**Title:**  Role of orbitofrontal cognitive map representations in flexible and inflexible behavior

**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. For example, subjects with a history of cocaine use exhibit repetitive and inflexible behavior5 during reversal learning, which correlates with impoverished representations of the task structure in OFC6 Such activity in OFC is thought to encode a cognitive map of state space4, however in a typical reversal learning task, features of the task that identify task-state specific information critical to cognitive mapping are confounded with actual changes in cue-reward relationships, temporal order, and behavioral inhibition, each of which are also 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 use an OS task to directly test whether a history of cocaine use causes inflexible behavior by disrupting cognitive map representations in OFC, and whether any such effects can be mitigated by dopamine D3 receptor (D3R) antagonists, which have been proposed as a novel pharmacotherapy strategy for treatment of cocaine use disorders.

**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. Analyses will focus on identifying correlates of cognitive mapping in OFC and testing whether they are disrupted by prior cocaine use.

**Aim 2. Test whether a novel D3R antagonist can mitigate impaired behavioral flexibility and associated changes in 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 D3R antagonist *R*-VK4-408,9. Analyses will focus on whether a novel drug therapy can improve behavioral flexibility, whether improvement is specific to cocaine-experienced rats, and how it relates to changes in neural correlates in OFC caused by cocaine use.

**Significance.** Disturbances to OFC function and deficits in reversal learning, a marker of behavioral inflexibility, are consistent features of many disorders of compulsivity in patients as well as translational animal models10–12. Drug-induced deficits in reversal learning in animal models of addiction are thought to reflect deficits in the creation and maintenance of cognitive map representations in OFC 4. 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 and inflexible neural representations in OFC5,6,14. However, in these tasks, the signal to switch to a new cognitive map - the reversal - is confounded with changes in the actual cue-reward associations. As a result, representations of cognitive maps in OFC cannot be disentangled from representations of the new associations, representation of value, or even response inhibition, each of which has also been argued to depend on OFC.

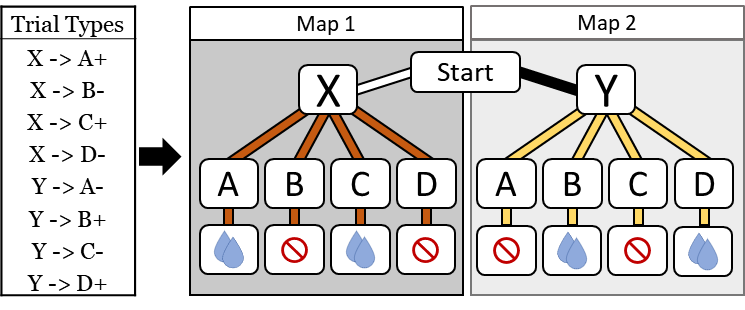
To resolve these confounds and more specifically identify whether OFC supports flexible behavior through a mapping function, it is necessary to use a behavioral approach that dissociates the cue that triggers changes in the task map being used from these other features. One such task is occasion setting (**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, except they are signaled by the X and Y cues, which isolate the information relevant to the alternative map states and are not confounded by changes in associative learning, value, or responding.

Here I will use an OS task modeled after the reversal task used in prior work to test whether reversal learning deficits in rats with a history of cocaine reflect impoverished cognitive map representations in OFC. If this is established, I will then test the efficacy of *R*-VK4-40, a promising D3R antagonist, to treat this behavioral inflexibility, and its neural correlates in OFC, in cocaine experienced rats. Drug compounds that target dopamine D3-receptor antagonists have been shown to have significant potential to treat aberrant behaviors in disorders of compulsivity that are also characterized by dysfunction of dopaminergic signaling1,8. *R*-VK4-40 is a highly selective D3R-antagonist that reduces psychostimulant use and relapse behaviors in rodent models8. However, its efficacy in treating long-term behavioral inflexibility caused by a history of cocaine use has not been tested. These findings will advance our understanding of how OFC dysfunction contributes to behavioral inflexibility in disorders of compulsivity, and how such dysfunction might be treated.

**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 representations in OFC compared to control rats.

******Procedure: Long Evans rats will undergo a standard cocaine (n = 8) or sucrose (n = 8) control self-administration protocol for 2 weeks followed by 30 days of withdrawal5. Rats will then be water deprived and given standard pretraining to become familiar with responding for odors and 10% sucrose reward in behavioral testing chambers. Next, drivable microelectrodes will be implanted in OFC to record neural activity according to established lab procedures15. Following recovery, rats will be trained with a novel set of cues on the OS task outlined in Figure 1. On each trial, the rat will initiate cue presentation by entering 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. 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. Criterion accuracy will be defined as 75% accuracy in a session, acquisition as criterion in 3 sessions in a row. Neural analyses will focus on sessions post-acquisition.

***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 (+) or no reward (-). *(Right)* Cognitive map illustration of task structure predicted in OFC representations.

Neural activity will be processed using methods established for analyzing activity in prior work in similar tasks6,16. Analyses will examine both single-unit and population level neural correlates of task features expected to reflect aspects of cognitive map representations. 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 predict which cue was presented on a given trial. It is difficult to discuss all the possible results from an electrophysiological experiment such as this, so only key predictions will be presented below.

**Expected Results.** Given the hypothesis that activity in OFC reflects cognitive map representations rather than isolated cue-reward value learning, I expect unique neural representations that discriminate between OS cues X and Y. Cues X and Y do not differ in predicting the next cue, responding, or whether the trial will be rewarded, so differential activity cannot reflect value or even simple associative information. Differences might reflect the distinct physical properties of X and Y; to rule out this possibility 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+. Differential activity to A+ vs A- must reflect information about future reward based on whether the previous cue was X or Y, i.e. a unique state/position along a path within a cognitive map. If activity to OS cues in OFC signals which cognitive map to use to correctly interpret the meaning of the target cues, then 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 analyses can 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, for each of the three neural correlates described above, stronger correlates of cognitive map representations should predict higher behavioral accuracy in a given session or portion of a session.

By contrast, if cocaine experience disrupts flexible behavior by affecting the mapping function of OFC, then cocaine experienced rats should require more sessions to learn the OS task to criterion accuracy compared to control rats, their post-criterion performance may be less accurate than controls, and the three neural correlates of cognitive maps in OFC will show reduced fidelity and/or correlations in cocaine rats compared to control rats.

**Experiment 2.**

Procedure: I will use the same procedure described in Experiment 1, except that sucrose and cocaine groups will receive an injection of either vehicle (25% 2-hydroxypropyl-β-cyclodextrin) or *R*-VK4-40 (3 and 10 mg/kg, i.p.) 15 mins prior to each OS session, i.e., four groups (n = 8 each), sucrose/vehicle, sucrose/ *R*-VK4-40, cocaine/vehicle, and cocaine/ *R*-VK4-40.

Expected results: In cocaine rats,*R*-VK4-40 is predicted to mitigate the behavioral inflexibility and underlying disturbances to cognitive map representations in OFC described in Experiment 1, making cocaine/ *R*-VK4-40 more similar to sucrose/vehicle. Of relevance in interpreting any effect will be whether *R*-VK4-40 affects behavior or cognitive map representations in sucrose control rats. This will indicate whether *R*-VK4-40 generally promotes behavioral flexibility, or if it is treating the specific impairments induced by a history of cocaine use. It is also possible that *R*-VK4-40 will only modify behavior but not disrupted OFC representations in cocaine rats. This would suggest that a different target system is being affected.

***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, I will also use this established reversal task to assess the treatment efficacy of *R*-VK4-40 in Exp 2.

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Intra-Institute Collaboration

The proposed experiments require collaboration between the labs of Dr. Geoffrey Schoenbaum (NIDA) and Dr. Amy Newman (NIDA). Dr. Geoffrey Schoenbaum will provide funding, space, and all necessary electrophysiological recording equipment and expertise. Dr. Geoffrey Schoenbaum will be the main mentor of Dr. Marios Panayi and will oversee all experiments. Dr. Amy Newman will provide the dopamine D3-receptor antagonist *R*-VK4-40, expertise in drug dose and timing parameters, as well as rats trained to self administer cocaine. Dr. Amy Newman, together with Dr. Geoffrey Schoenbaum, will oversee all experiments.