**Research Proposal.** Orbitofrontal cortex (OFC) dysfunction is a consistent neuropathology that underlies aberrant and inflexible behaviors that occur in many disorders of compulsivity such as cocaine addiction12, and obsessive-compulsive disorder3. OFC function is thought to represent a cognitive map of state space4, a mental map of the predictive relationships between cues, behavior, and outcomes that can guide behavior. Using a reversal learning task, subjects with a history of cocaine use exhibit repetitive and inflexible behavior5 and impoverished representations of task structure in OFC6. However, in a typical reversal learning task, features of the task that identify task-state specific information are confounded with other features such as changes in cue-reward relationships, temporal order, and behavioral inhibition, which have all been proposed as OFC functions7. Therefore, it is unclear whether cocaine use disrupts accurate representations of task states in OFC that are necessary for flexible behavior in reversal learning tasks. Occasion setting (OS) tasks share many features with reversal learning tasks but can be used to isolate the neural correlates of cognitive map representations by using explicit cues to signal cognitive map changes. Here I will address the question of whether a history of cocaine use disrupts cognitive map representations in OFC that are necessary for behavioral flexibility by combining electrophysiology with experimental manipulation of cocaine use during an OS task. Once established, I will test whether behavioral flexibility and OFC function can be restored using a novel pharmacotherapy for cocaine addiction. This will provide an important translational step towards understanding and treating disorders of compulsivity.

**Aim 1. Determine whether remapping of task representations in OFC during OS is disrupted in rats with a history of cocaine use.** Rats will undergo a cocaine self-administration procedure known to cause OFC dysfunction and behavioral inflexibility5,6, or a sucrose self-administration control. After a withdrawal period, these two groups of rats will be implanted with microelectrodes targeting OFC and trained on an occasion setting (OS) task. This approach will allow me to isolate cognitive map dependent task representations in OFC, test whether they are disrupted after cocaine use, and confirm that these representations are related to behavioral flexibility.

**Aim 2. Test whether a novel D3-antagonist can effectively recover impaired behavioral flexibility and its neural correlates in OFC in cocaine treated rats.** Rats will undergo the same procedure described in Aim 1, except that prior to each OS training session, half the rats in each group will receive injections of vehicle or the selective D3-antagonist VK4-1168,9. This will allow me to test whether a novel drug therapy can effectively recover impaired behavioral flexibility and its neural correlates in OFC caused by cocaine use.

**Significance.** Disturbances to OFC function and deficits in reversal learning, a marker of behavioral inflexibility, are both remarkably consistent features of many disorders of compulsivity in patients as well as translational animal models.

Compulsive and neurotypical behaviours do not occur in a vacuum, but instead are guided by informative cues in our environment. Activity in OFC is thought to represent the meaningful states signaled by these cues in terms of a cognitive map of state space used to navigate goal-directed behavior. OFC dysfunction is a common neuropathology in most disorders of compulsivity, and the behavioral inflexibility characteristic of these disorders is thought to reflect deficits in the creation and maintenance of these cognitive map representations in OFC. Key support for this comes from studies from this lab showing that rats with a history of cocaine use, known to cause compulsivity, exhibit inflexible behavior in reversal learning tasks and have impoverished cognitive map representations in OFC. In a typical reversal learning task, 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+. Changes in behavior after a reversal can be made more rapid by changing the underlying cognitive map to allow learning of the new relationship between cue and outcome states, without requiring unlearning of the old. However, since the reversal is signaled to the subject by a change in cue-reward associations, representations of cognitive maps in OFC cannot be disentangled from simple task features such as changes in reward. 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 features of reversal learning that makes it so popular is occasion setting (OS). In an OS task, subjects are presented with a sequence of two discrete cues, first an OS cue which indicates whether a second target cue predicts reward i.e. OS -> Target -> Reward (Figure 1). For example, target cue A is rewarded on X -> A+ trials, and non-rewarded on Y-> A- trials. This creates two cue-reward maps similar to the alternative maps that might be used in rapid reversal learning. However, both maps are learned from the start of the OS task and map switching on a trial-by-trial basis is signaled by an overt OS cue instead of by the act of reversing the cue-outcome associations i.e. X -> {A+, B-, C+, D-} and Y -> {A-, B+, C-, D+}. Therefore, depending on whether a trial starts with X or Y, the same target cue (e.g. A) signals two distinct states (A+ or A-) in separate cognitive maps. An OS task can therefore isolate neural representations of distinct map states unconfounded by changes in reinforcement. Notably, unlike reversal learning, all cues are followed equally by reward and non-reward.

Here I will manipulate a prior history of cocaine in rats to create a model of behavioral inflexibility and OFC dysfunction in disorders of compulsivity. Using in-vivo single-cell recording in OFC during an OS task I will answer the question: Do reversal learning deficits in rats with a history of cocaine reflect impoverished cognitive map representations in OFC?

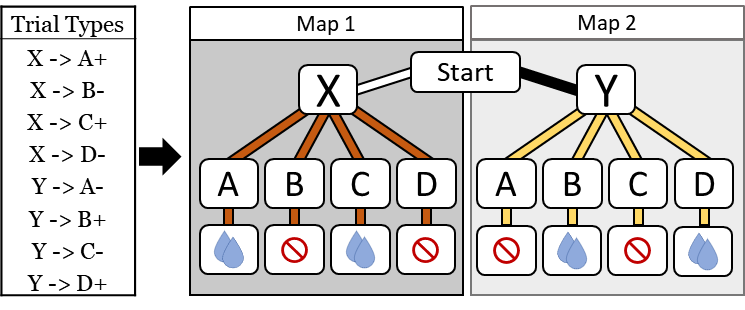
**Experiment 1.**

Hypothesis: A history of cocaine use causes behavioral inflexibility in tasks like reversal learning and OS by disrupting the formation and accuracy of cognitive map representations in the OFC.

Predictions: Behavioral accuracy in an OS task will correlate with the strength and fidelity of cognitive map representations such that more distinct cognitive map representations will predict higher behavioral accuracy. Furthermore, rats with a history of cocaine use will have lower behavioral accuracy and less distinct cognitive representations in OFC compared to control rats.

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. Rats will then be water deprived and given standard behavioral pretraining to become familiar with responding for odors and 10% sucrose reward in behavioral testing chambers. Next, a drivable bundle of microelectrodes will be implanted in OFC to record neural activity according to established lab procedures (ref). Following recovery, rats will be water deprived again and trained to with a novel set of cues on the OS outlined in Figure 1. On each trial, the rat will initiate cue presentation by entering and staying in the odor port, then a brief auditory cue (1000 ms) followed by an odor (500 ms) will be presented. On rewarded trials, responding to the food port below the odor port will be rewarded with a 10% sucrose solution. Correct performance will be defined as entering the food port on rewarded trials and withholding responding on non-rewarded trials. Each session will consist of 25 presentations of each trial type, presented in pseudorandom order, for a total of 200 trials. Criterion accuracy will be defined as 75% accuracy in a session. OFC activity will be recorded in all session, but neural analysis will focus on sessions with criterion accuracy.

The primary behavioral measure will be the number of sessions to reach criterion accuracy for 3 sessions in a row. Session to criterion will be compared between control and cocaine rats using a Poisson mixed-effects ANOVA model appropriate for count data.

******Neural activity will be processed using methods established for analyzing activity in prior work in similar tasks (REF). It is difficult to discuss all the possible results from an electrophysiological experiment such as this, so only key analyses and predictions will be presented below. Analyses will examine both single-unit and population level neural correlates of task features expected to reflect aspects of cognitive map representations. This will be achieved by comparing neural activity between trial conditions that require different cognitive maps i.e. differential activity during cues X and Y, as well as during cues A+ vs A-, B- vs B+, C+ vs C-, and D- vs D+. I will define the strength of unique state representations as, for example, the proportion of single units that selectively increase firing to one of these cue conditions, or the percentage accuracy of a classifier to accurately predict which cue was presented on a given trial. These measures represent two of the main methods to classify these neural correlates at the single-unit and population level.

***Figure 1.*** Proposed occasion setting (OS) task design. *(Left)* On each trial, OS cues X and Y uniquely identify whether the following Target cue (A-D) predicts reward. *(Right)* Cognitive map of task structure predicted in OFC representations.

**Predictions.** Given the hypothesis that activity in OFC reflects cognitive map representations rather than simply cue-reward value learning, I expect unique neural representations that discriminate between OS cues X and Y. This includes evidence that distinct subpopulations of neurons fire selectively to cue X or Y, or cues X and Y can be accurately decoded from population activity as unique trial. Cues X and Y do not differ in predicting whether the next cue is A, B, C, or D, or whether the trial will be rewarded. Therefore, differential representations of cues X and Y in OFC would not be predicted by expected outcome value or identity accounts of OFC function. However, while consistent with a cognitive map representation in OFC, these differences might simply reflect differences in the physical properties of cues X and Y. Next, to determine whether OFC represents the physical cue properties or the states within cognitive maps I will compare activity between the target cues that come after cues X and Y, that is A+ vs A-, B- vs B+, C+ vs C-, and D- vs D+. For example, when cue A is presented, any differences in representation must reflect information about future reward based on whether the previous cue was X or Y. Thus, differential activity to cue A on A+ vs A- trials reflects the unique state/position along a path within a cognitive map. Importantly, this would rule out the possibility that the OFC simply represents a cue’s physical properties or the cue’s predicted outcome value or identity. Furthermore, if OS cues signal which cognitive map to use to correctly interpret the meaning of the target cues, then I predict that more accurate/unique representations of OS cues X and Y will predict more accurate/unique representations of target cues A-D on rewarded vs non-rewarded trials. Together, these three analyses can be used to determine the strength and accuracy of the neural correlates of cognitive maps i.e. differential representation of (1) OS cues X vs Y and (2) target cues on rewarded vs non-rewarded trials (A+ vs A-, B- vs B+, C+ vs C-, D- vs D+), and (3) the correlation between them.

Additionally, if cognitive map representations in OFC reflect abstract task states, then I predict non-differential representations of different physical cues that share the same meaning within a cognitive map. Specifically, representations of cues A and C, as well as B and D, should not be distinct as they both predict reward and non-reward equally after X and Y.

Given the hypothesis that the accuracy of cognitive map representations in OFC are necessary for behavioral flexibility, I predict that behavioral accuracy for an individual rat on an individual session will be predicted by the fidelity of their cognitive map representations. That is, for each of the three neural correlates described above, stronger correlates of cognitive map representations will predict higher behavioral accuracy in a given session or portion of a session.

I predict that cocaine experienced rats will require more sessions to learn the OS task to criterion accuracy compared to control rats. This would be consistent with the hypothesis that reversal learning deficits in cocaine experienced rats reflects a general impairment in behavioral flexibility and not simply an inability to detect that cue-reward relationships change over time. Furthermore, during neural recording sessions with criterion levels of performance, I predict that the three neural correlates of cognitive maps in OFC will be significantly lower in cocaine rats compared to control rats. These neural correlates of cognitive maps should also correlate with accuracy on a given session, similar to previous reports in reversal learning [REF]. It is possible that cocaine rats will not show deficits behavioral flexibility in the OS task, or in the neural correlates of cognitive map representation in OFC. This would suggest that the deficits in reversal learning in cocaine experienced rats do not reflect deficits in cognitive map representations and instead reflect impairments such as value representations or inhibitory control. If this is found, the OS task conditions in this design allows for these alternative accounts to be tested.

Expt 2. Experiment 2 will test whether a novel D3-antagonist can treat the neural and behavioral deficits in behavioral flexibility caused by a history of cocaine use.

The D3 antagonist is expected to mitigate the behavioral inflexibility in cocaine rats, that is cocaine-vehicle rats are expected to take more session to reach criterion performance than cocaine-antagonist, sucrose-vehicle, and sucrose-antagonist groups. Of relevance in interpreting any effect will be whether the D3-antagonist affects control rats. Facilitation in controls might reflect a general facilitatory effect of the drug on information processing, learning, or behavioral control, whereas impaired performance might reflect a disruption of the balance between these systems. The neural analyses in Expt 1 will be used to relate the neural correlates of cognitive maps in OFC with behavioral flexibility. If the D3 antagonist mitigates the behavioral deficits observed in cocaine experienced rats via effects on OFC, then I predict that cognitive map representations in OFC and their relationship with behavior will match the control animals. Alternatively, it is possible that improvements in the behavioral deficit will not be reflected in normalized representations in OFC. This would suggest that a different target system is being affected by the drug. A prime neural candidate would be striatum which receives afferent projections from OFC which forms part of the common fronto-striatal neuropathology in cocaine addiction and other disorders of compulsivity.

***Alternative strategies, pitfalls and future directions.*** One possible outcomeis that rats pretreated with cocaine will not show behavioral deficits and/or their neural correlates in OFC. While this is unlikely, this finding would still provide interesting and meaningful information that address aim 1. In this scenario, subjects in Expt 1 will be retrained at the end of the OS task on a standard reversal learning task to replicate previous findings from the lab, as a positive control. If this is the case, then I will also use this established reversal task to assess the treatment efficacy of the D3-antagonist in Exp 2 while still addressing Aim 2.

* Drug: VK4-116 5 or 15 mg/kg i.p.
* Vehicle: 25% 2-hydroxyproply-β-cyclodextrin
* 15 mins prior to session

**References**

1. Volkow, N. D. & Fowler, J. S. Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. *Cereb. Cortex* **10**, 318–325 (2000).

2. Lucantonio, F., Stalnaker, T. A., Shaham, Y., Niv, Y. & Schoenbaum, G. The impact of orbitofrontal dysfunction on cocaine addiction. *Nat. Neurosci.* **15**, 358–366 (2012).

3. Graybiel, A. M. & Rauch, S. L. Toward a Neurobiology Review of Obsessive-Compulsive Disorder Dysfunction of the basal ganglia and associated cor. *Neuron* **28**, 343–347 (2000).

4. Wilson, R. C., Takahashi, Y. K., Schoenbaum, G. & Niv, Y. Orbitofrontal cortex as a cognitive map of task space. *Neuron* **81**, 267–279 (2014).

5. Calu, D. J. *et al.* Withdrawal from cocaine self-administration produces long-lasting deficits in orbitofrontal-dependent reversal learning in rats. *Learn. Mem.* **14**, 325–328 (2007).

6. Stalnaker, T. A., Roesch, M. R., Franz, T. M., Burke, K. A. & Schoenbaum, G. Abnormal associative encoding in orbitofrontal neurons in cocaine-experienced rats during decision-making. *Eur. J. Neurosci.* **24**, 2643–2653 (2006).

7. Stalnaker, T. A., Cooch, N. K. & Schoenbaum, G. What the orbitofrontal cortex does not do. *Nat. Neurosci.* **18**, 620–627 (2015).

8. Newman, A. H., Ku, T., Jordan, C. J., Bonifazi, A. & Xi, Z. X. New Drugs, Old Targets: Tweaking the Dopamine System to Treat Psychostimulant Use Disorders. *Annu. Rev. Pharmacol. Toxicol.* **61**, 609–628 (2021).

9. Kumar, V. *et al.* Highly selective dopamine D3 receptor (D3R) antagonists and partial agonists based on eticlopride and the D3R crystal structure: New leads for opioid dependence treatment. *J. Med. Chem.* **59**, 7634–7650 (2016).