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Niharika M. Dighe April 12, 2021

A circuit-specific role for ifenprodil in blocking cocaine-induced habits

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Abstract

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Goal-directed decision-making – i.e., performing actions based on their expected outcomes – is a critically important behavioral adaptation that requires using learned associations between actions and their outcomes or contingencies, to guide future choices. An over-reliance on inflexible habitual responding at the expense of goal-directed behavior represents a core feature of cocaine misuse. The orbitofrontal cortex (OFC), considered a part of the prefrontal cortex, is essential for updating actions based on changes to contingencies. Cocaine use impairs goal-directed behavior and eliminates dendritic spines in the OFC; these effects are more pronounced in adolescents. We have previously shown that mice that are resilient against developing escalatory cocaine seeking display decreased expression of the GluN2B subunit of NMDA receptors, a post-synaptic protein, in the OFC. Here, we show that ifenprodil, a GluN2B-selective antagonist that displays protective effects in animal models of alcohol, nicotine, and heroine relapse, preserves both goal-directed behavior and OFC dendritic spine densities in adolescent cocaine-exposed mice. Since ifenprodil prevents cocaine-induced loss of OFC dendritic spines, which are the principal post-synaptic sites of excitatory axonal projections, we hypothesized that dysregulation of inputs onto OFC neurons may be involved in the emergence of cocaine-induced habits. The OFC receives inputs from many brain regions, including major inputs from the basolateral amygdala (BLA), which plays a key role in memory consolidation in numerous memory systems. We have previously shown that chemogenetic inhibition of BLA→OFC connections during consolidation of novel contingency memories disrupts goal-directed behavior. Here, we demonstrated that ifenprodil administration failed to protect against cocaine-induced habits when BLA→OFC connections were inhibited, suggesting that the observed mechanism by which ifenprodil blocks cocaine-induced habits may act through BLA→OFC connections. It is possible that ifenprodil could prevent the emergence of durable neural adaptations in response to cocaine exposure in adolescence, that disrupt processes such as compositional changes to NMDA subunits and synaptic strengthening of BLA→OFC connections, which could impair decision-making in adulthood. These findings provide a deeper understanding of the effects of cocaine exposure on the adolescent brain and could lead to future treatment options to prevent long-term decision-making impairments.

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Table of Contents

Introduction

Goal-directed decision making

Cognitive control is a critical process that allows us to select actions from numerous possibilities based on our goals and knowledge. This process gives us cognitive flexibility, which is important for selecting actions that lead to a desirable outcome, known as "goal-directed decision making." Goal-directed behavior is also frequently updated based on changes to the relationship between actions and their outcomes (Gazzaniga, Irvy, and Mangun 2014) or "contingencies." For example, if an action that used to lead to a particular outcome no longer produces that desired goal, this new contingency information can be assimilated, and future choices would be updated to no longer engage in that action. Instead, future choices would be in line with actions that still predict desired goals, allowing us cognitive flexibility that is essential for complex behaviors (Balleine and O'Doherty 2010). In contrast to goal-directed decision making is habitual responding, which is stimulus-driven and does not depend on the relationship between actions and their outcomes. This decision making allows for quick responding and tends to be inflexible to changes in contingencies (O'Doherty, Cockburn, and Pauli 2017; Gazzaniga, Irvy, and Mangun 2014). While the difference between goal-directed and habitual behavior is graded and they can be flexibly engaged based on context, an inflexible deferral to habitual responding at the expense of goal-directed behavior is associated with deficits in cognitive control (Gazzaniga, Irvy, and Mangun 2014).

To test action-outcome learning, memory, and updating in mice, we use an instrumental behavioral paradigm called "instrumental contingency degradation." We first train mice in operant conditioning chambers to nose-poke on two distinct apertures for a food pellet reinforcer (Figure 1A). This training occurs over many days and is followed by a "reinforced" session during which

only one aperture is available and nose-poking at this aperture continues to result in reinforcer delivery (Figure 1B). Next, the mice undergo a "degraded" session when only the other aperture is available, but reinforcer delivery is not contingent upon nose-poking; instead, food pellets are delivered at regular intervals (Figure 1C). Therefore, in the reinforced session, the contingency of nose-poke action resulting in reinforcer delivery is maintained, while this contingency is violated in the degraded session. During a final choice test, mice are again given access to both apertures and we observe nose-poke responding at each aperture (Figure 1D). Goal-directed choice behavior is identified based on a significant preference for the previously reinforced vs. degraded aperture, indicating an updating of actions based on changes to contingencies. On the other hand, habit-biased behavior is characterized by equivalent responding on both apertures, indicating a failure to incorporate new contingency information.

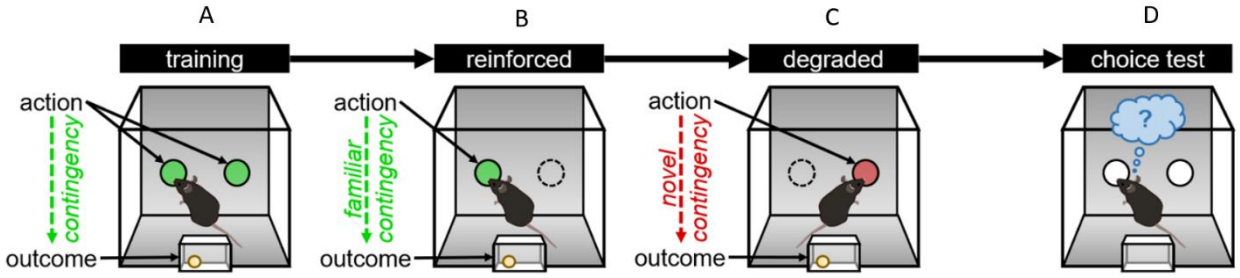

Figure 1: Instrumental contingency degradation task to assess action-outcome decision making in mice. (A) Operant conditioning chambers for training mice to associate nose-poking with food pellet reinforcers. **(B)** This is followed by a reinforced session in which the action-outcome relationship between nose-poking and delivery of the reinforcers is maintained for one aperture while the other is unavailable, thus presenting a familiar contingency. **(C)** Next, mice undergo a degraded session in which this action-outcome relationship is violated for the other aperture, thus presenting novel contingency information. **(D)** During a final choice test, mice are given access to both apertures and we measure activity at each aperture. Goal-directed mice are characterized by preferential activity at the reinforced *vs.* degraded aperture while habit-biased mice show no preference between the apertures.

The role of the orbitofrontal cortex in goal-directed behavior

While cognitive control requires coordinated function of many neural networks, the prefrontal cortex (PFC) plays an important role in supporting goal-directed behavior (Gazzaniga, Irvy, and Mangun 2014). Specifically, the orbitofrontal cortex (OFC) performs numerous functions to process outcome-predictive cues (Schoenbaum et al. 2003), track reward value (Gallagher, McMahan, and Schoenbaum 1999), and facilitate learning and updating the causal relationship between choice and reward (Noonan et al. 2017). Inactivation of the OFC impairs action-outcome decision making and leads to engagement in inflexible habitual responding (Zimmermann et al. 2017; Gremel and Costa 2013). The OFC is particularly important for flexibly updating behavior based on changes to contingencies (Li and Gourley 2020). Updating action-outcome association triggers activity-dependent dendritic spine plasticity on excitatory neurons in the OFC, a process necessary for mice to optimally select actions based on their likely outcomes (Whyte et al. 2019). This functional importance of the OFC in flexibly updating action-outcome behavior based on changes in contingencies makes it a neural target to explore in the context of cocaine-induced disruptions to goal-directed behavior (Everitt and Robbins 2016).

Maladaptive effects of cocaine on goal-directed behavior

Cocaine use affects millions of individuals around the world. According to the National Survey on Drug Use and Health (NSDUH), 57.2 million Americans (aged 12 or older) used illicit drugs in 2019, of which 5.5 million were users of cocaine. 97,000 of these users were adolescents (12-17 years of age) while 1.8 million users were young adults (18-25 years of age) (Han 2020). Cocaine-use has harmful physiological effects like disruptions in heart rate and blood pressure and can also cause anxiety, panic, and paranoia (NIDA 2020b).

Cocaine exposure can cause myriad changes in the brain including adaptations in the reward system and cognition that drive drug-seeking behaviors. Cocaine-induced cognitive impairments particularly affect decision making and adaptive learning by disrupting activity in the OFC (NIDA 2020a). Cocaine's maladaptive effect on cognition is thought to be a result of impaired cognitive control (NIDA 2020a), and also an inflexible shift from goal-directed to habitual decision making (Everitt and Robbins 2016), which both implicate the OFC. Many cocaine users exhibit reduced gray matter volume in the OFC (Franklin et al. 2002), which has been correlated with longer duration of drug dependence and compulsivity (Ersche et al. 2011). There is also distinct abnormal activity of the OFC with prolonged exposure to cocaine (Adinoff et al. 2001) and impaired performance on numerous OFC-dependent learning tasks (Lucantonio et al. 2012), including tasks similar to instrumental contingency degradation (Figure 1) (Ersche et al. 2016). Studies in mice have shown that cocaine exposure can also lead to reduced dendritic spine length and density in the OFC, which are crucial for plasticity and learning (Gourley et al. 2012).

Cocaine's disruptive effects on goal-directed behavior and dendritic spine dynamics are even more pronounced in adolescents who are more vulnerable to addiction and more susceptible to drug-dependence in adulthood (Chambers, Taylor, and Potenza 2003). Exposure to cocaine during adolescence can lead to simplification of dendritic arbors (DePoy et al. 2014) and also eliminate dendritic spines in the OFC (Gourley et al. 2012). A previous study from our lab showed that mice that were administered cocaine during adolescence engaged in habitual responding at the expense of goal-directed behavior in adulthood and also exhibited reduced dendritic spine density on excitatory OFC neurons (DePoy et al. 2017).

Dendritic spines are protrusions on dendrites that form the primary sites of excitatory synapses in the brain and are thus important for synaptic plasticity and learning (Nimchinsky, Sabatini, and Svoboda 2002). Therefore, they may play an important role in supporting decision making. Since dendritic spines are the principal post-synaptic sites of excitatory neurotransmission from axonal projections (Berry and Nedivi 2017), we hypothesized that dysregulation of inputs onto OFC neurons may be involved in the emergence of cocaine-induced habits.

Connections between the orbitofrontal cortex and the basolateral amygdala

An important input to the OFC is from the basolateral amygdala (BLA), which is a nucleus in the amygdala that is essential for regulating reward-related behavior (Yang and Wang 2017). It is important for understanding changes in reward value and contributes to adaptive learning (Stolyarova and Izquierdo 2017). The BLA and the OFC have strong bidirectional connections and their lesions produce similar deficits in reward-related behavior (Sharpe and Schoenbaum 2016). Furthermore, the BLA and the OFC are thought to co-regulate different aspects of cocaine-seeking behaviors (Lasseter et al. 2011). The BLA is thought to assign motivational (Corbit, Leung, and Balleine 2013) and emotional significance (Balleine and Killcross 2006) to events. Therefore, the BLA is considered to be important for supporting memory-modulatory functions such as consolidation, which leads to memory enhancement and stronger, more vivid memories (McIntyre et al. 2003; Bass and Manns 2015). Particularly in context of goal directed behaviors, the BLA is also important for consolidating action-outcome memories (Zimmermann et al. 2018). Rats with BLA lesions were unable to consolidate action-outcome contingencies such that they were unable to update behavior based on degraded contingencies (Balleine, Killcross, and Dickinson 2003). Since these behaviors are also supported by the OFC, this discovery implies a

functionally cooperative relationship between the BLA and the OFC in action-outcome decision making, that has yet to be fully explored.

In a prior experiment, we selectively inactivated BLA→OFC connections using Cre-recombinase-dependent inhibitory Gi-DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). These inhibitory receptors can only be expressed in the presence of a Cre-recombinase enzyme, which inverts part of the viral DNA containing the Gi-DREADD. These receptors can be activated by intraperitoneal administration of clozapine-N-oxide (CNO) and since the construct requires both the Gi-DREADD, infused into the BLA, and Cre-recombinase, infused into the OFC, to be active, we were able to selectively inactivate this projection at defined times (Figure 2A) (Zhu et al. 2016). We administered CNO immediately after the degraded session, thus silencing these neurons immediately following an experience when a new contingency context (*i.e.*, the degraded session) was introduced. We focused on this epoch, which can be thought of as the consolidation phase of new contingency learning, due to BLA's importance in consolidation processes (Balleine, Killcross, and Dickinson 2003; Bass and Manns 2015). This experiment revealed that inactivating BLA→OFC connections during new contingency learning disrupted subsequent goal-directed responding, leading mice to instead defer to familiar, habit-like responding (Figure 2B). We also found that inactivating BLA→OFC connections during the retrieval phase of this task, *i.e.*, the choice test, had no effect on goal-directed behavior (Figure 2C), suggesting that BLA→OFC connections are particularly essential for consolidating new action-outcome information (Li, Dighe, et al., in review).

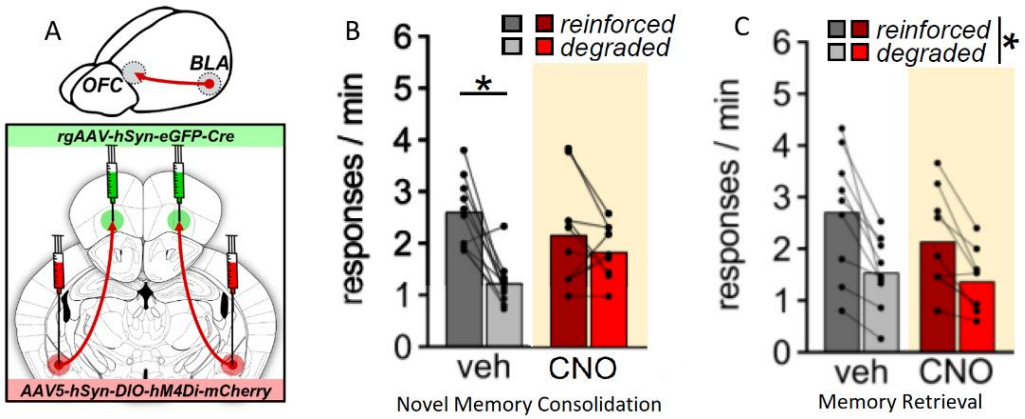

Figure 2: BLA→OFC connections are needed for novel memory consolidation in action-outcome-based decision making. (A) Selective chemogenetic inhibition of BLA→OFC connections using Cre-recombinase (infused in the OFC) and Cre-dependent Gi-DREADDs (infused in the BLA), by administration of CNO. **(B)** Inactivating BLA→OFC connections during novel memory consolidation led to a loss of goal-directed behavior. **(C)** In contrast, inactivating BLA→OFC connections during memory retrieval resulted in no disruptions in goal-directed behavior. Data from Li, Dighe, et., al., in review.

Understanding the role of NMDA receptors in cocaine-induced behaviors

To investigate cocaine's disruptive effect on goal-directed behavior, we investigated proteins that might be involved in synaptic plasticity. The N-methyl-D-aspartate receptor (NMDAR) was of particular interest, because it is a type of glutamate receptor that plays an important role in plasticity at excitatory synapses. NMDAR activity leads to an influx of calcium ions into dendritic spines and can induce multiple forms of synaptic plasticity such as long-term potentiation (LTP) and long-term depression (LTD) (Paoletti, Bellone, and Zhou 2013). NMDAR activity has also been linked to dendritic spine morphology and structure (Chen et al. 2011). The NMDAR is comprised of numerous subunits, including the GluN2A and GluN2B subunits, which are involved in synaptic plasticity, including during development (Ortinski 2014). The GluN2B subunit may be particularly important for synaptic plasticity since postsynaptic density of

GluN2B-containing NMDARs might lead to an increased influx of calcium ions into dendritic spines compared to GluN2A subunits in adolescent rodents (Shipton and Paulsen 2014).

Many studies that investigate NMDARs have used the non-competitive GluN2B-selective antagonist ifenprodil, which exhibited protective effects in animal models of alcohol (Vengeliene et al. 2005), heroin (Shen et al. 2011), and nicotine use (Gipson et al. 2013). Ifenprodil also blocked cocaine-induced behaviors in non-human primates (Fujiwara et al. 2007) and cocaine-induced increases in NMDARs in the nucleus accumbens (NAc) in mice (Schumann and Yaka 2009). DePoy et al. (2017) exposed mice to cocaine during adolescence and administered them ifenprodil in adulthood. Cocaine-exposed mice treated with ifenprodil engaged in goal-directed behavior, while cocaine-exposed mice deferred to habitual responding. Ifenprodil administration also led to higher dendritic spine densities in the OFC compared to cocaine-exposed mice that expressed markedly reduced levels of spine density. Another prior study in our lab revealed that mice that were resilient to cocaine-induced deficits in action-outcome-based decision making displayed reduced levels of GluN2B in the OFC (Li, Pitts, and Gourley, unpublished). Together, these studies suggest that the GluN2B subunit of NMDARs could be a mechanism by which cocaine induces habitual behavior and dendritic spine loss.

Hypotheses

To further test the role of GluN2B-mediated signaling in modulating action-outcome-based decision making in cocaine-exposed conditions, we co-administered cocaine and ifenprodil during adolescence and conducted the contingency degradation task in adulthood. We also conducted structural and morphological analyses to determine the effect of ifenprodil in preventing cocaine-induced loss of dendritic spine density**. I hypothesized that cocaine-exposed mice that were administered ifenprodil would engage in goal-directed decision making and also express typical dendritic spine densities on OFC neurons.**

We also investigated the role of BLA→OFC projections in ifenprodil's effect on goal-directed behavior in cocaine-exposed mice since dysregulation of inputs to the OFC appears to be a possible mechanism for cocaine-induced disruptions to action-outcome-driven behavior. We co-administered ifenprodil and cocaine to mice and then selectively inactivated the BLA→OFC projections during the consolidation period of the instrumental contingency degradation task (Figure 2). Here, **I hypothesized that ifenprodil's blockade of cocaine-induced disruptions to goal-directed behavior would require functionally intact BLA→OFC projections.**

Methods:

Experimental subject details

Multiple strains of mice were bred from Jackson Laboratories stock for use in this study. Cocaine and ifenprodil behavioral and BLA→OFC behavioral experiments used C57BL/6J mice (stock #000664; total n=70) while dendritic spine imaging was performed using B6.Cg-Tg (*Thy1*-YFP) HJrs/J mice (stock #003782; total n=44), back-crossed onto a C57BL/6 background, which express yellow fluorescent protein (YFP) in deep-layer pyramidal neurons (Feng et al. 2000).

Mice were housed on a 12-hour light cycle and provided food and water *ad libitum* prior to behavioral testing. Throughout food-reinforced instrumental conditioning, mice were food-restricted until body weights were reduced to 90-93% of baseline to motivate responding for food reinforcers. For all food-reinforced experiments, instrumental training began between postnatal day (P) 60-70. Separate groups of experimentally naïve mice were used for each experiment. All experimental procedures were performed in accordance with NIH Guidelines for the Care and Use of Laboratory Animals and were approved by the Emory University Institutional Animal Care and Use Committee (IACUC).

Behavioral testing apparatus and food-reinforcement training

All behavioral testing was conducted using operant conditioning chambers equipped with two distinct nose-poke apertures (left vs. right) and a retractable lever, along with a separate magazine for food pellet delivery or liquid dipper access (Med Associates). Mice were trained to nose poke for delivery of a 20 mg grain pellet food reinforcer (Bio-Serv). Initial training proceeded under a fixed ratio 1 (FR1) schedule of reinforcement in which each nose-poke response resulted in delivery of a single reinforcer. Mice underwent daily training sessions which lasted either 70 minutes or until mice earned 30 reinforcers from responding on each of the two apertures (60 total

reinforced responses per session), whichever occurred first. Overall, mice required between 5 and 7 training sessions to acquire 60 pellets within one session, at which point response training was considered completed.

Contingency degradation sessions

Following training, mice underwent the contingency degradation procedure consisting of two, 25-minute sessions on consecutive days. On the first day, only one aperture was available and nose-poking at this aperture continued to result in reinforcer delivery while the other was occluded. On the second day, the opposite aperture was occluded, but responding on the available aperture was no longer contingent on upon nose-poking action. Instead, pellets were delivered at a rate equal to what each mouse experienced during the previous day's reinforced session, independent of nose-poking responses. Thus, for one aperture, the association between nose-poking (action) and pellet delivery (outcome) was "reinforced", while for the other aperture, that contingency was "degraded". Which aperture (left vs. right) was designated to be associated with the degraded contingency was counterbalanced within and between groups. Mice were next placed back into the same chambers on a third day for a brief 15-minute choice test when both apertures were available, and nose-poke activity on either aperture was recorded. Goal-directed choice behavior is identified based on a significant preference for the previously "reinforced" vs. "degraded" aperture while habit-biased behavior is characterized by equivalent responding on both apertures, indicating a failure to incorporate new contingency learning.

Cocaine and ifenprodil administration

Cocaine (10 mg/kg in saline; Sigma-Aldrich) and ifenprodil (10 mg/kg in 2% DMSO and saline; Tocris Bioscience), were administered intraperitoneally from postnatal day (P) 31-35, with ifenprodil administered 30 minutes prior to cocaine.

Dendritic spine imaging and reconstruction

Thy1-YFP mice were anesthetized with a ketamine (100 mg/kg) and xylazine (10 mg/kg) and trans-cardially perfused with ice-cold phosphate-buffered saline (PBS) and 4% paraformaldehyde (PFA). Brains completed fixation in 4% PFA for 48 hours and were then transferred to 30% w/v sucrose. Fixed brains were sectioned into 50 µm coronal sections using a freezing microtome. Basal dendritic segments located 50-150 µm from the soma on deep-layer, YFP-expressing OFC neurons were identified using a Leica DM5500 microscope (Leica Microsystems) and imaged with spinning disk confocal (VisiTech International). Z-stack series containing dendritic segments were collected using a 100x 1.4 NA oil-emersion objective and a 0.1 µm step size. Three-dimensional reconstructions of dendritic spine morphologies were performed using the Filament Tracer module in Imaris software (Oxford Instruments). Dendritic spines along an approximately 20 µm dendritic segment were identified using a semi-automated auto-depth function.

Morphological classification of dendritic spines was performed using established parameters for pyramidal PFC neurons (Radley et al., 2013). For spines with a head:neck diameter ratio ≥1.1, those with head diameter ≥0.4 µm were classified as mushroom-type or otherwise considered thin-type. For spines with a head:neck diameter ratio <1.1, those with a spine length:neck diameter ratio ≥2.5 were also classified as thin-type or otherwise considered stubby-type. Only one dendrite was analyzed per cell to avoid over-representation within our dataset and

8 dendrites were analyzed per animal. Sections from branched dendrites with low background noise and optimal fluorescence were chosen, which were distributed equally across anterior and posterior regions of the OFC.

BLA→OFC selective chemogenetic manipulations

Intracranial surgeries were performed two weeks prior to the start of instrumental training to allow for expression of viral vectors. Mice were anesthetized with ketamine (80 mg/kg) and dexmedetomidine (0.5 mg/kg) and placed in a digitized stereotaxic frame (Stoelting). Surgeries were performed under aseptic conditions. Viral vectors were infused over the course of 5 minutes using a microliter syringe (Hamilton), which was left in place for an additional 10 minutes before retraction to restrict off-target viral vector spread. To achieve projection selectivity, a retrogradely transported Cre-recombinase construct (AAVrg-hSyn-HI-eGFP-Cre-WPRE-SV40; Addgene, deposited by James Wilson) was infused into the OFC alongside an anterogradely transported Cre-dependent chemogenetic receptor construct in the BLA (AAV5-hSyn-DIO-hM4D(Gi)-mCherry; Addgene, deposited by Bryan Roth (Krashes et al. 2011)).

Following behavioral testing, brains were fixed and prepared as described above, and GFP or mCherry expression was examined to determine the extent of Cre and hM4D(Gi) expression. The chemogenetic receptor ligand CNO (0.1 mg/kg in 2% DMSO and saline; RTI International) was administered intraperitoneally immediately following contingency degradation, thus modulating activity of chemogenetic receptor-expressing neurons during memory consolidation.

Statistical Analyses

Statistical analysis was performed using SPSS software (IBM) and with $\alpha<0.05$. For behavioral experiments, response rates were compared using ANOVA. The specific factors analyzed for each ANOVA differ based on experiments and are detailed in the figure legends. *Post-hoc* comparisons were performed following significant interaction effects or main effects distinguishing >2 conditions. Different *post-hoc* tests were used based on experiments as described in the figure legends.

Results

Ifenprodil co-administration with cocaine preserves goal-directed decision making

We first investigated whether attenuating GluN2B-mediated signaling could block cocaine-induced disruptions in action-outcome-based decision making. We first administered either ifenprodil (ifen) or a vehicle (veh) control followed by cocaine (coc) or saline (sal) using intraperitoneal injections during adolescence (P31-35). Four weeks later, mice were assessed for their capacity to use novel contingency information to update behavior and guide future choices using the instrumental contingency degradation task. Neither cocaine nor ifenprodil affected responding during training (Figure 3A). Cocaine-exposed mice failed to use action-outcome contingencies to guide choices following instrumental contingency degradation, instead deferring to habitual responding, and this effect was blocked by ifenprodil (Figure 3B). This outcome suggests that ifenprodil co-administration blocks cocaine-induced deficits in linking actions and outcomes, thus preserving goal-directed behavior.

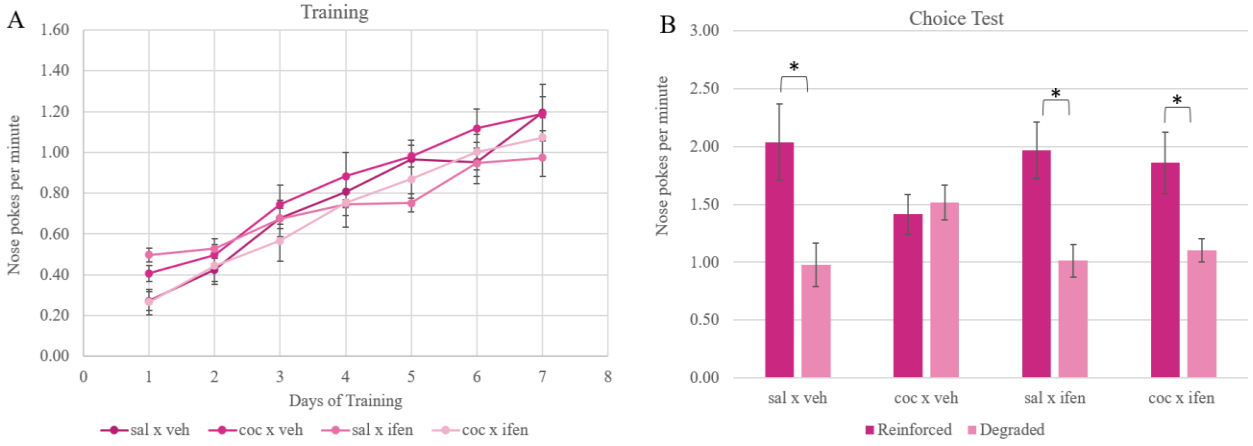

Figure 3: Blockade of cocaine-induced disruptions to goal-directed behavior with ifenprodil. (A) There is a significant effect for training days ($F_{6,198}$ = 74.115, p<0.001 for days) with no significant difference between groups ($F_{6,198}$ = 1.681, p>0.05, other Fs<1). **(B)** There was a significant main effect of contingency ($F_{1,33}$ = 44.714, p<0.001) and interaction between contingency x ifen x CNO ($F_{1,33}$ = 5.854, p=0.021). Post-hoc t-tests: sal x veh, p<0.01, sal x ifen, p<0.01, coc x veh, p>0.05, coc x ifen, p<0.05. Bars and symbols represent group means. All error bars show +/- SEMs. *p<0.05.

Cocaine-induced reduction in OFC dendritic spine density is prevented by ifenprodil

We next examined the density and structure of dendritic spines in the OFC, which are the primary sites of excitatory neurotransmission and plasticity in the brain, by performing high fidelity, three-dimensional dendritic reconstructions (Figure 4A). We focused on basal dendrites of pyramidal neurons in the OFC which project to and receive inputs from subcortical regions in the OFC (DeNardo et al. 2015) and are vulnerable to cocaine-induced structural changes (DePoy et al. 2017). Exposure to cocaine reduced total dendritic spine density in the OFC while ifenprodil prevented this spine loss (Figure 4 A&B).

We conducted morphological categorical analysis by focusing on three main dendritic spine subtypes: mushroom, thin, and stubby. Mushroom spines have large heads and contain most of the excitatory synapses; they are fully mature and important in maintaining synaptic plasticity. Thin spines are smaller and contain smaller excitatory synapses, whereas stubby spines are shorter and are less prevalent in adult cortices. Both of these subtypes are thought to be more immature (Berry and Nedivi 2017). This analysis showed that mushroom dendritic spine density was specifically reduced with cocaine exposure (Figure 4C).

We also conducted a correlational analysis between the total OFC dendritic spine density and the choice test preference ratio, which is the ratio between activity at the reinforced *vs.* degraded apertures during the choice test. A ratio of 1 indicates equal activity at both apertures, *i.e.,* no preference for either aperture, which is reflective of habitual responding, while a ratio greater than 1 indicates preferential activity at the reinforced aperture, reflective of goal-directed behavior. Therefore, the higher the preference ratio, the higher the engagement in goal-directed behavior. We found a positive correlation between dendritic spine density and engagement in goal-directed behavior (Figure 4D), suggesting that stable substrates for synaptic presence in the OFC

are crucial for action-outcome learning and memory. Overall, cocaine's disruptive effect on action-outcome decision making could be mediated by dysregulation of post-synaptic signaling and neural structure in the OFC, which is important for goal-directed behavior (Whyte et al. 2019).

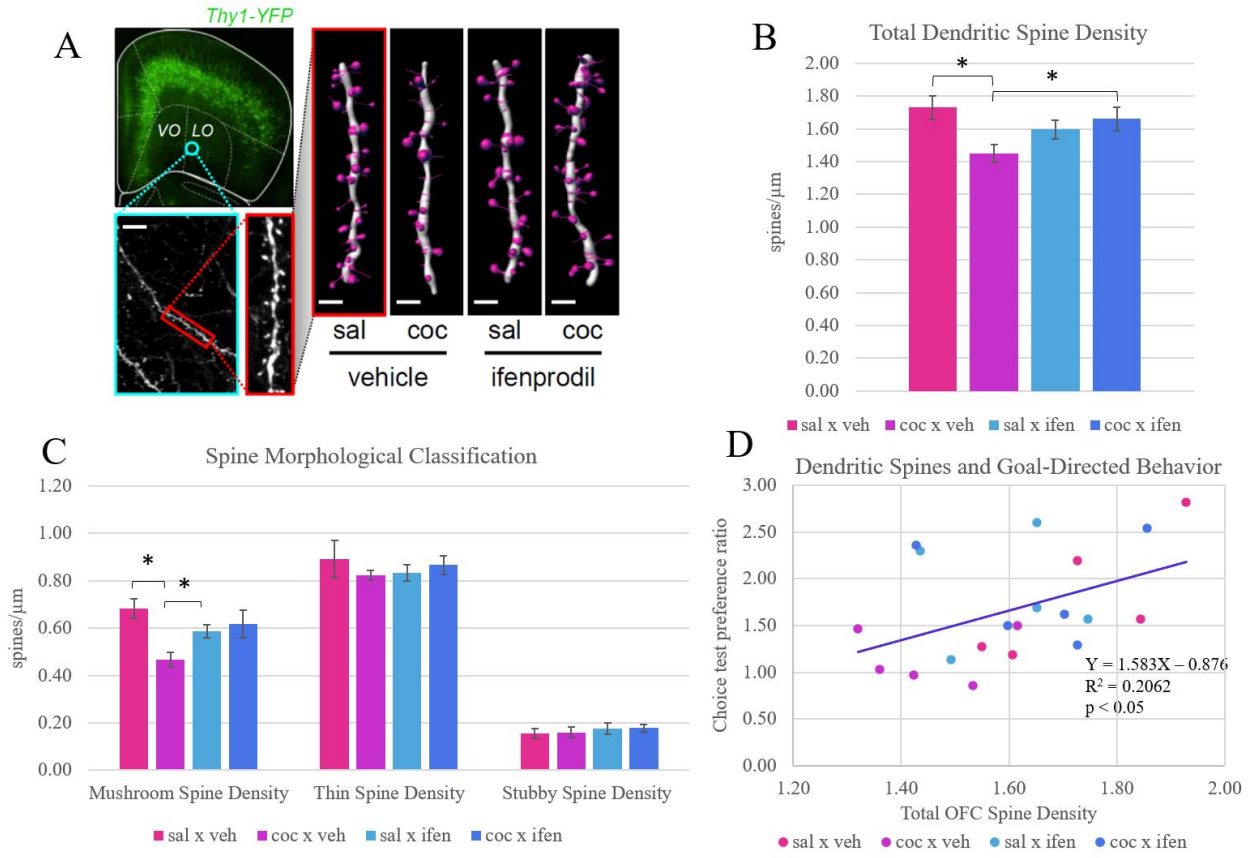

Figure 4: Ifenprodil prevents cocaine-induced loss of dendritic spine density. (A) Representative images of dendritic spines from *Thy1*-YFP mice. Upper left: Ventral and lateral sections of the OFC, Bottom left: Section of a basal dendrite, Inset: Dendritic spines on the basal dendrite, Right: 3D reconstructions of basal dendrites. **(B)** There was a significant interaction between groups in spine density (coc * ifen, $F_{1,16} = 7.333$, $p<0.05$). *Post-hoc* t-tests: sal x veh VS coc x veh, $p<0.05$ and coc x veh VS coc x ifen, $p<0.05$. **(C)** There was also a significant interaction between groups in mushroom dendritic spine density analyzed using 2-factor ANOVA (coc * ifen, $F_{1,16} = 8.903$, $p<0.01$). *Post-hoc* t-tests: sal x veh VS coc x veh, $p<0.05$, sal x ifen VS coc x veh, $p<0.05$, and coc x veh VS coc x ifen, $p=0.054$. No other comparisons reached significance. **(D)** Significant positive correlation between the choice test preference ratio ($R^2 = 0.2062$, $F_{1,18} = 4.675$, $p<0.05$). Bars represent group means. Symbols represent individual mice. All error bars show +/- SEMs, *$p<0.05$.

Correction of action-outcome learning and memory following cocaine requires functional BLA→OFC connections

Considering that cocaine appears to dysregulate the sites of input to the OFC, we next asked whether BLA→OFC projections are required for ifenprodil to correct cocaine-induced impairments to goal-directed behavior. We once again administered either ifenprodil (ifen) or a vehicle (veh) control followed by cocaine in all mice during adolescence (P31-35). After 4 weeks, mice underwent surgery for infusion of inhibitory chemogenetic constructs. We inhibited BLA→OFC projections during the consolidation process (CNO for inhibition and vehicle for control) as described earlier (Figure 2).

Response rates did not differ in training (Figure 5A). Ifenprodil once again preserved goal-directed behavior in cocaine-exposed mice that otherwise deferred to habitual responding. However, inhibiting BLA→OFC projections during novel contingency memory consolidation abolished this effect (Figure 5B). This outcome suggests that the mechanism by which ifenprodil preserves goal-directed behavior requires functional BLA→OFC connections.

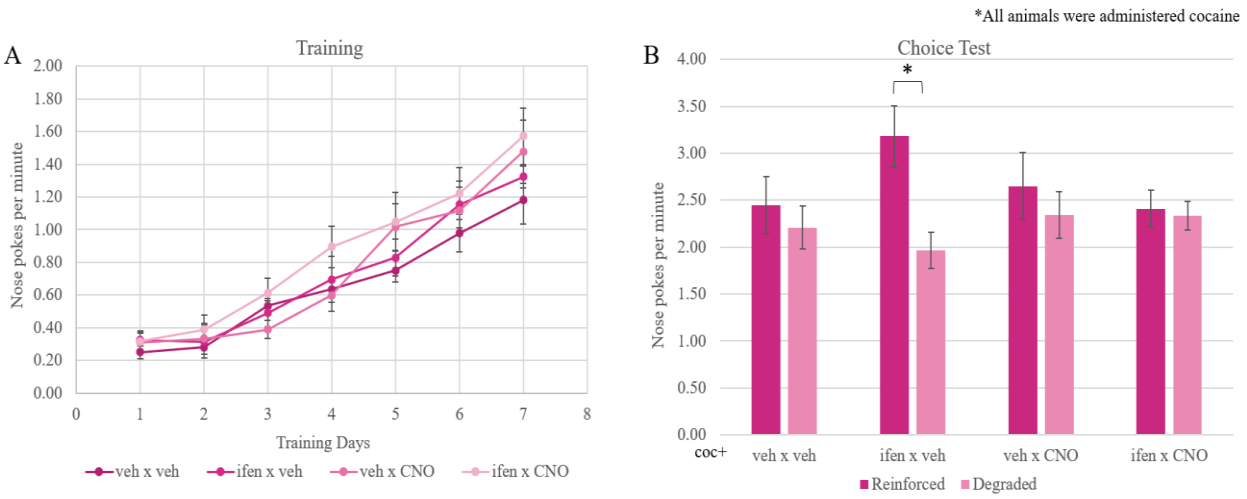

Figure 5: Ifenprodil's blockade of cocaine-induced disruptions to goal-directed behavior requires functionally intact BLA→OFC connections. (A) Significant effect of training days ($F_{6,174}$ = 100.035, p<0.001 for days) with no significant difference between groups (all other Fs<1). **(B)** There was a significant main effect of contingency ($F_{1,29}$ = 9.729, p=0.004) and interaction between contingency x ifen x CNO ($F_{1,29}$ = 4.243, p=0.048). *Post-hoc* t-tests: veh x veh, p>0.05, veh x CNO, p>0.05, ifen x veh, p<0.05, and ifen x CNO, p>0.05. Bars and symbols represent group means. All error bars show +/- SEMs, *p<0.05.

Discussion

Flexible goal-directed decision making is disrupted by cocaine exposure (Everitt and Robbins 2016) and also results in reduced dendritic spine density in the OFC, which is more pronounced in cases of adolescent exposure (DePoy et al. 2017). Here, we demonstrated that co-administration of the GluN2B-selective antagonist ifenprodil with cocaine can block deficits in goal-directed decision making and prevent cocaine-induced dendritic spine loss in the OFC. We also showed that functionally intact BLA→OFC projections are necessary for the ability of ifenprodil to preserve goal-directed behavior in cocaine-exposed mice, suggesting that the action of ifenprodil in mitigating cocaine-induced habits modulates either BLA→OFC connections, distributed brain networks that require BLA→OFC projections, or some combination thereof.

Ifenprodil blocks cocaine-induced disruptions in goal-directed behavior

Adolescence represents a vulnerable period for cocaine-induced dysregulation of NMDAR signaling, likely driven by GluN2 subunit compositional changes. For example, cocaine exposure increases expression of NMDARs in the ventral tegmental area (Schumann, Michaeli, and Yaka 2009) and disrupts postsynaptic glutamatergic signaling in the medial prefrontal cortex (mPFC) (Caffino, Messa, and Fumagalli 2018) of adolescent rats, which is not observed in adult rats. Cocaine-induced NMDAR disruptions during adolescence could be attributed to changes in GluN2 subunit composition and maturation of synapses during development. Early development is characterized by high levels of GluN2B-containing excitatory synapses, which are replaced by GluN2A subunits in adulthood (Ortinski 2014; Shipton and Paulsen 2014). This critical developmental change might account for synaptic maturation and can prime the adult brain for LTP induction (Smaga, Sanak, and Filip 2019), mediated by GluN2A subunits (Kohr et al. 2003).

Ifenprodil's selective inhibition of the GluN2B subunit could promote glutamatergic signaling through GluN2A-containing NMDARs, which could preserve goal-directed behavior in cocaine-exposed mice.

Another consideration is that we administered ifenprodil prior to cocaine. Huang et al. (2009) demonstrated that cocaine exposure during adolescence led to upregulation of GluN2B but not of GluN2A subunits in the nucleus accumbens (NAc). They hypothesized that cocaine could induce GluN2B-regulated silent synapses (NMDAR-dominant synapses that are less likely to conduct postsynaptic current (Paoletti, Bellone, and Zhou 2013)) in adolescence, which could have long-lasting changes in adulthood (Huang et al. 2009). This model of silent synapses was observed in the NAc, which constitutes mainly GABAergic medium spiny neurons and supports reward-related behaviors (Soares-Cunha et al. 2020); therefore, it might not be exactly applicable to the pyramidal neurons in the OFC and action-outcome-driven behavior in our study. However, Huang et al.'s results demonstrate that GluN2B regulation might be involved in acute or early synaptic responses to cocaine exposure and can trigger durable cocaine-elicited neural adaptations in adulthood, as we observe in our study with loss of goal-directed behavior and reduced dendritic spine density. In other words, preemptively inhibiting GluN2B prior to cocaine exposure during adolescence could lead to the preservation of goal-directed behavior and healthy dendritic spine density in adulthood by protecting against cocaine-induced silent synapses.

Ifenprodil preserves dendritic spine densities, despite cocaine exposure

We demonstrated that ifenprodil preserved dendritic spine density in the OFC, which was reduced with cocaine exposure. Cocaine administration can eliminate dendrites and dendritic spines on excitatory neurons in prefrontal regions such as the medial prefrontal cortex (Radley et al. 2015) and the OFC (DePoy et al. 2014; Gourley et al. 2012; DePoy et al. 2017; Radley et al.

2015). We also observed that the density of mushroom-shaped dendritic spines was particularly reduced with cocaine exposure, which are more important for synaptic plasticity and learning and memory processes (Berry and Nedivi 2017), while other spine types showed no significant changes. Ifenprodil's preservation of dendritic spine density could potentially be attributed to the boosting of GluN2A-mediated signaling which is thought to stabilize and increase maturation of excitatory synapses, specifically through increasing density of mature, mushroom dendritic spines (Dong and Nestler 2014), as observed in our results.

The relationship between dendritic spine density and action-outcome-based decision making

We demonstrated that mice with higher OFC dendritic spine densities had greater engagement in goal-directed behavior, with cocaine-exposed mice expressing low dendritic spine densities and poor engagement in goal-directed behavior. This outcome could indicate that higher OFC dendritic spine density could support engagement in goal-directed behavior, as dendritic spine synthesis in cortical and subcortical regions has been linked to different learning and memory systems (Moser, Trommald, and Andersen 1994; Leuner, Falduto, and Shors 2003; Restivo et al. 2009; Vetere et al. 2011; Bock et al. 2014; Kuhlman et al. 2014; Mahmmoud et al. 2015; Ma et al. 2016; Swanson, DePoy, and Gourley 2017). Whyte et al. (2019) demonstrated that learning and updating associations between actions and outcomes eliminated immature, thin dendritic spines, resulting in higher proportions of mature, mushroom-shaped dendritic spines in the OFC. This outcome suggests that morphological changes to dendritic spines are associated with learning and memory processes, as thin, immature dendritic spines are pruned away with experience to increase availability of mature mushroom-shaped dendritic spines, which are considered necessary for long-term memory formation (Kasai et al. 2010). Thus, a reduction in mushroom-shaped dendritic

spines (as with cocaine-exposed mice here) could in turn disrupt memory processes (linking actions and outcomes), resulting in a loss of goal-directed behavior.

The role of BLA→OFC projections in goal-directed action

We next examined the role of BLA→OFC connections in cocaine-induced disruptions to action-outcome-based decision making, demonstrating that ifenprodil was not able to preserve goal-directed behavior in cocaine-exposed mice with inactivated BLA→OFC connections. Cocaine exposure could dysregulate inputs into the OFC by reducing dendritic spine density, as observed in our results, since structural and morphological changes to dendritic spines have been linked to altered inputs on pyramidal neurons (Lang et al. 2004; Araya, Eisenthal, and Yuste 2006; Harvey et al. 2008). Since BLA→OFC connections are glutamatergic inputs that undergo synaptic strengthening during development (Cunningham, Bhattacharyya, and Benes 2002), it is possible that cocaine exposure could dysregulate BLA→OFC projection maturation in adolescence by disrupting dendritic spine dynamics. Ifenprodil's preservation of dendritic spine density could thereby preserve glutamatergic signaling at the BLA→OFC synapses, potentially through GluN2A-mediated synaptic maturation (Smaga, Sanak, and Filip 2019). An important consideration is that our dose of 0.1mg/kg of CNO is a low, sub-threshold dose that alone is not sufficient to impair flexible contingency learning in drug-naïve, DREADD-expressing mice (Li, Dighe, et al., in review). Therefore, loss of goal-directed behavior could be attributed to the inactivation of BLA→OFC connections during consolidation of novel contingencies, rather than an effect of global inactivation, which could thus be involved in ifenprodil's mechanism for preventing cocaine-induced habits.

One consideration is that the BLA→OFC connections might be modulating signals from other excitatory glutamatergic inputs to the OFC, such as inputs from the hippocampus, which has

strong connections with the OFC (Ross et al. 2013). It is possible that cocaine exposure could disrupt these joint inputs through decreased dendritic spine density, which might be preserved with ifenprodil administration. Inactivating BLA→OFC inputs could jointly dysregulate other inputs to the OFC too, thus abolishing goal-directed behavior.

Future directions

The results of this study underscore the importance of dendritic spine dynamics and glutamatergic signaling in action-outcome-based decision making, which can be affected by cocaine exposure. My results also reveal that functional BLA→OFC inputs are necessary for action-outcome-based decision making; thus, future studies should focus on how cocaine affects BLA inputs onto OFC neurons. Converging projections from other brain areas should also be considered. Since the hippocampus is also important for the consolidation of memories (Manns et al. 2007) and can interact with the OFC to guide flexible goal-directed behavior (Wikenheiser and Schoenbaum 2016), one future direction would be to comprehensively investigate whether inactivating hippocampus→OFC connections during the consolidation of action-outcome memories disrupts subsequent goal-directed behavior. An initial investigation in our lab indicated that inactivating hippocampus→OFC connections during memory consolidation processes can disrupt action-outcome behavior (Barfield and Gourley 2019). What information is being conveyed to the OFC is unclear, and whether these connections are affected by cocaine could be investigated. Given apparently similar functions of BLA→OFC and hippocampus→OFC connections, future experiments could also attempt to elucidate whether and how these connections jointly control goal-directed behavior, or if one of these connections plays a more significant role, while the other is more supportive in nature.

Conclusion

The disruptive effect of cocaine on decision making can be characterized, in part, by a maladaptive deferral to habitual responding (Ersche et al. 2016). Research in rodents shows that cocaine reduced dendritic spine density on excitatory neurons in the OFC (DePoy et al. 2017), which is important in updating action-outcome learning, and in the absence of dendritic spine plasticity, mice defer to habitual behavior (Whyte et al. 2019). Cocaine-induced decision-making biases are observed in humans as well as mice, thus investigating interventions to block cocaine-induced disruptions in goal-directed behavior in model organisms can have significant translational impacts. In this study, we showed that the GluN2B-selective antagonist ifenprodil can preserve goal-directed behavior and dendritic spine densities in adolescent cocaine-exposed mice. My results also highlighted the importance of BLA→OFC connections in goal-directed behavior, which underscores the importance of glutamatergic signaling and dendritic spine density on OFC neurons receiving subcortical input in day-to-day decision making.

My experiments used adolescent-onset cocaine exposure. Given the large adolescent population of cocaine users (NIDA 2020b) as well as the disproportionately harmful effects of cocaine in adolescents (DePoy et al. 2017), the results of this study importantly further our understanding of the effects cocaine on the adolescent brain. These results demonstrate that targeting glutamatergic signaling in adolescence can mitigate long-term cocaine-induced disruptions to goal-directed behavior. Although clinical trials of ifenprodil and other GluN2B-selective antagonists have thus far been unsuccessful because of detrimental side effects (Paoletti, Bellone, and Zhou 2013), these results could lead to future treatment options to prevent long-term decision-making impairments.

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