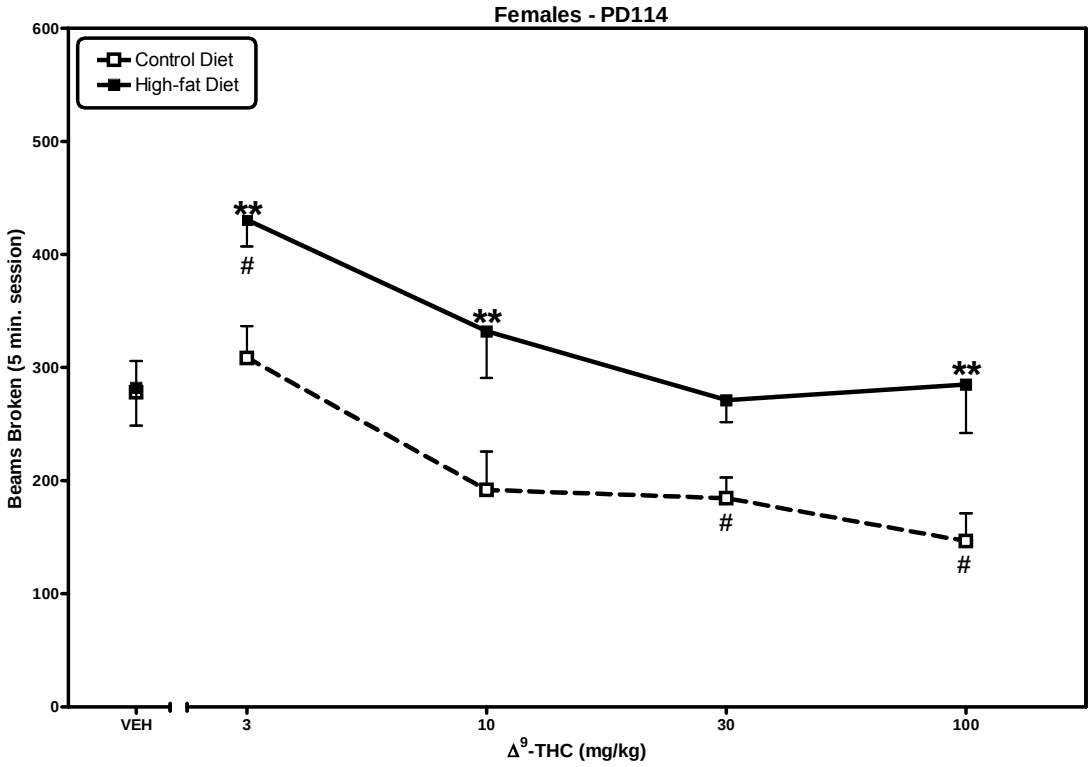

Figure 18. Locomotor activity for female rats at PD114. Number of beams broken (mean +/- SEM) by female rats after treatment with Δ^9-THC at postnatal day 114. A significant interaction between Δ^9-THC and diet on the number of beams broken was evident. Female rats maintained on the control diet broke significantly fewer beams than female rats maintained on a high-fat diet when treated with 3 mg/kg, 10 mg/kg and 100 mg/kg doses of Δ^9-THC. Female rats maintained on the control diet broke significantly fewer beams when treated with a 30 mg/kg and 100 mg/kg doses of Δ^9-THC than when treated with vehicle. Additionally, female rats maintained on a high-fat diet broke a significantly larger number of beams when treated with a 3 mg/kg dose of Δ^9-THC than with treated with vehicle. Data points highlighted with "#" are significantly different from the respective vehicle-treated conditions. Data points highlighted with asterisk are significantly different from each other. ("*****" indicates $p < 0.05$; "******" indicates $p < 0.01$.)

0.001 (Fig. 20) with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC reducing the number of beams broken ($p < 0.05$). Analysis of the locomotor activity tests conducted on male rats at postnatal day 37 failed to detect a significant main effect of diet the number of beams broken, $F(1, 15) = 0.0254$, $p = 0.876$. Likewise, there was not a significant interaction between type of diet consumed and the dose of Δ^9-THC, $F(4, 60) = 0.701$, $p = 0.595$.

When tested on postnatal day 44, changes in gross locomotor activity after treatment with Δ^9-THC were similar to what was seen on postnatal day 37. There was a significant main effect of Δ^9-THC the number of beams broken, $F(4, 60) = 6.131$, $p < 0.001$ (Fig. 21) with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC reducing the number of beams broken ($p < 0.05$). Analysis of the locomotor activity tests conducted on male rats at postnatal day 44 failed to detect a significant main effect of diet the number of beams broken, $F(1, 15) = 0.828$, $p = 0.377$. Likewise, there was not a significant interaction between type of diet consumed and the dose of Δ^9-THC, $F(4, 60) = 0.327$, $p = 0.859$.

At postnatal day 61, the significant main effect of Δ^9-THC on the number of beams broken during the locomotor sessions persisted, $F(4, 60) = 19.865$, $p < 0.001$ (Fig. 22) with 10 mg/kg, 30 mg/kg and 100 mg/kg doses of Δ^9-THC reducing the number of beams broken. No significant main effect of diet on the number of beams broken was noted, $F(1, 15) = 0.0851$, $p = 0.775$, and the interaction between the type of diet and dose of Δ^9-THC was not significant, $F(4, 60) = 1.137$, $p = 0.348$.

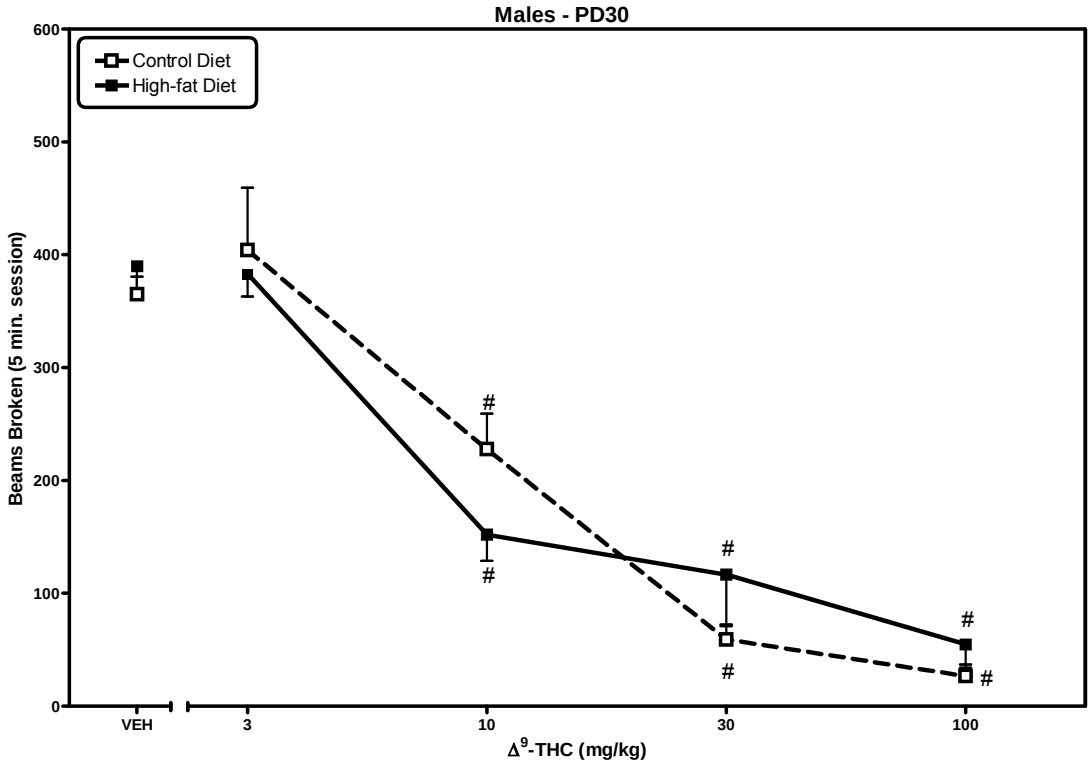

Figure 19. Locomotor activity for male rats at PD30. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 30. Delta-9-THC dose-dependently decreased locomotor activity in both groups. No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

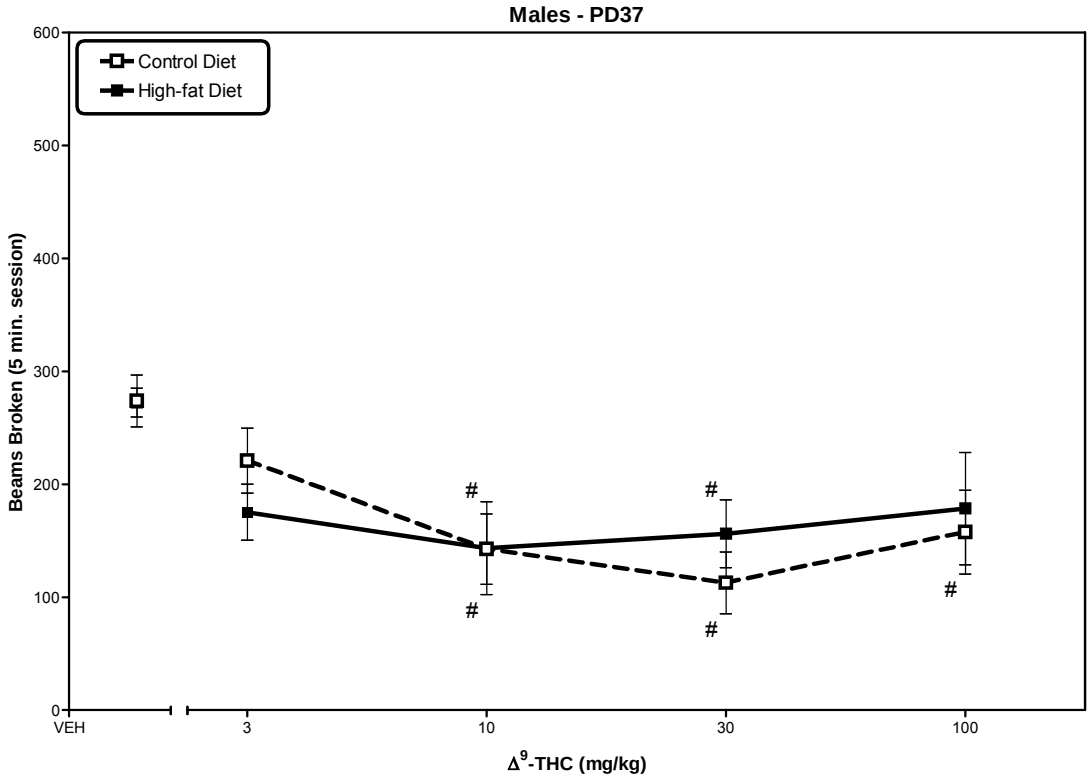

Figure 20. Locomotor activity for male rats at PD37. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 37. A significant main effect of Δ^9-THC on the number of beams broken was noted, No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

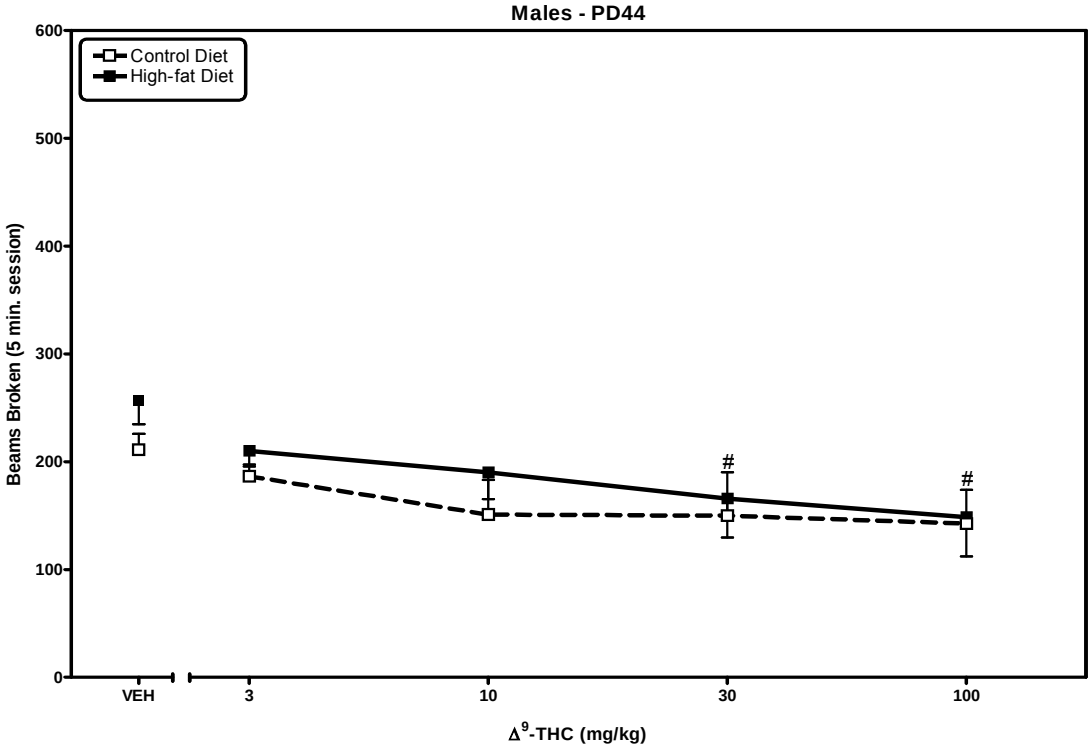

Figure 21. Locomotor activity for male rats at PD44. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 44. A significant main effect of Δ^9-THC on the number of beams broken was noted, No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

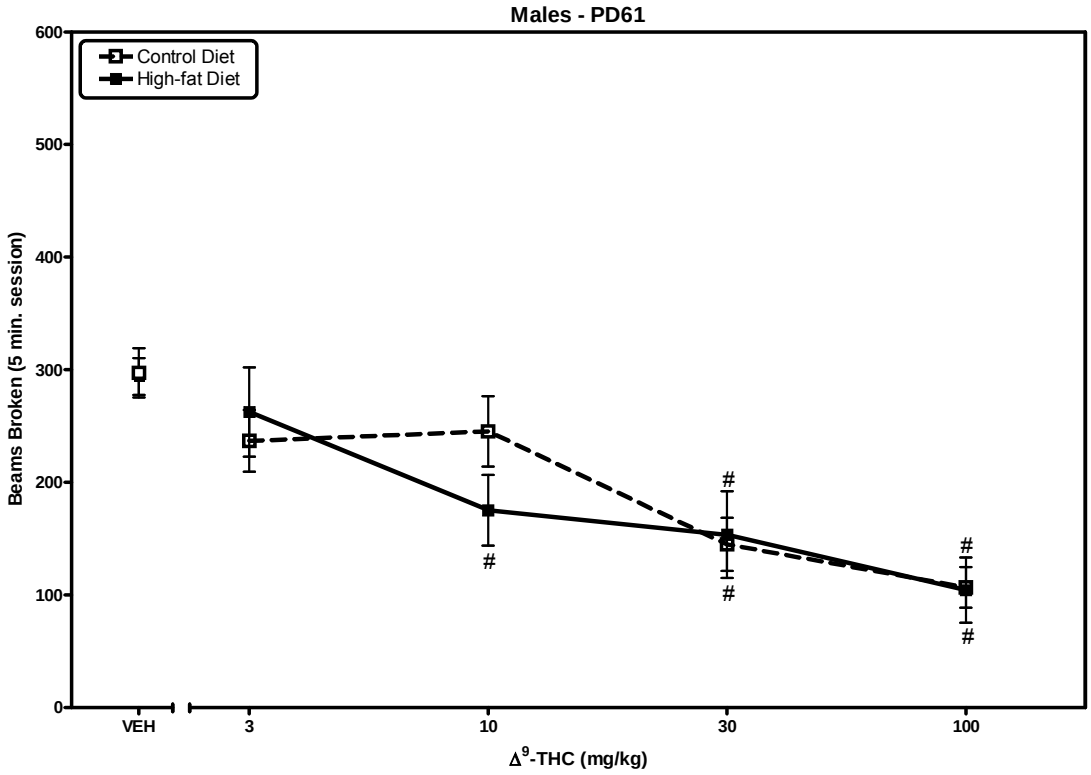

Figure 22. Locomotor activity for male rats at PD61. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 61. A significant main effect of Δ^9-THC on the number of beams broken was noted, No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

At postnatal day 68, there was still a significant main effect of Δ^9-THC on the number of beams broken during the locomotor sessions, $F(4, 60) = 18.558$, $p < 0.001$ (Fig. 23). Locomotor activity was inhibited significantly 30 mg/kg and 100 mg/kg doses of Δ^9-THC ($p < 0.05$ for both measures). No significant main effect of diet on the number of beams broken was noted, $F(1, 15) = 0.0380$, $p = 0.848$, and the interaction between the type of diet and dose of Δ^9-THC was not significant, $F(4,60) = 1.879$, $p = 0.126$.

Similarly, at postnatal day 114, there was a significant main effect of Δ^9-THC on the number of beams broken during the locomotor sessions, $F(4, 60) = 4.453$, $p = 0.003$ (Fig. 24). However, Δ^9-THC only inhibited locomotor activity significantly at a dose of 100 mg/kg ($p < 0.05$). Once again, no significant main effect of diet was noted, $F(1, 15) = 0.0995$, $p = 0.757$, and the interaction between the dose of Δ^9-THC and the type of diet consumed was not significant, $F(4, 60) = 1.541$, $p = 0.202$.

Hypothermia

Females. Female rats exhibited a significant main effect of Δ^9-THC on body temperature each age point tested (Table 5). Doses of Δ^9-THC that exceeded 30 mg/kg commonly induced significant hypothermia when compared to vehicle treatment ($p < 0.05$). In contrast, neither a significant main effect of diet nor a significant interaction between type of diet and Δ^9-THC on hypothermia were noted for at any age point. In female rats, the hypothermic effects of Δ^9-THC were indistinguishable across the groups, regardless of the age point when tested.

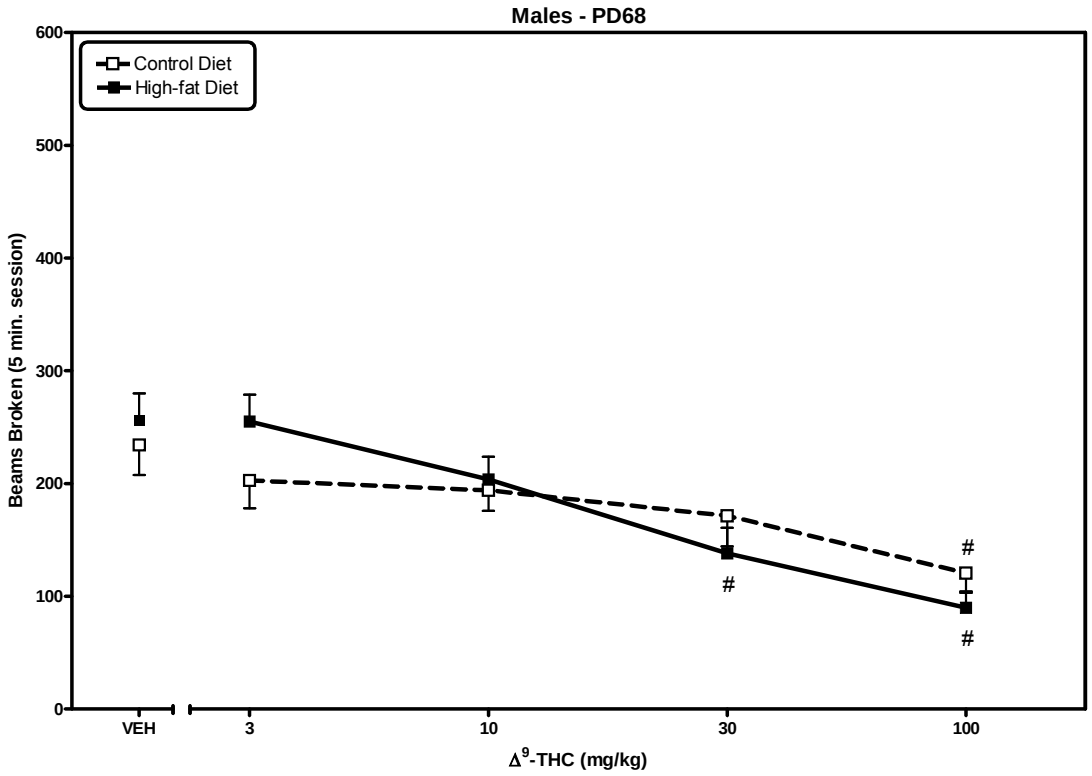

Figure 23. Locomotor activity for male rats at PD68. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 68. A significant main effect of Δ^9-THC on the number of beams broken was noted, No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

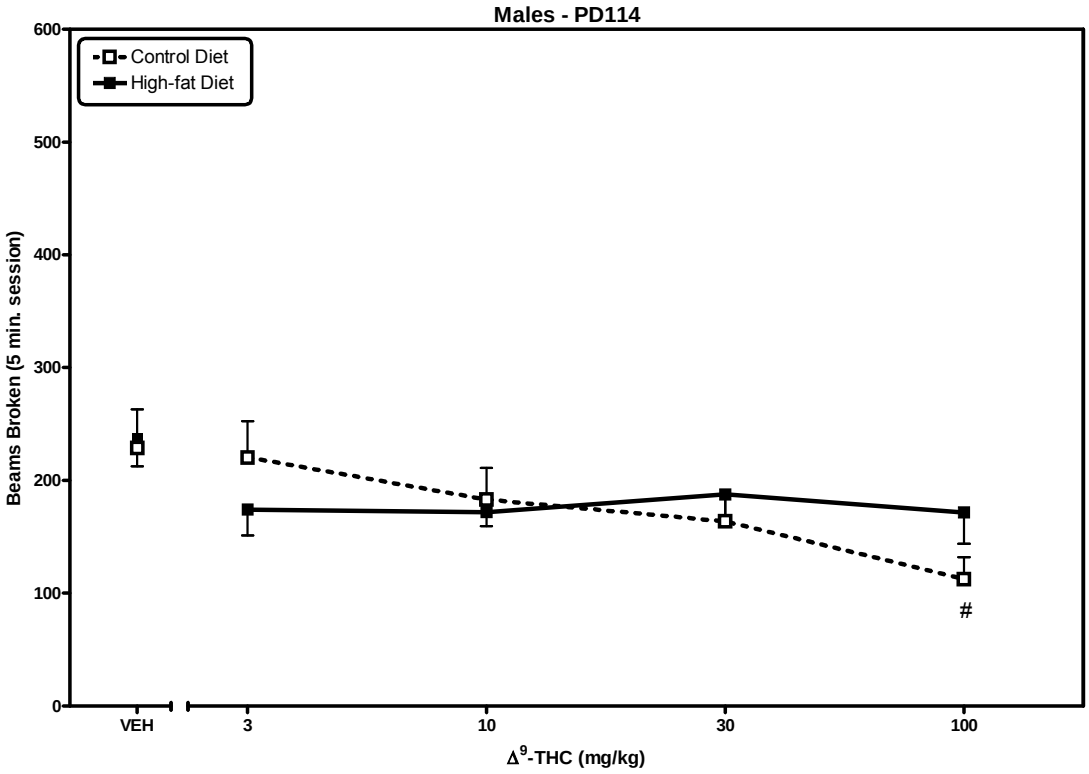

Figure 24. Locomotor activity for male rats at PD114. Number of beams broken (mean +/- SEM) by male rats after treatment with Δ^9-THC on postnatal day 114. A significant main effect of Δ^9-THC on the number of beams broken was noted, No between-group differences in drug response were detected. Data points highlighted with a "#" are significantly different from the respective vehicle-treated conditions.

Table 5

Effects of Δ^9-THC and type of diet on body temperature.

	Main Effect of Δ9-THC		Main Effect of Diet		Interaction of Δ9-THC and Diet	
Females	***F*-test**	***p*-value**	***F*-test**	***p*-value**	***F*-test**	***p*-value**
PD30	$F(4,64)=143.99$	< 0.01	$F(1,16)=1.42$	> 0.05	$F(4,64)=0.96$	> 0.05
PD44	$F(4,64)=98.35$	< 0.01	$F(1,16)=3.11$	> 0.05	$F(4,64)=1.77$	> 0.05
PD68	$F(4,64)=64.57$	< 0.01	$F(1,16)=0.22$	> 0.05	$F(4,64)=0.34$	> 0.05
PD114	$F(4,64)=115.72$	< 0.01	$F(1,16)=1.45$	> 0.05	$F(4,64)=0.41$	> 0.05
Males	***F*-test**	***p*-value**	***F*-test**	***p*-value**	***F*-test**	***p*-value**
PD30	$F(4,64)=109.08$	< 0.01	$F(1,16)=0.85$	> 0.05	$F(4,64)=0.52$	> 0.05
PD37	$F(4,60)=12.98$	< 0.01	$F(1,15)=0.14$	> 0.05	$F(4,60)=1.24$	> 0.05
PD44	$F(4,60)=8.35$	< 0.01	$F(1,15)=0.05$	> 0.05	$F(4,60)=1.82$	> 0.05
PD61	$F(4,60)=15.22$	< 0.01	$F(1,15)=0.12$	> 0.05	$F(4,60)=0.38$	> 0.05
PD68	$F(4,60)=26.53$	< 0.01	$F(1,15)=0.39$	> 0.05	$F(4,60)=0.95$	> 0.05
PD114	$F(4,60)=26.46$	< 0.01	$F(1,15)=0.02$	> 0.05	$F(4,60)=0.17$	> 0.05

Males. For the male rats, there was also a significant main effect of Δ^9-THC on body temperature each age point tested (Table 5). Again, doses of Δ^9-THC that exceeded 30 mg/kg induced significant hypothermia when compared to vehicle treatment ($p < 0.05$). Like the female rats, neither a significant main effect of diet nor a significant interaction between type of diet and Δ^9-THC on hypothermia were noted at any age point. Again like the female rats, the hypothermic effects of Δ^9-THC in male rats were indistinguishable across the groups, regardless of the age point when tested. It is noteworthy, however, that both groups of male rats exhibited an attenuated hypothermic

response to Δ^9-THC after the initial exposure and this pattern of diminished responses persisted through postnatal day 114.

Antinociception – Tail-flick

Females. Female rats exhibited a significant interaction between Δ^9-THC and diet on latency to withdraw the tail from a radiant heat source, $F(1, 16) = 9.075$, $p = 0.008$ (Fig. 25A). There was also a significant main effect of Δ^9-THC on latency to withdraw the tail from the source, $F(1, 16) = 386.570$, $p < 0.001$). In contrast, no main effect of diet was detected, $F(1, 16) = 0.0000684$, $p = 0.994$. However, female rats maintained on a high-fat diet exhibited a smaller drug-related change in latency to withdraw the tail from the heat source ($M = 3.636$, $SD = 0.603$) than did the females maintained on the control diet ($M = 4.963$, $SD = 1.159$), $t(16) = 3.050$, $p = 0.008$ (Fig. 25B).

Males. For male rats, there was a significant main effect of Δ^9-THC on latency to withdraw the tail from a radiant heat source, $F(1, 15) = 427.949$, $p < 0.001$ (Fig. 26A). A 30 mg/kg dose of Δ^9-THC significantly increased latency to withdraw the tail from the heat source than did treatment with vehicle ($p < 0.05$). There was not, however, a significant main effect of the type of diet on tail withdrawal latency $F(1, 15) = 0.0547$, $p = 0.818$. Additionally, no significant interaction between the type of diet and the dose of Δ^9-THC was noted $F(1, 15) = 0.418$, $p = 0.528$. Unlike female rats, however, male rats maintained on a high-fat diet exhibited a drug-related change in latency to withdraw the tail from the heat source ($M = 3.699$, $SD = 0.948$) that was indistinguishable from the drug-related change in latency exhibited by the males maintained on the control diet ($M = 3.934$, $SD = 0.462$), $t(15) = 0.635$, $p = 0.535$ (Fig. 26B).

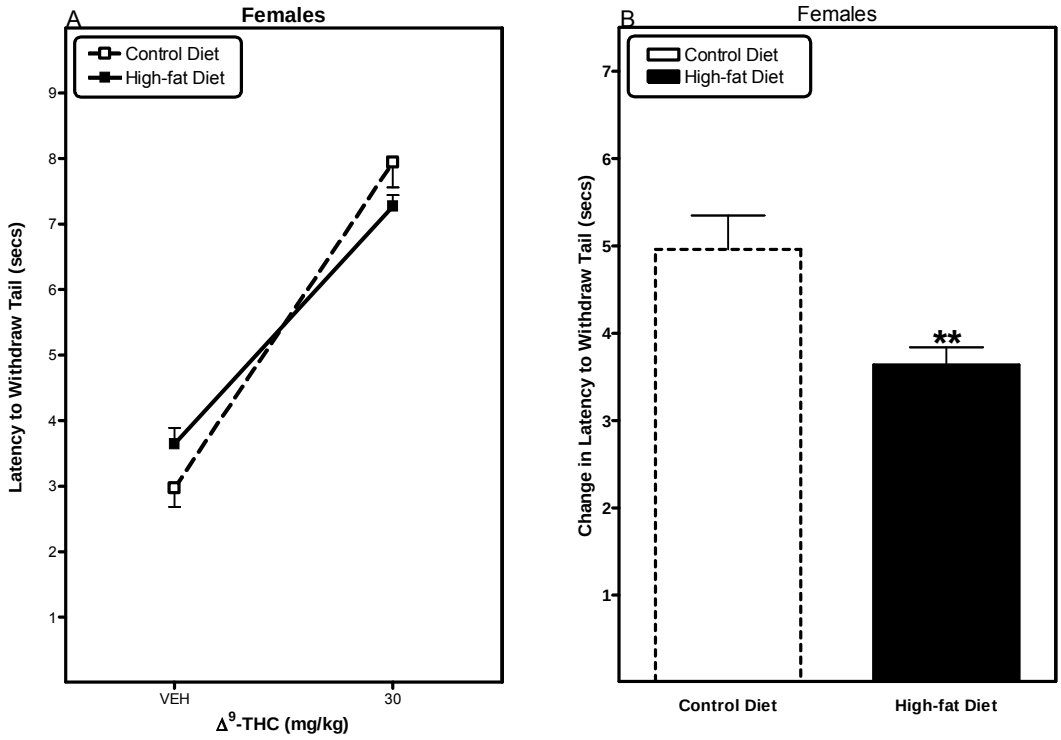

Figure 25. Antinociception in female rats. Changes in tail-flick latency produced by treatment with Δ^9-THC. Female rats demonstrated a significant interaction between diet and Δ^9-THC on tail-flick latencies (Fig 25A). A main effect of Δ^9-THC on tail-flick latency was also noted in female rats. Female rats maintained on a high-fat diet also exhibited significantly smaller changes in tail-flick latencies after treatment with a 30 mg/kg dose of Δ^9-THC than female rats maintained on the control diet (Fig. 25B; "**" indicates $p < 0.01$).

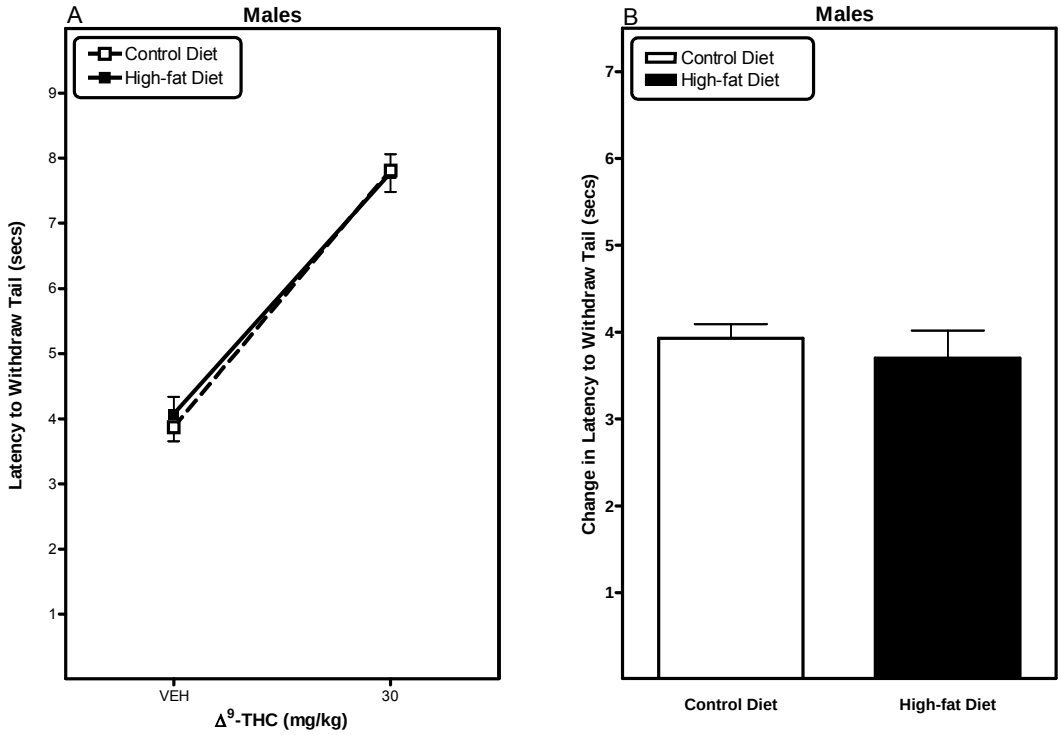

Figure 26. Antinociception in male rats. Changes in tail-flick latency produced by treatment with Δ^9-THC. Male rats demonstrated a significant main effect of Δ^9-THC on tail-flick latencies (Fig 26A). Unlike female rats, however, male rats did not exhibit an interaction between diet and Δ^9-THC on tail-flick latencies. Additionally, the changes in tail-flick latencies induced by treatment with a 30 mg/kg dose of Δ^9-THC were indistinguishable across the groups (Fig. 26B).

Food-intake

Females. During the first hour of a 3-hour feeding trial, female rats exhibited a significant main effect of Δ^9-THC on food intake, $F(5, 80) = 6.855$, $p < 0.001$ (Fig. 27). Food intake increased significantly when rats were treated with 1 mg/kg, 3 mg/kg and 10 mg/kg doses of Δ^9-THC when compared with vehicle. Analysis also revealed a significant main effect of diet on food intake, $F(1, 16) = 8.686$, $p = 0.009$, with female rats maintained on a high-fat diet consuming significantly more food during the feeding trial than the female rats maintained on the control diet. Despite this, no interaction between Δ^9-THC and diet on food intake was detected, $F(5, 80) = 0.809$, $p = 0.547$.

Over the course of the full 3-hour feeding trial, female rats exhibited a significant interaction of Δ^9-THC and diet on food intake, $F(5, 79) = 7.350$, $p < 0.001$ (Fig. 28). Analysis also revealed significant main effects of Δ^9-THC dose, $F(5, 79) = 9.915$, $p < 0.001$, and of diet on food intake, $F(1, 16) = 8.874$, $p = 0.009$. The female rats maintained on a high-fat diet ate significantly more than the female rats maintained on the control diet when treated with 10 mg/kg and 30 mg /kg doses of Δ^9-THC ($p < 0.05$ for both measures). Conversely, female rats maintained on the control diet ate significantly more than female rats maintained on a high-fat diet when treated with a 1 mg/kg dose of Δ^9-THC ($p < 0.05$).

In the same 3-hour experiment, female rats maintained on the control diet ate significantly more when treated with a 1 mg/kg dose of Δ^9-THC than with treated with vehicle, $p < 0.05$. Female rats maintained on the high-fat diet, however, ate significantly more when treated with 3 mg/kg and 10 mg/kg doses of Δ^9-THC doses than when treated

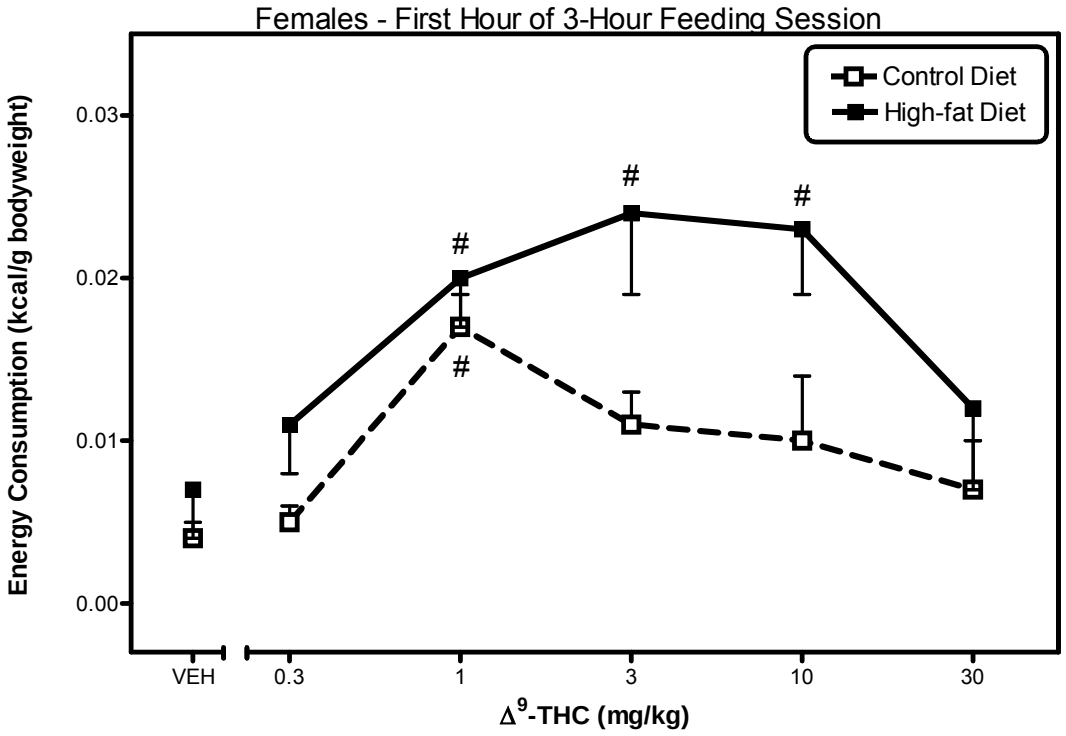

Figure 27. Relative food-intake over the first hour of a 3-hour feeding trial in female rats. A significant main effect of Δ^9-THC on food intake was noted. Neither an interaction between diet and Δ^9-THC on food intake nor a main effect of diet were detected. Compared to vehicle conditions, food intake was stimulated 1 mg/kg – 10 mg/kg in female rats maintained on a high-fat diet. Female rats maintained on the control diet ate significantly more when treated with a 1 mg/kg dose of Δ^9-THC than when treated with vehicle. Data points highlighted with "#" are significantly different from the respective vehicle-treated conditions.

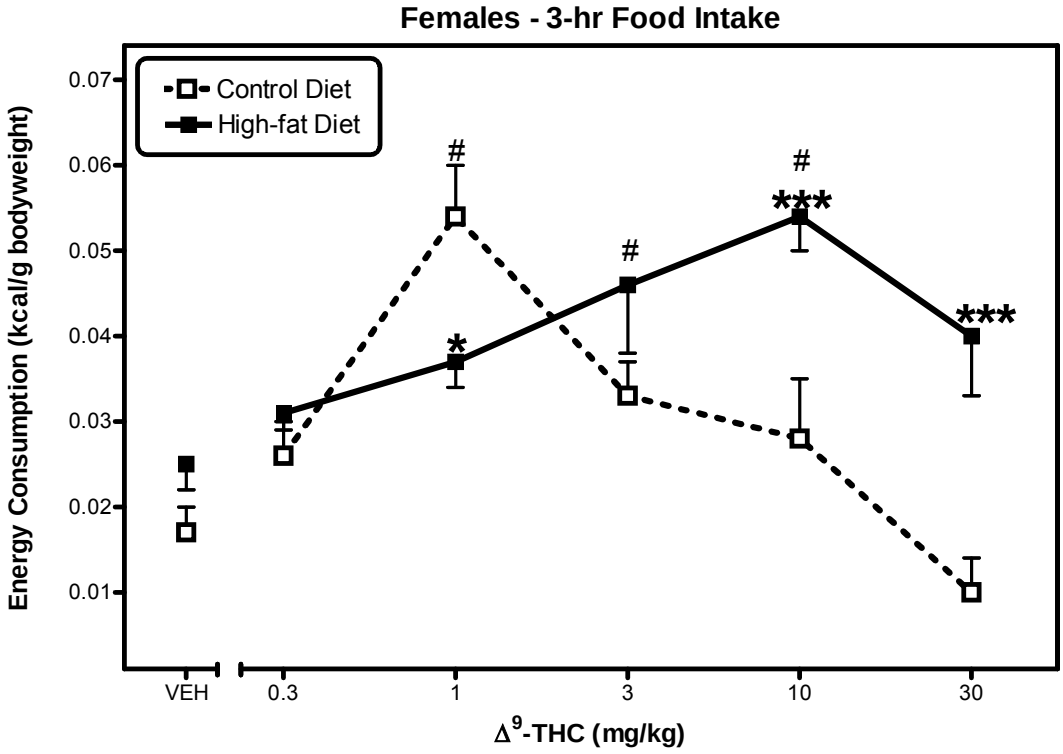

Figure 28. Relative food-intake over the course of a 3-hour feeding trial in female rats. A significant interaction between diet and Δ^9-THC on food intake was noted. Additionally, a main effect of dose and a main effect of diet were detected. Compared to vehicle conditions, food intake was stimulated at 3 mg/kg and 10 mg/kg doses of Δ^9-THC in female rats maintained on a high-fat diet. Female rats maintained on the control diet ate significantly more when treated with a 1 mg/kg dose of Δ^9-THC than when treated with vehicle. Data points highlighted with "**#**" are significantly different from the respective vehicle-treated conditions. Data points highlighted with an asterisk are significantly different from the control diet group ("*****" indicates $p < 0.05$; "*******" indicates $p < 0.001$).

with vehicle ($p < 0.05$ for both measures). Both the smallest dose (e.g., 0.3 mg/kg) and the largest dose of Δ^9-THC (e.g., 30 mg/kg) failed to produce changes in food intake in either group compared to vehicle.

Males. Over the course of a 1-hour feeding trial, the male rats exhibited a significant interaction between diet and Δ^9-THC on food intake, $F(4,60) = 3.693$, $p = 0.009$ (Fig. 29). Analysis also revealed significant main effects of Δ^9-THC, $F(4, 60) = 83.927$, $p < 0.001$, and diet on food intake, $F(1, 15) = 10.200$, $p = 0.006$. Male rats maintained on a high-fat diet ate significantly more than rats maintained on the control diet when treated with vehicle and with a 3 mg/kg dose Δ^9-THC ($p < 0.05$ for both measures).

Context-specific tolerance

In a test of context-specific tolerance, male rats exhibited a significant interaction between the drug exposure environment and Δ^9-THC on hypothermia, $F(4, 24) = 3.450$, $p = 0.023$. The male rats used in these experiments exhibited a main effect of Δ^9-THC on hypothermia when first exposed to the drug (PD30; Fig. 30A). Despite this, the rats that were treated with Δ^9-THC in two distinct environments (e.g., a behavioral pharmacology lab and the vivarium) seven days apart (PD30 and PD37) exhibited significantly less hypothermia on the second exposure than male rats that were treated with Δ^9-THC in the same environment at those same age points, $p < 0.05$ (Fig. 30B). At a 100 mg/kg dose, the group of males that been exposed to Δ^9-THC in two discrete locations exhibited less hypothermia than the group of males that had been exposed to Δ^9-THC in the same environment twice ($p < 0.05$). The mean baseline body temperatures of the two groups

were indistinguishable on PD30 (Lab/Lab group = 37.4°C, Lab/Vivarium group = 37.5°C) and on PD37 (Lab/Lab group = 37.6°C, Lab/Vivarium group = 37.3°C).

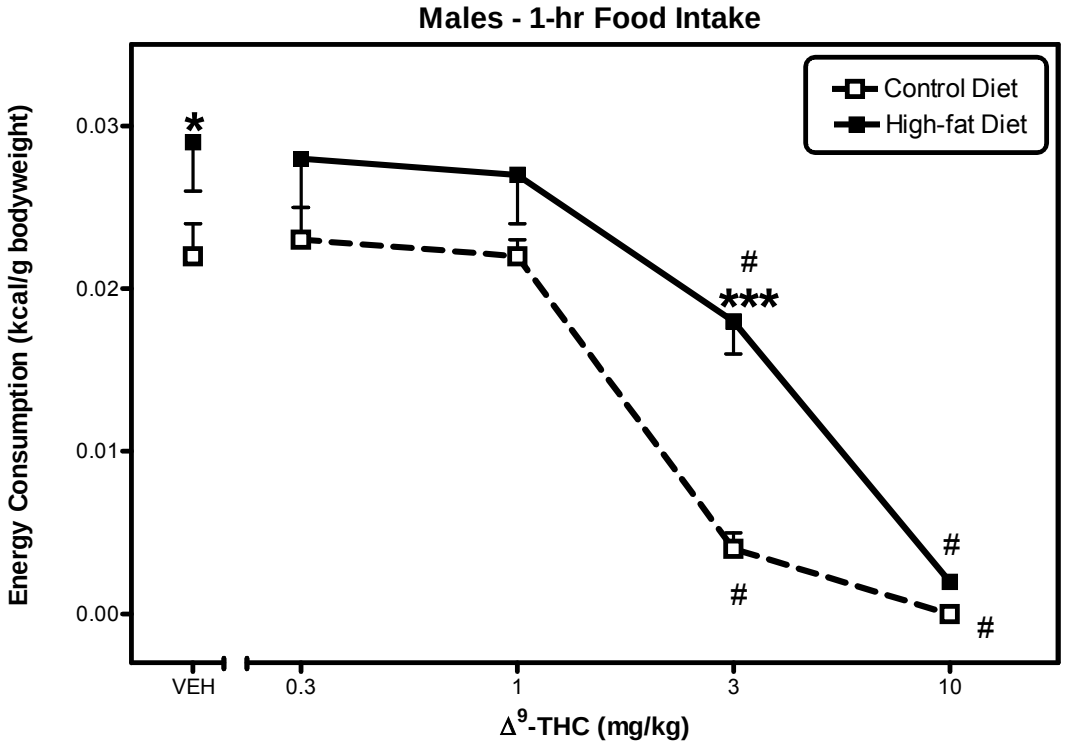

Figure 29. Relative food-intake over the course of a 1-hour feeding trial in male rats. A significant interaction between diet and Δ^9-THC on food intake was noted. Additionally, a main effect of dose and a main effect of diet were detected. Under vehicle conditions, male rats maintained on a high-fat diet ate significantly more than male rats maintained on the control diet. Compared to vehicle conditions, food intake was inhibited at 3 mg/kg and 10 mg/kg doses of Δ^9-THC in both groups of male rats. Data points highlighted with "**#**" are significantly different from the respective vehicle-treated conditions. Data points highlighted with an asterisk are significantly different from the control diet group ("*****" indicates $p < 0.05$; "*******" indicates $p < 0.001$).

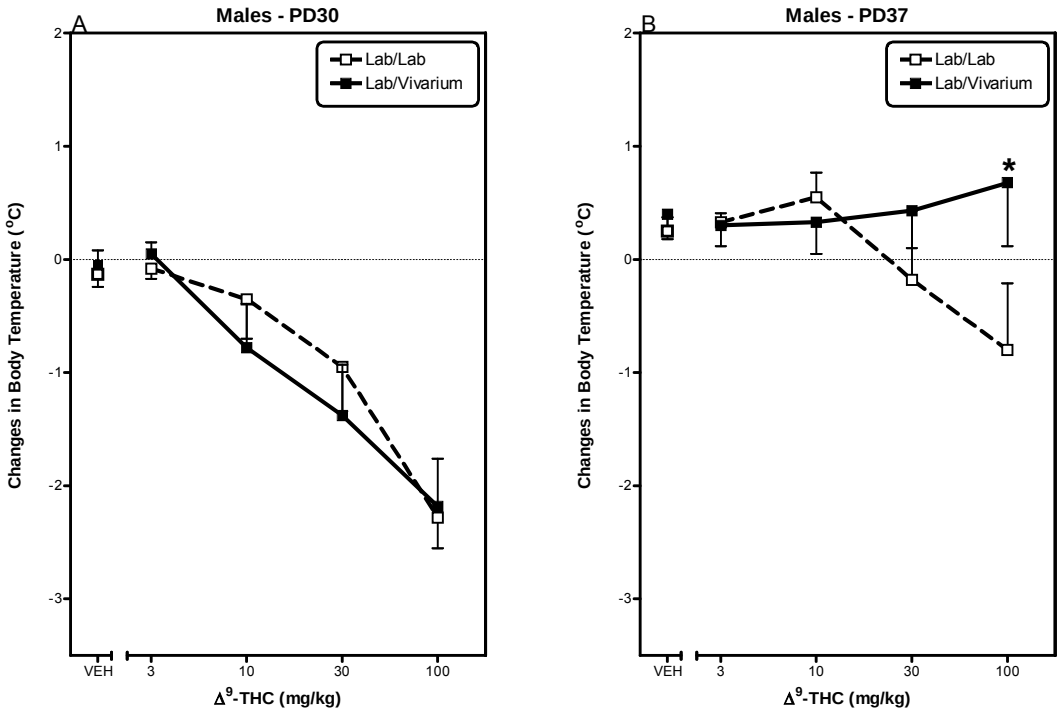

Figure 30. Context-specific tolerance to the hypothermic properties of Δ^9-THC. Upon initial exposure (PD30; Fig. 30A), a significant main effect of Δ^9-THC on hypothermia was evident in both groups. The initial exposure to Δ^9-THC took place in a behavioral pharmacology lab for both groups. On the subsequent exposure (PD37; Fig. 30B), a significant main effect of Δ^9-THC on hypothermia was not detectable. There was, however, a significant interaction between the location of the second exposure and Δ^9-THC on hypothermia. Data points highlighted with an asterisk are significantly different from the control diet group ("*****" $p < 0.05$).

Discussion

Females

The most striking finding from these experiments is that, in female (but not male) rats, consumption of a high-fat diet throughout adolescence resulted in reduced sensitivity to the psychomotor effects of Δ^9-THC. Within two weeks of the female rats being placed on the high-fat diet, a trend suggestive of cross-tolerance to the psychomotor effects of Δ^9-THC began to develop.

If present, this cross-tolerance may be the result of chronically elevated endocannabinoid signaling produced by the inhibition of anandamide hydrolysis. The type and quantity of polyunsaturated fatty acids in the diet can directly influence endocannabinoid levels (Berger *et al*., 2001; Watanabe *et al*., 2003). Diets rich in omega-6-polyunsaturated fatty acids may increase endocannabinoid levels by providing additional substrate for FAAH (Matias *et al*., 2006). It may be possible to inhibit the activity of by providing additional substrate because FAAH hydrolyzes the amide bond of several common amines formed from long-chain fatty acids, including anandamide (Schmid *et al*., 1985; Ueda *et al*., 1995).

By the time of the final assessment on postnatal day 114, Δ^9-THC produced smaller increases in the amount of time on the bar apparatus and did not inhibit locomotion in females maintained on the high-fat diet compared to the females

maintained on the control diet. Further, female rats maintained on this diet were less sensitive to increased food intake caused by Δ^9-THC. These changes in drug response are strikingly similar to changes seen in rats that are tolerant to Δ^9-THC (Fan *et al*., 1994).

The impression that female rats maintained on a high-fat diet became cross-tolerant to the psychomotor effects of Δ^9-THC was reinforced by other observations made during these trials. Female rats maintained on a high-fat diet appeared to be less sedated by moderate and high doses of Δ^9-THC (i.e., above 10 mg/kg) than female rats maintained on a control diet.

Other non-systematic observations made over the course of these experiments suggest that female rats maintained on a high-fat diet found high doses of Δ^9-THC (e.g., 100 mg/kg) to be less aversive than female rats maintained on the control diet. Doses of some drugs (i.e., naloxone, lithium chloride) that produce conditioned place aversion are associated with an increase in ultrasonic vocalizations in the 20kHz frequency range as compared to similar vocalizations made by vehicle-treated animals (Burgdorf *et al*., 2001). Tones in the 20kHz range are near the high-end of the audible frequency range for humans (Jauchem and Cook, 2007). When treated with a 100 mg/kg dose of Δ^9-THC, female rats maintained on the control diet commonly emitted sustained, high-pitched vocalizations in response to being handled. In contrast, female rats maintained on a high-fat diet rarely emitted vocal responses after being touched.

While suggestive of cross-tolerance to cannabinoids, however, the phenotype resulting from this dietary regimen is distinct from that observed in cannabinoid tolerant animals. For example, the female rats maintained on this diet also commonly exhibited a

repetitive, stereotyped series head movements when treated with high doses of Δ^9-THC, an effect that does not occur in cannabinoid-tolerant rats. It is unclear whether these head movements are a unique consequence of cannabinoid exposure after sustained consumption of a high-fat diet or if they are simple exaggerations of other common movements.

Notably, other cannabinoid-mediated effects, such as hypothermia and antinociception were unaffected or minimally affected. Importantly, it has been demonstrated that cannabinoid tolerance can develop in an asymmetric fashion. For instance, after treatment with the CB_1 agonist CP55, 940 for 4 consecutive days, rats developed tolerance to the antinociceptive effects of the drug while the effect on operant responding for food remained unchanged (DeVry *et al*., 2004). In this same study, a non-significant trend toward tolerance to the hypothermic effect of CP55, 940 was noted. In addition, these data suggest that the observed changes in sensitivity to the psychomotor effects of Δ^9-THC in female rats maintained on a high-fat diet is probably not attributable to differential metabolism or altered bioavailability of Δ^9-THC.

It could be also reasoned that, because Δ^9-THC is highly lipophilic and passes into fat cells easily, changes in sensitivity to the psychomotor effects of Δ^9-THC could be attributable to increased adiposity and a concomitant reduction in drug bioavailability. Once again, the lack of a main effect of diet on the hypothermic properties of Δ^9-THC argues against this conclusion.

Another interesting finding from these studies is related to observed changes in food intake after treatment with Δ^9-THC that occurred in female rats. The graphs of food

intake after treatment with Δ^9-THC depict a classical rightward shift in the dose-response curve. As such, it appears that the female rats maintained on a high-fat diet were tolerant to Δ^9-THC-induced changes in food intake. The feeding behavior exhibited by the female rats maintained on the control diet is consistent with Δ^9-THC-induced changes reported by other investigators (Williams *et al*., 1998). However, Δ^9-THC continued to stimulate food intake in the female rats that were maintained on a high-fat diet at doses that were inactive or reduced food intake in other studies (Koch and Matthews, 2001).

It is important to point out a potential confound inherent to the food-intake studies. Changes in food intake after treatment with Δ^9-THC were measured using the food that was normally consumed by each group. In other words, the female rats maintained on a high-fat diet ate high-fat chow during the feeding trials. Similarly, female rats maintained on the control diet ate standard rodent chow during their feeding trials. Diets were not changed during the feeding trials because of potential inter-group differences in the hedonic value of food or in the degree of neophobia. Though measuring food intake with a common diet would have eliminated one condition that varied systematically with drug treatment, it would have added at least one more.

It has been demonstrated that CP-55940, a potent CB_1 agonist, differentially enhances the reinforcing efficacy of corn oil as compared to vehicle (Ward and Dykstra, 2005), but it is not clear whether the high-fat diet used in these studies would be similarly appealing after treatment with cannabinoids. It is possible that the observed inter-group differences in food-intake are related to a differential influence of Δ^9-THC on the intake

of high-fat foods. An examination of the reinforcing efficacy of the high-fat diet used in these studies provides one possible future direction for this project.

Males

In contrast, male rats maintained on a high-fat diet across the same developmental interval were only affected to a moderate degree. Male rats maintained on a high-fat diet spent more time on the bar apparatus when treated with Δ^9-THC than male rats maintained on the control diet, but this difference only appeared after the high-fat diet had been consumed for 12 weeks and there were no overt differences in their level of sedation at higher doses. Although this observation was not obtained systematically, it stands in contrast to similar observations made during the trials with the female rats. Additionally, male rats maintained on the control diet ate significantly less than male rats maintained on a high-fat diet when treated with a 3 mg/kg dose of Δ^9-THC, though baseline differences in food intake between the groups complicate the interpretation of these data. Measures of gross locomotion, hypothermia and antinociception did not reveal any differences between the male rats maintained on a high-fat diet and male rats maintained on the control diet.

Unlike female rats, the male rats maintained on a high-fat diet never differed significantly from the male rats maintained on the control diet in terms of the number of beams broken. Although a dose-dependent relationship between Δ^9-THC dose and the number of beams broken persisted through postnatal day 114, locomotion was only significantly inhibited by large doses of Δ^9-THC (e.g., 100 mg/kg) at that age. The observation that both groups of male rats were less sensitive to the locomotor inhibition

caused by Δ^9-THC is intriguing, if not a bit puzzling. It is unlikely that these data can be explained in terms of pharmacokinetic tolerance because these rats had not been treated with Δ^9-THC in 45 days. Pharmacokinetic explanations are also inconsistent with the robust dose-dependent relationship between Δ^9-THC and the amount of time spent on the bar apparatus was apparent at postnatal day 114. This leaves open the possibility of context-specific tolerance to the locomotor effects of Δ^9-THC. Previous work has demonstrated that behavioral tolerance to the locomotor effects of cannabinoids can be produced after repeatedly pairing a cannabinoid agonist (HU210) with a locomotor chamber (Hill *et al*., 2004). It is also possible that there are sex differences in the rate at which these rats became habituated to the locomotor chamber (Bolivar *et al*., 2000).

Another intriguing finding from these experiments is the rapidity and degree to which young male rats developed tolerance to the hypothermic effects of Δ^9-THC. At postnatal day 30, a 100 mg/kg dose of Δ^9-THC administered under a cumulative dosing regimen reduced body temperatures by about 2.1^o Celsius. When the male rats were tested again at postnatal day 37, Δ^9-THC proved to be less efficacious at reducing body temperature in both groups. Although a dose-dependent relationship between Δ^9-THC and hypothermia continued through postnatal day 114, the maximum degree of hypothermia observed at any subsequent age point was about 1^o Celsius. This trend held true for both groups. Neither a main effect of diet nor an interaction of diet and dose of Δ^9-THC on hypothermia were observed in male rats at any age.

When a separate group of young male rats were exposed to a cumulative dosing protocol identical to the one used in these experiments, profound tolerance to the

hypothermic effects of Δ^9-THC developed after a single session. The tolerance that developed after the cumulative dosing session on postnatal day 30 was still evident on postnatal day 37 and was robust enough to eliminate the hypothermic response to even large doses of Δ^9-THC (100 mg/kg).

Even though there was no significant main effect of the dose Δ^9-THC on hypothermia at postnatal day 37, there was a significant interaction between the environment where the second test was conducted and the hypothermic effects of Δ^9-THC. In a most unusual twist, however, hypothermic responses to Δ^9-THC were least evident in the environment that had never been paired Δ^9-THC. At the very least, these results suggest that context-specific tolerance plays a very small part in the diminished hypothermic response that appeared after first cumulative dosing session.

Also unlike in the female rats, sustained consumption of a high-fat did not significantly alter the antinociceptive properties of Δ^9-THC in male rats. Under both vehicle conditions and drug conditions, tail withdrawal latencies were virtually identical for both groups. Likewise, both groups of male rats exhibited similar drug-related changes in the latency to withdraw the tail from the noxious stimuli caused by the radiant heat lamp.

Possible Mechanisms of Tolerance

While it cannot yet be concluded that the diet-related changes in sensitivity to the effects of Δ^9-THC observed in these studies involve receptor-level changes, there is no evidence that observed differences in sensitivity to Δ^9-THC are attributable to diet-related differences in pharmacokinetics. The data, however, are generally consistent with the

region-specific changes in CB_1 receptor density reported in elsewhere (Harrold, *et al*., 2002; Hayakawa, *et al*., 2007). In these studies, CB_1 receptor expression appeared to decline significantly in the striatum and nucleus accumbens while remaining essentially unaltered in the hypothalamus after sustained consumption of a high-fat diet.

It would be expected that diminished CB_1 receptor expression in the striatum would result in reduced sensitivity to the psychomotor effects of Δ^9-THC. Similarly, it would be expected that diminished CB_1 receptor expression in the nucleus accumbens would attenuate the augmentation of the hedonic value of food caused by Δ^9-THC. In contrast, unaltered CB_1 receptor expression in the hypothalamus would likely result in an unaltered hypothermic response to Δ^9-THC. Each of these expectations is consistent with the data derived from these experiments.

Sex-differences in Drug Response

Despite the evidence that a high-fat diet alters sensitivity to Δ^9-THC-induced changes in food intake and, to a lesser degree, the antinociceptive properties of Δ^9-THC, these studies do not allow for a direct examination of sex differences because of differing treatment histories for each sex. Male rats endured prolonged periods of inactivity (e.g., 3-9 months) between testing in the triad of measures that compose the experimental core of this project (e.g., hypothermia, gross locomotor activity, time on bar apparatus) and the beginning of the feeding trials and the tail withdrawal tests. The female rats, on the other hand, were moved from the triad of measures to the feeding trials and tail withdrawal tests as rapidly as practical. Accordingly, the food intake data and the measures of antinociception were conducted at different age points for each sex.

It is well-known that nutritional status can affect the menstrual cycle of humans (Frisch and McArthur, 1974). There is evidence of a positive correlation between the consumption dietary fats and plasma estrogen levels during some phases of the human menstrual cycle (Kaneda *et al*., 1997). Despite this, high dietary intake of polyunsaturated fats is not associated with changes in menstrual cycle length (Nagata, Oba and Shimizu, 2006).

Given the evidence derived from humans, it is at least possible that high-fat diets alter estrogen levels rodents. If present, such alterations could be important in the current context because there is some evidence that CB_1 receptor expression in the anterior pituitary gland fluctuates during the course of the rodent estrous cycle and is related to circulating levels of estrogen (Gonzalez *et al*., 2000). It is not clear, however, whether a similar relationship exists in other brain areas. While it is possible that high-fat diets modulate CB_1 receptor expression in the brain by altering circulating estrogen levels, there is scant evidence to support such a conclusion.

Recent evidence from experiments with ovariectomized mice, however, suggests that progesterone may be an important modulator of cannabinoid-induced catalepsy (Kalbasi Anaraki *et al*., 2008). Cannabinoid-induced catalepsy was unaltered when ovariectomized mice were treated with estrogen, but treatment with progesterone potentiated the cataleptogenic properties of cannabinoids. It is important to remember, however, that progesterone levels may decline in female rats that consume high levels of omega-6-polyunsaturated fats (Trujillo and Broughton, 1995). The corn oil used as a

source of dietary fat in this study is rich in omega-6-polyunsaturated fats (U.S. Department of Agriculture, Agricultural Research Service, 2005).

While there appears to be a relationship between the type of diet used in these experiments and low levels of progesterone levels in rats, there is no direct evidence that low levels of progesterone would attenuate catalepsy-like behavior induced by Δ^9-THC. The relationship between high-fat diets, progesterone levels and the cataleptogenic properties Δ^9-THC is one possible future direction for this project.

The experiments with the male rats were conducted in the summer and experiments with the female rats were conducted nearly fifteen months later in the late fall and early winter. As a consequence, potential seasonal differences in the data make a direct comparison of sex-differences in diet-related changes in sensitivity to Δ^9-THC inadvisable. Despite this, there was an obvious disparity in the diet-related changes in the sensitivity to Δ^9-THC that were observed within each sex. In male rats, there were very few diet-related changes in the psychomotor effects of Δ^9-THC, regardless of the diet consumed. As mentioned previously, however, female rats had been maintained on high-fat diet for at least thirty-eight days were much less sensitive to the psychomotor effects of Δ^9-THC than female rats maintained on the control diet.

The results of these experiments raise questions regarding the relationship between sustained consumption of a high-fat diet and the developing brain. It is not clear whether diet-related differences in sensitivity to Δ^9-THC are caused by the consumption of high-fat diets during a vulnerable period of brain development, or whether rats that are placed on high-fat diets in adulthood would eventually exhibit a similar type of

desensitization. These are compelling questions and they may be related to an important public health issue, but they cannot be answered without further experimentation.

Despite these limitations, the apparent sex differences in the effect of a high-fat diet on sensitivity to some of the pharmacological effects of Δ^9-THC are intriguing. As of yet, the degree to which sex hormones influence these differences is unclear. Clearly, parallel trials should be conducted with groups of overiectomized females and castrated males maintained on each of the diets. It is also not yet clear whether the diet-related differences in sensitivity to Δ^9-THC involve differences in CB_1 receptor sensitivity. Accordingly, CB_1 receptor function should be studied in drug-naïve rats of both sexes that have been maintained on each of the diets.

The degree to which the various phases of the estrous cycle influence sensitivity to Δ^9-THC in rodents is of fundamental importance, but there is a paucity of research in this area. The female rats used in these studies were housed separately in cages that were under positive airflow. This housing arrangement and the positive airflow environment of the home cages should have impaired estrous synchrony in these rats (Schank and McClintock, 1997).

The experiments detailed here were designed as part of a developmental study and employ methodologies that are distinct from those used in studies designed to explore sex-differences. While the phase of the estrous cycle was not considered during these experiments, it is improbable that the female rats maintained on a high-fat diet randomly expressed synchronized estrous cycles while being uniformly out-of-phase with the female rats maintained on the control diet. Female rats have an estrous cycle that lasts

between 4 and 5 days (Butterstein *et al*., 1997). Assuming a mean estrous cycle duration of 4.5 days, the probability of nine rats randomly expressing a synchronized estrous cycle while nine other rats were randomly asynchronous is (0.222^8 X 0.778^9) or 6.19 X 10^{-7}.

In conclusion, the data derived from these experiments suggest that, in female rats, sustained consumption of a high-fat diet throughout adolescence and into adulthood significantly reduces sensitivity to the psychomotor effects of Δ^9-THC when compared to female rats maintained on the control diet. Further, female rats maintained on the high-fat diet were less sensitive to the changes in food intake caused by Δ^9-THC than were female rats maintained on the control diet. In contrast, relatively few differences in the sensitivity to Δ^9-THC were noted in male rats maintained under the same dietary conditions throughout the same developmental period.

These findings raise questions regarding possible sex-differences in the pharmacokinetics of Δ^9-THC, as well as the relationship between dietary fats, sex hormones and Δ^9-THC. Further, it is not clear whether similar results would be seen in female rats that began eating a high-fat in adulthood. The mechanism(s) by which high-fat diets differentially alter sensitivity Δ^9-THC across the sexes has yet to be elucidated and the possibility of diet-related changes in endocannabinoid signaling must considered in the light of these results. All of these topics represent possible future directions for these studies.

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Vita

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