

# CHAPTER SEVEN

## General Discussion

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The current thesis deals with a broad range of issues relevant to the study of cannabis as a drug of abuse. Ultimately, the studies presented here may increase our understanding of the neuropharmacological and genetic bases of cannabis dependence and cannabis-induced anxiety in the human population. Cannabis is moderately addictive in humans, with only a small subset of users becoming dependent on the substance. In addition, only a small proportion of the human population appears to be susceptible to the anxiogenic effects of cannabis. One useful explanation for these observations is that certain individuals may be genetically predisposed to cannabis dependence and anxiety (Lyons et al., 1997). The current thesis addressed the issue of genetic susceptibility to the effects of cannabinoids by using different rat strains as experimental subjects. Specifically, it investigated why cannabinoids have been claimed to have uniquely rewarding effects on the Lewis strain of rat (Gardner et al., 1988; Lepore et al., 1996a).

The results of Chapters 2 and 3 provided evidence that Lewis rats may be less susceptible to the anxiogenic effects of the synthetic cannabinoid receptor agonist, CP 55,940, when compared to Wistar rats. This was clearly illustrated in three different animal models of anxiety: the social interaction test, the conditioned USV test and the open area avoidance test. Data gained from the predatory odour avoidance and light-dark emergence models of anxiety suggested a similar strain difference in the effects of CP 55,940. However, these models were plagued by confounding floor and ceiling effects in baseline levels of anxiety-like behaviour that limited any strong interpretation of the data. While beyond the scope of the current thesis, future experiments might attempt to circumvent these floor and ceiling effects before drug testing by manipulating different

conditions such as the level of light in the experimental room. Taken together, experiments in Chapters 2 and 3 provide strong evidence that Lewis rats are less susceptible to the anxiogenic effects of CP 55,940 than Wistar rats. This observation may help explain why Lewis rats are also distinctively affected by cannabinoids in terms of reward. That is, if you assume that the rewarding effects of cannabinoids trade-off with the simultaneous anxiogenic effects of cannabinoids, then Lewis rats may be more susceptible to reward because they are less susceptible to anxiety. However, in other strains of rat such as Wistar rats, the anxiogenic effects of cannabinoids may overshadow the rewarding effects.

Chapter 4 demonstrated that the strain differences in CP 55,940-induced anxiety observed in Chapters 2 and 3 correlated with strain differences in the effects of CP 55,940 on the pattern of neural activity, as measured by *c-fos* expression in brain areas involved in reward and anxiety. This study specifically tested whether CP, 55,940 had distinct effects on the pattern of Fos, the protein product of the IEG, *c-fos*, in the CNS of Lewis rats in comparison to Wistar rats. In general, Lewis rats showed significantly less CP 55,940-induced Fos-labeled cells compared to Wistar rats. CP 55,940 may have less affected Lewis rats than Wistar rats because of reduced CB<sub>1</sub> receptor activation or because CB<sub>1</sub> receptor activation was less effective in regulating the expression of the *c-fos* gene. When paying particular attention to areas implicated in cannabinoid-induced anxiety, such as the CEA, BNST, PVN and PAG, Lewis rats showed significantly less CP 55,940-induced *c-fos* expression in comparison to Wistar rats. Thus, these data are consistent with the notion that the Lewis rat's increased susceptibility to the rewarding effects of cannabinoids may be explained by their reduced susceptibility to the anxiogenic effects of cannabinoids.

In Chapter 4, Lewis rats, when compared to Wistar rats, unexpectedly showed fewer Fos-labeled cells in neural substrates implicated in reward, such as the NAS, VTA and PPTg. In light of the fact that DA is critical to cannabinoid-induced *c-fos* expression in the striatum (Miyamoto et al., 1996), and DA release in the ventral striatum is thought to be central to the rewarding actions of drugs of abuse (Koob, 1996; Koob & Le Moal, 1997;

Koob et al., 1998; Robbins & Everitt, 1999), it was hypothesised that Lewis rats administered CP 55,940 would show enhanced *c-fos* expression, at least in the VTA and NAS, in comparison to Wistar rats. The reasons why the opposite results were found are not clear. However, these results may have import in explaining why cannabinoids have only a minor rewarding effect, if any, on Lewis rats. This assertion is based on three findings. First,  $\Delta^9$ -THC only slightly modulates the rewarding efficacy of brain stimulation in comparison to other drugs of abuse, such as cocaine (Lepore et al., 1996a). Second, as reported in Chapter 6, the selective cannabinoid receptor antagonist, SR 141716, only subtly reduced the rewarding efficacy of brain stimulation in comparison to the D<sub>1</sub> receptor antagonist, SCH 23390. Third, as also reported in Chapter 6, CP 55,940 was found to have no effect on the rewarding efficacy of brain stimulation. Taken together these findings are consistent with the notion that Lewis rats may not find cannabinoids sufficiently rewarding to enable self-administration. Thus, it appears likely that the unmasking of a subtle rewarding effect of  $\Delta^9$ -THC (Lepore et al., 1996a) is more likely a consequence of Lewis rats being less susceptible to the anxiogenic effects of cannabinoids, rather than cannabinoids having more of an effect on the neural substrates of reward.

One possible explanation for the lesser *c-fos* expression observed in reward-relevant brain areas of Lewis in comparison to Wistar rats relates to the observation that Lewis rats are generally less affected by CP 55,940 than Wistar rats. Thus, it could be argued that Lewis rats are merely subsensitive to the effects of CP 55,940 in comparison to Wistar rats. This was demonstrated in Chapters 2, 3 and 4 when assessing the effects of CP 55,940 on anxiety, *c-fos* expression, catalepsy, body temperature and locomotor activity in Lewis compared to Wistar rats. This subsensitivity may arise for a number of reasons. Lewis rats may have fewer CB<sub>1</sub> receptors or fewer CB<sub>1</sub> receptor linked G<sub>i</sub> proteins when compared to Wistar rats. Lewis rats may express a unique cannabinoid-destroying enzyme that limits the bioavailability of CP 55,940. Or subtle differences may exist between Lewis rats and Wistar rats in the mRNA that encodes the binding domains of

the CB<sub>1</sub> receptor protein. All these tentative explanations provide testable hypotheses for future research.

However, observations made in the current thesis, and in previous research, are not always consistent with the assertion that Lewis rats are merely subsensitive to the effects of CP 55,940 in comparison to Wistar rats. In Chapter 3, Lewis rats administered CP 55,940 showed a dose-dependent reduction in the latency to fall from a rotarod in comparison to Wistar rats, who were unaffected by CP 55,940. Furthermore, prior research has shown that the dose of <sup>9</sup>-THC which facilitates self-stimulation in Lewis rats (1.0 mg/kg) is relatively low (Lepore et al., 1996a) and approximates the potency of the doses of CP 55,940 used in the present study. Taking such research into account, it appears that the assertion that Lewis rats are merely subsensitive to the effects of cannabinoids is questionable and further research is required. Findings presented here are equally consistent with the notion that the Lewis strain of rat is uniquely affected by cannabinoids such as CP 55,940.

Results of the present thesis bear similarity to a study that demonstrated a differential anxiogenic response to <sup>9</sup>-THC in the EPM across C57BL/6, DBA/2 and ICR strains of mice (Onaivi et al., 1996). This study concluded that only ICR mice showed heightened anxiety-like behaviours on the maze while under the influence of <sup>9</sup>-THC. This conclusion was problematic as other mouse strains exhibited higher baseline levels of anxiety-like behaviour regardless of drug treatment, and these near-ceiling effects made it difficult to discern an anxiogenic response to <sup>9</sup>-THC. This situation bears some similarity to the results reported in Chapters 2 and 3. In these chapters it was shown in the predatory odour avoidance model and the light-dark emergence test that strain differences in cannabinoid-induced anxiety were difficult to discern due to the high levels of baseline anxiety-like behaviours in Lewis rats. Regardless of interpretation problems surrounding the data of Onaivi *et al* (1996), their research revealed two novel and distinct CB<sub>1</sub> receptor mRNAs in the C57BL/6 strain of mouse, providing evidence for more than one subtype of

CB<sub>1</sub> receptor in this mouse strain. Interestingly, the C57BL/6 strain showed different hypothermic and analgesic responses to <sup>9</sup>-THC compared to ICR and DBA/2 mice. This suggests that the presence of different subtypes of CB<sub>1</sub> receptor may bestow differential sensitivity to the effects of cannabinoids. Using techniques such as the polymerase chain reaction (PCR) or immunohistochemistry, it would be of interest to determine whether Lewis rats also show the presence of distinct variants of the CB<sub>1</sub> receptor in specific brain regions, or whether Lewis rats show a lesser density of CB<sub>1</sub> receptors in areas implicated in anxiety such as the CEA, BNST, PVN and PAG.

The current thesis provides some testable hypotheses for future research which may attempt to manipulate rodents into self-administering cannabinoids. Self-administration studies may further test the hypothesis that Lewis rats are susceptible to the rewarding effects of cannabinoids because, in comparison to Wistar rats, they are less susceptible to the anxiogenic effects of cannabinoid compounds. Testing self-administration of <sup>9</sup>-THC or CP 55,940 in Lewis rats is clearly an experiment that needs to be performed.

However, it is apparent that Wistar rats could be subjected to neural manipulations that may lead them to self-administer cannabinoids. Lesions of neural substrates of anxiety, or the use of a CRH receptor antagonist may not increase the Lewis rats ability to self-administer a cannabinoid, because they do not have much cannabinoid-induced anxiety or CRH release to reduce (Sternberg et al., 1989a; Sternberg et al., 1989b). However, Wistar rats, or rats with high levels of cannabinoid-induced anxiety or CRH release, could be made to self-administer the drug if its anxiogenic effects are blocked. The unexpected results documented in Chapter 4 add weight to this hypothesis. That is, Wistar rats showed greater CP 55,940-induced *c-fos* expression in reward-relevant substrates compared to Lewis rats. This may indicate that CP 55,940 had more of an effect on reward in Wistar rats than in Lewis. However, Wistar rats still show an increased susceptibility to the anxiogenic effects of cannabinoids that overshadows any delineation of a rewarding effect. If the anxiogenic effects of cannabinoids on Wistar rats were limited by lesions or

pharmacological blockade, for example, by co-administering a benzodiazepine, then they may be more likely to self-administer cannabinoids .

Chapters 5 and 6 were concerned with the addictive potential of cannabinoids and their capacity to produce sensitization to other drugs such as cocaine. Thus, Chapter 5 attempted to construct an alternative and hitherto untested behavioural model of neuroadaptations that may underlie the addiction process. Further, it also provided a model of the so-called "gateway" hypothesis. Reinforcing the notion that Lewis rats are particularly sensitive to the effects of drugs of abuse, Lewis rats but not Wistar rats showed clear behavioural sensitization to cocaine. However, unlike cocaine, chronic intermittent exposure of small to moderate doses of CP 55,940 did not produce behavioural sensitization, with no gradual increase in locomotor activity observed over days in Lewis or Wistar rats. Further, pre-exposure to CP 55,940 did not increase the locomotor activating effects of cocaine in these strains. Conversely, Chapter 5 provided evidence that pre-exposure to cocaine did not modulate the effects of CP 55,940 on locomotor activity in Lewis or Wistar rats. Furthermore, when CP 55,940 was co-administered with cocaine in Lewis rats, it significantly reduced the locomotor activating effects of cocaine without blocking or enhancing the development of behavioural sensitization. Thus, behavioural cross-sensitization did occur between CP 55,940 and cocaine when tested using a variety of different methods. Based on this evidence it appears unlikely that CP 55,940 is able to induce sensitization of neural pathways thought to be pivotal to enhancing drug-seeking behaviour. Furthermore, the present experiment does not offer, at least on the behavioural level, a clear support for the "gateway" effect of cannabis.

Many future research opportunities exist in the search for an animal model of the "gateway" hypothesis. A recent study has shown that pre-exposure to  $\Delta^9$ -THC potentiated subsequent amphetamine-induced stereotypy and locomotor activity (Gorriti et al., 1999). This promising study underscores the need for future endeavours to utilise other cannabinoid receptor ligands and other drugs of abuse, such as amphetamine, heroin or

nicotine, before it can be definitively stated that cannabinoids cannot produce behavioural sensitization or cross-sensitization. In addition, it is possible that the chronic intermittent administration of cannabinoids produces cellular and molecular changes in the CNS that are not accompanied by any overt changes in behaviour. Therefore, cellular and molecular studies need to be performed to determine whether neuroadaptations do arise from the chronic intermittent administration of cannabinoids. For instance, a microdialysis study could assess whether an incremental rise in DA release occurs in the shell of the NAS with the chronic intermittent exposure of rats to cannabinoids. A study like this, if successful, would be able to show that cannabinoids do "sensitize" neural pathways central to addiction.

Finally, Chapter 6 attempted to extend previous research which has isolated a rewarding effect of  $\Delta^9$ -THC in Lewis rats (Lepore et al., 1996a). However, CP 55,940, a synthetic analogue of  $\Delta^9$ -THC, used throughout the thesis, was shown to have no clear effect on responding for electrical stimulation of the MFB. The reasons for this are unclear. However, previous experiments documenting the rewarding effect of  $\Delta^9$ -THC on brain self-stimulation behaviour only revealed this effect when variation in baseline responding over days was very small. Thus, the lack of reward modulation reported in the current thesis may simply be an artifact of a lack of stability in baseline responding over days, so preventing the delineation of a subtle rewarding effect of CP 55,940. Alternatively, slight differences do exist between the chemical structures of  $\Delta^9$ -THC and CP 55,940, and these might account for the lack of effect of CP 55,940 on reward. Therefore, it is possible that the effects of  $\Delta^9$ -THC may be more rewarding than the effects of CP 55,940. It is also possible that the effects of CP 55,940 may be more aversive than the effects of  $\Delta^9$ -THC. Future research could aim to test such hypotheses.

The finding that the selective cannabinoid receptor antagonist, SR 141716, dose-dependently reduced the rewarding efficacy of MFB stimulation is consistent with the involvement of endogenous cannabinoids in the neural substrate of reward. Similar to the

experiments conducted by Gardner and colleagues with  $\Delta^9$ -THC, the effect of SR 141716 on reward was quite small. The only dose that was significantly effective was a large dose of SR 141716 (20 mg/kg). This brought into question whether the effect of SR 141716 was specifically mediated by CB<sub>1</sub> receptors alone. A future experiment can test this by observing whether the coadministration of a cannabinoid receptor agonist, such as CP 55,940, is able to reverse the effect of 20 mg/kg of SR 141716. However, recent evidence suggests that this experiment may not easily solve the problem. Recent findings indicate that SR 141716 may not act "silently" to block endogenous cannabinoid tone (Rinaldi-Carmona et al., 1995; Rinaldi-Carmona et al., 1994). That is, SR 141716 may be an inverse agonist and have its own intrinsic effect upon the CB<sub>1</sub> receptor (Bouaboula et al., 1997; MacLennan et al., 1998).

The current thesis also provides a finding pertinent to strain differences in the effects of cocaine on behaviour. In Chapter 5, it was shown that Lewis rats were more susceptible to behavioural sensitization promoted by cocaine in comparison to Wistar rats. This is consistent with the notion that Lewis rats are more susceptible to acquisition of a cocaine self-administration habit which may rely on their enhanced susceptibility to the sensitizing effects of cocaine (De Vries et al., 1998; Kosten et al., 1997; Phillips & Di Ciano, 1996). Furthermore, the current thesis provides evidence of strain differences in drug-free behaviour and *c-fos* expression. Thus in Chapter 2 and 3, Lewis rats showed higher baseline levels of anxiety-like behaviours as assessed by the open area avoidance and light-dark emergence tests. Moreover, in Chapter 4 it was shown that Lewis rats exhibited lower baseline locomotor activity and reduced baseline *c-fos* expression in certain brain regions in comparison to Wistar rats.

It is interesting to speculate on which genes could be responsible for the divergent effects that CP 55,940 has on Lewis and Wistar rats. One method useful to answering such a question is the analysis of gene expression using oligonucleotide arrays (also known as "Gene Chips"). The advantage of this technique is that it allows inferences to be drawn

about how different genes might interact to create a complex trait such as anxiety. This is provided by the ability of the arrays to assay up to 10,000 genes at once (Sandberg et al., 2000). Oligonucleotide arrays would allow the measurement of whether differential gene expression exists between Lewis and Wistar rats at baseline and also after being exposed to CP 55,940 in different brain regions. In the future it is conceivable that this sort of information may aid in the isolation of genetic markers to help determine which people in the community are more susceptible to cannabinoid-induced anxiety or dependence.

## 7.1. Conclusion

The current thesis increases our understanding of the behavioural, neural and emotional effects of cannabinoids. It highlights the lack of a viable animal model of cannabis self-administration in rats. This continues to be a problem for behavioural pharmacologists working in the cannabinoid field. The current thesis shows that the alleged “cannabis-preferring” and “addiction prone” Lewis strain of rat may not in fact be more susceptible to the addictive or rewarding effects of cannabinoids. This is indicated by: 1) an absence of effects of both CP 55,940 and SR 141716 on self-stimulation, 2) a lack of CP 55,940-induced behavioural sensitization, and 3) an absence of CP 55,940-induced cross-sensitization with the effects of cocaine. However, the current thesis does show that the Lewis strain of rat is uniquely affected by cannabinoids. The various strain differences being observed in the effects of cannabinoids on anxiety-like behaviours, motor behaviours, *c-fos* expression and body temperature support this claim. Such findings contribute to the large body of evidence which indicates that genetic disposition contributes to the various ways animals respond to drugs. Results of the current thesis are consistent with a recent human twin study showing that genetic factors play an important role in determining the subjective responses to cannabinoids (Lyons et al., 1997).

The current research contributes towards the greater understanding of the effects of cannabinoids and the endogenous cannabinoid system. This may assist in elucidating the widespread use and abuse liability of cannabis. This research may also be valuable because cannabinoids are an emerging class of therapeutic agents yet more needs to be known about how cannabis affects the brain and behaviour.