THE LASTING NEUROBEHAVIOURAL EFFECTS OF CANNABINOIDS: A COMPARISON OF PERINATAL, ADOLESCENT, AND EARLY ADULT EXPOSURE

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PREFACE

Historically, few other drugs of abuse have provoked more controversy than cannabis. Cannabis use appears to have originated in Central Asia, and has a long history reported to date back centuries before the birth of Christ (for review see Fankhauser, 2002). It is not only one of the oldest medicinal plants known, but is considered an innocuous drug of leisure in most countries (ElSohly, 2002). Whilst use of cannabis has been reported to have a number of positive recreational (Health Council of the Netherlands: Standing Committee on Medicine, 1996) and health benefits (Joy, Watson, & Benson, 1999), negative psychological and physiological symptoms have also been reported (for review see Hall, Solowij, & Lemon, 1994).

The following research confirms that there are a number of negative effects associated with cannabis exposure. However, the author wishes to put these findings in perspective--In the author's mind, it is unclear as to whether cannabis use should be legalised or retain its illegal status. The exploration of this question is beyond the scope of the current research. It is clear however, that cannabis has attracted "bad press" in a historical sense.

The onset of this situation primarily occurred in 1937, when cannabis attracted an illicit status when the "Marihuana Tax Act" was passed by the United States of America Congress at the insistence of Harry Anslinger, then the Commissioner of the Federal Bureau of Narcotics (Goode, 1970). Interestingly, this is the same year in which Anslinger published an article titled: "Marijuana- Assassin of Youth" (Anslinger 1937), which portrayed cannabis as a drug responsible for inducing murders, suicides, and homicidal behaviour. At the point of publication, no experimental evidence to support

these accusations existed, and to this day, such claims remain unsupported. According to the legislators, a major reason drugs are criminalised and the cost of policing and legislating justified, is to curb violent behaviour. The irony of this situation is that the drug most likely to induce aggressive behaviour is legally available and sold by the state for profit (Hoaken & Stewart, 2003).

Movie Poster of the 1937 movie: "Marijuana- Assassin of Youth"– based on article by H.J. Anslinger, the U.S. Commissioner of Narcotics (first published in *The American Magazine*, July 1937).

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DECLARATION

I certify that the substance of this thesis has not already been submitted for any degree and is not currently being submitted for any other degree or qualification.

I certify that any help received in preparing this thesis, and all sources used, have been acknowledged in this thesis.

.....  .....

Signature

ABSTRACT

There is mounting evidence that chronic cannabis use might result in lasting neurobehavioural changes, although it remains unclear whether vulnerability diminishes with age. The current research examined the effects of cannabinoid exposure at salient developmental ages, namely, perinatal, adolescent, and young adult ages. The first study in the thesis assessed the effects of perinatal THC [(-)-Δ^9-tetrahydrocannabinol] exposure on learning. Twelve male Wistar rat pups were treated daily with THC (5 mg/kg, s.c.) or its vehicle between postnatal days (PND) 4 and 14. Rats were subsequently tested drug-free during young adulthood (PND 56) using a two-component food-motivated double Y-maze test. Each trial included distinct spatial discrimination and delayed alternation components, which permitted the simultaneous assessment of reference memory and working memory. Rats were tested for 30 trials per day, 5 days per week for 5 weeks. Results revealed no significant differences between THC- and vehicle-treated rats in the spatial discrimination task. However, compared to vehicle-treated rats, THC-treated rats committed significantly more errors, and required significantly longer to obtain 80% correct performance over 2 consecutive days in the delayed alternation task. These results suggest that neonatal THC exposure leads to a specific and lasting deficit in learning in adulthood, which is likely due to impaired working memory function.

Second, the remaining studies involved the systematic examination of cannabinoid exposure at perinatal, adolescent, or early adult ages. Twenty-four 4-day old (perinatal), twenty-four 30-day old (adolescent), and twenty-four 56-day old (young adult) male albino Wistar rats were injected with vehicle or

incremental doses of the cannabinoid receptor agonist CP 55,940 [(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol] daily for 21 consecutive days (0.15, 0.20 or 0.30 mg/kg for 7 days per dose, respectively). Following a 28-day drug-free period, working memory was assessed in an object recognition task. One week later, social anxiety and aggressive behaviour was assessed in a social interaction test. Two days later, generalised anxiety was assessed in an emergence test. Finally, drug-induced changes in basal neural activity were examined using *c-fos* immunohistochemistry. In the object recognition task, working memory was impaired in rats treated with CP 55,940 at all three developmental ages (perinatal, adolescent, adult). In the social interaction test, rats treated with CP 55,940 at all ages showed evidence of social anxiety. Further, reduced aggressive behaviours were evident in adolescent and adult CP 55,940-treated rats. In the emergence test, CP 55,940 had no effects in five of six emergence test measures, but a modest but significant reduction in anxiety was noted in one measure following adolescent exposure. These behavioural alterations were not accompanied by long-term drug-induced alterations in basal neural activity as determined using *c-fos* immunohistochemistry. However, differing baseline levels of *c-fos* expression dependent on age were observed in several brain regions. Results suggest that chronic cannabinoid exposure leads to long-term memory impairments and increased anxiety, irrespective of the age at which drug exposure occurred. Comparison with earlier work (O'Shea, Singh, McGregor, & Mallet, 2004) suggests that adult males are more sensitive to cannabinoid-induced behavioural deficits than are adult females.

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ABBREVIATIONS

2-AG.....2-arachidonylglycerol

5-HT.....5-hydroxytryptamine

ACTH.....adrenocorticotropic hormone

AIDS.....acquired immune deficiency syndrome

ANA.....anandamide

ANOVA.....analysis of variance

BDNF.....brain-derived neurotrophic factor

BNST.....bed nucleus of the stria terminalis

CB_1.....cannabinoid receptor 1

CB_1-KO..... CB_1 receptor knock-out

CB_2.....cannabinoid receptor 2

CBD..... cannabidiol

CBF.....cerebral blood flow

CBN.....cannabinol

CCD.....charged-coupled device

CEA .....central nucleus of the amygdala

CNS .....central nervous system

CP 55,940.....(-)-*cis*-3-[2-hydroxy-4-(1,1-dimethylheptyl)phenyl]-*trans*-4-(3-hydroxypropyl) cyclohexanol

CPU .....caudate putamen

CRF-41.....corticotropin releasing factor

DAT.....dopamine transporter

DMTS.....delayed-match-to-sample

DNA.....deoxyribonucleic acid

DRL.....differential reinforcement of low-rate responding

DSM-IV-TR.....Diagnostic and Statistical Manual of Mental Disorders: Fourth Edition, Text Revised

EEG.....electroencephalographic

ERP.....event-related potential

EW.....Edinger-Westphal nucleus

Fos-IR.....Fos-immunoreactivity

fMRI.....functional magnetic resonance imaging

GABA .....gamma amino butyric acid

GAD.....generalised anxiety disorder

GD.....gestational day

HPA.....hypothalamus pituitary adrenal

HPLC.....high performance liquid chromatography

HU-210.....(-)11-hydroxy-Δ^8-tetrahydrocannabinol-dymethylheptyl

IcjM.....islands of Calleja

i.p.....intraperitoneal

LTP.....long-term potentiation

LS.....lateral septum

LSD.....lysergic acid diethylamide

MBH.....medial basal hypothalamus

MDMA.....3,4-methylenedioxymethamphetamine

METH.....methamphetamine

mPFC.....medial prefrontal cortex

MRI.....magnetic resonance imaging

mRNA.....messenger RNA

NAC.....nucleus accumbens

PAG.....periaqueductal grey

PET.....positron emission tomography

PND.....postnatal day

REM.....rapid eye movement

SEM .....standard error of the mean

s.c.....subcutaneous

SR 141716.....N-piperidino-5-(4-chlorophenyl)-1-(2,4-dichlorophenyl)-4-methylpyrazole-3-carboxamide

T1.....trial 1

T2.....trial 2

THC.....(-)-Δ^9-tetrahydrocannabinol

VR_1.....vanilloid type 1 receptors

VTA.....ventral tegmental area

WAIS..... .....Wechsler Adult Intelligence Scale

WISC.....Wechsler Intelligence Scale for Children

WIN 55,212-2.....4,5-dihydro-2-methyl-4(4-morpholinylmethyl)-1-(1-naphthalenyl-carbonyl)-6H-pyrrolo[3,2,1-i,j]quinolin-6-one

WT.....wild-type

PRESENTATIONS, PUBLICATIONS, AND OTHER PUBLICITY RELATED TO THE CURRENT RESEARCH

Conference Presentations

Singh, M.E., O'Shea, M., Warty, N.A., McGregor, I.S. and Mallet, P.E. (November, 2002). Repeated exposure to a cannabinoid receptor agonist alters subsequent basal and morphine-induced Fos immunoreactivity. Presented at the Annual Meeting of the Society for Neuroscience, Orlando, Florida, USA. Abstract published: Society for Neuroscience Abstracts, 28.

O'Shea, M., Singh, M.E., McGregor, I.S. and Mallet, P.E. (November, 2002). Repeated cannabinoid exposure produces residual working memory impairments and anxiety in pre-pubescent rats. Presented at the Annual Meeting of the Society for Neuroscience, Orlando, Florida, USA. Abstract published: Society for Neuroscience Abstracts, 28.

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Publications

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O'Shea, M. and Mallet, P.E. (2005). Impaired learning in adulthood following neonatal Δ^9-THC exposure. Behavioural Pharmacology, 16, 5-6, 455-461.

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