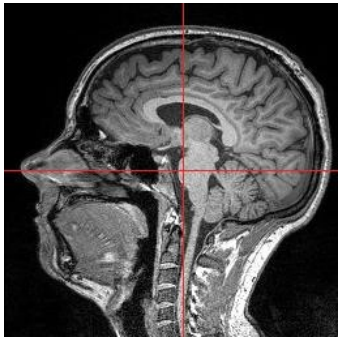

Biomedical & Life Sciences | Lancaster University

Preclinical (rodent) brain imaging: why and how?

SynaNet H2020

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Overview

- Why image the brain in rodents?
- Why ^{14}C-2-Deoxyglucose (^{14}C-2-DG) Functional Brain Imaging
- How: an overview of the ^{14}C-2-DG procedure
- One example of research from our lab

Why image the brain in rodents?

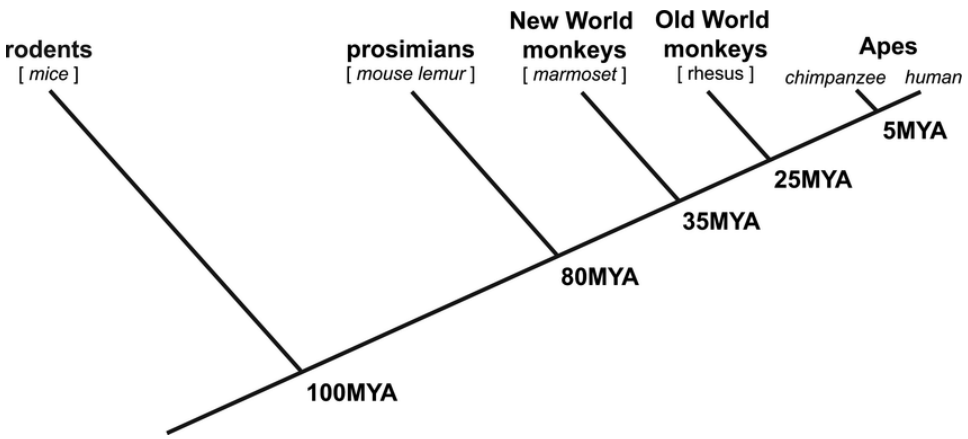
- Conservation between humans and rodents
  - Genetics

  (protein coding genes ~85% similar)
  - Neurotransmitter systems
  - Brain structure-function relationships

- Well controlled experiments in rodents

- Ethical considerations

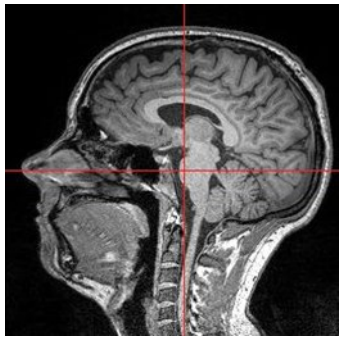
- Useful models for:
  - Human brain diseases (psychiatric/neurodevelopmental)
  - Mechanistic insight
  - Validating interventions/drug treatments

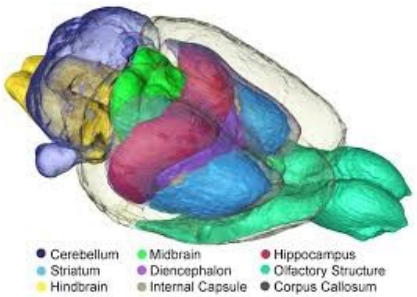


Last common Mammalian Ancestor 150 MYA

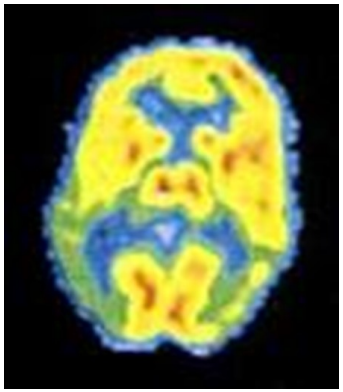
Types of imaging of the Human and Rodent Brain

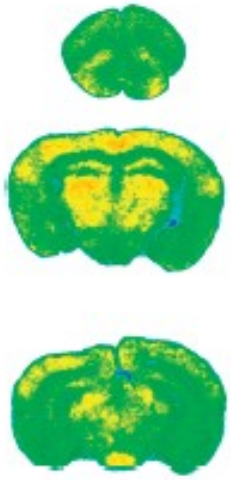
Structural



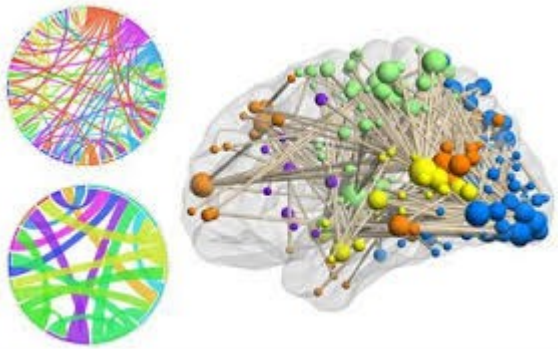


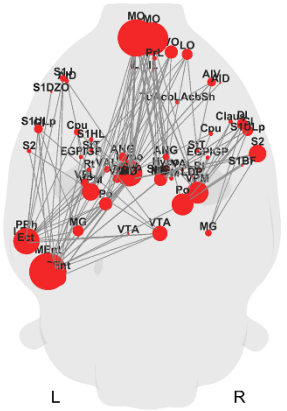
Functional





Connectomes





Why ^{14}C-2-Deoxyglucose functional brain imaging?

- Brain is 2% of body mass
- Consumes 20% of our daily energy intake
- Strongly prefers **glucose** as an energy source

^{14}C-2-DG functional brain imaging – origin & practicalities

Journal of Neurochemistry, 1977, Vol. 28, pp. 897–916. Pergamon Press. Printed in Great Britain.

THE [^{14}C]DEOXYGLUCOSE METHOD FOR THE MEASUREMENT OF LOCAL CEREBRAL GLUCOSE UTILIZATION: THEORY, PROCEDURE, AND NORMAL VALUES IN THE CONSCIOUS AND ANESTHETIZED ALBINO RAT[1]

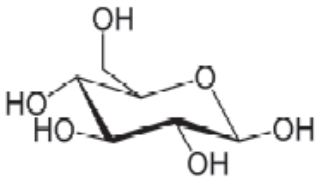
L. SOKOLOFF,[2] M. REIVICH,[4] C. KENNEDY,[2,3] M. H. DES ROSIERS,[2] C. S. PATLAK,[5] K. D. PETTIGREW,[5] O. SAKURADA[2] and M. SHINOHARA[2]

[2]Laboratory of Cerebral Metabolism, National Institute of Mental Health, Bethesda, MD 20014, U.S.A., [3]Department of Pediatrics, Georgetown University School of Medicine, Washington, DC 20007, U.S.A., [4]Department of Neurology, University of Pennsylvania School of Medicine, Philadelphia PA 19174, U.S.A.
and
[5]Theoretical Statistics and Mathematics Branch, Division of Biometry and Epidemiology, National Institute of Mental Health, Bethesda, MD 20014, U.S.A.

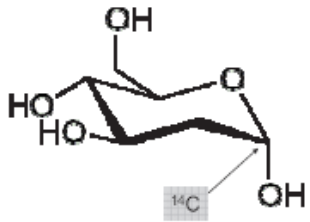
(*Received* 3 *November* 1976. *Accepted* 12 *January* 1977)

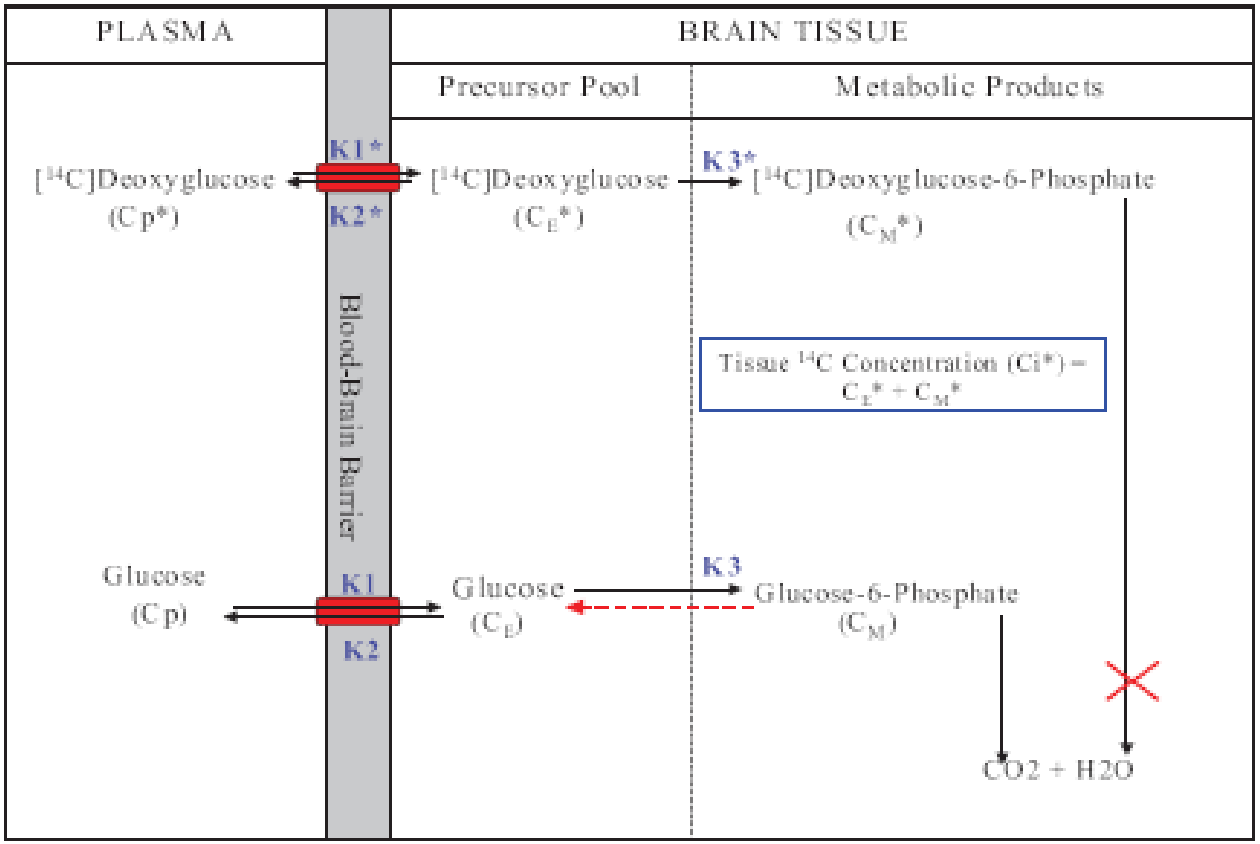
^{14}C-2-DG functional brain imaging – theoretical basis

D-glucose



2-deoxy-D-glucose

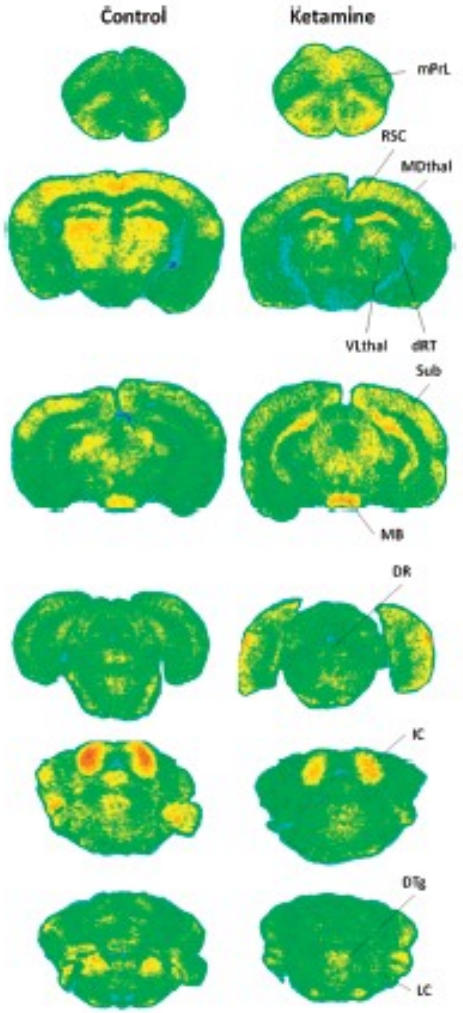




45 minute time period

2-DG-P >> 2-DG

^{14}C-2-DG functional brain imaging – adaptation for use in mice



- Sokoloff et al., method requires surgery (arterial and venous cannulation)
  - ^{14}C-2-DG injection
  - blood sampling
- Sokoloff *et al*, method requires some restriction of movement – animals can't engage in behaviours over the time course of the experiment
- Dawson *et al*., method adapted for application in freely moving, behaving mice

^{14}C-2-DG functional brain imaging

Step 1: Inject a tracer amount of ^{14}C-2-deoxyglucose (4.625 MBq/kg, i.p.)

Step 2: place animal into home cage/behavioural testing environment

Step 3: 45 minutes after tracer injection humanely sacrifice animal

Step 4: Rapidly dissect out brain and flash freeze at -45^{o}C in isopentane

Step 5: Collect a blood sample

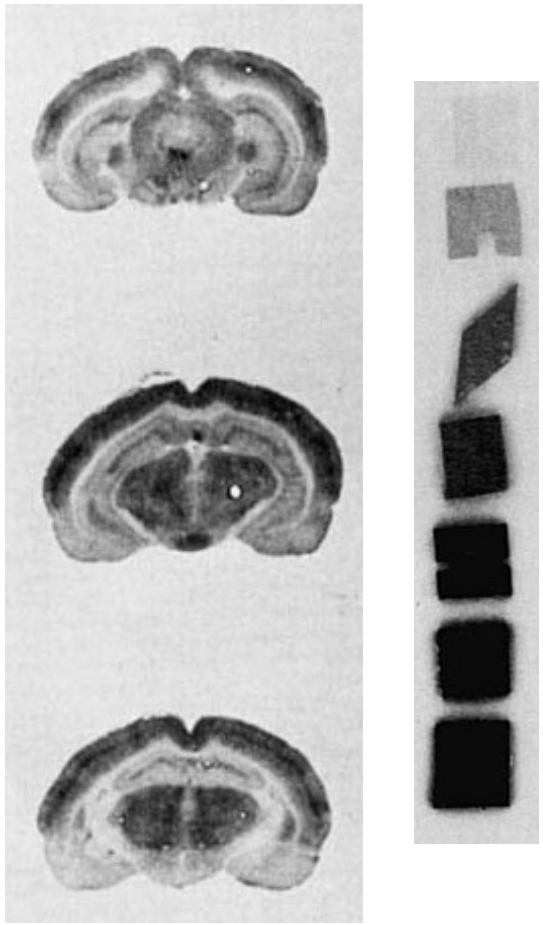
- measure circulating ^{14}C-2-DG levels (scintillation counter)
- measure circulating glucose levels (diabetes monitor)

Step 6: Section brain (20µm) and thaw mount sections onto glass cover slips

Step 7: Expose brain sections to x-ray film (7 days) along with calibrated ^{14}C-standards

Step 8: Analyse autoradiographic images using MCID+ software to determine ^{14}C-2-DG levels (reflecting rates of "local cerebral glucose utilisation (LCGU)") in brain "regions of interest (RoI)".

^{14}C-2-DG functional brain imaging – autoradiograms & applications



Sokoloff et al., 1977 – cited > 6000 times

- Translational rodent models (e.g. stroke, epilepsy, neurodegenerative diseases, psychiatric disease, neurodevelopmental diseases)
- Drugs (e.g. amphetamine, ketamine, antipsychotics, MDMA)
- Response to environmental stimuli (e.g. odours, whisker stimulation)
- Parallels and theory fed into the development of the ^{18}F-fluoro-2-deoxy-D-glucose (^{18}F-FDG) PET imaging in man (Phelps et al., 1979)

^{14}C-2-DG functional brain imaging

advantages

- Very well characterised
- Quantitative measure of brain region metabolism strongly linked to regional neuronal activity
- Excellent anatomical resolution (20 µm)
- Undertaken in conscious, freely moving (and sometimes behaving) animals - no anaesthesia required

disadvantages

- Limited temporal resolution – metabolism over 45 minutes
- One off measurement excludes longitudinal studies in same animal
- Measurement can be confounded – e.g. drug induced hyperglycaemia (measurable) or severe cerebral hypoperfusion (not easily determined in same animal)

An example from our lab

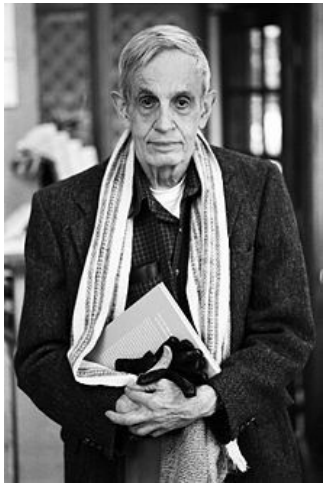
^{14}C-2-DG Imaging in mice with truncated *Disc1*

Schizophrenia : The Disease

- Chronic, debilitating disorder affecting approximately 1% of the world's population.
- **Positive Symptoms**: Hallucinations and Delusions
- **Negative Symptoms**: Avolition (a lack of motivation), reduced Affect (reduced emotional responsiveness) and Anhedonia (inability to experience pleasure)
- **Cognitive Deficits**: Executive Function (attentional processing, cognitive flexibility) and Memory deficits
- Current medication only effective in a subset of patients, and only for the positive symptoms

Famous Schizophrenia Sufferer's

Syd Barrett, Pink Floyd

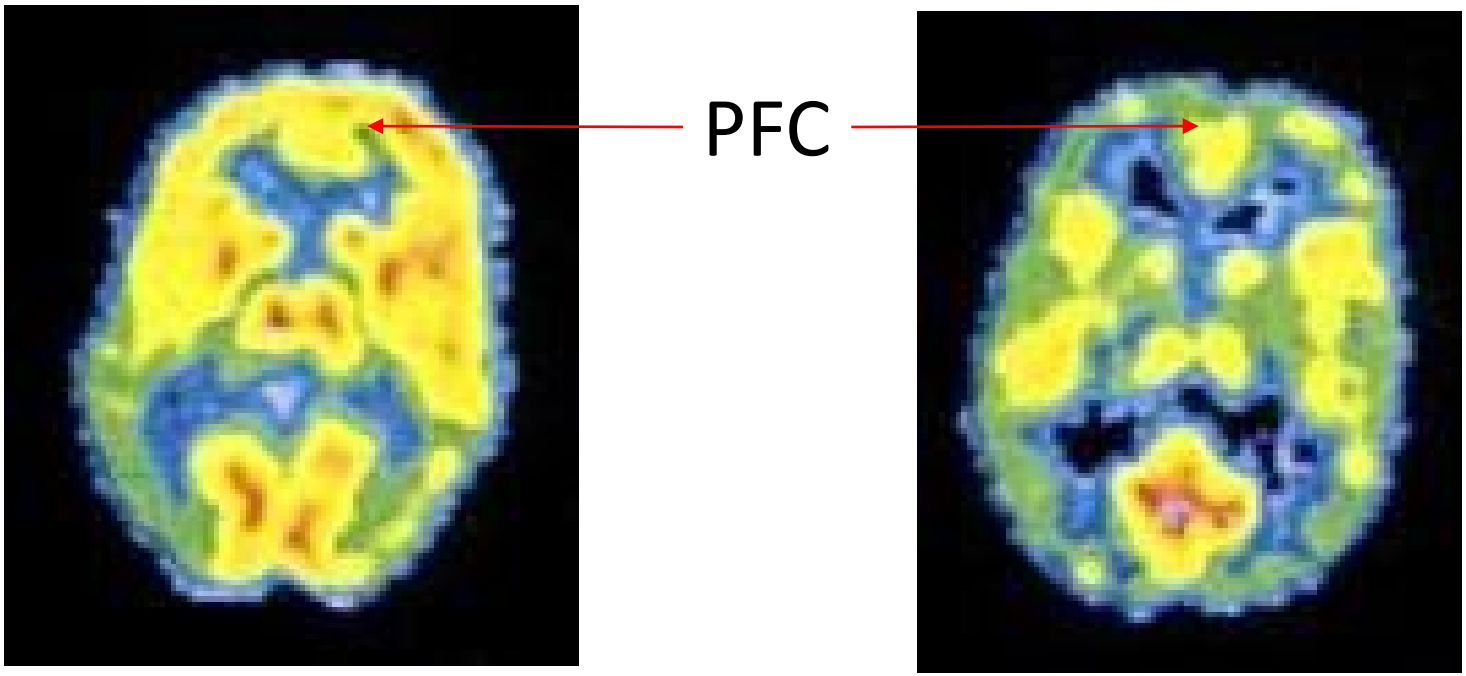


John Nash, Mathematician

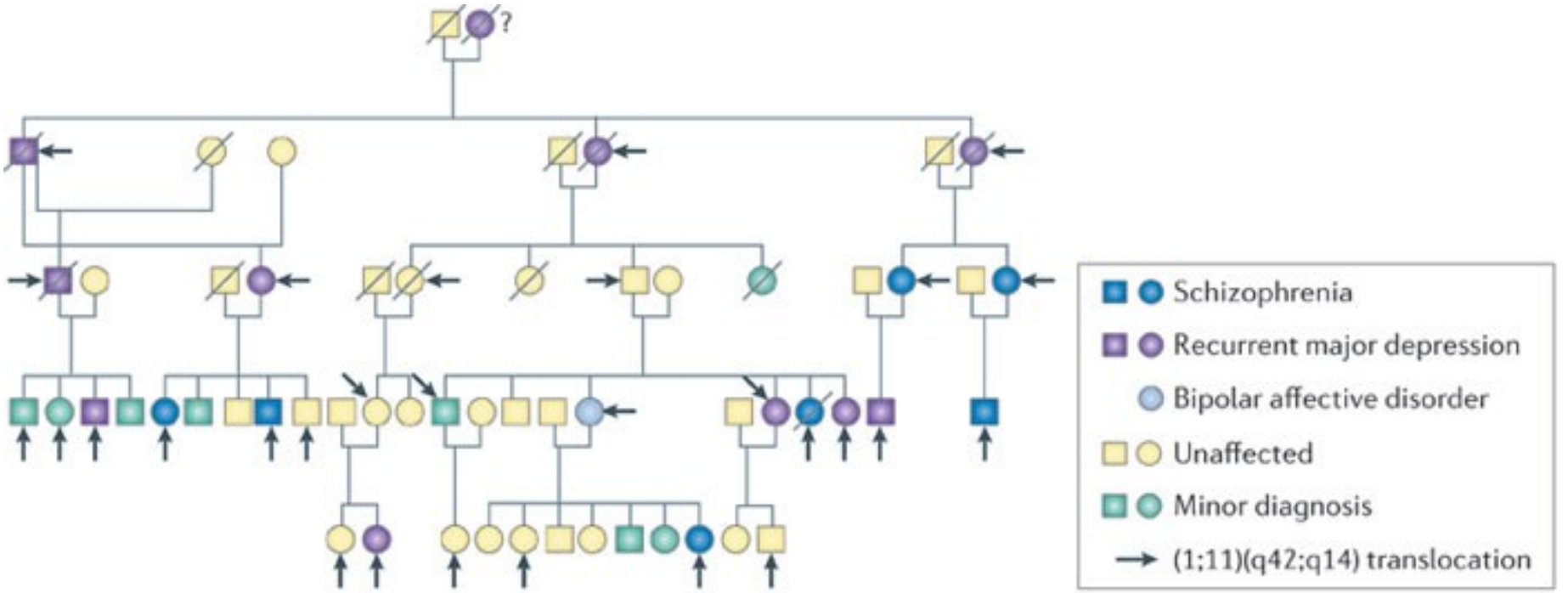
“Hypofrontality” in Schizophrenia

- Reduced rate of cerebral metabolism found in the prefrontal cortex (PFC) of people with schizophrenia
- Contributes to cognitive problems in the disorder
- Images below are from ^{18}F-FDG PET imaging

Healthy Control **Schizophrenic Patient**



Disrupted-in-Schizophrenia-1 (DISC1) – a risk gene for psychiatric disorders

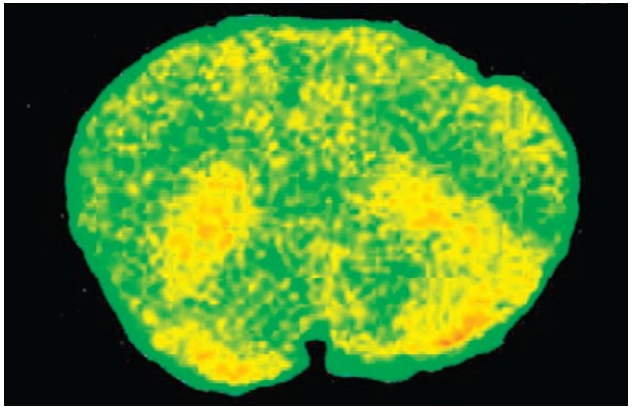


Nature Reviews | Neuroscience

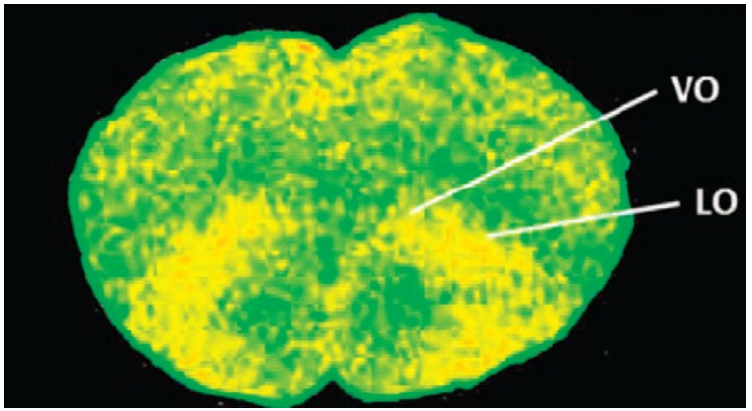
- Family tree of Scottish family with truncated *DISC1*
- Breakpoint between exon 8 and 9 of the *DISC1* gene

Brandon & Sawa, 2011. *Nature Reviews Neuroscience* 12:707

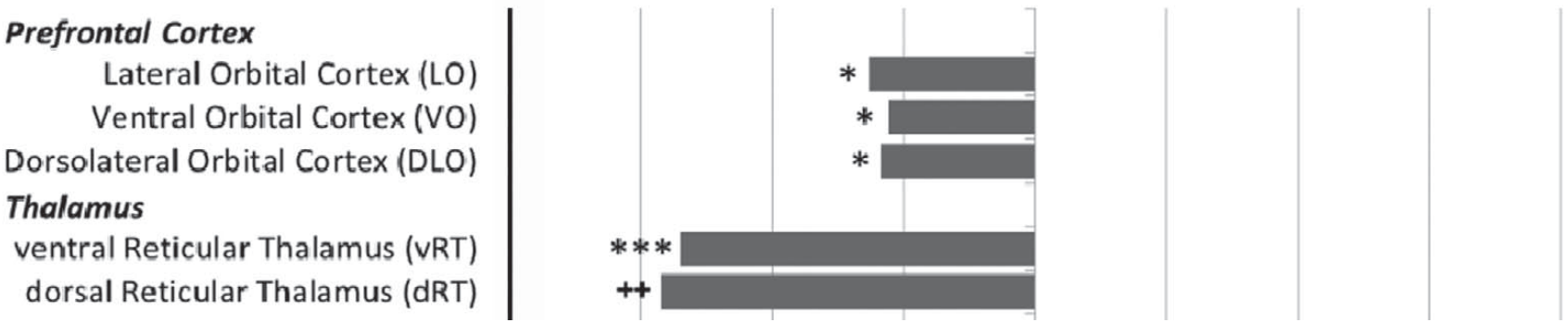
Mice with truncated *Disc1* show schizophrenia-like hypofrontality



Wild-type



Disc1 truncation



Dawson *et al.*, 2015

Summary

- ^{14}C-2-DG functional brain imaging:
  - Allows us to characterise cerebral metabolism in rodents
  - Cerebral metabolism indicates neuronal activity
  - An important tool in translational neuroscience research