V2.0

Compulsivity is present in many disorders such as obsessive-compulsive disorder and addiction which share many behavioral, neural, and neurochemical pathologies. Behavioural inflexibility, in which behaviour persists despite evidence that it is no longer effective or appropriate, is characteristic in patients of disorders of compulsivity (DOC) and depends upon the integrity of orbitofrontal cortex (OFC) and striatal dopamine function.

* Reversal learning is a promising task to model behavioural inflexibility
  + OFC activity tracks informative cues, and many of these neurons remap after cues reverse their meaning
* Cocaine history significantly impairs reversal behaviour, and significantly reduces the proportion of neurons that remap
* Impaired reversal learning and OFC function following cocaine history is a promising model to understand inflexible behaviours, and test pharmacological treatments to treat behavioural inflexibility.
* One prevailing issue is that interpretation of OFC remapping of cue meaning in reversal learning cannot be dissociated from other task features that correlate during reversal.
* To isolate neural activity associated with the meaning of cues from simply reflecting reward/non-reward or cue identity, an occasion setting task will be used.

OFC dysfunction in DOC has been implicated in reversal learning deficits, an experimental measure of behavioural inflexibility .

Reversal learning procedures have significant potential to further understanding and treatment of disorders of compulsivity (DOC) such as obsessive-compulsive disorder and cocaine addiction. Reversal learning tasks measure behavioural flexibility by changing the meaning of cues guiding when to act and when to withhold behaviour. Impairments in reversal learning are a reliable feature of both clinical and translational models of DOC. Reversal learning provides an important method to understand and test pharmacological treatments of DOC. A key neuropathology underlying DOC and causing reversal learning deficits is Orbitofrontal cortex (OFC) dysfunction. In reversal learning, distinct subpopulations of OFC neurons respond to cues that signal when to perform or withhold behaviour, and flexibly update firing when cue contingencies change. A history of cocaine use significantly impairs both flexible updating of behaviour and OFC activity in reversal learning.

Compulsive and neurotypical behaviours do not occur in a vacuum, but instead are elicited and informed by informative cues in our environment. Reversal learning procedures model this with discrete cues (e.g. odors) that indicate whether a behaviour (e.g. checking a location) will lead to a biologically meaningful outcome (e.g. food). In a typical procedure, subjects first learn to discriminate responding to a rewarded (A+) and non-rewarded (B-) cue, and then these cue-outcome relationships are reversed i.e. A- and B+. A reversal deficit is characterized by subject’s taking significantly longer to reach a threshold of behavioural accuracy following reversal. Distinct populations of neurons in OFC increase firing to rewarded and non-rewarded cues before and after reversal. This is consistent with a mental representation of task structure being updated in OFC, which is the general function proposed by current computational reinforcement learning models of OFC. Prior use of cocaine significantly impairs reversal learning behaviour and the flexibility of OFC activity to adapt to the new contingencies, suggesting that cocaine use impairs flexible updating of task representations in OFC. However, in this simple task the identity and value (average reward history) of each cue are significantly correlated. The meaning and identity of cues in the task can be separated by making cue-reward relationships conditional on other information/cues i.e. A is rewarded when preceded by cue X, but not cue Y (X->A+, Y -> A-), and B is rewarded when preceded by cue Y, but not X (X->B-, Y -> B+). Neural activity to cues A and B are now meaningful only in the context of preceding cues X and Y. A reversal of these contingencies (i.e. X->A- / Y -> A+ and X->B+ / Y -> B-), allows for an analysis of updating neural representations of task structure that can be dissociated from cue identity and value.

More generally, OFC activity is thought to reflect an internal cognitive model of a task/our environment. Prior history of cocaine use has been found to disrupt the flexible use and updating of these OFC representations, and optogenetic stimulation of OFC during learning effectively treats this impairment in rodents. This suggests that cocaine use impairs flexible updating of an internal model of the task within the OFC, leading to inflexible and persistent behaviour in reversal learning. However, in the reversal learning tasks commonly used, representations of internal cognitive maps cannot be disentangled from simple task features such as the presence or absence of reward. This is because the implementation designed to enforce the introduction of alternative maps utilizes the reward associations themselves. That is, the reversal is signaled to the subject by a change in cue-reward associations. Further, this approach confounds time and recognition of its passage, and leaves the decision whether to even create alternative maps up to the subject. The argument is that OFC function is necessary for rapid reversal learning because it facilitates the creation, maintenance or use of these alternative maps; however it could equally well be argued (and in fact has) that this deficit, viewed in isolation, is simply due to slower learning or deficits in response inhibition. To resolve these issues, it is necessary to use a behavioral approach that dissociates the cue that triggers changes in the map being used from these other features and requires mapping for successful performance. One such task that still shares many of the features of reversal learning that makes it so popular is an occasion setting task.

**Expt 1.** **Determine whether remapping of task representations in OFC during OS reversal learning are disrupted in rats with a history of cocaine use.**

Procedure: Long Evans rats (N = 16) will undergo intrajugular catheter surgery followed by a standard cocaine (n = 8) or sucrose (n = 8) control self-administration protocol for 2 weeks followed by 30 days of withdrawal. Next, a drivable bundle of microelectrodes will be implanted in OFC to record neural activity. Following recovery, rats will be water deprived and trained to discriminate odors that predict water reward outline in **Figure 1**. On each trial, the rat will initiate cue presentation by entering and staying in an odor port, then an odor or brief auditory cue followed by an odor will be presented indicating whether reward is available. Following a rewarded odor trial, responding to the food well below the odor port will be rewarded with water. Correct performance will be defined as entering the food port on rewarded trials and withholding responding on non-rewarded trials. Once behaviour reaches a criterion of 90% correct responding over 20 consecutive trials with OS cues (X, Y, A, B) and Simple cues (C, D), reversal learning manipulations will occur: first for simple and then OS cues in separate sessions. Each reversal manipulation will involve presenting the original odor-reward contingencies until criterion performance accuracy, and then a reversal of these odor-reward contingencies until behavior reaches criterion accuracy. This will allow a within-session comparison of acquisition and reversal behaviour and neural activity.

Reversal to simple odor cues C and D will replicate the established reversal learning procedure in which odor identity and reward are correlated before and after reversal. In OS trials, conditional cues X and Y are followed equally by target odor cues A and B, and lead to reward or non-reward equally over the reversal manipulation. Therefore, remapping of selective neural activity to rewarded and non-rewarded cues A and B in OS reversal will identify remapping of cue meaning independent of reward.

The primary behavioural measure will be the number of trials to reach criterion accuracy following reversal manipulations. Neural activity will be measured throughout behavioural training, and will focus on average baseline corrected firing rates during cue presentation. It is difficult to discuss all the possible results from an electrophysiological experiment such as this, so only a key analysis and prediction will be presented below. This prediction focusses on identifying cue selective neurons that reverse their cue preference in parallel with behavioural reversal in the task. The proportion of these neurons has been shown to correlate with reversal behaviour and is significantly reduced in cocaine experienced rats in simple reversals. Verification of electrode placement will occur post-hoc using blinded histological processing techniques. Verification of the long-term effects of cocaine history will be confirmed by testing for sensitized (i.e. increased) locomotor activity in cocaine rats relative to sucrose rats in response to ascending doses of cocaine (7.5, 15.0, and 30.0 mg/kg cocaine injected i.p.).

*Planned analyses and expected outcomes:* Cocaine rats are predicted to take significantly more trials to criterion accuracy than sucrose control rats following simple and OS reversal. I also expect OS reversals to be more difficult and take more trials to criterion accuracy than simple reversals in all rats. Trials to criterion following simple and OS reversals will be compared between sucrose and cocaine groups with a Poisson mixed-effects ANOVA model appropriate for count data. This finding is expected to replicate impaired behavioral flexibility caused by a

The primary correlate of neural flexibility during reversal learning will be changes in the proportion of cue selective neurons before and after reversal. In simple reversal activity (baseline corrected average firing rates) to the cues during acquisition (C+ and D-) and reversal (C- and D+) will be compared by ANOVA (cue x reversal). Cue selectivity will be defined as significantly higher activity to one of these cues. Changes in cue selectivity from acquisition to reversal will identify whether a neuron’s cue selectivity reverses, stays the same, is lost after reversal, or is newly acquired after reversal. The percentage of cue selective neurons that reverse selectivity is the neural correlate of behavioral flexibility in this task. Overall, rats with a higher percentage of reversing neurons are predicted to show faster reversal (i.e. fewer trials to reversal criterion), which will be tested by Pearson correlation. Furthermore, significantly fewer reversing neurons are expected in the cocaine experienced rats than the sucrose experienced rats, which will be tested by Pearson chi-square. These predictions are based on previous findings in the lab.

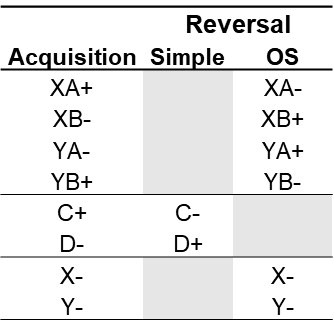
In the simple reversal, the meaning and expected reward value of the cues are correlated. OS reversals will allow me to classify reversal neurons that are selective for cue value or cue meaning. Cue selectivity will be classified by ANOVA comparing activity between cues A vs B, rewarded vs non-rewarded trials, during acquisition vs reversal. This will create a large number of potentially interesting classifications; however, two classifications are of particular interest: cue meaning neurons and cue value neurons. Odor cues A and B can take on 4 possible meanings in the OS reversal task, rewarded following X (X-> A+ / X -> B+), rewarded following Y (Y-> B+ / Y -> A+), non-rewarded following X (X-> B- / X -> A-), or non-rewarded following Y (Y-> A- / Y -> B-). For example, a “rewarded following X” neuron would show selective firing to odor A only on X->A+ trials during acquisition, and then selectively fire to odor B on X->B+ trials after reversal. If the OFC is necessary for representing and updating task structure, then I expect to find a small, but non-trivial percentage of cue selective OFC neurons will reflect this abstract cue meaning (5-10%) in sucrose rats. I predict that there will be significantly fewer cue meaning neurons detected in cocaine experienced rats. If these neurons reflect updated representations of the task structure following reversal, then I predict that rats with a higher percentage of these neurons will acquire reversal learning faster. Specifically, a significant negative Pearson correlation between the proportion of cue meaning neurons and the number of trials to criterion after reversal.

I predict that cue value neurons would show reversal of cue selectivity for neurons that selectively respond to rewarded vs non-rewarded trials. Similar to cue meaning neurons, I predict that there will be significantly more cue value neurons in sucrose rats than cocaine rats, and that they will correlate with the speed of behavioural reversal.

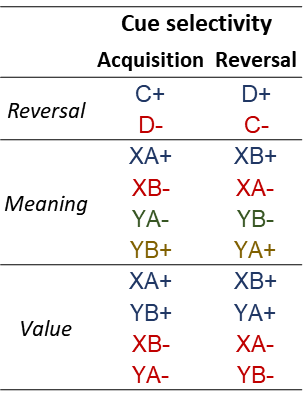
Alternative predictions are that: Cocaine will disrupt the flexible updating of cue value but not cue meaning in OFC.

Or cocaine will disrupt cue meaning but not cue value neurons in OFC.

If cocaine experience significantly reduces the number of cue value but not cue meaning neurons, then this would be consistent with the proposal that reversal learning deficits are caused by impaired cue-value learning.



***Table 1.*** *Experimental design. X and Y are auditory cues, cues A-D are odors, and pairs of cues reflect sequential presentation of an auditory cue followed by an odor. Rewarded Go trials are indicated by “+”, and non-rewarded NoGo trials are indicated by “-”.*



***Table 2.*** *Neurons that change cue selectivity from acquisition to reversal are expected to be the neural correlates of behavioral flexibility. Three categories of changes in cue selectivity (Reversal, Meaning, Value) are shown. Reversal neurons reverse cue selectivity for cues C or D from acquisition to reversal. Meaning neurons reverse selectivity for cue A or B to reflect whether they are rewarded following X or Y. Value neurons reverse cue selectivity for rewarded and non-rewarded odors independent of whether they are preceded by X or Y. Possible patterns of cue selectivity changes from acquisition to reversal are shown with matching colors.*

*Background: Reversal neurons in OFC correlate with behavioural flexibility (speed of reversal). Cocaine rats have reversal deficits (slower reversals) that correlate with a reduced proportion of Reversal neurons. Note that these differences are present even after criterion performance has been achieved. OFC representations are known to include expected cue value as well as the abstract meaning/structure of the task. Simple reversals conflate changes in cue value (rewarded->non-rewarded) with changes in cue meaning (go->nogo).*

*To dissociate neurons selective to task structure from cue value, an occasion setting (OS) task can be used. Variants of occasion setting tasks have been found to be OFC and striatum dependent, OFC neurons have been found to be selective to task structure in a manner that correlates with behavioural flexibility. A target cue can signal either reward on non-reward depending on the identity of the cue that preceded it i.e. cue A is rewarded after X and not Y, and cue B is rewarded after Y and not X. In this task, a neuron can show selectivity for cue identity (e.g. selectivity to A+ and A-), cue reward (e.g. selectivity to A+ and B+), or task specific structure (e.g. selectivity only to A+).* *Behaviourally, the speed of acquisition and reversal of these OS cues is a measure of behavioral flexibility.*

*Predictions:*

1. *Behaviourally Cocaine rats will require more trials to reach criterion accuracy during acquisition and reversal of OS.*
2. *Task specific neurons will be behaviourally relevant in control rats following acquisition and reversal.*
3. *Task specific neurons will be reduced in cocaine rats following acquisition and reversal.*
4. *These differences in task specific neurons will be present even though behaviorally accuracy will be matched, i.e. neurons will be classified from trials after criterion behavioral accuracy is reached.*

*Prediction:*

*Hypothesis 1: Meaning and Value neurons will both correlate with behavioural flexibility (speed of reversal).*

*Hypothesis 2: Cocaine rats will have fewer Meaning and Value neurons than sucrose rats, reflecting impaired behavioural reversal.*

*Alternative Hypothesis: Cocaine rats will show a dissociation between Meaning and Value neurons, potentially reflecting a lack of insight/coherence between knowledge about changes in the task structure and appropriate behaviour given that knowledge.*

**Expt 2. Determine the efficacy of D3-anatagonist to recover flexibility of behaviour and OFC activity cocaine rats with a history of cocaine use.**

<https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2391072/>