Addiction and the Brain
Isabel Smith - Rokos Award Internship Report

I spent two weeks in July under the supervision of Professor Jeff Dalley in the Psychology department at Cambridge. His work focuses on behavioural and neurobiological endophenotypes (behavioural phenotypes with genetic associations) and their application to psychiatric disorders, and individual differences in predisposition to drug abuse and addiction.

I got in contact with Professor Dalley after attending an interdisciplinary workshop where he discussed vulnerability to addiction. His lecture explored the question of why some people develop addictions and others do not. He described the development addiction as an interaction between the drug (all drugs of abuse influence dopamine release), the host (who may be vulnerable to addiction) and the environment. His research has shown that trait impulsivity is an endophenotype that confers risk for developing dependency, and that a decrease in dopamine D2 receptor density acts as a biomarker for addiction.

As this was a short-term placement, Professor Dalley organised for me to shadow a number of different researchers in his lab, working on different projects with each.

Quinpirole Sensitisation - research
My first task was to do some background reading into his lab’s work on Quinpirole. Unfortunately the project was postponed so I wasn’t able to shadow the researchers in the lab, but I learned a lot about dopamine receptors that was useful later in the placement.

Quinpirole is a psychoactive drug and a selective D2 and D3 receptor agonist. Chronic administration of quinpirole (e.g. in treatment of Parkinson’s disease) can lead to compulsive behaviour similar to that in OCD such as compulsive checking. Such symptoms may arise due to sensitisation to quinpirole (the Quinpirole Sensitisation Model of OCD; Szechtman et al., 1998); rats chronically injected with the substance show increased locomotion and excessively visit one or two objects in open field tests (i.e. they show compulsive checking). Quinpirole exerts its effects through the dopaminergic system in cortico-striatal regions of the brain, and I was asked to research how it affects D2 and D3 receptors at chronic versus acute doses in these areas.

The project was investigating reversal learning and discrimination performance in rats given acute or chronic doses of quinpirole. High doses of quinpirole have previously been shown to impair reversal learning. In initial tests, they showed that both acute and chronic doses impaired performances, but a single low dose enhanced performance. They wanted to further investigate this finding to establish whether it was spurious or if there was a causal link.

Regulation of compulsive behaviour - PCR
For the second half of my first week, I learned about PCR (a technique used to amplify DNA) and assisted another intern in the lab. The project that we were working on was investigating how compulsive responding is regulated. Specifically, they investigated the effect of cocaine versus sucrose on downregulation of dopamine (D1 and D2) and serotonin (HT-2) receptors in the dorsolateral striatum of the rat brain by comparing gene expression using mRNA.
I assisted with the mRNA extraction phase. To extract mRNA from the brain sample, we first used an ultrasonic cell disruptor to lyse the membranes. The samples were then homogenised with a buffer to enable purification of intact RNA, then centrifuged to separate it into phases. The product was a solution with two clear phases: an upper, colourless phase containing the RNA and a lower, pink organic phase.

Having learned about PCR at A-Level and read about it in many research papers throughout my degree, I enjoyed learning the practical skills behind the technique.

Myo-inositol - operant conditioning chambers
I then had the opportunity to shadow Dr. Bianca Jupp (a research associate of Professor Dalley) in the animal laboratories. She researches the neurobiology underlying the behavioural trait of impulsivity and related endophenotypes, and how these confer risk for developing drug abuse and addiction.

Before I joined her in the lab, I was asked to research the functioning of myo-inositol. Myo-inositol is a common form of inositol (a simple sugar that forms the basis of a number of important intracellular signaling molecules) that is involved in calcium signalling and the Krebs cycle. It is also implicated in psychiatric disorders; the inositol-depletion hypothesis suggests that the therapeutic effects of lithium treatment for Bipolar Disorder are mediated by a depletion in myo-inositol. Dr. Jupp’s previous research on impulsivity uncovered a link between myo-inositol and impulsivity. While performing MR-spectroscopy looking at metabolites (such as GABA, glutamate and myo-inositol) in the brain they found that highly impulsive rats showed a reduction in myo-inositol. They repeated the study to establish whether it was a spurious result, and replicated the findings. Myo-inositol may therefore be a cortical marker for impulsivity. They further investigated this by looking at gene expression in the infralimbic cortex using PCR. In order to publish their findings, they need to establish causation. Dr Jupp is currently working to show a causal relationship by demonstrating the effects of knocking out the gene for myo-inositol.

Firstly, Dr. Jupp showed me the operant conditioning chambers that we would be using and the computer programs that run them. Rats who are placed in the chambers are required to respond to flashes of light by pressing corresponding plates in order to receive a sucrose pellet. The computer program monitors whether they press correctly, too early or miss the press, and if they persevere in pushing the panel. Any incorrect behaviour is punished with a 5 second dark ‘time-out’. She explained that this is a useful task for assessing impulsivity, as highly impulsive rats press the panel before they are supposed to. To assess whether there is a link between impulsive responding and myo-inositol, she will selectively alter myo-inositol expression and observe the effects on impulsive responding.

I was assisting with the training phase, where she was repeatedly running trials with the rats to establish their initial levels of impulsivity. We retrieved the rats from a dark room where they are housed and transported them to the testing room, where we put them in the chambers for 30 minutes (100 training trials). Dr. Jupp explained that she did this daily for 6 weeks in order to train them to criterion level. Once the trials were complete, we re-housed and fed the rats. She then showed me around the rest of the animal labs, including where surgical procedures (such as lesioning and insertion of microdialysis probes) and rehabilitation occurs. I was fascinated to see some of the labs where the studies of addiction were taking place, including a study of heroin self-administration in which rats pressed a lever to self-administer heroin via a catheter to their jugular vein.
I thoroughly enjoyed shadowing Dr. Jupp on this study. Having learned about learning theory as part of my 2nd year course I was excited to gain hands on experience with the chambers and I was fascinated to explore the animal labs and to discuss Dr. Jupp's research and academic background with her.

**HPLC & micro-dialysis**

For the last few days of my placement, Professor Dalley taught me how to conduct High Precision Liquid Chromatography (HPLC). HPLC is a technique used to separate, identify and quantify each component in a solution. I was asked to run HPLC on a number of neurotransmitters and metabolites (dopamine, noradrenaline, serotonin, DOPAC, 5-HIAA and HVA) to create a baseline for them to compare the results of their study to. This involved calculating the required molar mass of each substance to reach the desired concentrations when diluted with PCA, then inserting each sample into the HPLC column. The substances have different retention times, so produce a signal at different times. The signal was displayed on a computer program (Chromeleon) as a graph from which I calculated the area under the curve for each signal spike. Initially I couldn’t get the right signal on the output, so Professor Dalley showed me how to calibrate the program to a partial injection loop and I switched to a finer pipette to avoid air bubbles being extracted into the column. After a few false runs, I managed to set up the equipment to produce a clear signal. Once I’d obtained a signal from each of the solutions I was able to plot a graph (using Graphpad Prism) to show the expected area under the curve from each concentration, to be used as a reference when the concentration of the metabolites is unknown. Lastly, I created a solution containing all of the metabolites. Two of the compounds had similar retention times (dopamine and 5HIAA) so their area-under-curve was difficult to establish. We ran the solution again with a soap to separate the curves.

![Diagram showing HPLC set-up (left) and micro-dialysis probe set-up (right)](image_url)

Having created these solutions, I was then taught how to use microdialysis probes by the Neurobiology lab manager. He makes each probe by hand, using individual fibres from dialysis pumps. The probes are minute - I could hardly see the component tubes, even when looking at them under a microscope. They are also incredibly delicate, as any leak renders them unusable. To test that the probes worked, I created an artificial cerebrospinal fluid buffer to act as the perfusate (which is injected into the probe), and inserted the probe into the neurotransmitter solution. The perfusate is infused through the length of the probe into the outlet tubing. The membrane around the tubing is partially permeable, so substances from the neurotransmitter solution diffuse through the membrane. The perfusate at the outlet is collected for analyte quantification by HPLC. I ran this process at various speeds to determine the optimal flow rate for the probes (at faster speeds, the gradient is constantly changing so there is a higher molar transfer rate but the outlet is more dilute). By comparing the signal to that of the control samples and calculating an extraction ratio, I showed that the probes functioned well.
Overall, I thoroughly enjoyed my time at Cambridge and I learned a number of experimental techniques and gained invaluable experience in a lab. I am fascinated by their research and excited that I had the opportunity to talk to the researchers about their work. I left on a very positive note, with Professor Dalley saying that he would be happy to consider me if I wanted to pursue postgraduate study in his lab.

| PCR - ultrasonic cell disruptor | Stained rat brain microscope slides (to check accuracy of probe location) | Chromeleon HPLC computer program output |