# The behavioural and neural effects of cannabinoids:

## *Studies using Lewis and Wistar strain rats*

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# Dedication

*To my sister*

**Alysia Arnold**

*1979-1992*

Woodcut of *cannabis sativa*, 1543 (left) and Charles Baudelaire (right)
from
Plants of the Gods (1992)
By Richard Evans Shultes
and Albert Hofmann.

# Abstract

Cannabis (known in its common forms as *Cannabis sativa* or *Cannabis indica*) is the most widely used illicit drug in the world and has been used for thousands of years for medicinal, religious and hedonistic purposes. In the last half of the 20th century the therapeutic uses of cannabis were largely ignored as most Western governments prohibited the use of the drug. Prohibition has come about largely as a result of the view that cannabis is a dangerous drug that poses major risks to both mental and physical health. However, this view is being increasingly challenged in recent years with a major popular movement towards decriminalization of cannabis occurring in some Western countries and a resurgence of interest in the medicinal properties of cannabis.

Since Mechoulam and colleagues first isolated $\Delta^9$-tetrahydrocannabinol ($\Delta^9$-THC) as the main psychoactive constituent of cannabis, considerable advances have been made in the pharmacology of cannabis and cannabis-like drugs (cannabinoids). Central and peripheral cannabinoid receptors have been isolated and two endogenous ligands have been discovered. In addition, two cannabinoid receptor antagonists have been developed. However, our knowledge of the behavioural, neural and emotional effects of cannabis and the cannabinoids has often lagged behind our understanding of basic cannabinoid pharmacology.

The present thesis attempts to further the understanding of the behavioural, neural and emotional effects of cannabinoids, using laboratory rats as subjects. A synthetic analogue of $\Delta^9$-THC (CP 55,940), is used as the primary pharmacological tool. The thesis offers a broad perspective with three major areas of investigation. These are: 1) the effects of CP 55,940 on anxiety-related behaviour (Chapters 2 and 3); 2) the effects of CP 55,940 on patterns of brain activation as indicated by *c-fos* expression (Chapter 4) and; 3) the addictive potential of CP 55,940 and its capacity to produce sensitization to the effects of other drugs such as cocaine (Chapters 5 and 6). A recurring theme throughout the thesis is that genetic factors may partially determine

the behavioural, neural and emotional response to cannabinoids. To this end, the thesis compares Lewis and Wistar strains of rat in a wide variety of assays. Previous research has isolated Lewis rats as an "addiction-prone" and a "cannabinoid-preferring" strain, as they are more sensitive to the rewarding effects of various drugs of abuse including cannabinoids. Conversely, cannabinoids appear to have aversive effects in Wistar rats.

A long-standing puzzle in cannabinoid research has been the question of why rats do not self-administer cannabis or cannabinoids. One likely reason is that cannabinoids have predominately aversive effects in rats. It is proposed here that these aversive effects arise because cannabinoids are anxiogenic agents in most rat strains. However some evidence indicates that the Lewis strain of rat are the only strain to find cannabinoids rewarding. It is hypothesised that Lewis rats may be more susceptible to the rewarding effects of cannabinoids because they are less susceptible to the anxiogenic effects of these compounds.

In Chapters 2 and 3 the anxiogenic effects of the synthetic cannabinoid agonist CP 55,940 were compared in Lewis and Wistar rats in several different animal models of anxiety. In Chapter 2, the predatory odour avoidance, open area avoidance and conditioned ultrasonic vocalization (USV) models were utilised. In the predatory odour avoidance model, rats were exposed to cat odour in a rectangular arena and given the opportunity to hide in a small box. Both Lewis and Wistar rats displayed high levels of hiding during odour exposure. In Wistar but not Lewis rats, 50 µg/kg of CP 55,940 (i.p.) enhanced this avoidance response. Unfortunately, Lewis rats showed exceptionally high avoidance of the cat odour making it difficult to discern the effects of CP 55,940. To avoid this problem a second experiment was conducted, where rats were tested in the same arena as in the first experiment but with no cat odour present. Again in Wistar, but not Lewis rats, 25 and 50 µg/kg of CP 55,940 (i.p.) increased the avoidance of the open space. In the third experiment, Lewis and Wistar rats were placed in a chamber in which they had previously received footshock. Wistar but not Lewis rats re-exposed under the influence of 10, 25 or 50

µg/kg CP 55,940 (i.p.) emitted significantly more USVs than vehicle-treated rats. Thus, CP 55,940 clearly increased anxiety-related behaviour in Wistar rats but not Lewis rats, supporting the notion of a genetic predisposition towards cannabinoid-induced anxiety.

In Chapter 3 the generality of the findings made in Chapter 2 were tested by utilising two further animal models of anxiety, the social interaction and light-dark emergence tests. From the results of Chapter 2, it could be claimed that Lewis rats were merely subsensitive to the effects of CP 55,940. Therefore a higher dose range (0, 25, 50 and 75 µg/kg i.p.) of CP 55,940 was employed in Chapter 3. In addition, the rotarod test was used to assess whether CP 55,940 has ataxic effects at these doses. In the first experiment, two unfamiliar rats were placed in a large arena and the time the rats spent socially interacting was recorded. CP 55,940 significantly reduced the total time rats spent socially interacting in Lewis (25 and 75 µg/kg) and Wistar rats (50 and 75 µg/kg). However, CP 55,940 has a significantly greater effect in Wistar rats compared to Lewis rats. In the second experiment, rats were placed in a small box within a large open arena and the latency to emerge from this box was measured. CP 55,940 increased emergence latency (at 75 µg/kg) and mean time per entry into the box (at 25 and 75 µg/kg) in Wistar but not Lewis rats. Furthermore, CP 55,940 caused a greater decrease in time spent in the open arena (at 25 and 75 µg/kg) and frequency of emergence (at 75 µg/kg) in Wistar rats in comparison to Lewis rats. In the third experiment, CP 55,940 (at 25, 50 and 75 µg/kg) caused mild incoordination only in Lewis rats as measured by the rotarod test. This finding argues against the assertion that the CP 55,940-induced anxiety-like behaviours in Wistar rats are merely a result of motoric impairment. Furthermore, it illustrates that Lewis rats are not generally subsensitive to the effects of CP 55,940. That is, when compared to other rat strains, Lewis rats may be more or less sensitive to the effects of CP 55,940 depending on what behaviour is being assessed.

From the results of Chapters 2 and 3 it can be seen that Lewis rats are less sensitive to the anxiogenic effects of CP 55,940 than Wistar rats. In Chapter 4 it was

hypothesised that in Lewis rats the effects of CP 55,940 on neural substrates of reward far outweigh the effects the compound has on neural substrates mediating anxiety. To examine this issue, the effects of CP 55,940 at a moderate (50 µg/kg i.p.) and high (250 µg/kg i.p.) dose were observed on *c-fos* expression (a measure of neural activation) and behaviour in Lewis and Wistar rats. CP 55,940 dose-dependently inhibited locomotor activity and reduced body temperature with Lewis rats being significantly less affected than Wistar rats. The 250 µg/kg dose caused significant catalepsy in both strains with a significantly greater effect in Wistar rats. These strain differences in the effects of CP 55,940 on body temperature and motor behaviour clearly correlated with *c-fos* expression in various regions and subregions. In general, Lewis rats showed significantly less Fos-labeled cells in comparison to Wistar rats. These strain differences in the effects of CP 55,940 on *c-fos* expression appeared unique to cannabinoids, as cocaine (15 mg/kg i.p.) had equivalent effects on *c-fos* expression in Lewis and Wistar rats.

CP 55,940 promoted *c-fos* expression in areas not previously assessed, such as the median preoptic nucleus (MnPO), medial preoptic nucleus (MPO), anterior hypothalamic area (AH), islands of Calleja (ICjM), periaqueductal gray (PAG) and the pedunculopontine tegmental nucleus (PPTg). The strain differences uncovered in Chapters 2 and 3 correlated well with strain differences in the effects of CP 55,940 on *c-fos* expression in areas implicated in cannabinoid-induced anxiety, such as the central nucleus of the amygdala, bed nucleus of the stria terminalis, paraventricular nucleus of the hypothalamus and PAG. However, the effects of CP 55,940 on *c-fos* expression in a neural circuit which may underlie reward, which includes the shell of the nucleus accumbens (NAS) and PPTg, were also less in Lewis rats in comparison to Wistar rats. Future investigations must address whether the reduced effects of CP 55,940 on the Lewis rat are due to pharmacokinetics or pharmacodynamics. In addition, future studies must reconcile the pattern of *c-fos* expression observed here with prior reports of the Lewis rat being a unique “cannabinoid-preferring” strain.

In Chapter 4, CP 55,940 administration promoted *c-fos* expression in areas of the brain thought to play a critical role in behavioural sensitization such as the ventral tegmental area and NAS. This is interesting because it is possible that *c-fos* is involved in promoting neuroadaptations that underlie drug addiction. To examine this idea, Chapter 5 investigated a behavioural assay of the long-term neural adaptations that may occur with the chronic administration of cannabis, namely, behavioural sensitization. This chapter also examined an animal model of the "gateway hypothesis", that is, the hypothesis that prior exposure to cannabis increases an individual's vulnerability to using other drugs. This animal model is known as cross-sensitization. First it was shown that Lewis, but not Wistar rats, given cocaine (15 mg/kg i.p.) every second day over a two week period displayed a progressively greater locomotor response to the drug over days indicating behavioural sensitization. When CP 55,940 (0, 10, 25 or 50 µg/kg i.p.) was administered under a similar regime, no such sensitization was observed in either strain. Rather, the two highest doses of CP 55,940 (25 and 50 µg/kg) caused locomotor suppression that lasted throughout administration. When Lewis or Wistar rats pre-exposed ten times to CP 55,940 were challenged with cocaine (15 mg/kg), no exaggerated locomotor response to cocaine was evident relative to non pre-exposed rats. When these rats were subsequently re-tested with CP 55,940, it continued to produce a dose-dependent suppression of locomotor activity. Finally, when CP 55,940 (50 µg/kg) was co-administered with cocaine in Lewis rats, it significantly reduced the locomotor hyperactivity produced by the drug but did not block the development of behavioural sensitization to cocaine. These results show that CP 55,940 does not sensitize locomotor activity with repeated administration in the same way as cocaine, and that pre-exposure or concurrent exposure to CP 55,940 does not enhance sensitivity to the subsequent behavioural effects of cocaine. Therefore, unlike Chapters 2, 3 and 4 where strain differences were observed in CP 55,940–induced anxiety, hypothermia, catalepsy, *c-fos* expression and ataxia, there were no strain differences with respect to behavioural sensitization.

Landmark studies by Gardner and colleagues showed that Lewis rats are particularly susceptible, in comparison to other rat strains, to the rewarding effects of $\Delta^9$-THC on: 1) medial forebrain bundle (MFB) self-stimulation behaviour and; 2) dopamine (DA) efflux in the NAS. However, in Chapter 4 Lewis rats were less susceptible than Wistar rats to CP 55,940-induced *c-fos* expression in the NAS. Further, Lewis rats showed no behavioural sensitization to the chronic administration of CP 55,940. In light of these findings, Chapter 6 assessed whether CP 55,940 *does* have a rewarding effect on MFB self-stimulation behaviour in Lewis rats. Lewis rats were trained to self-stimulate the MFB using a rate–frequency paradigm and then administered CP 55,940 (0, 10, 25 and 50 µg/kg i.p.). CP 55,940 had no effect on MFB self-stimulation behaviour as assessed by the $M_{50}$, the stimulation frequency at which half-maximal response rates were obtained. This result calls into question previous assertions that Lewis rats are a "cannabis-preferring" strain of rat.

Previous studies utilising the cannabinoid $CB_1$ receptor antagonist, SR 141716, have shown that the endogenous cannabinoid system may have some involvement in the rewarding effects of cocaine, morphine, sucrose and alcohol. Thus, Chapter 6 also assessed the effects of SR 141716 (0, 1, 3, 10 and 20 mg/kg i.p.) on MFB stimulation in Lewis rats. The role of DA in MFB stimulation reward has already been established, so for comparison purposes the effects of the DA $D_1$ receptor antagonist SCH 23390 (0.06 mg/kg i.p.) was also assessed. Only a very high dose of SR 141716 (20 mg/kg) caused a significant inhibition of the rewarding efficacy of the stimulation with all other doses (1, 3, and 10 mg/kg) being ineffective in modulating the rewarding impact of brain stimulation. This was seen as an increase in $M_{50}$. By comparison, a relatively low dose (0.06 mg/kg) of SCH 23390 caused a large increase in $M_{50}$. These results indicate a relatively modest influence of the endogenous cannabinoid system on reward-relevant neurotransmission in the self-stimulation paradigm.

Chapter 7 concludes the thesis and discusses the implications of the results obtained. The main findings of the current thesis are: 1) that the suggested "addiction-prone" Lewis strain of rat is less susceptible to cannabinoid-induced anxiety in

comparison to Wistar rats; 2) Lewis rats show less cannabinoid-induced *c-fos* expression in comparison to Wistar rats (including in brain regions implicated in cannabinoid-induced anxiety and reward); 3) cannabinoid-induced *c-fos* expression exists in a number of brain regions never previously assessed such as the MPO, ICjM and PPTg; 4) behavioural sensitization does not occur with the repeated administration of CP 55,940; 5) cannabinoid pre-exposure or co-administration does not increase the sensitivity of the locomotor–activating effects of cocaine; 6) the endogenous cannabinoid system, at most, only has a minor influence on the neural substrate of brain stimulation reward and; 7) that there are previously unreported strain differences in cannabinoid-induced hypothermia, catalepsy and ataxia. These results add to our understanding of the effects of the behavioural, emotional and neural effects of cannabinoids and the endogenous cannabinoid system.

# Abbreviations

2-AG ……2-arachidonyl glycerc
5-HT ……..5-hydroxytryptamin
ACTH ……..adrenocorticotropic hormon
AH ……..anterior hypothalamic are
AIDS ……acquired immunodeficiency syndrom
ANOVA ……analysis of varianc
BNST ……bed nucleus of the stria terminali
cAMP ……cyclic adenosine monophosphat
CEA ……..central nucleus of the amygdal
cm ……..centimeter
CNS ……central nervous syster
CPU ……caudate-putame
CRF ……continuous reinforcemer
CRH ……..corticotropin releasing hormon
DA ……dopamin
DNA ……..deoxyribonucleic aci
DSM ……...Diagnostic and Statistical Manual of Mental Disorder
EPM ……..elevated plus-maz
FI ……fixed interva
g ……..gram
GABA ……..gamma amino butyric aci
GP ……globus pallidu
h ……hour
HPA ……hypothalamo-pituitary-adrena
i.p. ……..intraperitonea
ICD ….International Statistical Classification of Diseases and Related Health Problem
ICjM ……islands of Callej
IEG ……immediate early gen
LH ……lateral hypothalamic are
LS ……..lateral septur
m ……..meter
mA ……milliamp
MDMA……..……3,4 methylenedioxymethamphetamine
MFB ……..medial forebrain bundl
min ……minute
MnPO ……..median preoptic nucleu
MPA ……medial preoptic are
MPC ……..medial prefrontal corte
MPO ……medial preoptic nucleu
mRNA ……..messenger ribonucleic aci
NAS ……..nucleus accumben
NMDA ……..N-methyl-D-aspartat
PAG ……periaqueductal gre
PBS ……phosphate buffered salin
PCR ……polymerase chain reactio
PET ……positron emission tomograph
PPTg ……pedunculopontine tegmental nucleu
PV ……..paraventricular nucleus of the thalamu
PVN ……..paraventricular nucleus of the hypothalamu
r.p.m. ……revolutions per minut

REM …….random eye movemer
SCh ……..suprachiasmatic nucleu
sec ……second
SEM ……..standard error of the mea
SN ……substantia nigr
SO ……..supraoptic nucleu
TH ……tyrosine hydroxylas
$\Delta^9$-THC ……..$\Delta^9$-tetrahydrocannabinc
U.S.A. ……United States of Americ
USV …….ultrasonic vocalizatio
VEH …….vehicl
VTA ……..ventral tegmental are
W ……watt

# Publications and Submissions

**J. C. Arnold**, A. N. Topple, G. E. Hunt and I. S. McGregor (1998). Effects of pre-exposure and co-administration of the cannabinoid receptor agonist CP 55,940 on behavioural sensitization to cocaine. ***European Journal of Pharmacology***, 354, 9-16.

**J. C. Arnold**, G. E. Hunt, I. S. McGregor. Antagonism of central cannabinoid $CB_1$ receptors reduces the rewarding efficacy of medial forebrain bundle self-stimulation in rats (submitted).

**J. C. Arnold**, R. A. Dielenberg and I. S. McGregor. Strain differences in cannabinoid-induced anxiety: studies in Lewis and Wistar strain rats (submitted).

**J. C. Arnold**, A. N. Topple, P. E. Mallet and I. S. McGregor. Strain differences in the effects of the synthetic cannabinoid receptor agonist CP 55,940 on *c-fos* expression, body temperature and motor behaviour. Manuscript in preparation.

**J. C. Arnold**, G. E. Hunt and I. S. McGregor (1999) The central endogenous cannabinoid system: a subtle role in the reinforcing efficacy of brain stimulation? Paper presented to the Postgraduate conference, Department of Psychology, University of Sydney, October 1999. This abstract was published in the ***Journal of Applied Health Behaviour***, 1-2, 1999.

**J. C. Arnold**, E. Campbell, A. N. Topple, S. L. Booker and I.S. McGregor. The continuing search for strain differences in the reinforcing effects of cannabinoids in rats. Paper presented to the International Cannabinoid Research Society conference, Atlanta, Georgia, June 1997.