Institute of Psychiatry

at The Maudsley

MRC Social, Genetic and Developmental Psychiatry Centre

KING'S College LONDON

University of London

Molecular mechanisms underlying the behavioural effects of cannabis in mice

- modelling a risk factor in schizophrenia

Dr Cathy Fernandes

RCUK Academic Fellow

Social, Genetic & Developmental Psychiatry Centre

Institute of Psychiatry

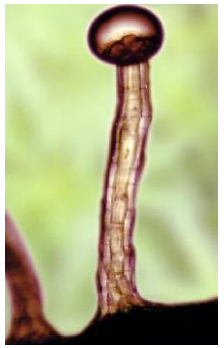
Origins of Cannabis

Cannabis sativa

- one of the first plants used as a medicine, in religious ceremonies and recreationally (> 6000 yrs ago)
- Napoleonic troops brought it to Europe from Egypt (1800's)
- WB O'Shaughnessey's tincture of hemp (cannabis in alcohol) used as an analgesic and muscle relaxant (1839)
- introduced to Western medicine from 1842
- human experimentation with cannabis in 1845 (psychiatrist, JJ Moreau) – 16 g of cannabis extract!

Origins of Cannabis

cluster of flowers on the female cannabis plant are white in appearance due to the outgrowths or trichomes in which cannabinoids accumulate



main active constituent identified by Gaoni & Mechoulam (1964)

[**delta-9-tetrahydrocannabinol**; Δ9-THC]

a high-affinity binding site for cannabinoids (CB_1 cannabinoid receptor) was cloned from rat brain by Matsuda et al (1990)

endogenous ligand for cannabinoid receptor (Devane et al, 1992)

- **anandamide** (endocannabinoid)

The Evil Weed?

one of the most commonly used illicit drugs

- 19% of 16-24 yr olds; 9.0% of 11-15 yr olds (England/Wales 2008/09)

generally considered to be a safe drug

- pleasurable effects of relaxation and euphoria
- altered sensory perception & mildly hallucinogenic
- increased appetite, antiemetic, analgesic

harmful side-effects

- long-lasting cognitive impairment, anxiety attacks
- dependence and addiction (1 in 9 users; gateway drug ?)
- cannabis psychosis
- modest increase in risk of developing psychosis

The Evil Weed?

increased risk (x2) of developing schizophrenia

- 45,570 Swedish conscripts (Andreasson et al, 1987)
- six fold increased risk if heavy users (Zammit et al, 2002)

particular risk if cannabis use in adolescence

- Andreasson et al (1987)
- Fergusson et al (2003)
- Arseneault et al (2004)
- Stefanis et al (2004)
- Konings et al (2008)

worrying trends....

The Evil Weed?

- increasing **THC** in cannabis (Potter et al, 2008)
  - more potent forms of cannabis (sinsemilla or skunk)

2-6% THC in resin

16-20% THC in skunk

  - THC-dominant plant (low or no **cannabidiol**, De Meijer et al 2003)

- age of first-time cannabis users is rapidly decreasing
  - 40% of 15 year olds in the UK have tried cannabis (Hickman et al, 2007)
  - increased problematic cannabis use (2-2.5 % of young adults in UK using cannabis on a daily basis)

The Evil Weed?

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Review

Cannabis use in young people: The risk for schizophrenia

Paola Casadio[a,*], Cathy Fernandes[b], Robin M. Murray[b], Marta Di Forti[b]

[a] Mental Health Department, AUSL Ravenna, Via Baliatico 3, Faenza (RA), Italy
[b] Institute of Psychiatry, De Crespigny Park, London SE5 8AF, UK

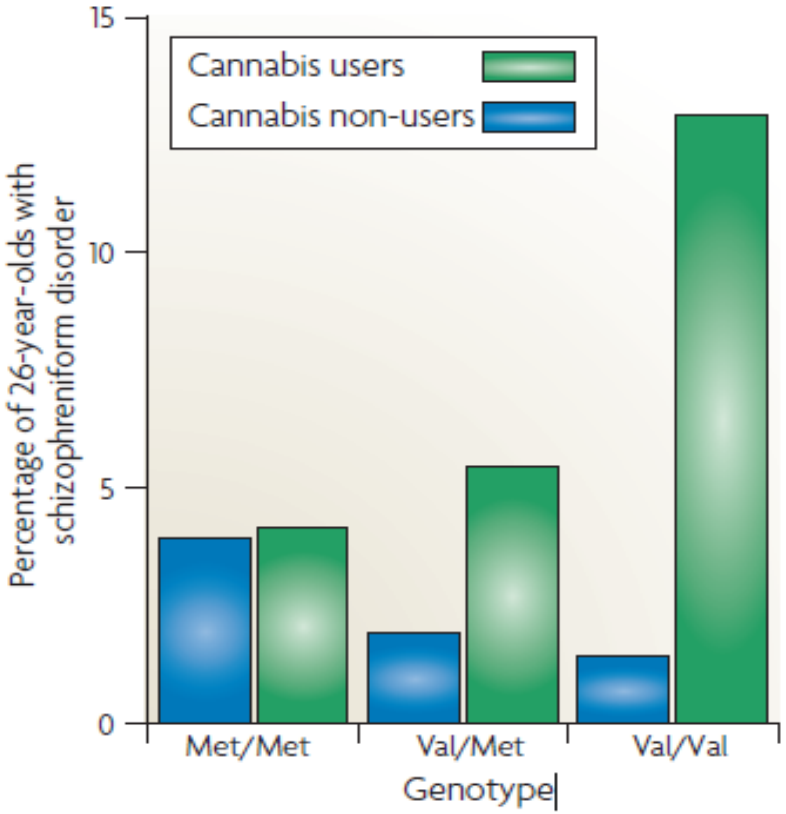
- many (heavy) cannabis users do not develop psychosis
  - **age of first use of cannabis**
  - **degree of cannabis exposure** (Di Forti et al, 2009)
  - **genetic factors** (Caspi et al, 2005)

The Evil Weed?

Individually vulnerability

- many (heavy) cannabis users do not develop psychosis
- psychosis prone?
- genetic link

Caspi et al, 2005



Modulation of the effect of adolescent cannabis use on psychosis by COMT genotype. Owing to a functional polymorphism that involves a Val-to-Met substitution at codon 158, the gene for catechol-O-methyltransferase (COMT) has two common allelic variants that influence the efficiency with which dopamine is broken down in the prefrontal cortex.

Cannabis use in adolescence

move beyond epidemiological studies and associations

effects of cannabis during development

- neuronal networks are still under development (Romeo, 2003)
- role of the endocannabinoid system (Fernandez-Ruiz et al, 2000)

focus on prenatal/perinatal periods (Viveros et al, 2005)

- impaired executive function (Fried et al, 1998), impaired social behaviours and emotional reactivity (Trezza et al, 2008)

studies on the effects of adolescent exposure

- cannabis extract or THC exposure during adolescence results in cognitive impairments (Stiglick & Kalant, 1982, 1985)

Cannabis use in adolescence

synthetic cannabinoid agonist exposure during adolescence

- deficits in recognition memory, sensory motor gating & anhedonia (Schneider and Koch, 2003)
- reduced anxiety (Biscaia et al, 2003)
- no effect on spatial learning (Cha et al, 2006)
- puberty (late adolescence) more vulnerable period than prepuberty (early adolescence), Schneider et al (2005)

Cannabis
- modelling vulnerability

(1) What is the impact of THC exposure during development on adult behaviour?

Schizophrenic patients have a broadly defined, but severe social deficit and deficits in a range of cognitive functions, including working and episodic memory (Keefe, 2000; Cirillo & Seidman, 2003)

(2) To what degree are the effects of THC on behavioural processes genetically influenced?

(3) What molecular mechanisms may be mediating any altered sensitivity to THC ?

Cannabis
- modelling vulnerability

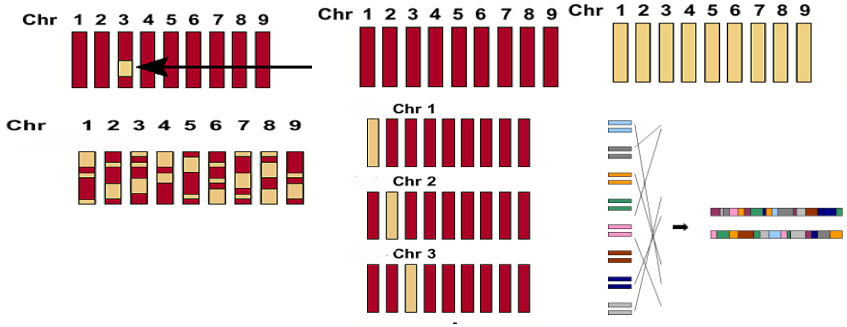
Using **genetically informative** mouse models to determine the effect of δ-THC exposure during adolescence on

- behaviour (cognitive & social endophenotypes)
- gene expression profiles
- DNA methylation (epigenetic process)

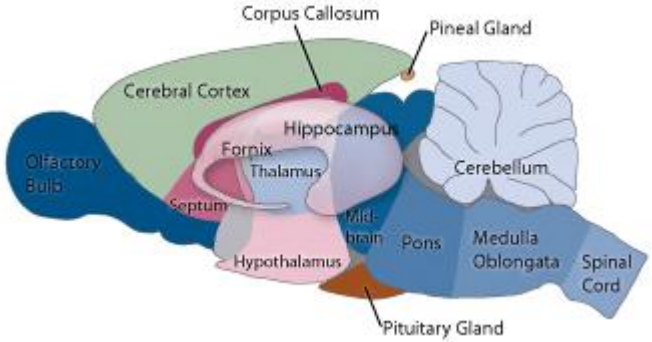
identify specific genes and the downstream molecular events associated with exposure to δ-THC

Mouse - a model organism

diverse range of genetic manipulations



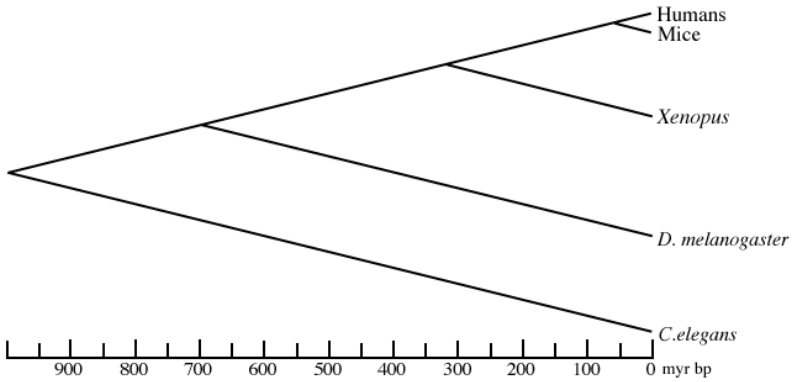
physiological & anatomical similarity



numerous behavioural models available

Our commensal cousins

Genetic similarity: evolution

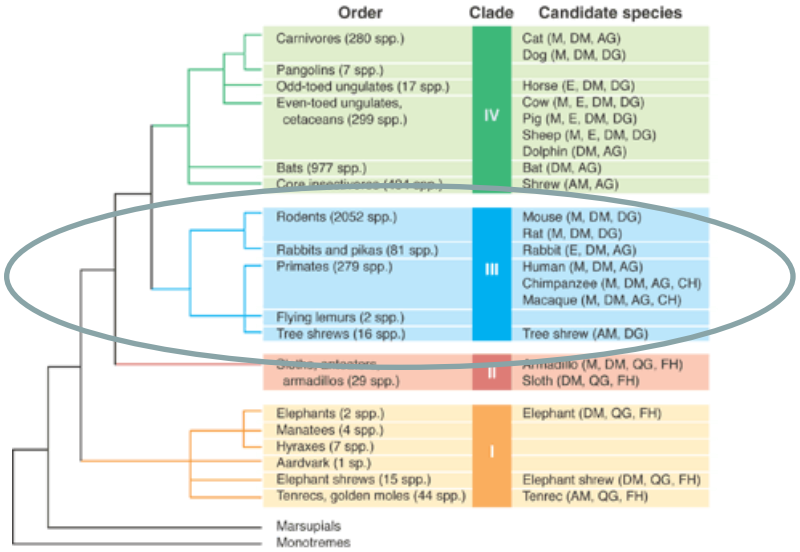


- humans and mice are ten times more closely related to each other than either is to flies or nematodes

(Silver, 1995)

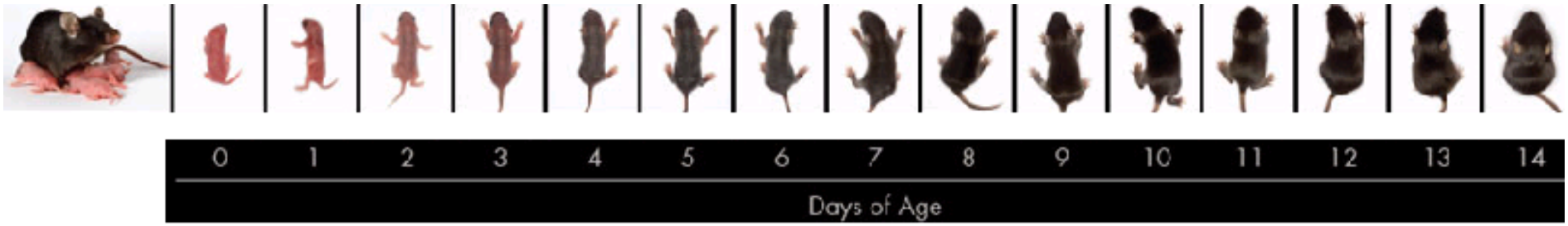
Genetic similarity: mammalian phylogeny

O'Brien et al, 2001 Science 292:2264



90% rodent genes have a human homolog

Developmental stages



http://jaxmice.jax.org/images/literature/pupsposter-large.jpg

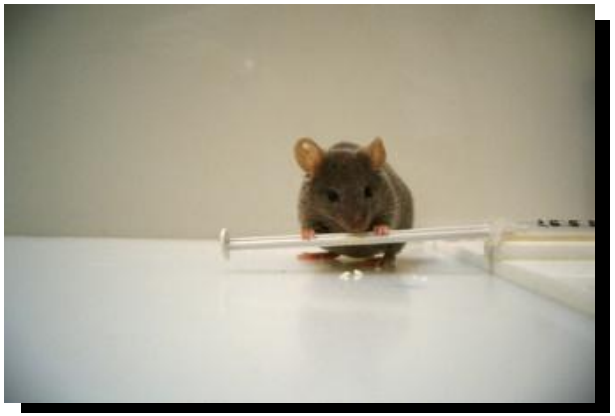
childhood (> pd 7-28) → adolescence (pd 28-49) → adulthood (> pd 56)

puberty (pd 35-56)

- critical postnatal ontogenetic stages
  - neurophysiological and hormonal processes (Spear, 2000)

Study design

- **Δ9 -tetrahydrocannabinol (δ-THC)**
  - 10 mg/kg (i.p.) or vehicle
  - once daily for 14 days
  - (injection naive group)

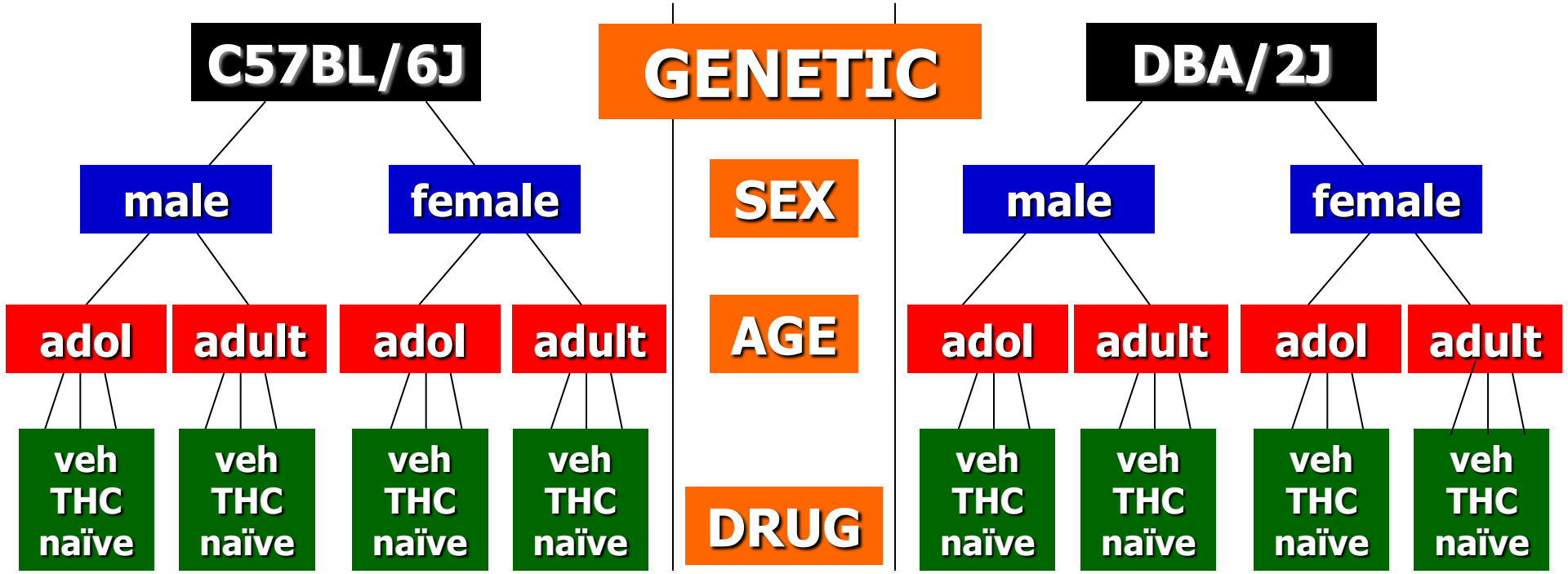


- **two different inbred strains of mice** **C57BL/6J** **DBA/2J**

(male and female mice,
n=7-8 per strain per sex)

- **developmental stages**
  - adolescent (4 weeks old) versus adult (8 weeks old)

Study design



- behavioural phenotyping
  - drug-free (at least 2 weeks)
  - 12 wks old
  - battery of tasks

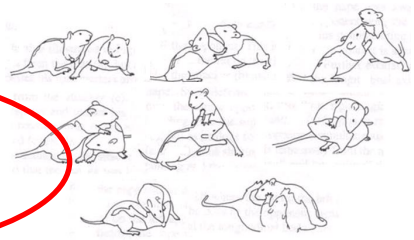
Behavioural test battery

EMOTIONALITY TASKS

Open field

Light/dark box

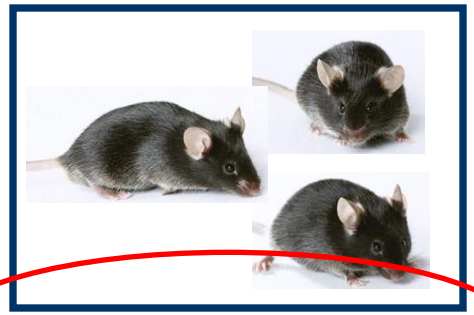
SOCIAL BEHAVIOURS



Social investigation

COGNITIVE TASKS

Novel object discrimination

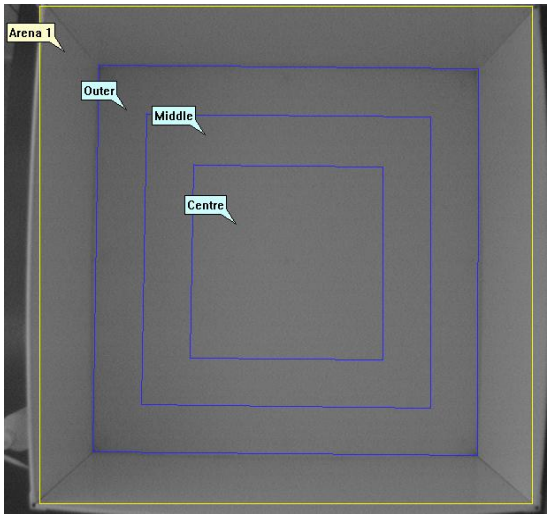


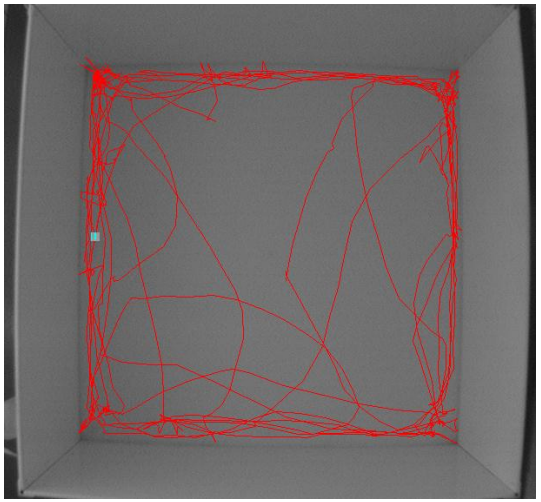
Social discrimination

Morris water maze & DMP task

Behaviour
- anxiety & activity

Behaviour: anxiety in the Open Field

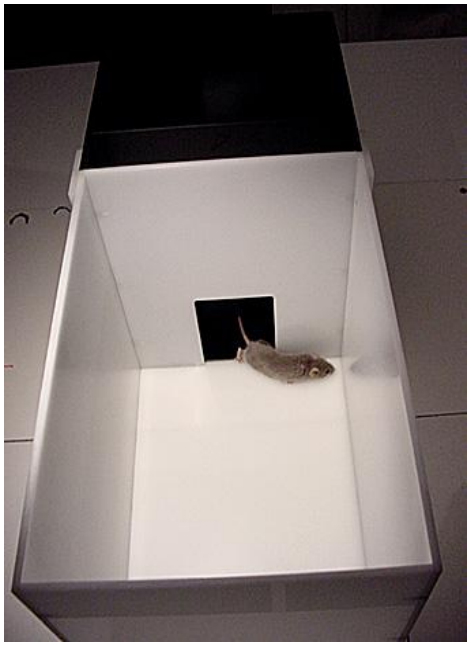




- 10 min exposure to the arena
- time spent in the central zone (anxiety measure)
- distance moved in the outer zone (activity measure)

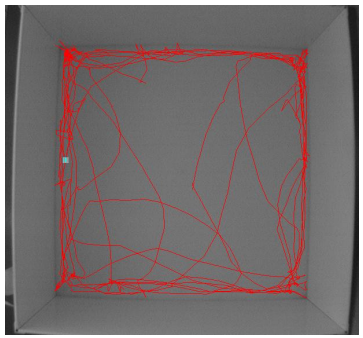
Behaviour
- anxiety & activity

Behaviour: anxiety in the Light/Dark Box



- 5 min exposure to the arena
- time spent in the light area (anxiety measure)
- distance moved in the dark area (activity measure)

Behaviour
- anxiety & activity



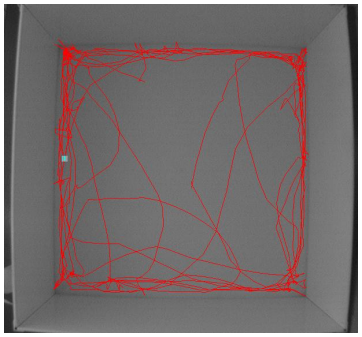
Open Field

adolescent-treated mice

(♀ & ♂)

δ-THC exposure during adolescence does not alter anxiety (or locomotor activity) in either open field or light/dark box

Behaviour
- anxiety & activity

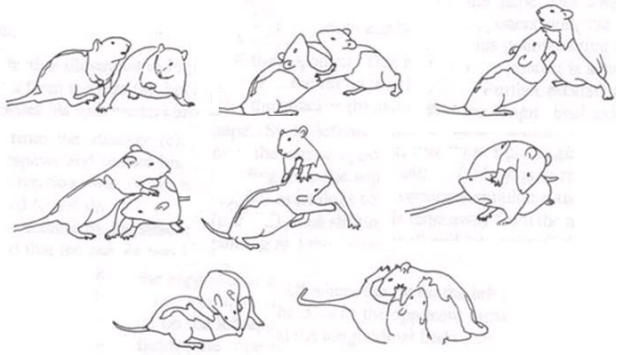


<u>Open Field</u>
adult-treated mice
(♀ & ♂)

δ-THC exposure during adulthood does not alter anxiety (or locomotor activity) in either open field or light/dark box

Social investigation

Behaviour
- social investigation



(strain and sex matched juvenile conspecifics)

- 1 hr habituation to the arena
- 5 min exposure to conspecific
- social behaviours: social and anogenital sniffing and following conspecific
- allogrooming and aggressive behaviours (chasing, biting, wrestling) towards a conspecific

Behaviour
- social investigation

Vehicle versus THC treated mice:

- δ-THC exposure did not significantly alter the profile of social behaviours

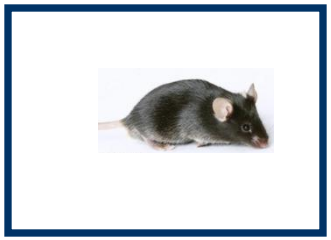
Behaviour - social investigation (♀ & ♂)

δ-THC exposure during adolescence reduces social investigation in DBA/2J mice only

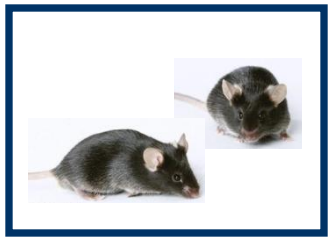
Behaviour - social investigation (♀ & ♂)

δ-THC exposure during adulthood does not alter social investigation

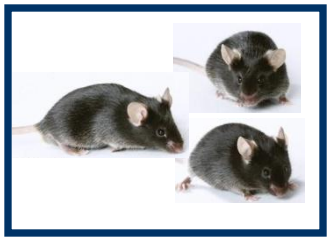
Behaviour - social discrimination (♀ & ♂)



(1 hr)



(24 hours)



δ-THC exposure does not alter social discrimination (long-term memory)

Behaviour - summary

(1)What is the impact of THC exposure during development on adult behaviour?

δ-THC exposure during adolescence caused a long lasting, specific reduction in social behaviour (δ-THC exposure did not alter anxiety, locomotor activity, spatial learning or social discrimination at 24 hr)

THC or synthetic cannabinoids reduce social behaviour in rats but conflicting results regarding the developmental effects on social behaviour (O'Shea et al, 2004 & 2006; Quinn et al, 2008)

(2) To what degree are the effects of THC on behavioural processes genetically influenced?

Effect of δ-THC exposure during adolescence on social behaviour was only seen in DBA/2J mice

Molecular mechanisms underlying the effects of δ-THC ?

Genetic influence on the effects of δ-THC

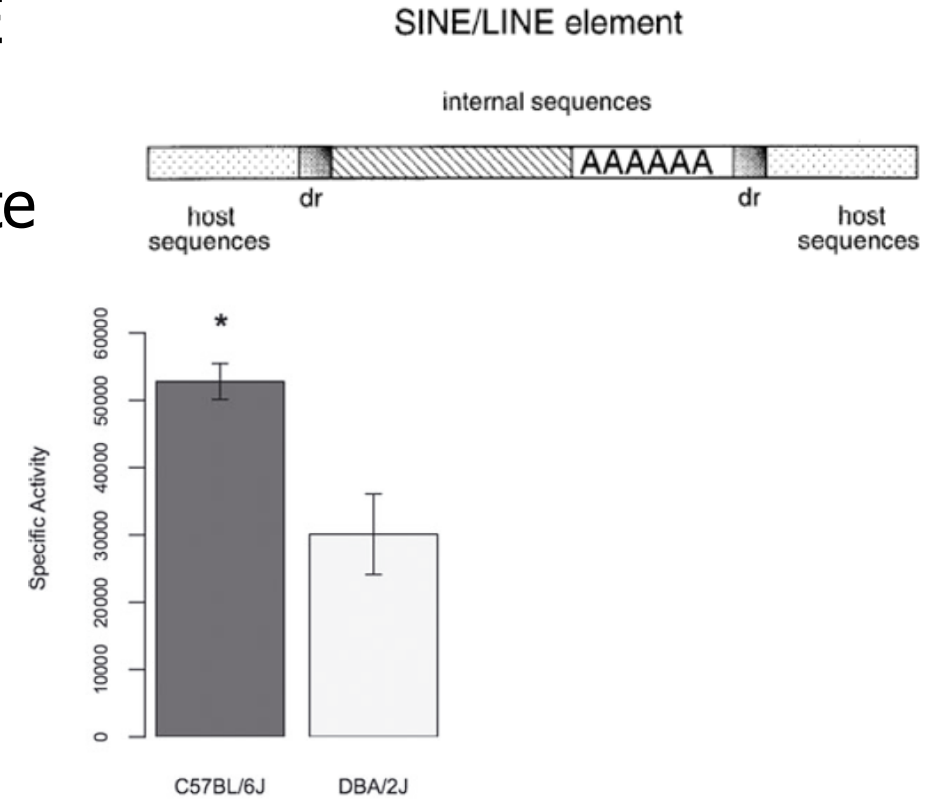
- individual susceptibility to the harmful effects of cannabis
- genetic factors (Harrison & Weinberger, 2005)
- COMT (catechol-O-methyltransferase)
  - Val158Met polymorphism associated with the increased risk of psychosis incurred by adolescent-onset cannabis use (Caspi et al, 2005)
  - Val158 carriers appear to be more sensitive to the psychotic experiences and cognitive impairments following administration of δ-THC (Henquet et al, 2006)

Genetic influence on the effects of δ-THC

- mutation in Comt between DBA/2J and C57BL/6J mice (Kember et al, 2010)

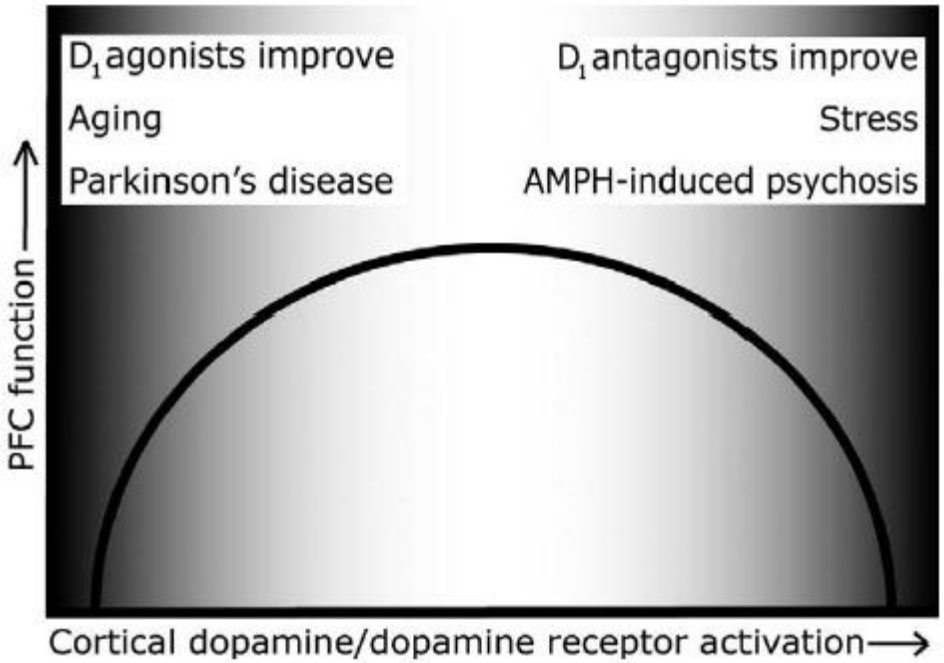
- identified as a B2 short repeat element (SINE)
  - promoters in B2 SINE
  - regulatory elements
  - micro RNA binding site
  - methylation

- COMT enzyme activity



Comt and the prefrontal cortex

- THC increased cognitive and social deficits seen in Comt knockout mice (O' Tuathaigh et al, 2010)
- but not a clear direction of effect (low Comt comparable to human MET carriers but it is VAL carriers at risk)



(taken from Tunbridge, Harrison & Weinberger, 2006)

Molecular mechanisms

- **identify downstream molecular events**
  - gene expression profiling using Affymetrix microarrays (chips)
  - DBA/2J mice selected based on social investigation behaviour (n=7-8/group; 30 arrays in total)
  - mRNA extracted from the prefrontal cortex
  - Affymetrix Mouse Exon St 1.0 arrays
    - mapped to 1 million exon clusters and over 80,000 transcript clusters (build May 2004)
  - signal values analysed after filtering out signals below background

(no gene expression values survived multiple testing correction – 30 array study underpowered)

Molecular mechanisms

Increased mRNA levels in 3 dopamine receptor types in the mice given THC during adolescence

Molecular mechanisms

Ingenuity pathway analysis (top 200 genes VEH v THC adolescent)

- dopaminergic pathway one of the top altered pathways

Summary

- **Is there a robust deficit in social behaviour?**
- **What is the best (translatable) dose of THC?**
  - strain differences in acute response to THC
    B6> DBA hypomotility, catalepsy & ataxia
  - pharmacokinetic analyses (Liz Tunbridge, University of Oxford)
- **Pinpoint the critical developmental period**
  - late adolescence (puberty), Schneider et al (2005)
- **Refined behavioural phenotyping**
- **Identify downstream molecular events**
  - valid the gene expression changes in the dopaminergic system
  - genomewide DNA methylation profiling

Acknowledgements

Jelle Mostert
Vitantonio Insalata
Henry Goodfellow

Rachel Kember
Leo Schalkwyk
Elizabeth Tunbridge

David Collier
Robin Murray

Funding

- Research Councils UK
- NARSAD