The Role of the Orbitofrontal Cortex in Alcohol Preference, Seeking and Consumption

THE ROLE OF THE ORBITOFRONTAL CORTEX IN ALCOHOL PREFERENCE, SEEKING AND CONSUMPTION

A Dissertation Presented

by

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Submitted to the Graduate School of the
University of Massachusetts Amherst in partial fulfillment
of the requirements for the degree of

DOCTOR OF PHILOSOPHY

September 2019

Neuroscience and Behavior Program

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DEDICATION

To:

My amazing and supportive husband, David Layman
Dr. Sandra Peterson for being both an academic and personal mentor of the highest caliber.
my cousins and best supporters, Jenny Verdooren and her husband Ben Verdooren
The amazing members of the IMSD program for their support

Thank you all for your love and support!

ACKNOWLEDGMENTS

I would like to thank Dr. David Moorman for funding my research, guiding my scientific training and helping me develop into a scientist. I appreciate the guidance and support of Drs. Luke Remage-Healey, Heather Richardson and Elena Vazey for helping me develop sound scientific approaches, research design and data analysis. I truly appreciate all of your guidance.

I am very grateful to Jeffrey Tarantino, Taryn Rahman, Annalise Binette and Rachel Siegel for their day-to-day support of my experimental studies. You all were so helpful, upbeat and made working in the lab a joy! I also want to thank Jessica Cabellero, Beata Kaminska and Dr. Chris Perk for their support, feedback and genuine lab comradery. You all made the lab so awesome to be a part of!

I want to extend my appreciation for Dr. Sandra Peterson and Vanessa Hill for their support financially through the NEAGEP fellowship – I don't think I could have gotten through this Ph.D without your support!

I also want to thank the animal care staff, you all made working in the lab so much easier and your genuine care for the animals gave me peace of mind.

Lastly, I want to thank my loving and supportive husband! For being patient with all of those late nights working in lab or analyzing data, I always felt that I could do everything because you were by my side! Excelsior!

ABSTRACT

THE ROLE OF THE ORBITOFRONTAL CORTEX IN ENCODING ALCOHOL PREFERENCE, SEEKING AND CONSUMPTION

SEPTEMBER 2019

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Structural and physiological abnormalities in the frontal cortex are strongly correlated with alcohol use and misuse – a worldwide biomedical concern. One frontal cortical region that has been understudied in terms of alcohol use and misuse is the orbitofrontal cortex (OFC). The OFC is critical for encoding the predictive value of appetitive outcomes (rewards), and OFC activity is correlated with the propensity of an individual to seek that reward. Although studies have examined the role of the OFC in cocaine preference and seeking, few studies have examined whether OFC activity encodes the value of alcohol rewards or even if the OFC is necessary for the expression of alcohol seeking. Results demonstrated that OFC neurons do encode the relative behavioral preference for alcohol rewards and that this encoding is relatively stable. Additionally, I investigated whether OFC neurons were necessary for alcohol seeking and consumption using inhibitory chemogenetics and discovered that OFC neurons are selectively necessary for reinstating palatable alcohol as well as sucrose seeking. The findings support that the OFC encodes relative preference for

alcohol but is only necessary for modulating behavior on cued-reinstatement, suggesting that the OFC is a target brain region for intervention for alcohol relapse.

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CHAPTER 1

GENERAL INTRODUCTION AND BACKGROUND

1.1 The functional role of the prefrontal and orbitofrontal cortex

The prefrontal cortex is not critical for performing simple or automated behaviors, but instead is important in cognitive control when individuals use previous experiences, internal goals and the consequences of actions to guide behaviors (for review, see Miller & Cohen, 2001). One of the first studies ever performed examining the newly termed "prefrontal region" was conducted in 1884 by David Ferrier and Gerald Yeo (Ferrier & Yeo, 1884) where lesions of non-human primates produced profound negative impacts on individual behavior, playfulness and social behavior. Since then, the prefrontal cortex (PFC) has been studied in humans, non-human primates, rats and mice specifically for its role in behavior. In humans, damage to the prefrontal cortex (PFC) suppresses an individuals' ability to monitor impulsive responses and results in an inability to learn and alter strategies in rule-based tasks (Milner, 1963; Shallice & Burgess, 1991; Aron et al., 2004). Lesioning or inactivating the PFC in non-human primates produces similar behavioral phenotypes (Brozoski et al., 1979; Dias et al., 1996). The prefrontal cortex contributes to social learning, impulse control and decision-making through its interconnections with virtually all sensory and motor systems as well as a wide range of subcortical structures (Goldman-Rakic, 1987; Pandya & Barnes, 1987; Barbas & Pandya, 1991; Dembrow & Johnston, 2014). Specifically, prefrontal brain regions have connections with somatosensory, premotor, sensory cortices as well as the amygdala, striatum, perirhinal and entrorhinal cortex in humans, non-human primates and rodents

(Carmichael & Price, 1995; Öngür & Price, 2000; Heidbreder & Groenewegen, 2003; Uylings et al., 2003; Asher & Lodge, 2012; Kondo & Witter, 2014; Heilbronner et al., 2016a). There are extensive homologies between the human, non-human primate and the rodent prefrontal cortex (Uylings et al., 2003; Seamans et al., 2008; Heilbronner et al., 2016b) suggesting that particular prefrontal brain regions (e.g., medial prefrontal cortex, mPFC, or orbitofrontal cortex, OFC) are valuable targets in translational research.

Within the PFC, the OFC is directly implicated in influencing behavior and decision-making. The OFC has connections with both olfactory and taste cortex that play a critical role in linking sensory signals with their outcomes (Schoenbaum & Eichenbaum, 1995; Critchley & Rolls, 1996; Rolls, 1996; Hoover & Vertes, 2011; Schoenbaum et al., 2007). Neurons in the OFC respond to odors, temperatures, visual and auditory stimuli that are associated with food and drink (Rolls, 2015). Neurons in the orbitofrontal cortex form associations between stimuli and their outcomes to encode rules or relative value in experiences that ultimately modulate behavior (Shimamura, 2000; Padoa-Schioppa, 2011; Wallis, 2011; Wingerden et al., 2012; Bartra et al., 2013; Clithero & Rangel, 2013; O'Doherty, 2014). Natural environments are rich in sensory information (e.g., odors, visual cues, sounds, colors, etc.,) that the OFC then ties with outcomes directly related to survival (e.g., food, shelter, predators, etc.,) allowing individuals to learn and make flexible choices (for review, see Schuck et al., 2018).

In healthy humans, the OFC is key in representing the value of rewards and the sensory information the predicts them, which is key for decision-making and expectation (Kringelbach, 2005). Humans patients with OFC lesions struggle to behaviorally adapt to changes in stimulus-outcome associations (Harlow, 1868; Berlin et al., 2004), use

previous experiences to inform future consequences (Bechara et al., 1994) and process outcomes of social behavior (Jonker et al., 2014). In rodents, the lateral OFC is suggested to play a critical role in incentive learning and decision-making, evaluating the relative value of outcomes and adjusting behavior when task rules are altered (Murray et al., 2007; Wallis, 2007; Burke et al., 2009; Balleine et al., 2011; Padoa-Schioppa, 2011; Walton et al., 2011; Young & Shapiro, 2011). OFC also plays a role in encoding imagined outcomes (Takahashi et al., 2009, 2013), suggesting that disruption of normal OFC function may result in an individual that struggles to anticipate appropriate outcomes. OFC also represents a cognitive map of task space (Wilson et al., 2014; Schuck et al., 2016) which in turn is used to determine the value of a reward (Tolman, 1948). An additional key function of the OFC is its ability to encode cue-outcome associations for rewarded and non-rewarded stimuli (Schoenbaum et al., 2003; Riceberg & Shapiro, 2012; Moorman & Aston-Jones, 2014; Rolls & Deco, 2016).

Being able to discriminate between stimuli that predict rewarded and non-rewarded outcomes (i.e., discrimination learning) is a key feature of animal behavior that is essential for survival (Phillmore, 2008). The OFC is able to encode stimuli that predict rewarded and non-rewarded outcomes in both rodents and in humans (Schoenbaum et al., 2003; Moorman & Aston-Jones, 2014; Cheng et al., 2016). Specifically, individual OFC neurons of rats display distinct patterns of firing in response to cues depending on whether or not the cue predicts a rewarding outcome (Schoenbaum et al., 2003). The OFC is more modulated in response to cues and actions that predict rewarded outcomes compared to cues and actions that predict non-rewarded outcomes (Moorman & Aston-Jones, 2014). In humans, the medial and lateral subregions of the OFC are functionally

connected with other brain regions to encode rewarded and non-rewarded outcomes, respectively (Cheng et al., 2016). OFC neurons also encode non-rewarded stimuli that lead to aversive outcomes in rodents, non-human primates and humans (see Rolls, 2000; Schoenbaum et al., 2003; Berlin et al., 2004; Atlas et al., 2016), which is key for updating behavior to avoid negative consequences. The OFC's ability to properly encode rewarded outcomes affects reward motivation (Patil et al., 2017) and supports the claim that OFC neurons encode the relative value of rewards (Tremblay & Schultz, 1999).

OFC neurons are well-known for encoding the subjective value of natural rewards and the stimuli that predict them (G Schoenbaum & Eichenbaum, 1995; Tremblay & Schultz, 1999; Roesch et al., 2006; Levy & Glimcher, 2012; DE Moorman & Aston-Jones, 2014; Stalnaker et al., 2014; Howard et al., 2015; Nogueira et al., 2017; Sarlitto et al., 2018). In primates, OFC neurons encode the relative value of rewards that is consistent with their behavioral preference (Tremblay and Schultz, 1999; Wallis and Miller, 2003). In rodents, OFC neurons are also able to distinguish between high- and low-valued natural rewards (Roesch et al., 2007; Burton et al., 2014) as well as relative preference for drug rewards such as cocaine and heroin (Sell et al., 2000; Guillem et al., 2017; Guillem and Ahmed, 2018). Alcohol consumption in heavy drinkers induces opioid release in both the orbitofrontal cortex and the nucleus accumbens (NAc), suggesting that connectivity between the OFC and NAc contributes to opioid regulation of alcohol intake in high-alcohol preferring individuals (Mitchell et al., 2012). NAc neurons are suppressed by OFC stimulation (Asher and Lodge, 2012) and OFC lesions suppress outcome value signaling of NAc neurons in response to cues (Cooch et al., 2015). Finally OFC is implicated in modulating NAc, specifically to enhance poor decision-making and

facilitate compulsive craving in alcohol addiction (Seo and Sinha, 2014). The functions of the OFC outlined thus far suggest that the OFC encodes the relative value for natural and drug rewards, potentially including alcohol, and therefore for the purposes of my dissertation research I focused on alcohol.

1.2 Examining the relationship between the orbitofrontal cortex and alcohol

In recent years, many studies have identified the OFC as a brain region disrupted in individuals suffering from drug addictions (see Schoenbaum et al., 2006), including alcohol use and abuse disorders (Moorman, 2018). Alcoholic patients exhibit poor control over impulse behavior, leading to detrimental and risky decisions (Lejuz et al., 2010; Verdejo-Garcia et al., 2008). Alcohol users perform poorly on the Iowa Gambling Task, a reward-based decision-making task, by reliably selecting highly valued but disadvantageous outcomes (Bechara et al., 2001; Cantrell et al., 2008; Dom et al., 2006; Mazas et al., 2000; Dom et al., 2005; Noel et al., 2007). Individuals suffering from alcohol misuse/abuse disorders exhibit deficits in behavioral flexibility tasks, in particular reversal learning and during attentional set-shifting tasks such as the Wisconsin Card Sort Task (Jenkins & Parsons, 1979; Kish et al., 1980; Parsons, 1983; Yohman et al., 1985; Beatty et al., 1995; Tivis et al., 1995; Rourke & Grant, 1999; Fillmore & Rush, 2006; Vanes et al., 2014). Alcohol users also perform poorly on reward-based decision-making tasks, such as the Iowa Gambling Task, in which participants reliably choose disadvantageous high-reward, high-penalty outcomes (Mazas et al., 2000; Bechara et al., 2001; Fein et al., 2004; Dom et al., 2006; Noël et al., 2007; Cantrell et al., 2008; Miranda et al., 2009).

Alcohol exposure also has direct effects on OFC neuron activity. *In vitro*, alcohol exposure to regular spiking neurons in the mouse OFC results in a glycine receptor-dependent decrease in OFC neuron excitability and suppresses NMDA excitatory post-synaptic currents, suggesting OFC influences neuronal function (Badanich et al., 2013). Interestingly, chronic exposure to ethanol (i.e., CIE) vapor increases excitability of OFC neurons (Nimitvilai et al., 2016). After inducing ethanol dependence using CIE in mice, ethanol seeking increased along with an increase in mRNA for GluN1 and GluN2A subunits of NMDA receptors thus increasing evoked NMDA-receptor mediated currents in medial OFC neurons (Radke et al., 2017). In alcohol naïve mice, dopamine, norepinephrine and serotonin decrease current-evoked spiking in lateral OFC neurons via inhibitory GIRK channels (Nimitvilai et al., 2017). However, in CIE-treated mice, the inhibitory effects of these monoamines were absent, suggesting that chronic ethanol disrupts normal OFC function. Although alcohol has a diverse impact on OFC function, chronic alcohol also impacts OFC-dependent behavior.

Alcohol-induced alterations to OFC function and activation are also observed in rodents. Adult mice undergoing CIE exhibit decreased performance on a set-shifting task (Badanich et al., 2011) and exhibit deficits in a maze-based attentional set-shifting task (Kroener et al., 2012; Hu et al., 2015). Chronic alcohol use during adolescence results in elevated risky decision-making (Nasrallah et al., 2009, 2011; Clark et al., 2012; McMurray et al., 2014; Schindler et al., 2014; McMurray et al., 2015). Chronic alcohol exposure also exacerbates risk-taking behaviors, which appears to be encoded in OFC neurons (Roitman & Roitman, 2010; Schultz et al., 2011) or is influenced by OFC neurons (Bechara et al., 2001; Bechara, 2004; Hsu et al., 2005). In alcohol-dependent

mice, OFC DREADD inhibition resulted in increased alcohol consumption compared to sham or vehicle treated groups, even when aversive quinine was added to the alcohol (Den Hartog et al., 2016). These data suggest that alcohol not only impacts OFC function, but also that OFC function can in turn affect alcohol seeking and consumption behaviors. Finally, during adolescent alcohol treatments, neuroinflammation and neurodegeneration is observed in the OFC (Crews & Boettiger, 2009; Coleman et al., 2011, 2014; Qin & Crews, 2012a, 2012b; Crews et al., 2013). Together these findings suggest that the OFC may play an important role in influencing alcohol preference, alcohol-related decisions and ultimately may contribute to alcohol relapse in response to alcohol cues or consumption.

1.3 Expanding on the role of the orbitofrontal cortex and encoding natural and drug rewards

It is clear that the OFC is not *necessary* for the expression of reward-guided behavior. OFC lesioned rats does not disrupt an animal's ability to learn instrumental tasks (Fuchs et al., 2004; Burke et al., 2008). Optogenetic inhibition of the OFC of rats also does not affect economic choice between rewards of different flavors and sizes (Gardner et al., 2017; Gardner et al., 2018; Miller et al., 2018). In primates, OFC inactivation does not disrupt reward preference during selective food satiation (West et al., 2011). Finally, OFC inactivation does not disrupt outcome preference for rewards – rats maintained a high preference for sucrose over a calorically equal but less preferred polycose (Keiflin et al., 2013). Together, these findings support an alternative view of the OFC suggesting that it supports choice behavior only indirectly, by contributing to

learning itself (Schoenbaum et al., 2009; Padoa-Schioppa, 2011; McDannald et al., 2012; Takahashi et al., 2009). In particular, the OFC is directly implicated in updating the value or outcome of rewards.

OFC neurons track changes in stimulus-outcome contingencies, alterations in expected outcomes and changes in reward value (Iversen and Mishkin, 1970; O'Doherty et al., 2001; McAlonan and Brown, 2003; Burke et al., 2009; Takahashi et al., 2009; West et al., 2011; Takahashi et al., 2013; Dalton et al., 2016; Gourley et al., 2016; Izquierdo et al., 2017; Hervig et al., 2019). The OFC is necessary for reversal learning, as OFC lesioned rats and non-human primates display significantly suppressed ability to update stimulus-outcome changes (Schoenbaum et al., 2003; Iversen and Mishkin, 1970; McAlonan and Brown, 2003; Burke et al., 2009; West et al., 2011; Dalton et al., 2016; Gourley et al., 2016; Izquierdo et al., 2017; Hervig et al., 2019). When rewards are devalued using satiation, OFC lesion resulted in mice and rats that were unable to update behavior for devalued rewards (West et al., 2011; Gourley et al., 2016). In humans the OFC is less activated in response to cues predicting a devalued reward compared to a non-devalued reward, suggesting the OFC encodes when rewards are devalued (Gottfried et al., 2003). The OFC's role in encoding changes to reward value is also important during drug abstinence.

During abstinence from drugs, the relative value of both natural or drug rewards, and the cues that predict them, are enhanced (Spring et al., 2003; Freeman et al., 2012; Preller et al., 2013; Reitzel and Leventhal, 2014; Li et al., 2017). During alcohol abstinence, alcohol cue presentation actives OFC neurons in alcohol abstinent individuals (Kim et al., 2014; Bach et al., 2015). The medial OFC is activated during alcohol cue

presentation, and positively predicts alcohol craving and relapse in individuals carrying the kainite receptor subunit GRIK1 (Bach et al., 2015). In rodents, the OFC is necessary for the expression of cue- and context-induced cocaine reinstatement (Fuchs et al., 2004; Lasseter et al., 2009; Lasseter et al., 2011) and OFC neurons are activated in response to alcohol cues during prolonged alcohol abstinence (Jupp et al., 2010). OFC inactivation also suppresses context-induced reinstatement of alcohol seeking (Bianchi et al., 2018). Although the OFC appears to play a role in responding to alcohol cues during alcohol abstinence and is necessary for context-induced reinstatement, it is still unclear if the OFC is necessary for the expression of alcohol preference, seeking and consumption.

In summary, the OFC encodes relative reward value, rewarded and non-rewarded outcomes, OFC neurons are activated by alcohol cues during abstinence and OFC neurons are necessary for cue and context-induced reinstatement of reward seeking for cocaine and alcohol, respectively. Therefore the purpose of this dissertation was to assess the OFC's role in encoding alcohol preference and alcohol-seeking behaviors. **I predict that the OFC encodes alcohol preference, seeking and consumption and that it plays a direct role in modulating preference, seeking and consumption.** In order to test these hypotheses, I developed two projects: 1) Examine whether the OFC encodes alcohol preference using *in vivo* recordings during operant alcohol seeking and consumption and 2) Examine whether the OFC is necessary for the expression of alcohol preference, seeking and consumption. As outlined above, cues predicting alcohol are able to induce alcohol craving and relapse, posing a significant health and financial setback to those suffering from alcohol use and abuse disorders. If the OFC encodes alcohol

preference, seeking and consumption *and* is necessary for the expression of alcohol preference, seeking, consumption and reinstatement of alcohol seeking, these findings would be key for developing therapeutic treatments for alcohol use and abuse disorders.

CHAPTER 2

ORBITOFRONTAL CORTICAL NEURON ACTIVITY ENCODES PREFERENCE FOR ALCOHOL

Submitted for publishing to Neuropsychopharmacology

2.1 Abstract

Orbitofrontal cortex (OFC) activity represents reward value, preference, and seeking. OFC function is disrupted in drug abuse and dependence, but its role in alcohol use disorders has been less well studied. In alcohol-dependent humans OFC activity is increased by alcohol cue presentation. Ethanol also modulates OFC neuron excitability *in vitro*, and OFC manipulation influences ethanol seeking and drinking in rodents. To understand the relationship between OFC function and alcohol use, we recorded OFC neuron activity during ethanol self-administration, characterizing the neural correlates of individual preference for alcohol. After 1 month of homecage access to 20% ethanol, male Long-Evans rats were trained on a cued reward seeking task for 20% or 10% ethanol or sucrose. OFC neuronal activity was recorded and associated with task performance and ethanol preference. Rats segregated into high and low ethanol drinkers based on homecage consumption and operant seeking of 20% ethanol. Motivation for 10% ethanol and sucrose was equally high in both groups. OFC neuronal activity was robustly modulated during sucrose and ethanol seeking and consumption. Modulation was strongest for sucrose in all rats, and strength of ethanol modulation was associated

with individual preference for 20% ethanol. Ethanol-associated OFC activity was more similar to sucrose-associated activity in high vs. low ethanol drinkers. The results show that OFC neurons are activated during alcohol-associated behaviors based on individual preference, strongly supporting this brain region as an area underlying alcohol use and alcohol use disorder and demonstrating how OFC neuronal circuits can drive motivation for alcohol.

2.2 Introduction

In humans, non-human primates, and rodents, the orbitofrontal cortex (OFC) regulates reward seeking and cognitive strategies associated with optimizing outcomes (Dalley et al., 2004; Kringelbach & Rolls, 2004; Kringelbach, 2005; O'Doherty, 2007; Rolls & Grabenhorst, 2008; Geoffrey Schoenbaum et al., 2009; Mainen & Kepecs, 2009; Balleine et al., 2011; Padoa-Schioppa, 2011; Takahashi et al., 2011; Wallis, 2011; Walton et al., 2011; Noonan M et al., 2012; Rudebeck & Murray, 2014; McDannald et al., 2014; Stalnaker et al., 2015; Rolls & Deco, 2016; Izquierdo, 2017). The OFC is activated during craving and seeking of drugs of abuse and in response to drug-associated cues (Garavan et al., 2000; Risinger et al., 2005; Baeg et al., 2009; Guillem et al., 2010, 2017; Guillem & Ahmed, 2018), and OFC hypoactivity is associated with impulsivity and drug use disorders (Whelan et al., 2012). Based on these and other results, OFC disruption has been hypothesized to be a major factor underlying drug addiction (London et al., 2000; Porrino & Lyons, 2000; Volkow & Fowler, 2000; Dom et al., 2005; Winstanley, 2007; Everitt et al., 2007; Schoenbaum & Shaham, 2008; Lucantonio et al., 2014; Fettes et al., 2017). However, comparably fewer studies have investigated the role of OFC in alcohol

use.

There is some evidence for a role for OFC in alcohol motivation and dependence (Moorman, 2018). Activation of OFC in humans has been associated with alcohol-related craving (Myrick et al., 2003, 2008; Lukas et al., 2013; Schacht et al., 2013a, ; Schacht et al., 2013b; Schacht et al., 2014). Connectivity between OFC and the striatum is altered in abstinent alcoholics (Volkow et al., 2007). Chronic alcohol (ethanol, EtOH) results in cognitive deficits associated with OFC dysfunction (Lejuez et al., 2010; Badanich et al., 2011). EtOH consumption and reinstatement increases Fos and □FosB expression in rodent OFC (Li et al., 2010; Jupp et al., 2010). Acute EtOH application *in vitro* inhibits excitability and synaptic function in OFC neurons (Badanich et al., 2013), and in mice withdrawn from chronic EtOH vapor, OFC neurons display increases in spine density and basal excitability and a diminished inhibitory response to acute EtOH (McGuier et al., 2015a; Nimitvilai et al., 2016). OFC lesions or DREADD inhibition increased alcohol drinking in rats and EtOH vapor treated mice (Den Hartog et al., 2016; Ray et al., 2018), and inactivation decreased context-induced reinstatement in rats (Bianchi et al., 2018).

A critical outstanding question is how alcohol seeking and use are reflected by OFC neuron ensemble activity, and whether this activity differs across individuals. Motivation for alcohol varies across both human and animal subjects, and individuals exhibiting greater euphoric or stimulating effects of alcohol may be at greater risk for misuse and dependence (King et al., 2011; Sharko et al., 2013; Momeni & Roman, 2014; Marcia et al., 2015; Moorman et al., 2016; Moorman et al., 2017). Given the strong association between OFC neuronal function and individual preferences for natural rewards, cocaine, and heroin (Tremblay & Schultz, 1999; Schultz et al., 2011; Guillem et

al., 2017; Guillem & Ahmed, 2018), it is logical to expect that differential OFC activity may reflect individual alcohol preferences. Here we characterized individual preference for EtOH in outbred Long-Evans rats and identified OFC correlates of this preference by recording neuronal activity during operant EtOH or sucrose seeking. Our results indicate that OFC neurons are robustly, but differentially activated during EtOH and sucrose seeking, and that degree of activation is associated with individual EtOH preference.

2.3 Materials and Methods

2.3.1 Animals

Male Long-Evans rats (n = 24; ~200-300g upon arrival; Charles River Laboratories, Wilmington, MA) were kept in temperature- and humidity-controlled conditions under reversed light/dark cycle (7:00 AM off to 7:00 PM on). Water was available *ad libitum* and rats were restricted to 25g of rat chow given after daily operant sessions once they reached 300g. Behavioral testing occurred between 9:00 AM and 5:00 PM. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Massachusetts Amherst and were conducted in compliance with the National Institutes of Health *Guide for the Care and Use of Animals*.

2.3.2 Experimental Design

Animals were initially trained to drink 20% ethanol (EtOH; diluted with tap water from 95% EtOH (Fisher Scientific, Pittsburgh, PA) in their home cages using the intermittent access to EtOH paradigm (Wise, 1975; Simms et al., 2008; Moorman & Aston-

Jones, 2009; Carnicella et al., 2014; Moorman et al., 2016; Moorman et al., 2017), with ad lib access to food and water. After 1 month of intermittent access, rats were trained to perform operant EtOH or sucrose seeking on an FR1 schedule. Behavioral testing occurred in operant chambers (Med Associates, Fairfax, Vermont) equipped with a house-light, nosepoke, and reward delivery port containing a spigot to deliver three rewards (15% surcrose, 10% EtOH, 20% EtOH) separately. Rats were initially trained on FR1 nosepoke responses for a sucrose cue (5 kHz tone, 1 sec) and 0.1 ml sucrose delivery through the spigot. Once rats reliably consumed sucrose in less than 2 sec after nosepoke, we increased the pre-cue nosepoke duration to 100 msec and decreased the post-cue response time (leaving the nosepoke and going to the spigot) to 500 msec. Sucrose was used as an initial reward to facilitate rapid task learning. Training sessions were 1 hour. After rats reached a criterion of 85% successful trials (i.e., retrieving sucrose within 500 msec post-cue onset) rats were prepared for surgical implantation of recording arrays.

After recovery, rats were re-trained on nosepoke-cue-sucrose task for 2 days. We then recorded the activity of OFC neurons while rats performed the nosepoke-cue-outcome task, where the cue-outcome pairings were 5 kHz tone-15% sucrose, 1 kHz tone-20% EtOH or 10 kHz tone-10% EtOH. Recording sessions consisted of either blocked trials (where all trials were one cue-outcome pairing) or interleaved trials (where two cue-outcome pairing trials were pseudorandomly interleaved – sucrose/20% EtOH,). All trials were self-initiated, and animals were free to consume or not consume rewards after trial initiation. All sessions lasted 1 h.

2.3.3 Surgery

Surgical methods were similar to our previous work (Moorman & Aston-Jones, 2014). Prior to surgery, rats were given minocycline HCl (Henry Schein, Melville, NY) at a dose of 100 mg/L *ad libitum* 2 days prior to and 5 days post array implantation. Anesthesia was induced using isoflurane in a closed container and maintained with 1.5-2.5% isoflurane through a nose-cone throughout surgery. Meloxicam (Metacam; Henry Schein, Melville, NY) was administered 1.36 mg/kg s.c., and initial incisions were treated with 2% lidocaine. Under isoflurane anesthesia, two separate arrays of 16 recording electrodes (50 μm nichrome wires, 200μm spacing center-to-center) were implanted in medial OFC (mOFC: A/P 3.6 to 4.6, M/L 0.7, D/V -5.0 to -5.2 mm from bregma) and lateral OFC (lOFC: A/P 3.6 - 4.6, M/L 2.6 to 3.0, D/V -5.0 to -5.2 mm from bregma). Arrays were secured to the skull with dental cement and 3 skull screws, one of which was connected to array ground.

2.3.4 Electrophysiological recordings

Electrophysiological recordings were performed using a Neuralynx Digital Lynx system (Neuralynx, Bozeman, MT). OFC neurons were recorded on each blocked and interleaved session for 2 days. Wideband signals were filtered 300-3000 Hz and thresholded to identify well isolated action potentials (≥4 SD from mean of peak height of noiseband), which were manually sorted in Offline Sorter (Plexon, Dallas, TX). Well-isolated units that fired throughout each recording session were included in analyses. After the final recording, recording electrode tips were marked with electrolytic lesions, OFC was extracted, and recording sites were confirmed with histological analysis of lesion location.

2.3.5 Histology

After the final recording, rats were anesthetized with 1.5-2.5% isoflurane and constant current (25 μA) was delivered to each recording wire for 15 sec to produce lesions to mark the tips of recording electrodes. One day later, rats were perfused with 0.9% NaCl solution followed by 4% paraformaldehyde. Brains were post-fixed overnight with 4% paraformaldehyde and cryoprotected in a 20% sucrose/0.1% sodium azide solution. Forty micron sections were cut, mounted on Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA), and tissue was stained with neutral red to confirm electrode placement.

2.3.6 Data analysis

Rats were identified as high or low drinkers (HD/LD) if mean EtOH consumption on the final three days of homecage intermittent access was greater or less than 3.5 g/kg/24hour respectively (George et al., 2012; Momeni & Roman, 2014; Spoelder et al., 2015). Operant behaviors analyzed included total number of rewards received, percent rewarded trials, and average duration of reward consumption, and latency to acquire reward. Behavioral data were analyzed using standard parametric or nonparametric tests depending on normality, described in Results using Prism (GraphPad, San Diego, CA) or Matlab (MathWorks; Natick, MA), and neurophysiological data were analyzed using Matlab.

Electrode placements were difficult to categorize into lOFC and mOFC, therefore all neurons were grouped together for analysis as OFC neurons. However, tentative differences in activity between lOFC and mOFC were analyzed based on stereotaxic

electrode placement (see Figure S3). Timestamps of sorted spikes and behavioral events were imported into Matlab, where custom analyses were used to assess the relationship of neuronal activity to behavior. Neuronal activity was grouped in 50 msec bins and aligned to task events. Spike density functions were generated by Gaussian smoothing. Event-related neuronal activity was Z-score normalized against baseline activity preceding trial initiation by subtracting mean and dividing SD of baseline. OFC response strength was calculated with the index:

$$\frac{\text{(Behavioral epoch activity – baseline activity)}}{\text{(Behavioral epoch activity + baseline activity)}}$$

where activity was number of spikes during a given behavioral epoch or matched baseline epoch during inter-trial intervals. Three main test epochs were studied: post-cue/pre-seeking (cue to cue+100 msec), reward seeking (400 msec pre-reward acquisition to reward acquisition), and reward consumption (first rewarded lick to last rewarded lick, with a trial-matched baseline epoch). Population results were visualized using population spike density functions and Wilcoxon signed-rank tests were used to measure significant shifts from zero in distribution plots for all indices (Roesch et al., 2012; Takahashi et al., 2013). All analyses were considered significant at $\alpha = 0.05$

2.4 Results

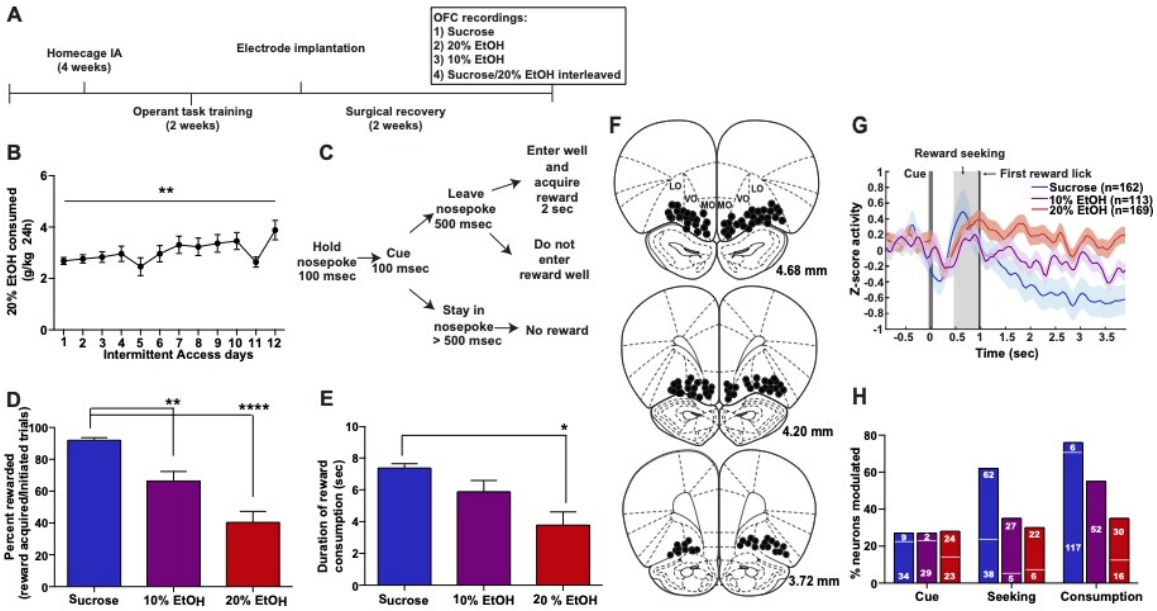

Figure 2.1. Experimental design and baseline reward seeking and consummatory behavior. (A) Experimental timeline. (B) Rats received 12 days homecage intermittent access to EtOH. Consumption escalated significantly over days. (C) Operant task diagram. Rats nosepoked to receive cues predicting sucrose or EtOH. (D) Rats acquired significantly more sucrose than EtOH rewards. (E) Rats consumed significantly more sucrose than 20% EtOH based on duration of reward consumption. (F) Placement of electrode wire tips in the OFC. (B) Z-scored average OFC activity aligned on cue presentation. In each session (sucrose, 10% EtOH, 20% EtOH) OFC neurons showed dramatic changes during cue presentation, reward seeking, and outcome consumption, but activity was variable across each condition. (G) Numbers of neurons significantly modulated during each epoch in each condition. Numbers of significantly excited and inhibited neurons are presented above and below the white line respectively. All neurons significantly modulated during 10% EtOH consumption (n=52) were inhibited. Substantial numbers of OFC neurons responded in all conditions, but more neurons responded during sucrose seeking. See main text for statistical details. *p < 0.05, ** p < 0.01, **** p < 0.0001.

20% EtOH consumption escalated across days during homecage intermittent access (Figure 2.1B; $H(11) = 25.58$, $p = 0.0075$, Kruskal-Wallis). During operant testing, rats nosepoked to receive a tone cue predicting one of three outcomes (20% EtOH, 10% EtOH or sucrose; Figure 2.1C). Following cue presentation, rats withdrew from the nosepoke and acquired reward from a spigot directly below the nosepoke.

Reward motivation, measured as number of trials in which rats acquired reward divided by number of initiated trials., was significantly modulated by reward type ($H(2) = 26.54$, $p < 0.0001$; Figure 2.1D). Rats acquired significantly more sucrose than 20% EtOH rewards ($p < 0.0001$, Dunn's MCT) and 10% EtOH rewards ($p = 0.0072$). Rats also acquired fewer 20% EtOH than 10% EtOH rewards on the whole, though this difference was not significant. We also quantified consumption via licking duration, measured from first lick during reward delivery to the final lick of that trial, for each outcome as a measure of preference. As with reward acquisition, reward consumption (Figure 2.1E) was significantly different across conditions ($H(2) = 9.16$, $p = 0.010$) and was greater for sucrose than 20% and 10% EtOH, with the difference between sucrose and 20% EtOH being significant ($p = 0.0078$, Dunn's MCT). Taken together, these results suggest that rats exhibit a hierarchy of overall preference for sucrose and EtOH at different concentrations.

Neurons recorded from OFC spanned mOFC and lOFC (Figure 2.1F). Although we targeted individual electrodes to mOFC and lOFC, we were not able to fully associate specific electrodes with OFC subregions based on histological analysis. We therefore analyzed all recordings as OFC activity for most analyses. Preliminary analysis of mOFC vs. lOFC results based on stereotaxically-targeted electrode placement is shown in Figure 2.8A.

We analyzed OFC neuronal activity during three epoch: cue presentation, reward seeking, and reward acquisition/consumption (Materials and Methods; Figure 2.1G, H).

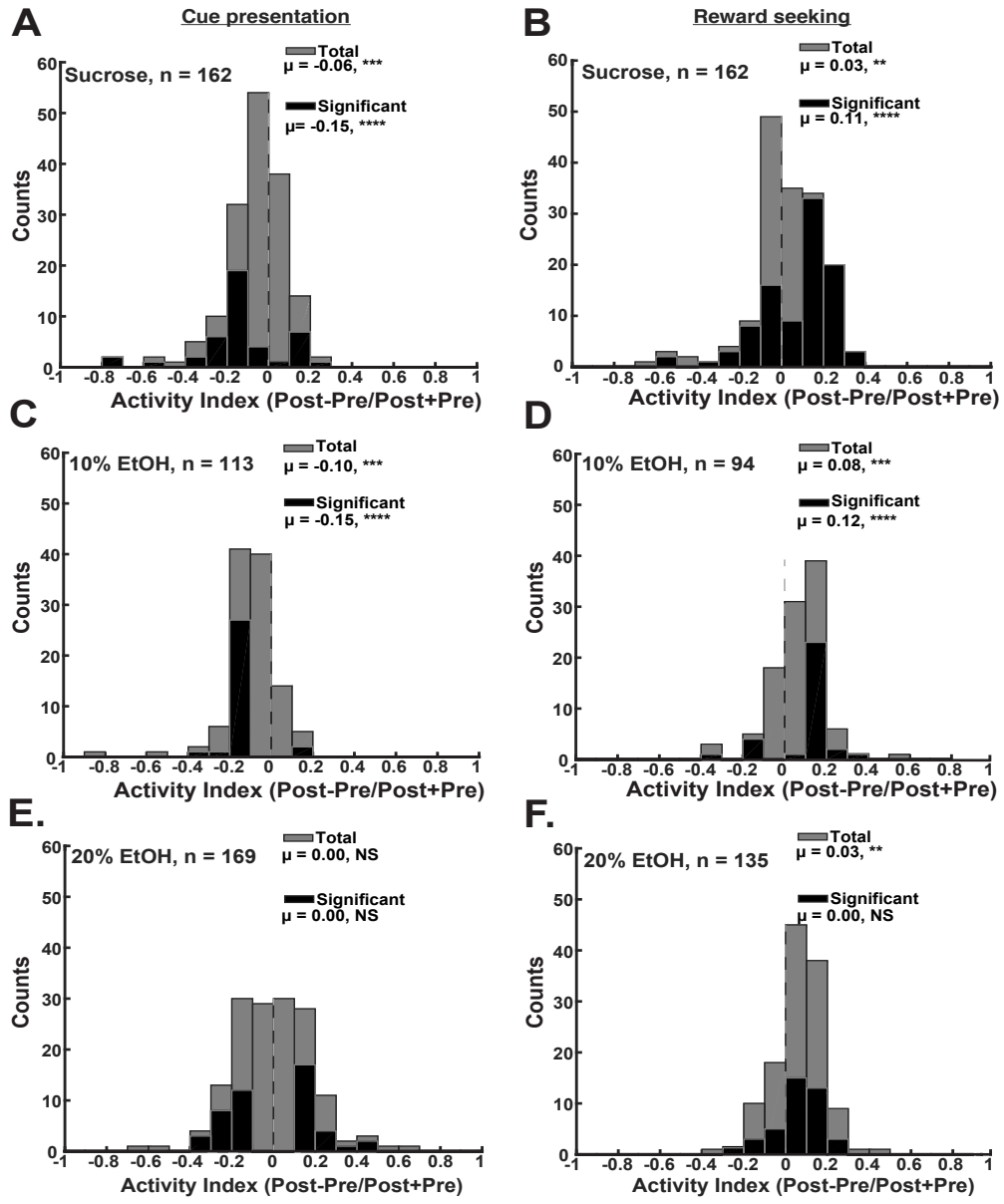

Figure 2.2. Electrophysiological index calculations during cue presentation, reward seeking and consumption. Index calculations for OFC neuron activity during cue presentation (A, C, E) and reward seeking epochs (B, D, F), during sucrose (A, B), 10% EtOH (C, D), and 20% EtOH (E, F) sessions. Indices calculated as in Materials and Methods. Note similarities in patterns of excitation vs. inhibition for sucrose and 10% EtOH vs. 20% EtOH sessions. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

During sucrose sessions, OFC neurons were suppressed during cue presentation, largely activated during reward seeking and again suppressed during reward consumption

(Figure 2.1G, H, blue), consistent with our previous report (Moorman & Aston-Jones, 2014), although there were examples of individual neurons exhibiting different profiles (Figures 2.1H, blue, and Figures 2.2, 2.3). During 10% EtOH trials, OFC neurons exhibited a similar pattern to that seen during sucrose seeking and consumption (Figures 2.1G, H, purple, and Figures 2.2, 2.3), but with slightly reduced proportions of responses and a stronger bias towards excitation during seeking. During 20% EtOH sessions, OFC neurons showed similar overall proportions of significant responses as during 10% EtOH sessions, but the proportions of excitation vs. inhibition during cue presentation and consumption were strikingly different (Figures 2.1G, H, red, and Figures 2.2, 2.3), particularly during consumption in which the strong bias towards inhibition seen in sucrose and 10% EtOH was reversed and more neurons exhibited excitation. The differences in proportions of significantly modulated neurons shown in Figure 2.1 were consistent with distributions of response indices of single neurons shown in Figure 2.2 (cue and seeking) and Figure 2.3 (consumption, see analysis below). During the cue presentation epoch, neuronal activity was significantly suppressed in both sucrose (significantly-modulated neurons: $z = -4.25$, $p < 0.0001$; all neurons: $z = -5.00$, $p = 0.00079$; Wilcoxon) and 10% EtOH (sig: $z = -7.12$, $p = 0.00042$; all: $z = -3.94$, $p < 0.0001$) trials, whereas activity was not significantly biased in 20% EtOH trials (sig: $z = -0.46$, $p = 0.77$; all: $z = -0.29$, $p = 0.65$). During reward seeking, activity was biased towards excitation in sucrose (sig: $z = 4.77$, $p < 0.0001$; all: $z = 3.16$, $p = 0.0084$), 10% EtOH (sig: $z = 3.59$, $p = 0.00038$; all: $z = 5.46$, $p < 0.0001$) and 20% EtOH (sig: $z = 1.55$, $p = 0.12$; all: $z = 4.29$, $p = 0.0061$) trials. Thus in both significantly-modulated populations and across the population as a whole, OFC neurons exhibited differential

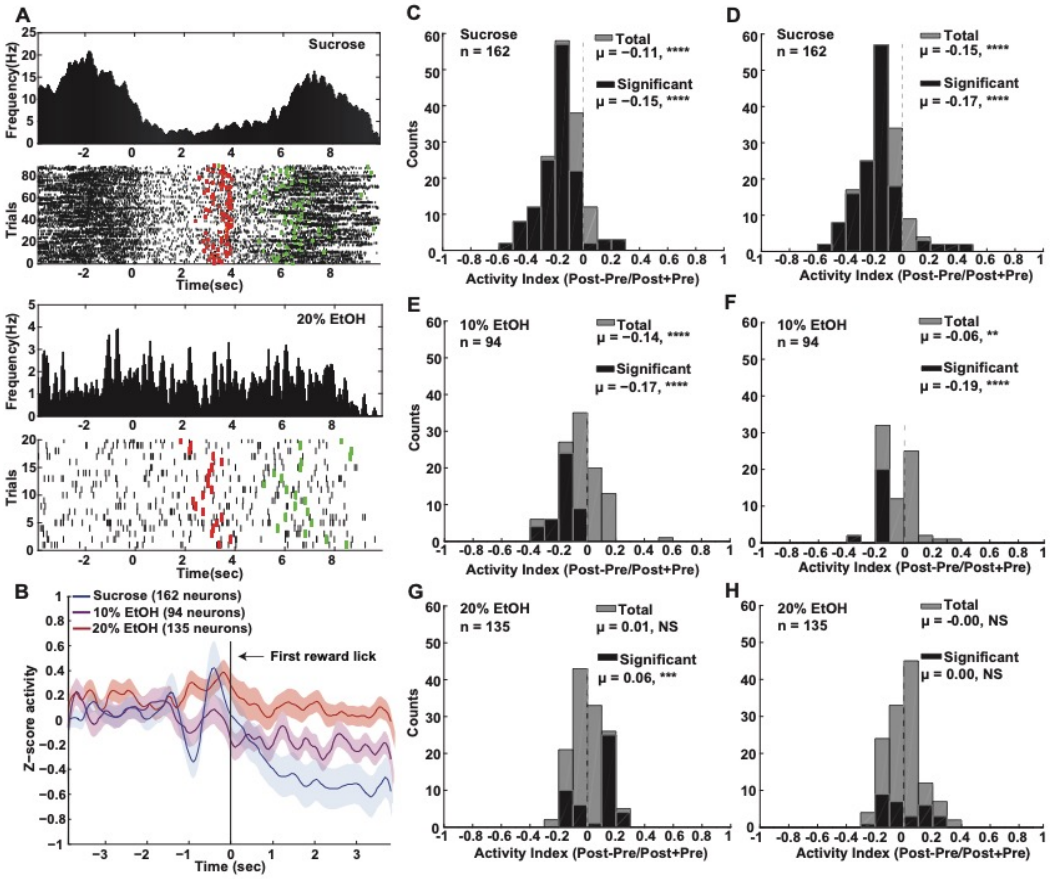

Figure 2.3. OFC activity during reward consumption. (A) Example activity of a single OFC neuron aligned on onset of sucrose consumption (top). Note the increased activity during the seeking epoch before reward at time 0, followed by dramatic suppression of activity during consumption. Activity was also suppressed during licking after sucrose pump offset (red points) and persisted until end of licking (green points). This finding was consistent across the majority of neurons modulated during sucrose seeking. In contrast, during 20% EtOH consumption, most neurons did not exhibit suppression as shown in a different OFC neuron (bottom). (B) Z-scored average activity of all recorded neurons aligned on onset of reward consumption. Note the strong suppression of activity during sucrose consumption, the moderate suppression during 10% EtOH consumption, and the absence of suppression during 20% EtOH consumption. (C-H) Index values calculated for each neuron (see Materials and Methods) during reward consumption for all neurons in each condition. C, E, and G show index data during the entire reward consumption epoch (first to last lick) whereas D, F, and H show index data during the period between sucrose offset and last lick. Almost all neurons were inhibited during sucrose consumption (C,D), many neurons were inhibited during 10% EtOH consumption, particularly those significantly modulated (E, F), and neurons were mostly not inhibited during 20% EtOH consumption and, in fact, many were significantly excited (G, H). ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

outcome classification, based on proportions of excitatory and inhibitory responses, at different task stages.

During reward consumption, OFC activity was suppressed in relation to overall reward preference. OFC neuronal activity was most suppressed during sucrose consumption (Figure 2.3A, top and 2.3B blue), in line with our previous results (Moorman & Aston-Jones, 2014). Here we measured individual licks during reward consumption, permitting us to extend previous findings to show that suppression of OFC activity persisted beyond the end of reward delivery and continued, on average, until the point of the last lick of the rewarded bout, even though reward was no longer delivered at this point (Figure 2.3A, red to green trial markers, and Figure 2.3D, F, H). These data demonstrate a novel aspect of OFC signaling: activity is suppressed during actions involving reward consumption even when there is no reward present (i.e., after sucrose is no longer delivered). In contrast, OFC neurons were suppressed neither during 20% EtOH reward delivery nor during licking after reward delivery ceased (Figure 2.3A, bottom, and 2.3B, red). OFC activity during 10% EtOH consumption was intermediate to that seen during 20% EtOH and sucrose (Figure 2.3B, purple), in line with the hierarchy of preferences shown behaviorally. We quantified strength of activity during reward consumption across neurons using baseline-normalized response indices (Materials and

Methods).

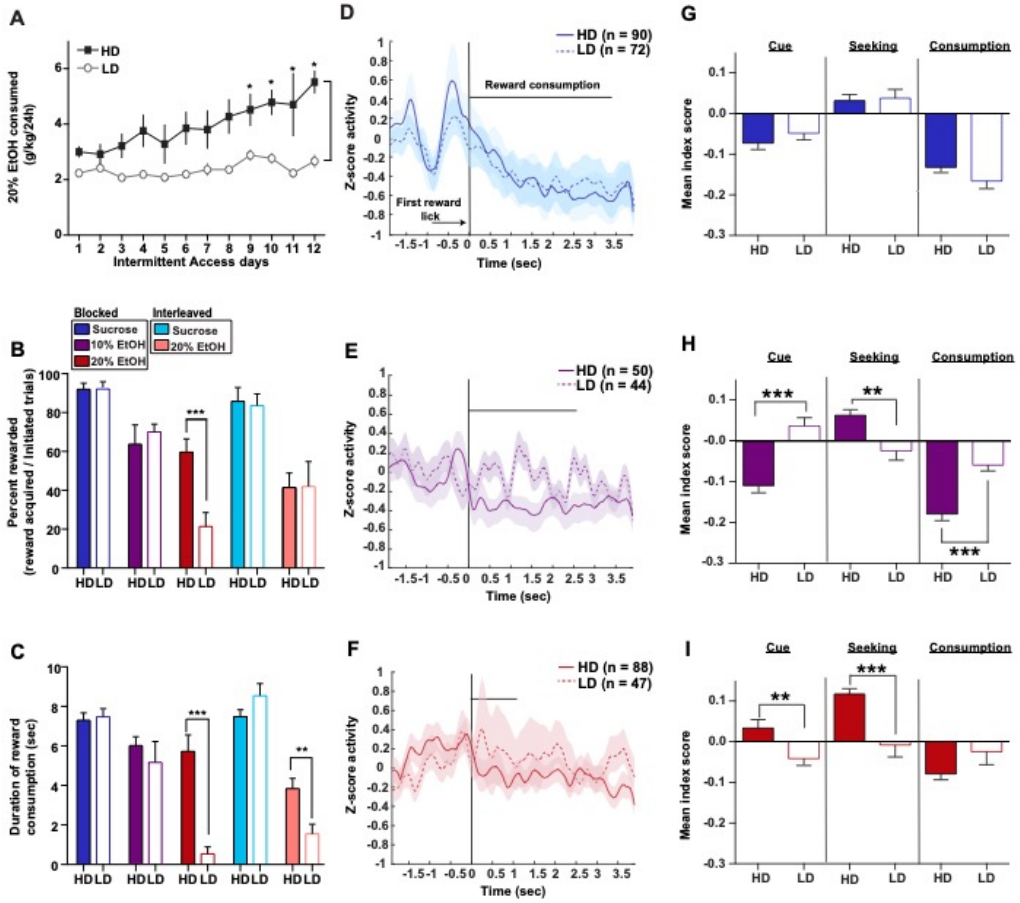

Figure 2.4. Reward consumption behaviors and OFC activity of HD and LD rats was statistically distinct for alcohol rewards. Rats were separated into high drinkers (HD) and low drinkers (LD) based on homecage EtOH consumption (see Materials and Methods). (A) HD rats exhibited significant escalation over the course of homecage intermittent access to EtOH whereas LD rats did not. (B) HD rats completed significantly more rewarded trials for 20% EtOH than LD rats, but there were no differences for sucrose or 10% EtOH rewarded trials. (C) HD rats consumed significantly more 20% EtOH than LD rats, measured by lick duration, but there were no differences in consumption of sucrose or 10% EtOH. OFC neuronal activity in HD vs. LD rats was similar during sucrose cues, seeking, and consumption (D, G), but was significantly different during cues, seeking, and consumption of 10% EtOH (E, H) and during cues and seeking of 20% EtOH (F, I). Overall strength of OFC signaling (either excitation or inhibition) was suppressed in LD rats relative to HD rats in EtOH sessions. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

As shown in Figure 2.4B, OFC activity was significantly reduced during consumption of sucrose (significantly-modulated neurons z = -8.94, p < 0.0001; all neurons: z = -7.56, p < 0.0001; Wilcoxon) and 10% EtOH consumption (sig: z = -7.32, p < 0.0001; all: z = -6.27. p < 0.0001). In contrast, during 20% EtOH consumption, few neurons were significantly suppressed, and more neurons actually exhibited increased activation. This excitation bias was significant for significantly modulated neurons (z = 3.06, p = 0.00022) but not across the whole population (z =0.87, p = 0.385). On the whole, similar effects were seen in neural activity during the epoch spanning the offset of sucrose delivery and the final lick (Figure 2.3D, F, H), the major difference being a lack of a significant shift towards excitation during 20% EtOH trials. Taken together, these data support the proposal that OFC encodes consumption of reward outcomes in a hierarchical

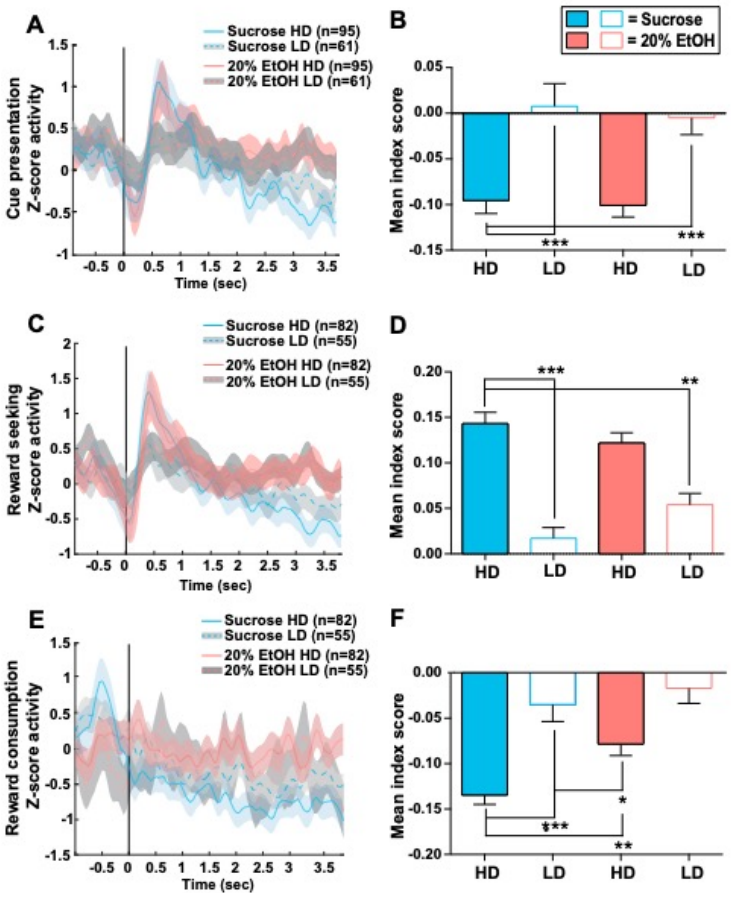

Figure 2.5. OFC activity from HD and LD rats during interleaved reward self-administration sessions reliably represented behavioral reward preference. During sessions in which sucrose and 20% EtOH trials were interleaved, activity of OFC neurons in LD rats was again suppressed relative to activity of those in HD rats. This was true for neuronal responses to the cue (A-B), during reward seeking (C-D), and during sucrose/EtOH consumption (E-F). *p < 0.05, **p < 0.01, ***p < 0.001.

manner in line with behavioral preference and that this encoding is present even when consumption-associated behavior continues to occur in the absence of reward.

Although seeking-related activity was similar across outcomes with respect to proportion of neurons excited vs. inhibited (Figures 2.1 and 2.2), the relationship of OFC

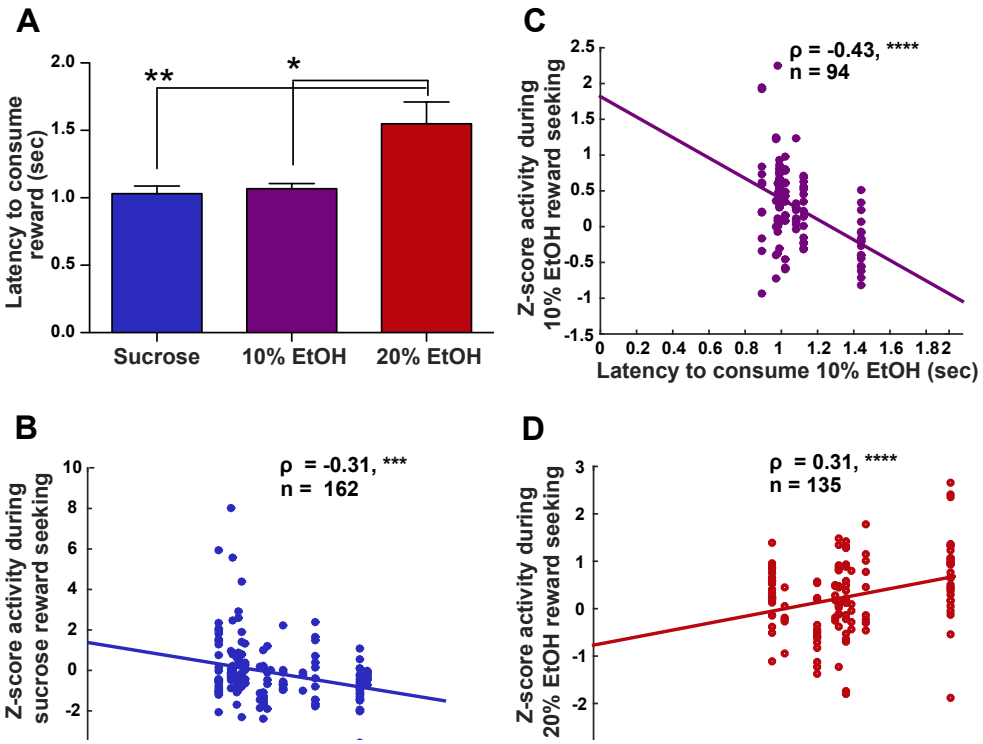

Figure 2.6. OFC activity during reward seeking significantly positively predicted the latency to retrieve palatable rewards. (A) Response latencies to consume reward (time from nosepoke exit to first reward lick) were significantly higher in 20% EtOH trials vs. sucrose or 10% EtOH trials. (B, C) Average z-score neuronal activity was significantly negatively correlated with latency in sucrose (B) and 10% EtOH (C) conditions. Each point represents mean z-scored activity of a single neuron during the seeking epoch. (D) In contrast, neuronal activity in 20% EtOH conditions was positively correlated with consumption latency. *p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

activity to seeking behavior differed depending on outcome. The latency to consume reward, measured as the time between nosepoke exit and first reward lick, was significantly longer in 20% EtOH sessions compared to 10% EtOH or sucrose sessions

(Figure 2.6A; $H(2)=13.23$, $p = 0.0013$, Kruskal-Wallis). OFC neurons also encoded seeking behavior differently in 20% EtOH vs. other conditions. In sucrose and 10% EtOH sessions, OFC neuronal activity was negatively correlated with seeking latency (sucrose, Figure 2.6B, $\rho = -0.31$, $p < 0.0001$; 10% EtOH, Figure 2.6C, $\rho = -0.43$, $p = 0.00042$, Spearman). In contrast during 20% EtOH sessions, OFC activity during reward seeking was positively correlated with latency (Figure 2.6D, $\rho = 0.31$, $p < 0.0001$). These results indicate that OFC activity differentially encodes reward seeking depending on outcome as well as the influence of outcome on acquisition-associated behaviors.

As noted in Materials and Methods, we were not completely confident in our ability to analyze differences in the activity of mOFC vs. lOFC neurons based on histological reconstruction of electrode placements. However, as a preliminary analysis we separated our recordings into tentative mOFC vs. lOFC based on stereotaxic placements of electrode bundles (Figure 2.8A). Based on this grouping, we found no significant differences in mOFC vs. lOFC signaling during sucrose or 10% EtOH sessions (Figure 2.8B, C), nor during cue or seeking epochs during 20% EtOH sessions (Figure 2.8D). However, we did identify a significant difference whereby the strength of inhibition was greater in mOFC vs. lOFC neurons during consumption of 20% EtOH ($U = 1482$, $p = 0.019$, Mann-Whitney). These data are intriguing in the light of previous reports of differential value/preference coding by mOFC vs. lOFC neurons (Burton et al.,

2014; Lopatina et al., 2016), but conclusions based on these data are tentative due to incomplete histological confirmation.

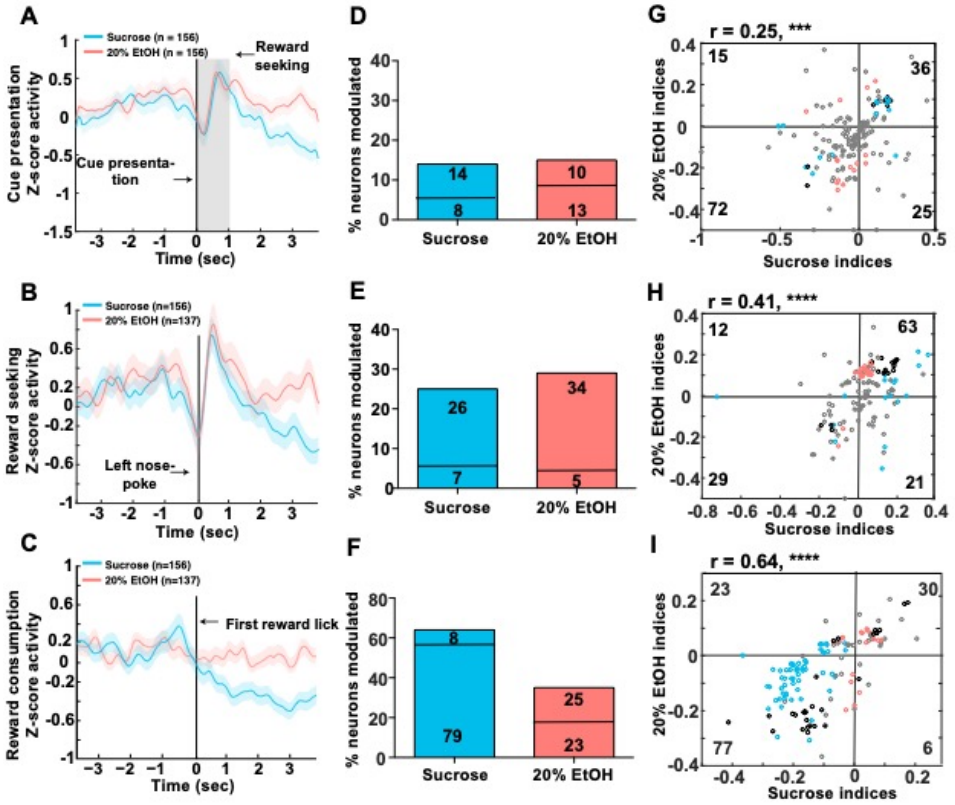

Figure 2.7. The same OFC neurons distinctly encode reward preference that reflects behavioral preference in a given self-administration session. Activity patterns of the same neurons recorded during interleaved trials of sucrose and 20% EtOH seeking. Across all recorded neurons, activity patterns were similar to those seen during blocked sessions when aligned on cue (A), seeking initiation (B), and reward consumption (C). The numbers of significantly excited or inhibited neurons were also similar as during blocked sessions (D-F). Recording the same neuron in both conditions allowed characterization of sucrose/EtOH index profiles for each neuron (G-I). Indices were calculated as in blocked conditions. Light blue, pink, black, and gray dots represent neurons significantly modulated by sucrose only, 20% EtOH only, both, and neither condition. Note that, despite the fact that activity was correlated across conditions, most neurons exhibited significant activity for one or the other outcome. *** $p < 0.001$, **** $p < 0.0001$.

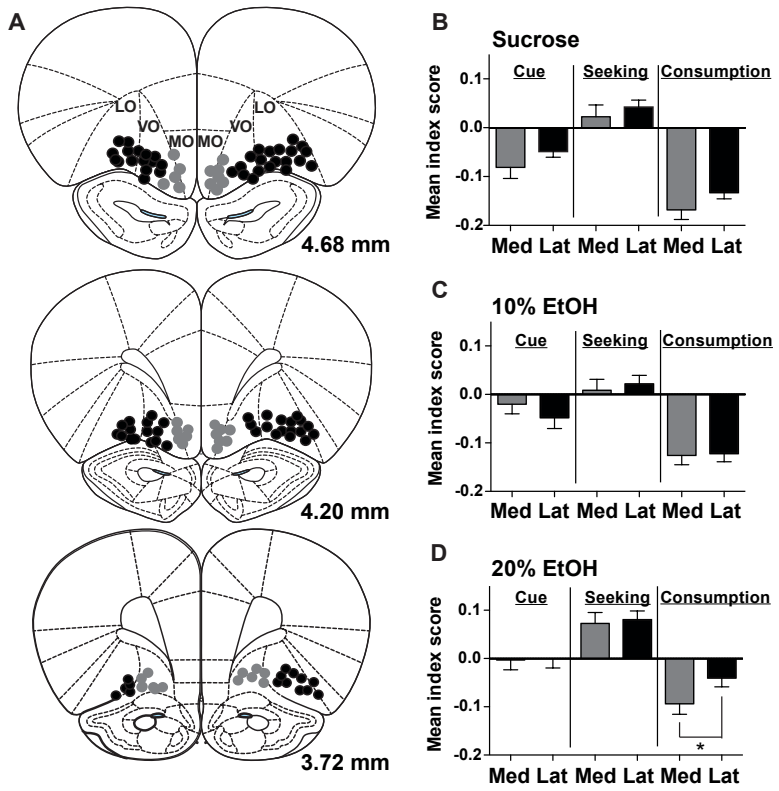

Figure 2.8. mOFC neurons encode 20% EtOH consumption similar to how all OFC neurons encode sucrose consumption. Preliminary analysis of mOFC vs. lOFC activity based on stereotaxic electrode placement (A). Gray and black dots represent placements localized to mOFC and lOFC respectively. (B-D) There were no significant differences in mOFC and lOFC neuron activation in most epochs of sucrose seeking (B) or 10% EtOH seeking (C). Activity was significantly different during 20% EtOH consumption (D), with mOFC neurons exhibiting significantly stronger suppression of activity relative to lOFC neurons. * $p < 0.05$.

In studies of neuronal activity involving chronically implanted electrodes, it is often difficult to assess whether the same neurons are recorded across days. In order to characterize how the same OFC neurons fired during sucrose and EtOH seeking, we recorded OFC activity from a subset of rats during sessions in which sucrose and 20% EtOH trials were pseudorandomly interleaved (Materials and Methods). During interleaved sessions activity was significantly modulated in a subset of neurons during cue, seeking, and reward consumption epochs (Figure 2.7). Percentages of significant neurons (Figure 2.7D-F) were similar during interleaved and blocked conditions (compare proportions in Figure 2.7D-F to those in Figure 2.1H), and the proportions of excitatory vs. inhibitory responses were largely preserved (though note a shift towards excitatory responses in interleaved sucrose trials, Figure 2.7D vs. Figure 2.1H). Across the population, response

indices were highly correlated in sucrose and EtOH trials across all neurons (Figure 2.7G-I). However, most individual neurons exhibited significant selectivity for either sucrose or EtOH (see colored points in Figure 2.7G-I). Although a subset of neurons exhibited significant modulation in both sucrose and EtOH trials (Figure 2.7G-I, black dots**:** 8 in response to cue, 17 during seeking, and 29 during consumption), the majority of significantly-modulated neurons exhibited selectivity for sucrose (Figure 2.7G-I, blue dots: 14 cue, 16 seeking, and 58 consumption) or EtOH (Figure 2.7G-I, red dots: 15 cue, 22 seeking, and 19 consumption). Across the population, neurons exhibited strong, significant biases towards inhibition during cue presentation (Figure 2.7G**;** $\chi^2(1) = 27.71$, $p = 0.00082$; Chi-square), reward seeking (Figure 2.7H**:** $\chi^2(1) = 24.01$, $p < 0.0001$) and reward consumption (Figure 2.7I: $\chi^2(1) = 40.51$, $p < 0.0001$). Thus, although the overall OFC population treated sucrose and EtOH as points in a continuum of rewards, as evidenced by the strong correlations, the population as a whole exhibited epoch-specific responses, and individual neurons exhibited selective activity for sucrose or EtOH.

Rats were separated into high drinkers (HD) and low drinkers (LD) based on average g/kg 20% EtOH consumed during homecage intermittent access (see Materials and Methods). HD rats escalated 20% EtOH consumption across intermittent access sessions, whereas LD rats did not (Figure 2.4A; 2-way ANOVA; main effect of day: $F(1,11)=2.77$, $p =0.002$; main effect of HD/LD: $F(1,1) = 127.3$, $p < 0.0001$; interaction $F(1,11) = 2.93$, $p = 0.0011$). Based on mean 24-hour consumption in the last three days of homecage intermittent access, HD rats consumed significantly more EtOH than LD rats (HD: 5.0 ± 0.45 g/kg); LD: 2.35 ± 0.13 g/kg; $U = 5$ $p < 0.0001$, Mann-Whitney).

During operant testing, HD and LD rats exhibited significant differences in EtOH seeking and consumption (Figure 2.4B). HD rats acquired significantly more 20% EtOH rewards than LD rats during blocked sessions (Figure 2.4B, red**;** $U = 5$, $p = 0.0038$), but there were no differences in seeking for sucrose or 10% EtOH (Sucrose: $U = 31$, $p = 1$; 10% EtOH: $U = 14$, $p = 0.42$). Preference and seeking were also related at the individual level: average g/kg of 20% EtOH consumed on the final 3 days of intermittent access was positively correlated with percentage of 10% and 20% EtOH rewarded trials during EtOH-only sessions (20% EtOH: $r = 0.71$, $p = 0.0025$, 10% EtOH: $r = 0.59$, $p = 0.03$, Pearson) but not with sucrose ($r = -0.25$, $p = 0.722$). Results were similar when correlating average g/kg of 20% EtOH consumed on the final 3 days of intermittent access and licking behavior during each rewarded trial (20% EtOH: $r = 0.82$, $p < 0.0001$; 10% EtOH: $r = 0.51$, $p = 0.007$; Sucrose: $r = 0.15$, $p = 0.99$). HD rats also consumed more 20% EtOH than LD, measured by the duration of licking during both blocked sessions (Figure 2.4C, red; $U = 3$, $p = 0.00064$, Mann-Whitney) and interleaved sessions (Figure 2.4C, pink**:** $U = 9$, $p = 0.007$). There were no differences between HD and LD rats with respect to consumption of sucrose or 10% EtOH (Sucrose: $U = 29$, $p = 0.837$; 10% EtOH: $U = 20$, $p = 0.159$).

OFC neural activity was significantly different between HD and LD rats during EtOH seeking. There were no significant differences in OFC activity during sucrose cue presentation, reward seeking and consumption in HD vs. LD rats (Cue: $U = 3128$, $p = 0.710$; Seek: $U = 3126$, $p = 0.701$; Reward: $U = 3010$, $p = 0.439$, Mann-Whitney; Figure 2.4D, G). In contrast, there were significant differences in OFC activity between HD vs.

LD rats during both 10% and 20% EtOH seeking, and OFC neurons in HD rats exhibited response profiles more similar to that seen during sucrose seeking (Figure 2.4E-I). These differences were significant for all epochs in 10% EtOH sessions (cue: $U = 639$, $p = 0.00074$; seeking: $U = 870$, $p = 0.0014$; consumption: $U = 416$, $p < 0.0001$), and during cue and seeking epochs during 20% EtOH sessions (cue: $U = 2204$, $p = 0.001$; seeking: $U = 1028$, $p = 0.00042$; consumption: $U = 1633$, $p = 0.4437$). Consumption differences during 20% EtOH sessions exhibited a similar HD/LD pattern as during 10% EtOH sessions, but were statistically underpowered during consumption for LD rats due to a limited number of 20% reward acquisitions by this population (see Figure 2.4B).

We also separated the subset of rats recorded during sucrose/20% EtOH interleaved sessions into HD and LD and again observed striking differences between these populations (Figure 2.5). In HD rats, OFC activity during sucrose and 20% EtOH trials followed a similar pattern: inhibition during cue presentation, excitation during seeking, and inhibition during consumption, similar to that seen during blocked sessions. In contrast, LD rats exhibited suppressed responses during all epochs. During cue presentation there was a significant main effect of preference ($F(1, 135) = 20.51$, $p < 0.0001$; 2-way mixed ANOVA). During the seeking epoch, there was a significant main effect of preference ($F(1, 135) = 40.99$, $p < 0.0001$) and significant interaction effect between preference and reward type ($F(1, 135) = 13.41$, $p = 0.0003$). Finally, during the reward consumption epoch, there was a significant main effect of preference ($F(1, 135) = 19.73$, $p < 0.0001$), a significant main effect of reward type ($F(1, 135) = 17.56$, $p < 0.0001$), and a significant interaction effect ($F(1, 135) = 4.61$, $p = 0.0336$). These results demonstrate a clear relationship between EtOH preference and OFC activity in HD vs.

LD rats. OFC neurons in HD rats responded more similarly for sucrose and EtOH, encoding both as palatable rewards. In contrast, OFC neurons in LD rats treated both sucrose and EtOH as less palatable, indicating an overall suppressive effect of the presence of EtOH during interleaved sessions.

2.5 Discussion

Here I characterized OFC neuronal activity during alcohol seeking in comparison with natural reward (sucrose) seeking. OFC neurons were significantly modulated during alcohol seeking, but the features of their responses were directly related to alcohol palatability and individual preferences for alcohol. OFC neurons were most strongly modulated by sucrose cues, seeking, and consumption, followed by 10% EtOH and 20% EtOH, in line with behavioral preferences. Furthermore, OFC neurons in HD rats fired more robustly during EtOH seeking than in LD rats, supporting the hypothesis that individual differences in relative preference for natural and drug rewards are encoded in OFC (Tremblay & Schultz, 1999; Schultz et al., 2011; Guillem et al., 2017; Guillem & Ahmed, 2018) and demonstrating this in the context of alcohol seeking. Our results indicate that OFC function is fundamentally different in high vs. low alcohol-preferring individuals. They further argue that one factor that may underlie alcohol use disorder is altered OFC function: OFC neurons in AUD-prone or -diagnosed individuals may respond more robustly to alcohol and alcohol cues, conferring enhanced value and driving enhanced alcohol motivated behavior.

My data are well-aligned with previous work. In humans, OFC is activated during alcohol craving (Myrick et al., 2003, 2008; Lukas et al., 2013; Schacht, Anton,

Randall, et al., 2013; Schacht et al., 2013; Schacht et al., 2014), and endgenous opioid release is induced by alcohol consumption in heavy drinkers (Mitchell et al., 2012). Previous studies have also demonstrated a role for OFC in alcohol use in behaving rodents. Inactivation of OFC in mice exposed to chronic EtOH vapor increased consumption of quinine-adultrated EtOH (Den Hartog et al., 2016), and lateral OFC lesions increased alcohol consumption in rats (Ray et al., 2018), both of which suggest a regulatory role for OFC in alcohol use. This is supported by the observations that chronic EtOH disrupts goal-directed behavior and suppresses OFC firing *in vitro*, and that DREADD activation of OFC activity restores goal-directed behavior (Renteria et al., 2018). OFC inactivation in rats decreases cued or context-driven reinstatement of EtOH seeking, arguing that OFC activity may facilitate EtOH seeking (Hernandez et al., 2017; Bianchi et al., 2018). These latter results indicate that OFC contributes to EtOH seeking, in line with preference-associated differences observed here. The exact relationship of OFC function to EtOH seeking – whether it induces or suppressess it – may depend on a number of factors such as species, withdrawal state, and OFC subregion, revealing a number of lines of important future research to identify details of this prominent influence.

There is also a clear impact of both acute and chronic EtOH on structure and function of OFC neurons. Chronic EtOH increased spine density in OFC neurons (McGuier et al., 2015), but see (Holmes et al., 2012; DePoy et al., 2013). Acute EtOH decreased (Badanich et al., 2013) and chronic EtOH exposure increased (S Nimitvilai et al., 2016; Sudarat Nimitvilai, Lopez, et al., 2017) or decreased (Nimitvilai et al., 2017; Renteria et al., 2018) OFC neuronal excitability. As with behavioral studies, there is

some variability across species and paradigms, but there is a clear influence of EtOH on OFC structure and function, in line with the behavioral physiological results reported here.

By using EtOH as a reinforcer that produces different levels of motivation across individuals, our results also addressed an important issue related to OFC coding mechanisms more broadly. Previous studies have reported OFC function associated with reward preference/value (Tremblay & Schultz, 1999; Izquierdo & Murray, 2004; Padoa-Schioppa & Assad, 2006; van Duuren et al., 2008, 2009; Rudebeck et al., 2013; Rolls, 2015; Howard et al., 2015; Rich & Wallis, 2016), but neuronal activity encoding outcomes independent of value has also been demonstrated (Furuyashiki et al., 2008; Stalnaker et al., 2014, 2015; Wilson et al., 2014; Schuck et al., 2016; Sadacca et al., 2018). Here I observed that OFC neurons encoded both outcome and preference in an interdigitated/multiplexed fashion in two ways. First, during blocked sessions, OFC neuronal responses exhibited dynamic response properties, in which proportions of excitatory vs. inhibitory responses changed depending on task epoch (Figure 2.1H). During the reward seeking epoch, OFC neurons fired more similarly for 10% and 20% EtOH than sucrose outcomes, categorizing outcomes (sucrose vs. EtOH) independently of preference. In the cue and consumption epoch, OFC activity was more similar for preferred (sucrose and 10% EtOH) than less preferred outcomes (20% EtOH), thereby demonstrating preference coding. Thus, OFC neurons multiplex outcome category and preference information at different timepoints during behavioral performance. Second, during interleaved recording sessions, OFC neurons tended to respond selectively to sucrose or EtOH, firing more strongly for one or the other, thereby demonstrating

outcome category representation (Figure 2.7**)**. However, at the same time, the population of OFC neurons preferentially responded to sucrose, and EtOH responses were largely stronger in HD vs. LD animals, thus showing that population signals from OFC reflected outcome preference, again most prominently during consumption. Taken together, our results demonstrate that OFC neuronal activity integrates both value/preference and outcome identity at both the level of the single neuron via dynamic encoding changes, and at the level of the population where proportions of outcome selective neurons reflected preference. Understanding the multifaceted nature of OFC representation of specific outcomes vs. preferred outcomes was not an initial goal of our study, so more work needs be done to investigate this multiplexing of information.

The fact that individual rats exhibited differential preference for EtOH revealed a number of details regarding the relationship between OFC function and EtOH use specifically, and behavior more generally. OFC neuronal activity was significantly different in HD and LD rats. During blocked 10% EtOH trials, HD and LD rats exhibited no significant differences in EtOH seeking or consumption behavior (Figure 2.4B, C), but in HD rats OFC neurons fired more strongly, and the patterns of responses were similar in sucrose and 10% EtOH conditions (Figure 2.4G, H). This suggests that OFC activity encodes some aspect of preference that is not necessarily represented by behavior – otherwise LD rats would either show lower seeking behavior or greater OFC activity. Exactly why OFC activity differed in HD and LD rats during 10% EtOH seeking remains somewhat mysterious and may relate to other aspects of subjective preference not measured using our operant task. In contrast, the effects of preference on OFC activity during 20% EtOH seeking mapped clearly onto behavior – HD rats were more highly

motivated than LD rats during both blocked and interleaved 20% EtOH trials, and OFC activity changes were stronger in HD than LD rats during these sessions. One possible difference between 20% and 10% EtOH may be the fact that intermittent access was performed using 20% EtOH, thus enhancing the relative palatability of 10% EtOH, which elicited more seeking and consumption across individuals. This positive contrast effect upon receipt of 10% EtOH may have driven similar EtOH seeking behavior in HD and LD rats via non-OFC neural systems, but OFC still reflected relative preference of EtOH. Contrast effects were also seen during interleaved trial sessions. In this case, HD and LD rats exhibited strong behavioral differences during EtOH sessions, and these behavioral differences were reflected by differences in OFC activity in HD vs/ LD rats (pink bars in Figures 2.4 and 2.5). However, during sucrose trials, HD and LD rats exhibited similar seeking and consumption behavior (blue bars in Figure 2.4), but OFC activity in LD rats was significantly suppressed during sucrose seeking (blue bars in Figure 5). Here again, I saw a dissociation between behavioral preference (HD and LD rats both were motivated for sucrose) and OFC activity (OFC activity was suppressed in LD rats during sucrose seeking). One possible explanation may be that subjective value of sucrose may have been compromised for LD rats by the presence of EtOH trials during sucrose sessions and that this change in value was reflected in altered OFC function, but not behavior, which may be driven by alternate neural pathways. These conclusions, while speculative, indicate interesting lines of future research in which outcome identity and value should be probed using different behavioral regimens, to dissect subjective experiences vs. behavioral report, in line with recent work on OFC and upshifts/downshifts in value

(McDannald et al., 2014; Stalnaker et al., 2014; Lopatina et al., 2015;Lopatina et al., 2016).

In summary, my data support my hypothesis that OFC activity encodes alcohol seeking and preference. Across all individuals, behaviors associated with sucrose and low alcohol concentrations more strongly drove OFC activity. However, OFC alcohol-related activity in high-preferring individuals was stronger than in low-preferring individuals, and was more in line with sucrose-like signaling. These data have significant implications for a potential mechanism whereby alcohol becomes highly valued in individuals with AUD and further establish the OFC as a major brain system involved in driving heightened alcohol use in such individuals. Further studies using, for example, dependence models, will likely reveal important new changes in OFC dynamics, supporting a further focus on this influential system.

CHAPTER 3

ORBITOFRONTAL CORTEX INACTIVATION SELECTIVELY IMPACTS ALCOHOL SEEKING DURING REINSTATEMENT

3.1 Abstract

Orbitofrontal cortex (OFC) neurons encode subjective reward value, preference and reward-seeking. In addicts experiencing withdrawal or abstaining from drug use, OFC neurons are activated during presentation of drug cues accompanied by self-reported craving. Inactivation of OFC is known to suppresses reinstatement, a behavior thought to underlie relapse in drug use, for cocaine-seeking in rodents. To date, is unclear whether the OFC is necessary for cued-reinstatement of alcohol or even sucrose seeking. Male and Female Wistar rats were trained to drink 20% ethanol in their homecage and were subsequently trained on a FR1 cued reward seeking task for 20% ethanol, 10% ethanol and sucrose. In another experiment Male and Female wistar rats were trained to only drink sucrose. 36 rats (18 males and 18 females) had hM4Di DREADD receptors injected into their OFC and 12 rats (6 males and 6 females) had OFC neurons transduced with GFP. Rats that were exposed to homecage ethanol were separated into high or low drinkers based on homecage drinking and the effects of OFC inactivation (CNO injection and saline/DMSO as control) on reward seeking and consumption were examined during homecage, FR1 operant and reinstatement testing.

Overall, females consumed more 10% and 20% ethanol than males in a homecage environment but not in an operant setting. OFC inactivation did not affect rewarded sucrose or ethanol seeking in a homecage or operant environment. In rats whose neurons were transduced with hM4Di DREADDs, OFC inactivation suppressed reinstatement of reward seeking for both 10% ethanol and sucrose regardless of sex or alcohol preference. CNO treatment had no effect on 10% EtOH cued-reinstatement behavior of rats whose OFC was transduced with GFP. My results suggest that OFC is not necessary for the expression of ethanol or sucrose seeking, but instead is neccessary for encoding the cue-outcome association of ethanol and sucrose during reinstatement, suggesting that OFC may be a therapeutic target for drug relapse.

3.2 Introduction

The prefrontal cortex has a number of connections between the limbic, striatal and cortical brain regions (Seamans et al., 2008) that contribute to encoding relative reward value (Öngür & Price, 2000; Heilbronner et al., 2016b; Lichtenberg et al., 2017; Murphy & Deutch, 2018), especially for drugs such as alcohol (Everitt & Robbins, 2005). Within the prefrontal cortex, the orbitofrontal cortex (OFC) functions to regulate goal-directed behaviors by encoding motivational parameters such as how appetitive a reward is (Tremblay & Schultz, 1999; Metereau & Dreher, 2015), the timing to reward delivery (Winstanley et al., 2004; Roesch et al., 2006) and even the general quantity of rewards delivered (Takahashi et al., 2013). The encoded value of a drug reward in the OFC can be subsequently used, along with other brain regions, to promote motivated behaviors (Baxter et al., 2000; Arana et al., 2003; Chudasama & Robbins, 2003; Rudebeck et al.,

2006; Wallis, 2007; Takahashi et al., 2009, 2013). OFC inactivation studies are useful for evaluating the role of the OFC in encoding drug rewards. Reversible DREADD activation of the lateral OFC (lOFC), in alcohol-dependent mice, results in enhanced ethanol consumption, when compared to non-dependent mice (Den Hartog et al., 2016). Together, these studies suggest that the OFC is a target for modulating reward-guided behaviors specifically for individuals struggling with addiction.

A key component of addiction is the high rate of relapse elicited through the presentation of environmental cues that induces increased craving, heightened drug seeking and consumption of highly valued drug rewards (Sinha et al., 2009; Seo & Sinha, 2014). One particular trigger for relapse is drug context-induced reinstatement of drug seeking (Wikler, 1973; Janak & Chaudhri, 2010; Bianchi et al., 2018). Drug-associated environments result in anticipation of drug self-administration leading to craving and often relapse. Additionally, cues associated with drug intake are strong enough to induce craving (See, 2005; Kondo & Witter, 2014; MacNiven et al., 2018; Kvamme et al., 2019) and thus result in relapse. Reversible inactivation of the lOFC suppresses cue-primed reinstatement for cocaine seeking (Fuchs et al., 2004) and context-induced reinstatement for alcohol (Bianchi et al., 2018). In humans, activation of the OFC was observed during presentation of alcohol-related cues (Kim et al., 2014) as well as during presentation cocaine-related stimuli for abstinent cocaine abusers (Grant et al., 1996). The OFC of rhesus monkeys are also activated in response to cocaine-associated cues (Baeg et al., 2009). Although the OFC is implicated in responding to cocaine and alcohol-associated cues, it is unclear whether OFC is necessary for cued reinstatement of alcohol-seeking – a key process in alcohol relapse.

To address this issue we chemogenetically inactivated the OFC of rats in their homecage and in an operant environment to examine alcohol drinking as well as during alcohol cued-reinstatement. Specifically, I examined how OFC inactivation affects 10% and 20% EtOH as well as sucrose drinking. Overall, rats prefer 10% EtOH over 20% EtOH (Hernandez & Moorman, 2019) which would allow me to examine how individual ethanol preference as well as general population preference is modulated by OFC inactivation. I additionally used sucrose as a palatable reward to examine whether OFC inactivation affects sucrose and drug reward seeking equally. I also injected a group of rats with a virus containing GFP in the OFC and examined the same drinking behaviors. Finally, I injected hM4Di into the OFC of rats that were only exposed to sucrose to examine how OFC inactivation modulates sucrose-only homecage, operant and cued-reinstatement sucrose consumption and seeking behaviors in order to examine whether OFC inactivation impacts reward seeking and reinstatement of natural rewards in a cohort of rats with no exposure to alcohol.

3.3 Materials and Methods

3.3.1 Animals

All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) at the University of Massachusetts Amherst and were designed and conducted in compliance with the National Institutes of Health *Guide for the Care and Use of Animals*.

3.3.2 Experimental design

Three experimental cohorts were used in this study. Cohort 1 consisted of 12 males and 12 females. Cohort 2 consisted of 6 males and 6 females and finally cohort 3 consisted of 6 males and 6 females. From cohort 1 and 2, male and female Wistar rats (~200-300 g upon arrival; Charles River, Wilmington MA Laboratories; n = 36) were initially trained to drink 20% ethanol (EtOH: diluted with tap water from 95% EtOH (Fisher Scientific, Pittsburgh, PA) in their home cages using the intermittent access to EtOH paradigm (Wise, 1975; Simms et al., 2008; Moorman & Aston-Jones, 2009; Carnicella et al., 2014; Moorman et al., 2016; Hernandez & Moorman, 2019). EtOH was given in home cages with ad lib access to food and water. Cohort 3 (n = 12, 6 males and 6 females) were not exposed to intermittent EtOH in their homecages but instead were used in a sucrose control experiment (see below).

3.3.3 Surgery

For viral expression studies, animals from cohort 1 and 3 were injected at a rate of 60 nL/min with 600nL with inhibitory DREADD (hM4Di) using an adeno-associated virus (AAV) driving the hM4Di-mCherry expression under the human synapsin promoter (pAAV-hSyn-hM4D(Gi)-mCherry, viral concentration 100μL at titer ≥ $3X10^{12}$ vg/mL; Addgene: Watertown, MA). For animals in cohort 2, animals were injected with eGFP using AAV virus under the human synapsin promoter (pAAV-hSyn-EGFP, viral concentration 100 μL at titer ≥ $3X10^{12}$ vg/mL; Addgene: Watertown, MA). For all surgeries, the following coordinates for injection were used in the OFC: A/P 3.6 - 4.6, M/L 2.6 to 3.0, D/V -5.0 to -5.2 mm from bregma).

3.3.4 Behavioral training

One week after recovering from surgery, rats from Cohort 1 and Cohort 2 were trained to self-administer 10% EtOH, 20% EtOH and 15% sucrose (Figure 3.1F) in an operant chamber (Med Associates, Fairfax, Vermont) on an FR1 schedule (Hernandez & Moorman, 2019). A separate cohort was only trained to self-administer 15% sucrose in an operant chamber. Self-administration training was conducted during 2 hr sessions during rats' dark cycle. To signal the beginning of trials and subsequent reward availability, the operant houselight would turn off. FR1 nosepokes then produced an auditory cue for 500 msec that predicted the availability of one of three outcomes in the spigot below the nosepoke: 15% sucrose, 10% EtOH or 20% EtOH. Rats were initially trained on FR1 nosepoke responses for a 10% EtOH cue (1 kHz played for 4 seconds) and 100 μL 10% EtOH delivered through a spigot as a reward. Once rats reliably consumed 10% EtOH less than 2 sec after nosepoke, the time spent in nosepoke was increased to 100 msec. Rats were required to enter the reward well below the nosepoke in order for the reward to be delivered. After reward delivery ended, the operant houselight turned on to signal an inter-trial timeout where any subsequent nosepoke was not rewarded until the light was turned off again, signaling a new trial. Training sessions were 1 hr. Once rats reached a criterion of 85% successful trials (i.e., retrieving sucrose within 2 seconds post-cue onset), rats were trained on FR1 sucrose and 20% EtOH self-administration. Cue outcome pairings were as follows: 1 kHz for 10% EtOH, 5 kHz for sucrose and 10 kHz for 20% EtOH. Once rats reliably entered the reward port after presentation of sucrose and 20% EtOH cues in a 2 hr session (>80% rewarded trials), inactivation studies commenced.

3.3.5 Inactivation experiments

Once rats were trained to self-administer each reward, OFC was inactivated using Gi-coupled hM4Di DREADs in 3 distinct reward-seeking environments: in a homecage setting, in an operant setting and during cued-reinstatement.

3.3.5.1 Homecage OFC inactivations

For homecage drinking inactivation testing, rats were given a sipper tube filled with 40 mL of either sucrose, 10% EtOH or 20% EtOH along with water and food. On Mondays and Tuesdays, rats consumed the reward for 2 hours without any injection to get baseline reward consumption. On Wednesdays and Fridays, 30 mins prior to behavioral testing to rats were either given an injection of CNO (3 mg/kg, i.p.; Yau & McNally, 2015) or saline /DMSO (solvent for CNO), in a counterbalanced fashion, to allow CNO to bind to DREADDs. On Thursdays, rats were not given any injection before receiving reward. This experiment took 3 weeks, with each week inactivations being conducted for one of the three rewards.

3.3.5.2 Operant OFC inactivation

OFC was inactivated using a similar approach described above. On Mondays and Tuesdays, rats were not injected with CNO or saline in order to observe baseline reward-seeking and consumption. On Wednesdays and Fridays rats were either injected with CNO (as described above) or saline/DMSO in a counterbalanced fashion. On Thursdays rats were not injected and were able to operantly self-administer rewards. This experimental procedure was done for all rats using sucrose, 10% EtOH and 20% EtOH as individually-delivered rewards.

3.3.5.3 Extinction training and cued-reinstatement inactivation

After operant inactivation experiments concluded, rats underwent daily 1 hr extinction sessions. Cohort 1 and Cohort 2 underwent this testing, each to test cued-reinstatement for a palatable reward: 10% EtOH or sucrose. During these sessions nosepokes had no consequences, however nosepokes and well entries were recorded during these sessions. Rats were trained on extinction for at least 2 weeks until nosepoking was <20 in a 2 hr session. Upon meeting criterion, rats were randomly selected to receive CNO or saline/DMSO on a reinstatement task. During this task (Figure 3.1G), a house light was illuminated until trials began. Once rats entered the nosepoke, a 10% EtOH or sucrose reward-predicting cue would play, depending on the cohort (see above) but no reward was delivered from the spigot. Again, all nosepokes and well entries were recorded during these sessions. After the first reinstatement session, rats were again trained on extinction until they met criteria and were re-tested on reinstatement using the counterbalanced injection treatment.

3.3.6 Histology

After the final reinstatement test, rats were perfused with 0.9% NaCl solution followed by 4% paraformaldehyde. Brains were post-fixed overnight with 4% paraformaldehyde and cryoprotected in a 20% sucrose/0.1% sodium azide solution. For rats injected with hM4Di-mCherry, 40-micron thick sections in the coronal plane were collected. To stain for mCherry, sections were blocked in 3% normal donkey serum (vol/vol; Jackson ImmunoResearch, West Grove, PA) in phosphate buffered saline with Triton and incubated overnight with primary (rabbit∝DsRed, 1:500; Takara Bio, Kusatsu,

Japan). Tissue was incubated with secondary antibodies (donkey∝rabbit, 1:250; Jackson ImmunoResearch) for 2 hrs followed by mounting onto Superfrost Plus slides (Fisher Scientific, Pittsburgh, PA) using CitiFluor (Electron Microscopy Sciences, Hatfield, PA). Rats injected with GFP were blocked similarly with overnight incubation of primary (chicken anti-GFP (1:2000; Abcam, Cambridge, MA). The next day tissue was incubated for 2 hr in secondary (488 anti-Chicken, 1:500; Jackson ImmunoResearch) following mounting to Superfrost Plus slides using Citifluor. Rats injected with the AAV construct containing hM4Di or GFP were removed from data analysis if viral expression was exclusively found in pyriform cortex (n = 1 from cohort 1) or majority of expression was in pyriform cortex (n = 2 from cohort 1) or if no DREADD expression was observed (n = 3 from cohort 1), leaving 16 rats (females: n = 8, males: n = 8) for data analysis from cohort 1. In cohort 2, all rats (females: n = 6, males: n = 6) had GFP expression exclusively in OFC and in cohort 3, 2 rats did not have DREADD expression in OFC and one rat died during surgery, leaving 9 animals (females: n = 5, males: n = 4).

3.3.7 Data analysis

Rats were identified as high or low drinkers (HD/LD) based off of mean EtOH consumption on the final three days of homecage intermittent access greater than or less than 3.5 g/kg/24 hr respectively (George et al., 2012; Momeni & Roman, 2014; Spoelder et al., 2015; Hernandez & Moorman, 2019). Operant behaviors analyzed were the total number of nosepokes, total number of reward received, percent rewarded trials, average latency to initiate trials and average latency to initiate reward consumption after hearing the cue. Depending on the normality, statistical tests to compare CNO and saline treatments were conducted using parametric or nonparametric t-tests using Prism

(GraphPad, San Diego, CA) and ANOVAs were used when comparing nosepoke and reward seeking behavior in response to CNO and saline treatment during reinstatement to nosepoke and reward seeking behavior during the final day of extinction. 2-way ANOVAs were conducted with CNO/Saline treatment and Male/Female as factors.

3.4 Results

Alcohol intermittent access and identification of HD/LD rats

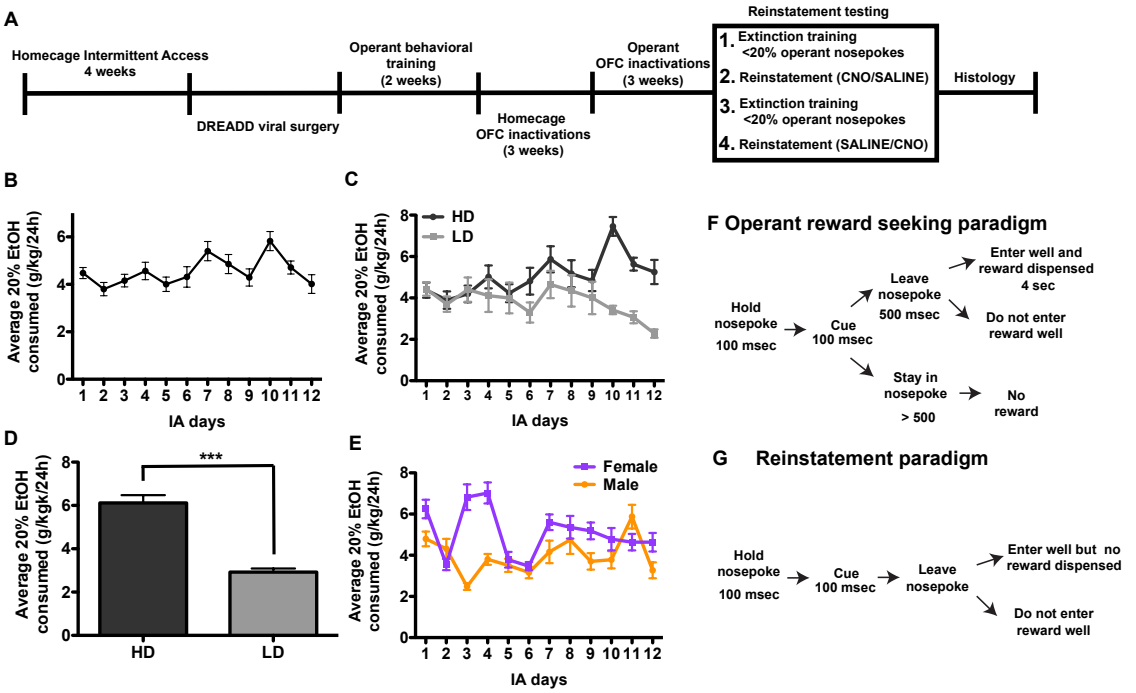

Figure 3.1. Experimental outline, behavioral design and alcohol consummatory behavior. (A) Experimental timeline. (B) Rats received 12 days homecage intermittent access (IA) to EtOH and consumption escalated significantly over days. (C) By the end of IA, HD rats consumed significantly more EtOH than LD rats (D) HD consumed significantly more EtOH on the final 3 days of IA compared to LD rats (E) No significant differences were observed between males and females across IA (F) Operant task diagram. Rats nosepoked to receive cues predicting sucrose or EtOH and were required to enter well for the reward to be dispensed. (G) In reinstatement testing, after nosepoke, either 10% EtOH or sucrose cues were presented, depending on the experiment, but no reward was delivered in the reward port. See main text for statistical details. ***$p < 0.001$.

On average, rats from cohorts 1 and 2 consumed 20% EtOH over 4 g/kg/24h across all days of intermittent access (IA) (Figure 3.1B), with significant differences in alcohol preference ($F(1,1) = 26.82$, $p < 0.001$) and an interaction effect ($F(1,11) = 2.465$, $p < 0.01$; 2-Way ANOVA) between HD and LD on the final 3 days of intermittent access (**D10**: μHD = 7.456±.451 g/kg/24h, μLD = 3.418±.204 g/kg/24h, $t = 4.828$, $p < 0.001$; **D11**: μHD: 5.633±0.309 g/kg/24h, μLD = 3.067±.287 g/kg/24h, $t = 3.069$, $p < 0.05$; **D12**: μHD = 5.253±0.585 g/kg/24h, μLD = 2.28±.201 g/kg/24h, $t = 3.555$, $p < 0.01$; Bonferroni post-tests; Figure 3.1C). HD rats consumed (μ = 6.114±0.3616 g/kg/24h) significantly more ($U = 0$, $p < 0.0001$; Mann-Whitney) 20% EtOH than LD rats (μ = 2.922±0.1693 g/kg/24h). Male and females exhibited similar patterns of drinking during IA (Figure 3.1E), with females drinking overall more in the first 6 IA sessions but exhibiting similar patterns of drinking for the final 6 sessions.

Homecage inactivation studies

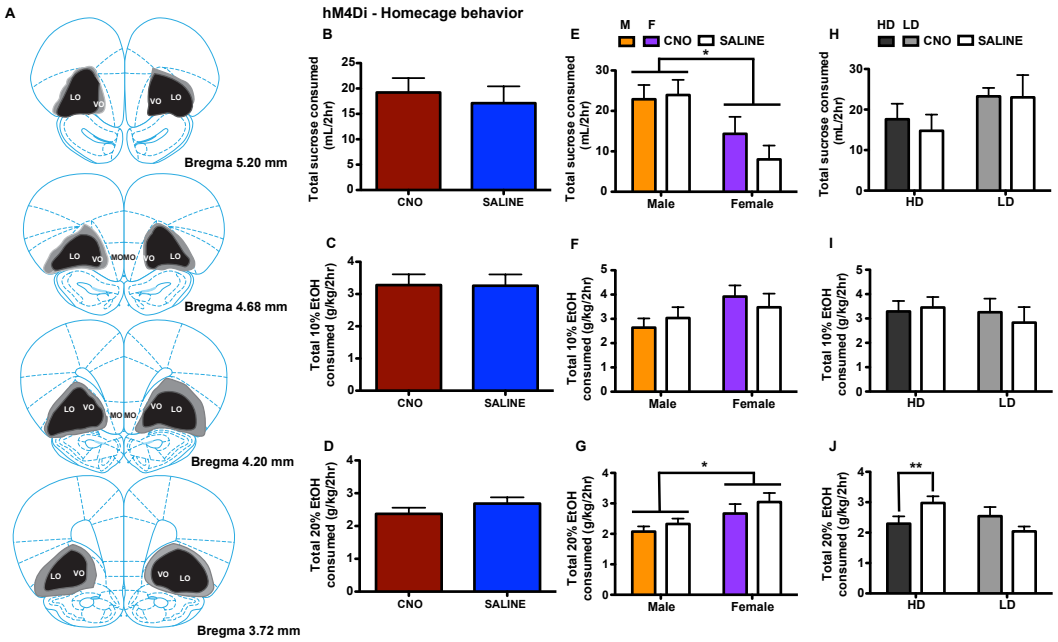

Figure 3.2. OFC DREADD expression coordinates and the impact of OFC inactivation on homecage sucrose, 10% EtOH and 20% EtOH consumption. (A) Average spread of hM4Di-mCherry overlapping expression across rats, with black areas identifying lowest expression and gray areas identifying highest expression areas. OFC inactivation did not modulate sucrose (B), 10% EtOH (C) or 20% EtOH consumption (D) in a homecage environment. Males consumed significantly more sucrose than females in a homecage environment (E). However females consumed more 10% EtOH (F) and significantly more 20% EtOH (G) than males. Operant task diagram. Rats nosepoked to receive cues predicting sucrose or EtOH and were required to enter well for the reward to be dispensed. (G) In reinstatement testing, after nosepoke, either 10% EtOH or sucrose cues were presented, depending on the experiment, but no reward was delivered in the reward port. See main text for statistical details. *p < 0.05, ** p < 0.01.

The OFC was inactivated during homecage drinking experiments. OFC inactivation did not impact sucrose ($p = 0.6091$, Figure 3.2B), 10% EtOH ($p = 0.6233$, Figure 3.2C) or 20% EtOH (0.1688, Figure 3.2D) consumption. However, we observed sex differences in 20% EtOH homecage drinking ($F(1,14) = 5.71$, $p = 0.0315$, 2-way ANOVA) with females consuming significantly more 20% EtOH (2.857±.188 g/kg/2h, Figure 3.2G) than males (2.199±.124 g/kg/2h) as well as a small and consistent, but insignificant difference in 10% EtOH homecage drinking ($F(1,14) = 4.18$, $p = 0.063$)

where females consumed more 10% EtOH (3.968±.221 g/kg/2h, Figure 3.2F) than males (2.838±.199 g/kg/2h). In a homecage environment, females consumed (11.167±3.167 mL/2hr, Figure 3.2E) significantly less 15% sucrose ($F(1,12) = 6$, $p = 0.0307$) than males (23.406±0.531). OFC inactivation did not alter HD and LD during homecage sucrose consumption (Figure 3.2G; $F(1,1) = 1.18$, $p = 0.2987$, 2-Way repeated measures ANOVA) and 10% EtOH consumption (Figure 3.2H; $F(1,1) = .4046$, $p = 0.535$). However, I observed an interaction effect where in HD rats, CNO injection significantly suppressed ($t = 3.569$, $p < 0.01$, Bonferroni) 20% EtOH consumption ($\mu = 2.295 \pm 0.241$g/kg/2h) compared to saline injection (2.976±.218 g/kg/2h; Figure 3.2I; $F(,1,1) = 11.96$, $p = 0.0038$, 2-way ANOVA). In rats with OFC neurons expressing GFP, I did not observe any effect of CNO treatment on 15% sucrose ($p = 0.7813$), 10% EtOH ($p = 0.4705$) or 20% EtOH ($p = 0.3013$) drinking. However, I did observe that overall females consumed (2.781±0.23 g/kg/2h, Fig 3.3B) more 10% EtOH ($F(1,10) =$

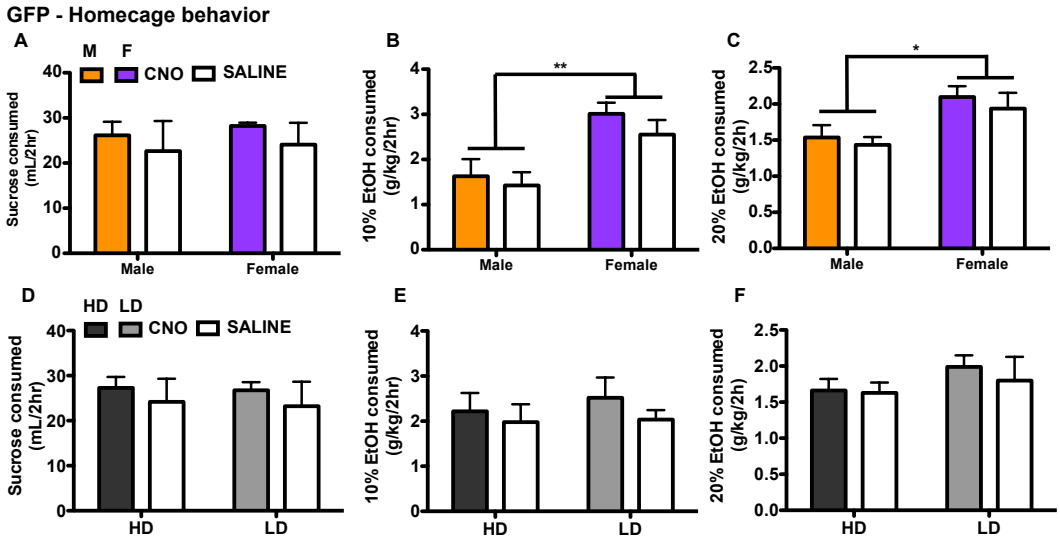

Figure 3.3. CNO treatment did not modulate homecage consummatory behavior in rats whose OFC neurons were transduced with GFP. CNO treatment did not impact sucrose (A), 10% EtOH or 20% EtOH, but females consumed significantly more 10% EtOH (B) as well as 20% EtOH (C) than males regardless of treatment. CNO treatment did not differentially impact HD vs. LD in sucrose (D), 10% EtOH (E) or 20% EtOH (F) consumption. *p < 0.05, **p < 0.01.

12.63, p = 0.0052) than males (1.526±0.102 g/kg/2h). Females also consumed significantly more 20% EtOH (2.017±0.081 g/kg/2h, Fig 3.3C) than males (1.485±0.051 g/kg/2h; F(1,10) = 7.24, p = 0.0226). No sex differences were observed in homecage sucrose consumption (Figure 3.3A; F1,1) = 0.1403, p = 0.7122, 2-way ANOVA) and although HD/LD rats consumed significantly different amounts of 20% EtOH during IA, no differences between HD and LD were observed during sucrose (Figure 3.3D), 10% EtOH (Figure 3.3E) or 20% EtOH (Figure 3.3F).

Operant inactivation studies

In the operant self-administration studies, OFC inactivation did not affect sucrose seeking (Figure 3.4A, p = 0.979, paired t-test) or consumption (Figure 3.4B, p = 0.8211), 10% EtOH seeking (Figure 3.4G, p = 0.1741) or consumption (Figure 3.4H, p = 0.346)

and finally 20% EtOH seeking (Figure 3.4M, p = 0.2256) or consumption (Figure 3.4N, p = 0.2107). Additionally, no significant differences in

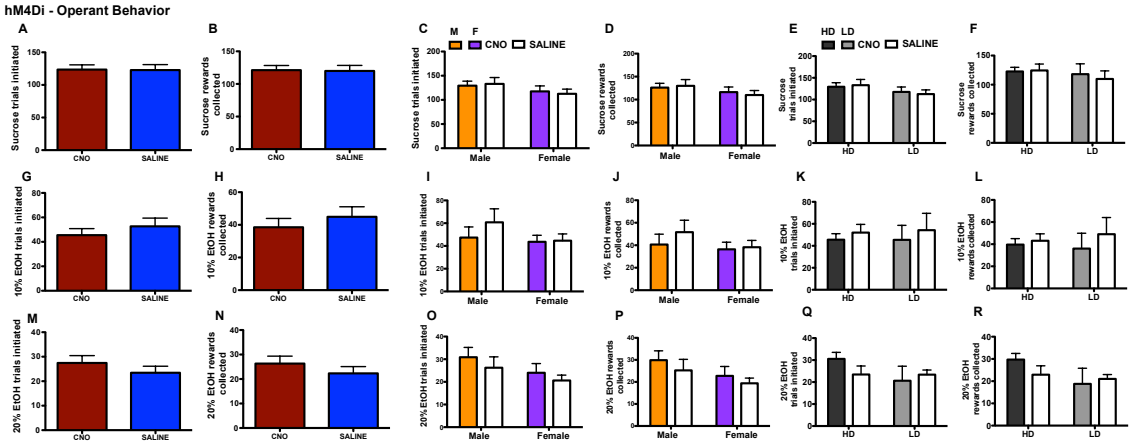

Figure 3.4. CNO treatment did not modulate operant reward-seeking and consummatory behavior in rats whose OFC neurons were transduced with hM4Di. In rats whose OFC was transduced with inhibitory DREADDS, CNO treatment did not modulate sucrose, 10% EtOH or 20% EtOH trials initiated (A, G, M), rewards collected (B, H N) in an operant environment. No significant differences were observed between males and females for trials initiated (C, I, O) or rewards collected (D, J, P). HD and LD rats were not differentially impacted by CNO treatment on trials initiated (E, K, Q) or rewards collected (F, L, R).

sucrose trials (p = .2265) and rewarded well entries (p = 0.2606) were observed between females and males (Figure 3.4C, D). Additionally, no sex differences were observed in the total number of initiated 10% EtOH trials (p = 0.3865) or rewarded well entries (p = 0.4232; Figure 3.4I, J) as well as initiated 20% EtOH trials (p = 0.1983) or rewarded well entries (p = 0.2005; Figure 3.4O, P). Latencies to initiate trials or enter reward ports after cue presentation were also not significantly affected by CNO/Saline treatment in sucrose, 10% EtOH or 20% EtOH operant sessions (data not shown). No significant differences in reward seeking or consumption of sucrose, 10% EtOH or 20% EtOH were observed after CNO/Saline treatment in between HD and LD (Figure 3.4E, F, K, L, Q, R). In rats whose OFC was transfected with GFP, CNO treatment did not affect sucrose (Figure 3.5A),

10% EtOH (Figure 3.5D) or 20% EtOH (Figure 3.5G) rewards collected. Additionally, CNO treatment did not influence reward seeking based on sex (Figure 3.5B, E, H) or drinking preference (Figure 3.5C, F, I).

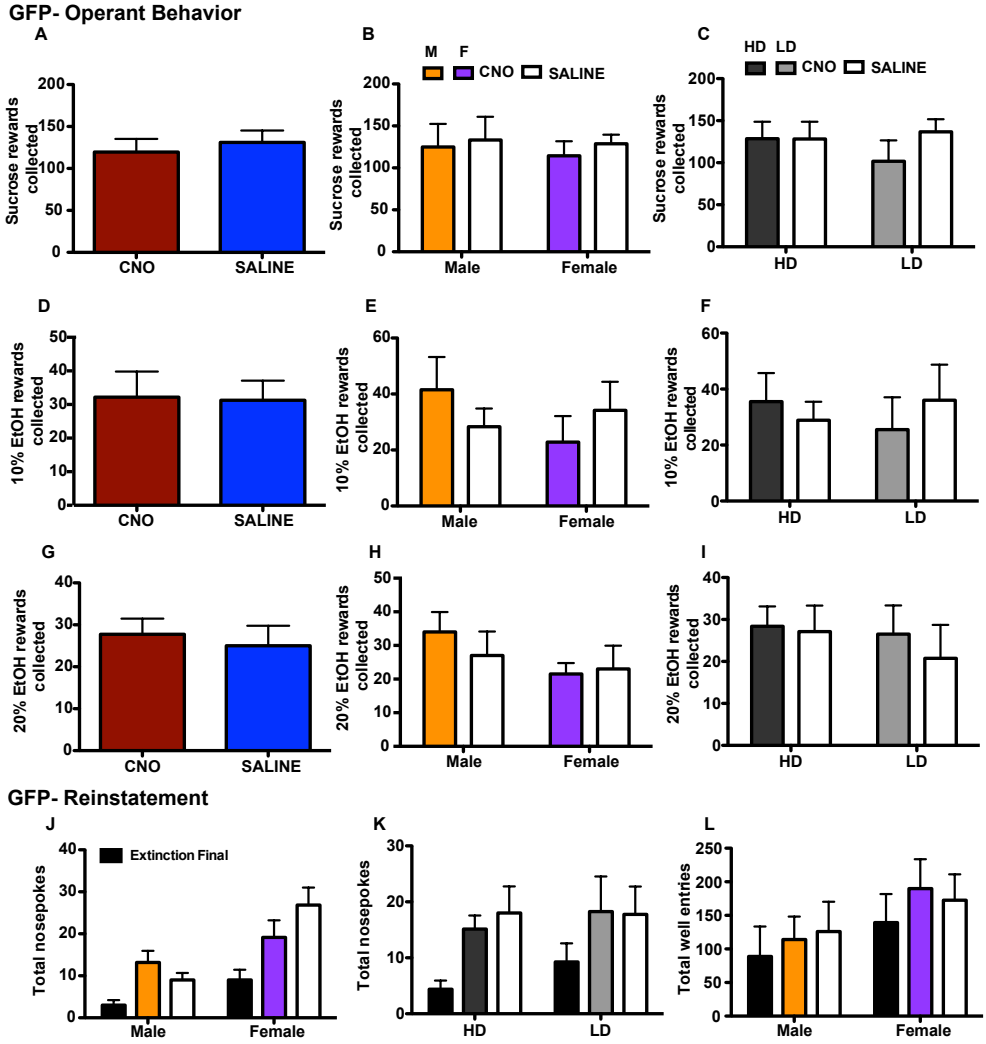

Figure 3.5. CNO treatment did not modulate operant or reinstatement behavior in rats whose OFC neurons were transduced with GFP. In an operant environment, CNO treatment did not affect rewards collected for sucrose (A), 10% EtOH (D) or 20% EtOH (G) in GFP transduced rats. For sucrose, 10% EtOH and 20% EtOH rewards collected, males and females were not differentially affected (B, E, H, respectively), HD and LD rats were not different (C, F, I, respectively) as well. During reinstatement, CNO treatment did not significantly alter total nosepokes between males and females (J), HD/LD rats (K) and did not impact total well entries between the sexes (L).

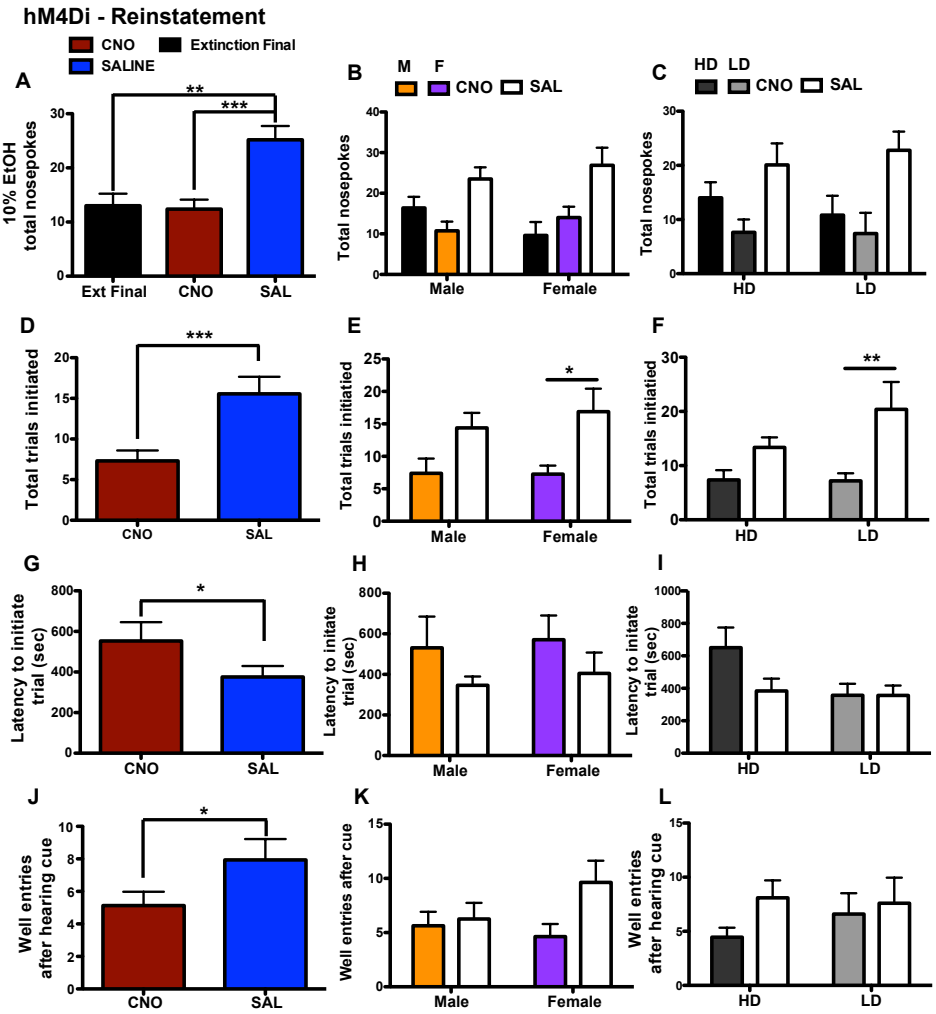

Figure 3.6. CNO treatment significantly decreased reinstatement reward-seeking and consummatory behavior in rats whose OFC was transduced with hM4Di. OFC inactivation significantly suppressed total nosepokes during reinstatement (A) and trial initiations (D). OFC inactivation significantly increased the latency to initiate a trial (G) and suppressed the well entries after cue presentation (J). No significant differences were observed between males/females (B) or HD/LD rats (C). OFC inactivation did significantly suppress female trial initiations (E) as well as LD trial initiations (F). OFC inactivation did not significantly alter latencies to initiate trials between males and females (H) and HD/LD (I). No sex (K) or drinking preference (L) differences were observed in well entries after cue presentation. *$p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

After rats met criterion for extinction training, reinstatement was measured as increased trial initiations or reward seeking when 10% EtOH cues could be once again

presented after nosepoking. Rats significantly reinstated nosepoke behavior for 10% EtOH ($\mu = 25 \pm 2.2556$; Figure 3.6A, $t = 4.031$; $p = 0.0002$, 1-way RM ANOVA) compared to the final extinction testing day ($\mu = 13 \pm 2.249$) as well as CNO treatment ($\mu = 12.38 \pm 1.751$). No differences in sex (Figure 3.6B**)** or alcohol preference (Figure 3.6C) were observed in reinstatement total nosepokes. In reinstatement trials, houselights were illuminated during timeout and turned off when a trial started and a rat could initiate a trial via nosepoke to elicit tone playback. OFC inactivation suppressed (Figure 3.6D, $W = -132$, $p = 0.0007$, Wilcoxon signed rank test) the number of trials initiated ($\mu = 7.313 \pm 1.28$) compared to saline treatment ($\mu = 15.56 \pm 2.09$). Trial initiations were suppressed with OFC inactivation in both males and females (Fig 3.5E, $F(1,1) = 14.15$, $p = 0.0021$, 2-way repeated measures ANOVA), as well as HD and LD ($F(1,1) = 18.43$, $p = 0.0007$). However, females' trial initiations were significantly suppressed by CNO injection (Figure 3.6E; $t = 3.08$, $p < 0.05$, Bonferroni post-test) and LD rats had significantly suppressed trial initiations after OFC inactivation (Figure 3.6H; $t = 3.559$, $p < 0.01$, Bonferroni post-test). OFC inactivation did not impact the total number of well entries ($F(1,2) = 2.37$, $p = 0.08$, data not shown). However, OFC inactivation significantly suppressed (Figure 3.6J, $W = -77$, $p = 0.0486$) cue-evoked well entries ($\mu = 5.125 \pm 0.8509$) compared to saline treatment ($\mu = 7.938 \pm 1.286$). No sex differences (Figure 3.6K) or alcohol preference differences (Figure 3.6L) were observed in well entries after cue presentation after OFC inactivation. OFC inactivation also decreased motivation to seek 10% EtOH (Figure 3.6G, $U = 74$, $p = 0.036$) measured by longer latencies to initiate trials ($\mu = 552.5 \pm 92.31$ sec) than in the saline condition ($\mu =$

375.4±54.34 sec). Males and females both had longer latencies to initiate trials (Figure 3.6H**)**, however the increased latency to initiate trials appears to be predominantly because of HD animal's behavior (Figure 3.6I**).** Between saline and CNO conditions, no significant differences were observed in the latency to enter the reward port after cue presentation ($t = 0.09$, $p = 0.9286$). Finally, reinstatement for 10% EtOH seeking was suppressed after OFC inactivation in both males and females (total nosepokes: $F(1,1) = 0.002$, $p = 0.984$; well entries after cue presentation: $F(1,1) = 0.477$, $p = 0.5$). OFC inactivation also did not alter motivational parameters such as latency to initiate trial ($F(1,1) = 0.21$, $p = 0.1186$) or latency to enter well after cue presentation ($F(1,1) = 0.51$, $p = 0.481$) between males and females. In rats whose OFC neurons were transfected with GFP, during reinstatement, CNO treatment did not significantly affect total nosepokes in males and females (Figure 3.5J), in HD or LD (Figure 3.5K) and finally did not alter the total number of well entries between sexes (Figure 3.5L) or drinking preferences (data not shown).

In the final experiment, rats were trained to self-administer only sucrose in a homecage and operant environment. OFC neurons were inactivated in a homecage environment and no significant differences in sucrose consumption were observed

(Figure 3.7A, t =0.36, p = 0.7276, paired t-test) between CNO (μ = 20.22±5.123 mL) and saline (μ = 21.33±4.936 mL).

In operant FR1 testing, OFC inactivation did not affect the total number of trials initiated (Figure 3.7C, t = 0.37, p = 0.721, paired t-test), the number of rewarded well entries (Figure 3.7E, t = 0.34, p = 0.743), latency to initiate trial (t = 0.7974, p = 0.4482) or latency to enter reward port after cue presentation (t = 1.191, p = 0.2677). No sex differences were observed in operant trials initiated (Figure 3.7D, F(1,1) = 0.0246, p = 0.8798; 2-way ANOVA), or rewarded trials (Figure 3.7F, F(1,1) = 0.3957, p = 0.545). Additionally, no sex differences were observed in latencies to initiate trials (F(1,1) = 0.0708, p = 0.7978) and consume reward (F(1,1) = 3.127, p = 0.1203).

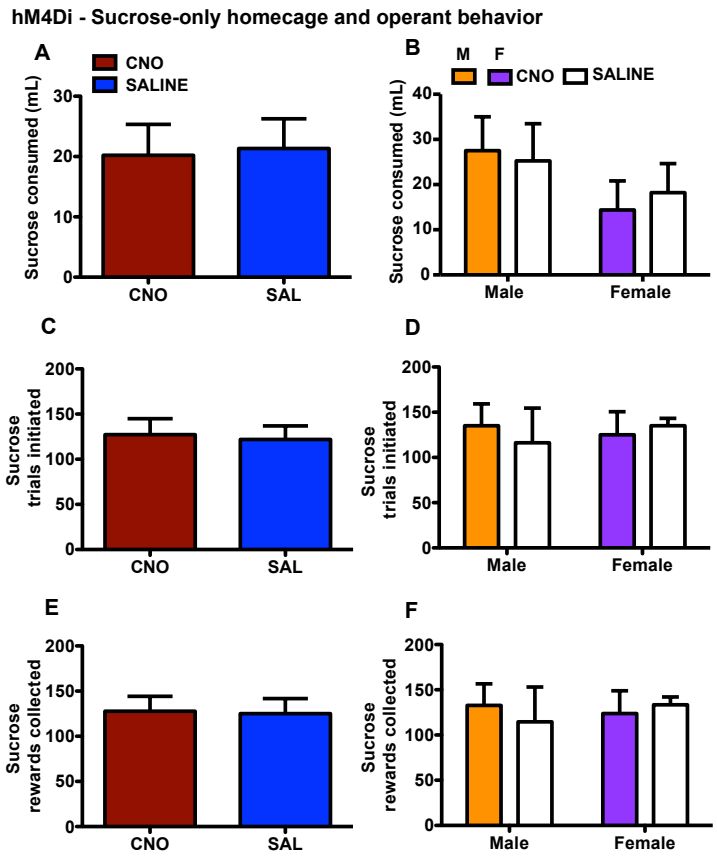

Figure 3.7. CNO treatment did not modulate homecage or operant sucrose seeking in rats whose OFC was transduced with hM4Di. OFC inactivation did not alter sucrose consumption levels (A) between males and females (B) in a homecage environment. Sucrose trials initiated (C) between males and females (D) additionally were not significantly affected by OFC inactivation, as well as sucrose rewards collected (E, F).

In sucrose reinstatement inactivation studies, OFC inactivation significantly suppressed reinstatement (Figure 3.8A, $F(1,2) = 8.331$, $p = 0.0033$; RM ANOVA). Furthermore, OFC inactivation suppressed (Figure 3.8D, $t = 2.971$, $p = 0.0171$, paired t-test) trial initiations ($\mu = 10.22 \pm 2.857$) as well as well entries after cue presentation (Figure 3.8H, $\mu = 6.667 \pm 2.427$; $t = 2.579$, $p = 0.0327$) compared to the saline condition (μ trials initiated $= 15.56 \pm 3.051$; μ cue-elicited well entries $= 12 \pm 2.708$). No sex differences were observed in total initiated trials (Figure 3.8E) or cue-initiated well entries (Figure 3.8I). Latencies to initiate trials (Figure 3.8F, $U = 24$, $p = 0.2766$; Mann-Whitney) and latency to enter well after cue presentation (Figure 3.8G, $U = 25$, $p = 0.8639$) were not significantly different between CNO and saline conditions. No significant differences were observed between males and females in total nosepokes ($F(1,1) = 0.5071$, $p = 0.4994$), initiated trials ($F(1,1) = 0.0246$, $p = 0.8798$), latency to

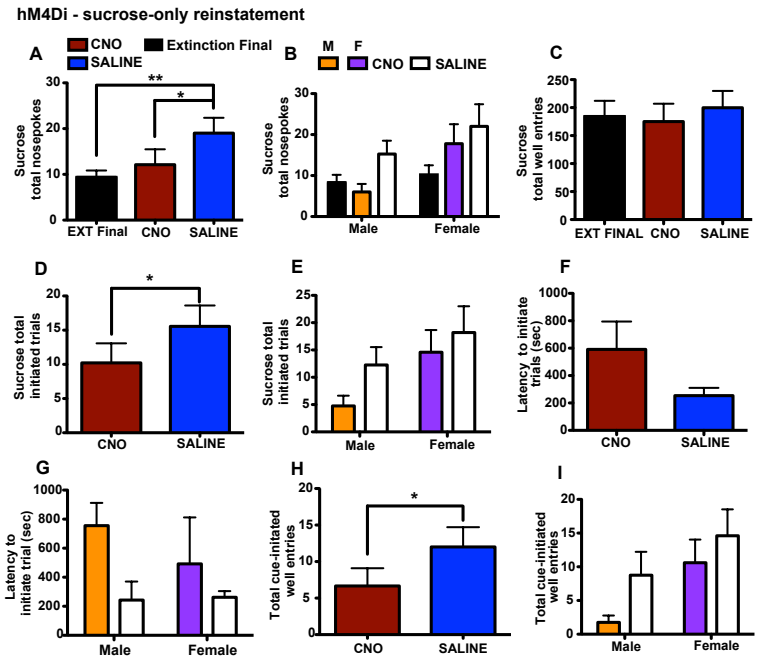

Figure 3.8. CNO treatment significantly suppressed sucrose reinstatement behavior in rats whose OFC was transduced with hM4Di. During sucrose reinstatement, OFC inactivation significantly suppressed total nosepokes (A), trial initiations (D) and cue-initiated well entries (H). OFC inactivation did not affect males differently than females in total nosepokes (B), initiated trials (E), latency to initiate trials (G) or cue-initiated well entries (I).

initiate trial ($F(1,1) = 0.07084$, $p = 0.7978$) or latency to enter well after cue presentation ($F(1,1) = 3.445$, $p = 0.1058$).

3.5 Discussion

In this study, I examined whether the OFC activity is necessary for alcohol seeking, consumption and reinstatement of alcohol-seeking behavior. The data shows that inactivation of the OFC does not alter homecage or operant sucrose, 10% EtOH reward seeking or consumption, but did significantly suppress 20% EtOH consumption in a homecage environment only for HD rats (Figure 3.2J). Interestingly, in alcohol-dependent mice, OFC inactivation results in enhanced alcohol drinking (den Hartog et al., 2016), suggesting that alcohol exposure may impact OFC's role in the expression of alcohol preference. It is possible that the lack of effects we see on homecage or operant sucrose, 10% EtOH drinking is due to our non-specificity of inhibition. OFC inactivation was sufficient to suppress cue-induced reinstatement of both 10% EtOH and sucrose-seeking. Specifically, OFC inactivation appears to suppress overall motivation to initiate trials and enter reward well ports after cue presentation in reinstatement sessions (Figures 3.6, 3.8) as well as latency to initiate trials. We did not observe this effect in rats whose OFC neurons were virally transfected to produce GFP instead of hM4Di DREADDS (Figure 3.5J-L).

Interestingly, we observed that females consume more 10% and 20% EtOH than males in a homecage environment (Figure 3.2F,G; Figure 3.3B,C), but not in an operant environment. These data support previous findings (Juárez & Tomasi, 1999; Priddy et al., 2017) suggesting that the environment where alcohol can be self-administered plays a

strong role in sex differences in alcohol consumption. However, male rats consumed more sucrose compared to females (Figure 3.2E, Figure 3.7B), which has been demonstrated in previous findings (Sclafani & Abrams, 1986) where males have also been shown to prefer lower concentrations of sucrose than females (Asarian & Geary, 2013).

Over the course of drug self-administration, drug effects are associated with conditioned stimuli or passive contextual discriminative stimulus exposure. Drug-associated discriminative stimuli are known to induce reinstatement for drug-seeking after abstinence (Childress, 1987b, 1987a; Jupp et al., 2010) or extinction of self-administration (Bouton & Bolles, 1979; Katner et al., 1999; Zironi et al., 2006). Inactivation of OFC, along with ipsilateral or contralateral basolateral amygdala (BLA), results in suppressed active lever pressing for cocaine during context-induced reinstatement (Lasseter et al., 2010) and suppressed alcohol-seeking is observed when OFC is inactivated during context-induced reinstatement (Bianchi et al., 2018). My data showed that OFC inactivation suppresses cued-reinstatement for 10% EtOH (Figure 3.6) and 15% sucrose (Figure 3.8). Additionally, I demonstrated that OFC inactivation not only affected 10% EtOH and sucrose seeking behavior during reinstatement sessions (i.e., nosepoking; Figure 3.6A,D and Figure 3.8A,D, respectively) but also suppresses "taking" behavior (i.e., entering reward delivery port after hearing cue during reinstatement; Figure 3.6J and Figure 3.8H). Rats were trained to enter the reward port after cue presentation, in order for reward to be delivered, so a suppression of reward "taking" behavior suggests OFC contributes to not just cue reactivity but also response-outcome contingencies when no rewards were delivered.

Economic choice involves comparing options when an actor (individual making a decision) has to determine the subjective value between two options based off of sensory cues and internal states (Padoa-Schioppa, 2011). Many studies have examined whether the OFC tracks value during economic choice by examining single unit activity or BOLD response (Padoa-Schioppa & Assad, 2006; Padoa-Schioppa, 2009; Levy & Glimcher, 2012; Xie & Padoa-Schioppa, 2016). However, recent studies have shown that medial (Gardner et al., 2018) as well as lateral (K. J. Miller et al., 2018) OFC inactivation is insufficient for choice behavior or economic choice. Interestingly, when rewards are devalued, OFC inactivation or removal in primates disrupt the ability to select valued rewards (Izquierdo & Murray, 2004; West et al., 2011; Gardner et al., 2017). Additionally, OFC inactivation also hinders the ability to alter behavior in reversal learning tasks (Dias et al., 1996; Chudasama & Robbins, 2003; Schoenbaum et al., 2003; Izquierdo et al., 2013). Traditionally, rodents or primates are trained to learn cue-outcome contingencies (e.g., a 1 kHz tone predicts sucrose flavored with grape and a 10 kHz tone predicts sucrose flavored with vanilla) in either a pavlovian or operant setting. Once animals have learned the cue-outcome contingencies, the outcome of each cue is reversed (e.g., the 1 kHz tone now predicts vanilla flavor and the 10 kHz tone now predicts grape flavor delivery). One hypothesis on the role of the OFC is that it guides behavior not just through encoding the value of rewards but also through updating the value of a reward when internal (e.g., satiation) and external (e.g., reward size does not meet expectations) states change (O'Doherty et al., 2000; Takahashi et al., 2009; Takahashi et al., 2013; Murray et al., 2015; Lopatina et al., 2016). In our study, rats were extinguished in the same environment that they were able to seek and consume sucrose and ethanol rewards,

thus suppressing active reward seeking (e.g., nosepokes). When rats were exposed to a cued-reinstatement environment, reward seeking behavior was re-established only when OFC neurons were not inactivated. When the OFC is not active cue-outcome associations are not relayed to the rest of the brain thus suppressing cue-induced seeking of rewards in a reinstatement setting, potentially through a lack of value encoding for alcohol and sucrose rewards. Together these findings suggest the OFC may play an important role in modulating addiction-related, as well as natural reward seeking, behavior by influencing an animals' ability to respond to cues that predict drug and palatable natural rewards.

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CHAPTER 4

GENERAL DISCUSSION

4.1 Main Findings

This dissertation consisted of two main aims. The first aim was to identify whether orbitofrontal cortex (OFC) neurons encode the innate preference an animal displays for alcohol and whether OFC activity tracks alcohol seeking and consumption. Results from Chapter 2 show that OFC neurons encode the relative preference for alcohol rewards and, in our experimental design, that this preference manifests specifically in OFC activity during cue presentation, reward seeking and consumption. OFC activity during 10% EtOH seeking and consumption mimics OFC activity during sucrose seeking and consumption. OFC neurons recorded from high-alcohol consuming rats during alcohol seeking and consumption were more robustly modulated than in low-alcohol consuming rats, supporting the role of the OFC in encoding alcohol preference. Interestingly, no significant differences in OFC activity, or in behavior, between high-alcohol consuming and low-alcohol consuming rats were observed in sucrose seeking or consumption. On interleaved sessions, overall activity from OFC neurons were consistent with behavioral preferences for sucrose and alcohol. However the presence of sucrose enhanced HD activity for 20% EtOH and the presence of 20% EtOH suppressed HD activity for sucrose. Together these findings suggest that the OFC is a site, at least in rats, for encoding alcohol preference.

The second aim examined whether the OFC is necessary for the expression of alcohol preference, seeking and consumption. Chapter 3 illustrates that chemogenetic inactivation of the OFC does not impact 10% alcohol consumption in a homecage or in an operant setting. Interestingly, OFC inactivation selectively inhibited homecage 20% EtOH consumption in HD rats. However, after alcohol and sucrose seeking was extinguished in an operant setting, OFC inactivation significantly suppressed cued-reinstatement for 10% alcohol and sucrose rewards. These findings imply that although OFC neurons track alcohol preference, seeking and consumption, OFC neurons appear to be *necessary* for updating behavioral responses to cues that once predicted palatable drug and natural rewards. Consistent with previous findings, OFC neurons appear to be necessary for modulating behavior when the value of rewards, or stimuli that predict rewards, are altered.

4.2 Proposed function of the OFC

The OFC is a brain region that is known for encoding the value of rewards via their olfactory, taste and pleasurable properties. Based on the present studies, along with previous literature, my research demonstrates that OFC neurons encode the relative value of alcohol rewards alongside natural rewards but also contributes to the modulation of goal-directed behavior in a relatively nuanced fashion. According to previous literature, OFC plays a stronger role in model-based value encoding, which allows an individual to make decisions based off of learned cue-outcome associations. I am able to make the claim that my findings support, both through awake-behaving electrophysiological recordings as well as the effects of chemogenetic inactivation, that the OFC encodes the

innate preference for alcohol rewards but is only necessary in alcohol reinstatement conditions.

4.2.1 Proposed mechanism #1: Subjective value of a reward is modulated during operant extinction learning, leading the OFC to contribute to behavioral changes only during reinstatement testing

It is clear in the literature that OFC neurons are *not* necessary for pavlovian or instrumental learning to occur (Schoenbaum et al., 2002, 2003; Bohn et al., 2003b, 2003a; Izquierdo & Murray, 2004; Orsini et al., 2015; Jean-Richard-Dit-Bressel & McNally, 2016; Izquierdo, 2017). However it is clear that OFC neurons do encode the outcome of cues in either pavlovian or instrumental tasks (for review, see Schoenbaum et al., 2009). Recent studies have begun to examine how the OFC interacts with other brain regions to modulate reward-seeking and responsiveness to cue presentation (e.g., Lichtenberg et al., 2017), but in the context of a pavlovian-instrumental transfer (PIT). Rats were trained that tones predicted delivery of sucrose (1.5 kHz) or grain pellets (white noise, 75 db). After rats reliably entered the reward port for those rewards, rats were exposed to the same cues while levers were available to press – assessing the influence of CS on action performance and selection in the novel lever choice scenario, the crux of PIT learning. Using chemogenetic inactivation, OFC→BLA and BLA→OFC connections were inactivated and only inactivation of BLA→OFC projections significantly disrupted PIT learning. Lesioning the connectivity between OFC and BLA also did not affect motivation to consume rewards, but did prevent alterations in behavior, in a satiated state, after rewards were devalued (Zeeb & Winstanley, 2013).

This is true for cocaine reinstatement as well. Unilateral OFC inactivation, with baclofen + muscimol ($GABA_A$ and $GABA_B$ antagonists), has no impact on cocaine cued-reinstatement, but inactivation of BLA with unliateral or contralateral OFC suppressed cued-reinstatement for cocaine (Lasseter et al., 2010). Finally, brain derived neurotropic factor expression in OFC neurons is neccessary for encoding food devaluation and cocaine place preference (Gourley et al., 2013). Many studies examining OFC function demonstrate that the OFC encodes the value of rewards in humans, primates and rodents (Tremblay & Schultz, 1999; Goldstein & Volkow, 2002; Arana et al., 2003; G Schoenbaum et al., 2003; Burke et al., 2008; Ernst et al., 2014; Bradfield et al., 2015; Howard et al., 2015). However, OFC is selectively *necessary* for modulating behavior only when the value of reward-predicting stimuli *or* reward-predicting environments is modulated either externally (e.g., the reward is devalued or the probability of reward delivery is decreased; Gallagher et al., 1999; Stopper et al., 2014; Ward et al., 2015; Gardner et al., 2017) or internally (e.g., satiation; O'Doherty et al., 2000; Zeeb & Winstanley, 2013; Murray et al., 2015).

As described above, many studies examine the effects of OFC inactivation on flexible, goal-directed behavior. In part, this is because the OFC is considered a brain region that encodes stimulus-outcome updating (Gallagher et al., 1999; Schoenbaum et al., 2002; Chudasama & Robbins, 2003; McAlonan & Brown, 2003; Roberts, 2006; Riceberg & Shapiro, 2012). OFC activity is modulated by the size, inherent preference and even time of delivery of rewards (Schoenbaum & Eichenbaum, 1995; Schoenbaum et al., 2003; Wallis & Miller, 2003; Roesch & Olson, 2004, 2005; Roesch et al., 2006; Moorman & Aston-Jones, 2014; Izquierdo, 2017). However, relatively few publications

have demonstrated, or examined, whether OFC inactivation directly modulates behavior driven by action-outcome contingencies (e.g., operant behavior). OFC neurons fire in accordance with relative value of rewards (i.e., size and workload to obtain rewards) but transient inactivation of OFC only degrades reward choice when rewards of similar values were presented together in the same self-administration session (Setogawa et al., 2019). Specifically, after OFC inactivation with muscimol, monkeys would choose lower value rewards (small size and increased workload) more often when the alternative choice was similar in value. The data from the second half of this dissertation provides one of the first examples that OFC neurons are key for driving cue-induced reward seeking for sucrose and 10% alcohol rewards.

4.2.2 Proposed mechanism #2: Alcohol consumption during intermittent access alters the function of OFC neurons to encode alcohol reward and thus modulates behavior

Alcohol intermittent access is a reliable mechanism to induce and even escalate alcohol consumption (Wise, 1975; Simms et al., 2008, 2010; Griffin 3rd et al., 2009; J. Li et al., 2010; Carnicella et al., 2011, 2014; Badanich et al., 2011; Lindell et al., 2017) and has long-term negative impacts on brain function. In primates that escalated alcohol consumption throughout a 12-month free alcohol access exposure, compared to non-drinking and low-alcohol consuming individuals, exhibited significantly decreased connectivity between decision-making brain regions and enhanced connectivity between the cingulate cortex (Telesford et al., 2015), a brain region implicated in alcohol craving

(Breese et al., 2011). In adolescent rats, ethanol exposure concomitantly enhances risky behavior along with altered patterns of activity in the OFC in adolescence (Nasrallah et al., 2009, 2011; Clark et al., 2012; McMurray et al., 2014), in adulthood (McMurray et al., 2015), can induce deficits in neurogenesis (Crews et al., 2006) and even enhance neurodegeneration in many brain regions (Nixon et al., 2010). Adolescent exposure to alcohol disrupts development of spatial working memory such that adult memory formation is more susceptible to alcohol-induced impairments (White et al., 2000). Finally, chronic intermittent ethanol exposure during adolescence also reduces an animals' ability to conduct OFC-dependent behavior (i.e., reversal learning; Badanich et al., 2011), suggesting that alcohol exposure is able to negatively impact prefrontal-dependent behavior (for review see Jadhav & Boutrel, 2018). In humans suffering from alcohol addiction and experiencing detoxification, orbitofrontal cortex is disrupted which is hypothesized to underlie impaired inhibitory control and impulsivity leading to relapse (Koob & Volkow, 2009). Finally, OFC metabolic activity is also decreased 8-15 days after alcohol detoxification, with recovery occurring in the subsequent months after (Volkow & Fowler, 1994). In **Chapter 2**, I demonstrate that OFC activity during alcohol cue presentation, reward seeking and consumption of 10% EtOH is statistically distinct between HD and LD animals (Figure 2.4E, H). HD rats' OFC activity during 10% EtOH seeking and consumption closely mimics that of both HD and LD rats' OFC activity during sucrose seeking and consumption (Figure 2.4D, G). During 20% EtOH cue presentation and reward seeking OFC activity is distinct between HD and LD, but not during reward consumption (Figure 2.4F, I). These data support my hypothesis that innate alcohol preference modulates OFC activity consistent with natural reward

preference. In rats whose OFC neurons were transfected with hM4Di DREADDs, both HD and LD, OFC inactivation suppressed cued reinstatement for 10% EtOH and sucrose seeking behavior (Figure 3.6C). Furthermore, OFC inactivation did not suppress alcohol or sucrose consumption for HD or LD during homecage or operant seeking (Figures 3.2 and 3.4). These data support the proposed explanation in **Chapter 4.2.1** that suggests that the OFC does not directly modulate goal-oriented behavior but instead only influences behavior when outcomes/goals are altered.

4.2.3 Functional significance of OFC contributing to reinstatement of palatable drug reward seeking

The present studies contribute to the field by first establishing that the OFC encodes relative innate alcohol preference, OFC inactivation suppresses homecage 20% EtOH consumption in HD rats, and suppresses cued reinstatement for 10% EtOH. For human alcoholics, the greatest threat to sobriety is relapse that is accompanied by a cognitive preoccupation with alcohol (Koob & Moal, 1997) often brought on by stress and alcohol cues (Becker, 2017) that leads to poor decision-making (Antoine Bechara et al., 2001; Seo & Sinha, 2015) leading to relapse. According to a 2003 study by SAMSHA "Alcohol and Drug Services Cost Study", the biomedical cost for addiction-related treatment was $15.6 Billion with treatments such as detoxification costing upwards of $121,381.07/person and diminished productivity in individuals suffering from alcohol use and abuse disorders (~$180,419,200). A key strategy to reduce both the biomedical costs, health repercussions as well as productivity issues is to decrease the frequency of relapse experiences in those suffering from alcoholism. Other studies have shown that the

OFC is necessary for both context and cued reinstatement of cocaine, alcohol and morphine seeking (Fuchs et al., 2004; Hodges et al., 2016; Bianchi et al., 2018; Fakhrieh-Asl et al., 2019). My data suggest that in a non-stressed state, that the OFC may be a key brain region for modulating drug seeking responses to stimuli associated with alcohol. In human cigarette smokers, smoking cessation occurs concomitantly with decreased activation of medial OFC (mOFC) during cue reactivity tasks in functional magnetic resonance imaging (fMRI; Franklin et al., 2011). Repetitive transcranial magnetic stimulation (rTMS) of the dosrsolateral prefrontal cortex (DLPFC) reduces cue-elicited craving for nicotine in smokers Gorelick et al., 2014). Additionally rTMS of the DLPFC significantly suppresses contralateral mOFC activity in response to nicotine cues (Li et al., 2017), suggesting that the OFC is part of the brain network responding to drug-predicting cues and thus contributes to motivation for drug seeking.

4.3 Concluding Remarks

The results of the experiments in this dissertation support the assertion that the OFC plays a significant role in drug use and abuse disorders. Results from my studies reveals individual differences in alcohol consumption in an operant setting and OFC firing activity during reward-seeking and consumption directly represents individual preferences for alcohol. Furthermore, individual variability in alcohol preference in blocked sessions is stable in interleaved sessions (i.e., sucrose and 20% EtOH) with few representations of preference overlap between alcohol and sucrose rewards. Although the OFC does not play a direct role in rewarded drug seeking, OFC is necessary for reinstatement/relapse-related behavior for not just 10% EtOH but also sucrose.

This dissertation contributes to the understanding of how the OFC functions to modulate behavior that is both consistent with the current literature but also sheds light on the subtle role of OFC in flexible behavior. Future work investigating the role of the OFC in alcohol preference and seeking behavior can expand these studies by also looking at how OFC activity during alcohol self-administration differs when stress is introduced or attenuated to both increase and decrease, respectfully, the value of alcohol to more closely mimic the circumstances by which alcoholics relapse or enhance alcohol consumption.

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