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Gracy Trinoskey-Rice April 8, 2019

Elucidating the role of β1-integrin-expressing neurons in corticostriatal regulation of goal-directed behavior

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Abstract

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Abstract: Flexible goal-directed behavior can depend on the ability to form action-outcome associations and update these associations in dynamic contexts. Corticostriatal circuits regulate some goal-directed behaviors via orbitofrontal cortex (OFC) modulation of striatal neurons. Meanwhile, β1-integrins are cell adhesion receptors necessary for dendrite and dendritic spine structure and stability. This study aims to elucidate whether β1-integrin-expressing neurons in the OFC regulate goal-directed action via projections to CaMKII-expressing neurons in the dorsomedial striatum (DMS).

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Introduction

Corticostriatal regulation of goal-directed behavior:

Certain behaviors and decision-making strategies are thought to be motivated by the ability of humans and rodents to associate actions with their resultant outcomes, and with the positive or negative value of these outcomes. Goal-directed behavior is characterized by the selection of an action based on its association with a positive outcome, or in order to avoid a negative outcome. In some cases, after repetition of a reinforced behavior, goal-directed actions can become stimulus-bound or "habitual," and thus, less sensitive to changes in action-outcome relationships. While both goal-directed and habitual actions are needed for adaptive and efficient behavior, neuronal changes that shift the balance between the two strategies, toward habit-dominated behavior, can lead to behavioral patterns associated with diseases such as addiction and obsessive-compulsive disorder (OCD). A task termed "instrumental contingency degradation" can be used to assess the ability of rodents and humans to select actions based on goal-directed or habitual response strategies. Upon degradation of a familiar action-reward association, reduced responding provides evidence of response updating typical of goal-directed behavior. Unaltered responding after contingency degradation indicates insensitivity to the change in action-outcome contingencies, and the dominance of a habit-based response.

Corticostriatal circuits regulate the balance between goal-directed and habitual behavior via the integration of reward, motor, associative, and somatosensory pathways[1,2,3]. Striatal neurons are recruited during instrumental task training, and neuronal firing develops over time to reflect action-reward associations[4,5,6]. Specific populations of neurons within these circuits are differentially involved in instrumental behavior[3], which has been observed through analysis of

neuron activity patters. Dorsolateral striatum (DLS) neurons become less active during goal-directed behavior and more active during habitual behavior[1, see also 7]. Neurons of the orbitofrontal cortex (OFC) and dorsomedial striatum (DMS) become more active during goal-directed behavior[1]. Further, the magnitude of activity changes in OFC neurons in response to change in outcome value is proportional to the level of goal-directed behavior[1]. Additionally, Nonomura et al. revealed that direct and indirect pathways of the dorsal striatum are differentially involved in goal-directed behavior, further reinforcing the essential function of the DMS[8]. While DMS direct pathway neurons encode reward outcomes associated with a chosen action, DMS indirect pathway neurons encode no-reward outcomes and next action selection[8].

Corticostriatal projections are largely (though not entirely) ipsilateral and organized topographically, with limbic areas and OFC neurons innervating the DMS and central striatum[2,3,9] (Figure 1) and sensorimotor cortex neurons projecting to the DLS[3] (for a review of rodent and human corticostriatal homologies, see Balleine and O'Doherty, 2010[2]). The topographic organization of projections, combined with analysis of neuron activity patterns, suggests function-specific circuitry between specific regions of the OFC and dorsal striatum. Goal-directed behavior is likely mediated, at least in part, by the DMS and central striatum via input from the OFC, while the DLS coordinates habitual actions via input from the sensorimotor cortical areas[1,2,7,10,11,12]. Furthermore, a growing body of inactivation studies have determined

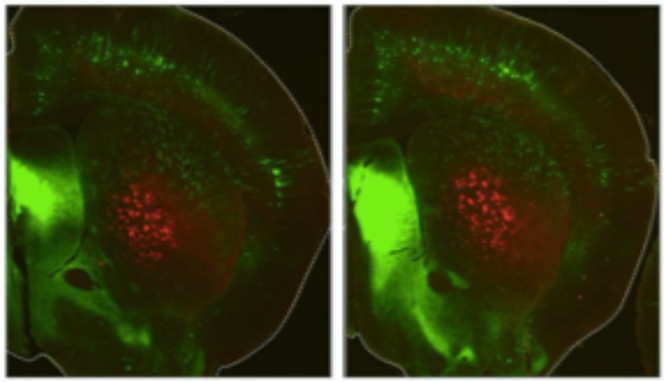

Figure 1: Representative fluorescent imaging of OFC inputs into the DMS after mCherry tagged viral vectors were infused into the OFC.

the necessity of specific corticostriatal projections[13]. For example, DMS inactivation reduces sensitivity to outcome devaluation and contingency degradation[7], and disconnection of the OFC from the ventral lateral striatum causes similar deficits[10].

Optimal goal-directed action selection likely involves continual updating of associations between contexts, likely outcomes, and action options[8]. OFC-striatal circuits play a role not only in recalling action value and selection between actions, but also controlling flexible action selection based on predictions of outcome value that are dependent on the current context, or "state" of a task[3,14]. The OFC is thought to mediate recognition of the current state of a task by integrating information from other brain areas like the hippocampus and amygdala in order to predict the most likely outcome of an action (For discussion, see Sharpe et al. 2019[14]). OFC activity reflects this role of updating associations based on distinct states or context. For example, Schoenbaum et al. showed that distinct populations of neurons within the OFC were active when different odor cues were used to indicate whether the same task would result in a rewarding or unrewarding outcome, and that activity of these distinct populations changed upon contingency reversal[15]. DMS neuron activity also reflects state-specificity, and this activity has been shown to be dependent on OFC-DMS connections[16]. Thus, OFC-DMS connections play a role in recalling outcome value as well as response updating and goal-directed action selection in the face of changing action-outcome contingencies.

Dynamic behavior and dendritic spine plasticity:

Action-outcome encoding and updating has been associated with structural changes in neuronal dendritic spines. Dendritic spines are the primary sites of excitatory synapses in the brain, and their structural plasticity in response to stimuli is related to changes in synaptic

strength as well as cognitive processes such as learning and memory[17,18]. Dendritic spines and the molecular mechanisms supporting their stability have been implicated in many instances of neuroplasticity, including hippocampal-dependent learning and memory[20]. Dendritic spines are the sites of long-term potentiation and long-term depression, and stimulation during learning directly influences spine anatomy[19]. During instrumental behavior training, the development of neuronal firing patterns occurs along with changes in neuroanatomy[4,5,6]. Interestingly, changes in gene expression in the striatum mediated by histone deacetylase occur concurrently with habit formation[21], further reinforcing the relationship between neuronal plasticity and instrumental behavior. More recently, dendritic spine plasticity in the OFC specifically was shown to be triggered by updating of reward-related expectations[18].

During adolescence, dendritic spines in the prefrontal cortex undergo a maturation process characterized by the pruning of some dendritic spines and the stabilization of others[22,23]. In mice, typical dendritic spine maturation during adolescence is disrupted by exposure to drugs of abuse: For instance, cocaine exposure in mice during adolescence results in a loss of dendrite complexity and dendritic spine density that persists into adulthood[23,24]. Behavioral changes are also observed following drug exposure: In both humans[25,26,27,28,29] and rodents[23,30], drug exposure has repeatedly been shown to shift the balance of goal-directed and habitual action systems toward habitual behavior. Because of the disruption of dendritic spine structure and stability following drug use and closely related behavioral deficits, the processes involved in the stability and plasticity of dendritic spines have been implicated as possible mechanisms that support typical goal-directed behavior.

Addiction is broadly characterized by maladaptive decision making, a loss of control over drug use and habit-like drug seeking despite adverse consequences[30]. In humans, a majority of

drug users initiate drug use during adolescence[31], and adolescents who take drugs are at high risk for addiction and relapse: 90% of active drug addicts began using in adolescence[32]. Furthermore, drug exposure initiated during adolescence has neuroanatomical effects which persist into adulthood even in the absence of continued drug use[29], including changes in mature dendritic spine density in the cortex[23]. Thus, it is important to understand the mechanisms by which goal-directed and habitual decision-making strategies are balanced, particularly mechanisms that are active during adolescence when many individuals are first exposed to drugs of abuse. Specifically, understanding the process of developmentally-regulated cortical dendritic spine plasticity, which has been shown to be affected by drugs of abuse and is potentially involved in instrumental learning[18], may help us to understand how drug exposures during adolescence create lasting changes in neuroanatomy and behavioral phenotypes.

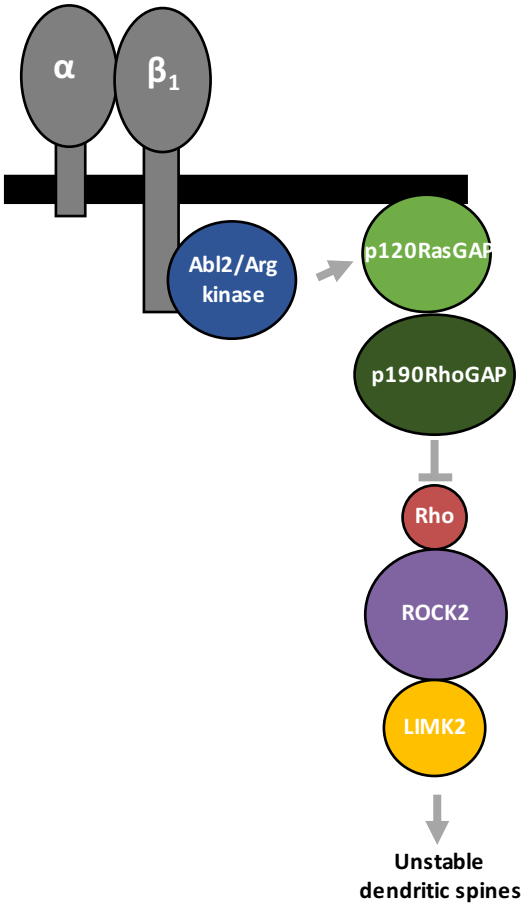

Figure 2: Extracellular matrix proteins bind to α/β1-containing integrin receptors. Once activated, β1-integrin stimulates Abl2/Arg kinase, which can then stimulate p190RhoGAP (forming a complex with p120RasGAP). Activation of p190RhoGAP inhibits Rho. When Rho is active, it stimulates ROCK2, which activates LIMK2. Rho signaling has been associated with dendrite retraction in multiple biological systems, thus inhibition stabilizes dendrite structure. (Fig. adapted from Shapiro et al., 2017)[40].

Integrins are heterodimeric extracellular matrix receptors involved in neuronal development and migration, synapse maturation and plasticity, and structural support via integrin-mediated cell adhesion[33]. The β1-integrin subunit (encoded by the gene *Itgb1*) mediates dendritic spine maturation via Arg kinase signaling[33] (Figure 2). The β1-integrin-

Arg-Rho-kinase 2 (ROCK2) signaling pathway stabilizes dendritic arbors via Rho GTPase activation[20] and this pathway is critical for synapse maintenance[33,37,20]. For instance, in a knockout model, *Arg*$^{-/-}$ mice have lower hippocampal synapse densities, coincident with impaired hippocampus-dependent learning and memory[20]. β1-integrins in the cerebral cortex control synapse and dendritic spine formation and stability[33,34], and inhibiting β1-integrin-mediated signaling leads to dendrite retraction[33]. Furthermore, changes in expression of β1-integrin and its downstream signaling partner Arg kinase influence both dendritic spine stability and behavioral reactions following cocaine exposure[35,22,33].

β1-integrins are currently being investigated in our lab as mediators of dendritic spine plasticity that can influence action-outcome decision making. In the hippocampus, β1-integrin deficiency leads to synapse loss, as well as impaired hippocampus-dependent learning and memory, first detectable during adolescence[33]. Also, inhibition of the β1-integrin substrate Arg kinase slows OFC-dependent instrumental reversal learning and potentiates responding for reward-related cues[22]. Current work in the Gourley lab reveals that β1-integrins are critical to dendritic spine maturation in the OFC during adolescence[38], and that conditional knockdown of *Itgb1* in the OFC during adolescence impedes decision making that is dependent on an OFC-amygdala connection[38] (also, Whyte and Gourley, unpublished). Additionally, pharmacological stimulation of the β1-integrin-Arg-Rho-kinase 2 (ROCK2) pathway (Figure 2) is sufficient to restore action-outcome decision making in mice with diminished β1-integrin in the OFC (Whyte and Gourley, unpublished) or in intact mice with exposure to extended response training, which induces habitual behavior[39].

This study aims to elucidate whether β1-integrin-expressing neurons in the OFC regulate goal-directed behavior via interactions with CaMKII-expressing medium spiny

neurons (MSNs) in the DMS. Corticostriatal interactions regulate several goal-directed behaviors. Meanwhile, β1-integrins are critical for dendritic spine stability on OFC neurons involved in goal-directed behavior, and *Itgb1* knockdown in the OFC starting in adolescence impedes decision making[38]. OFC projections to the DMS are necessary for goal-directed behavior[1], but whether goal-directed behavior is regulated via β1-integrin-expressing neurons in the OFC interacting with MSNs in the DMS *specifically* is still unknown.

Here, we use an adaptation of surgical disconnection procedures. Classically, one would ablate OFC-DMS interactions via contralateral lesions of the OFC and DMS; given that projections are largely (though not entirely) ipsilateral, this procedure "disconnects" OFC-striatal interactions. Here, "disconnection" was achieved through unilateral placement of inhibitory Gi-coupled DREADDs (Designer Receptors Exclusively Activated by Designer Drugs) within the DMS, combined with unilateral infusion of Cre-Recombinase in the OFC of 'floxed' *Itgb1* mice to create local *Itgb1* knockdown (see Methods, Intracranial Surgeries). Again, due to the largely ipsilateral arrangement of OFC-DMS projections, ipsilateral OFC-DMS infusions would be expected to disrupt one OFC-DMS connection, leaving this connection intact in the opposite hemisphere (Figure 3, top left). Contralateral infusions leave the OFC intact in one hemisphere, and the DMS intact in the opposite hemisphere, but disrupt OFC-DMS connections (Figure 3, top right): β1-integrin-deficient OFC neurons project to intact striatal neurons in one hemisphere, and in the other hemisphere, intact OFC neurons will interact with inactivated striatal neurons upon administration of the DREADDs ligand. While not a classical disconnection, this procedure will be referred to as a "disconnection" for simplicity in this manuscript. Because *Itgb1* expression in the OFC is critical for OFC-dependent decision-making[38], this approach allows for

observation specifically of β1-integrin-dependent OFC-DMS *interactions* on action-outcome decision making.

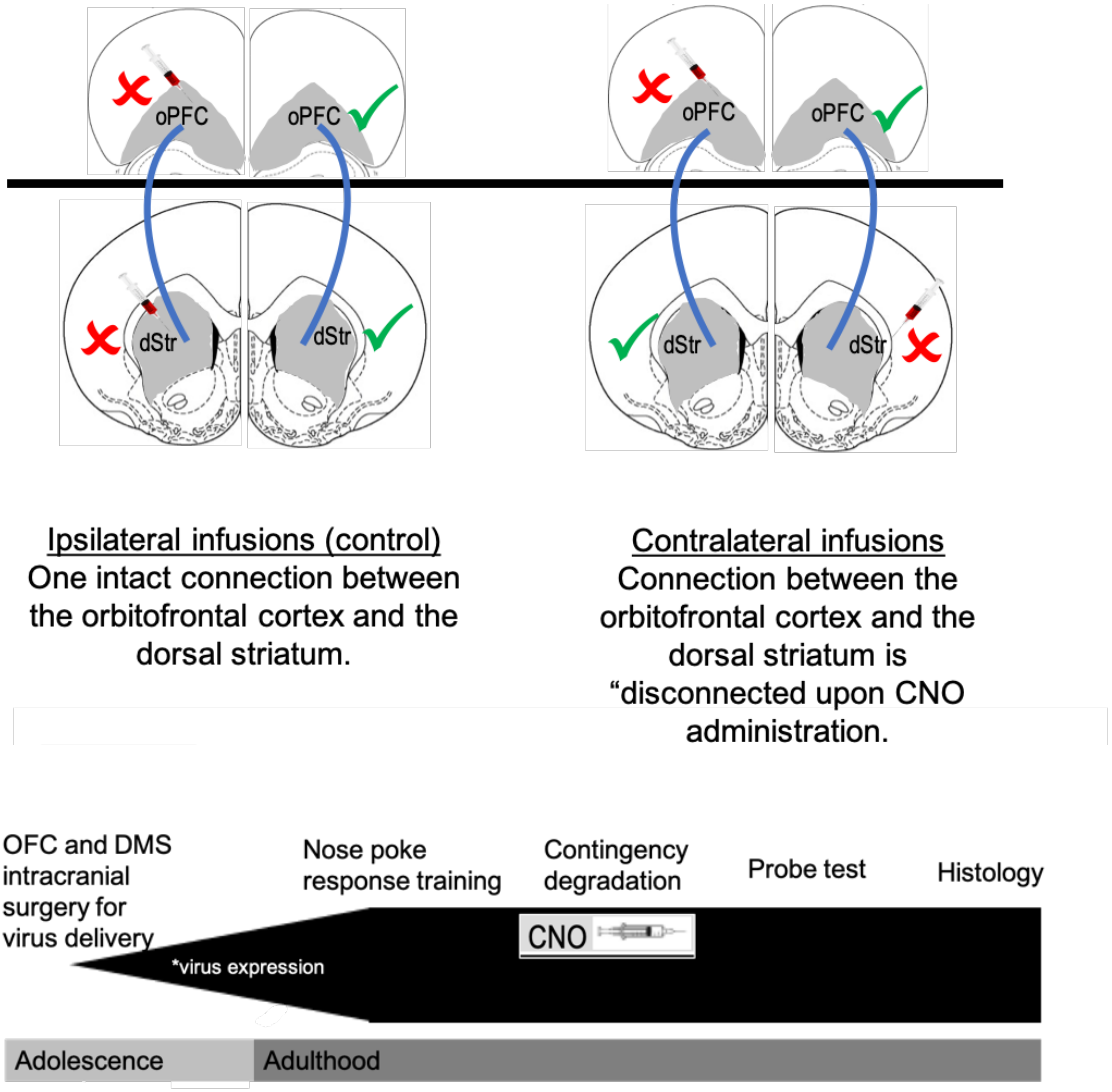

Figure 3: OFC selective *Itgb1* knockdown and DMS inactivation
Viral vector-mediated *Itgb1* knockdown in the OFC, coupled with inhibitory Gi-DREADDs in the DMS, allows for "disconnection" of *Itgb1*-dependent OFC-striatal connections upon administration of CNO. Viral vector infusion occurs during adolescence to achieve *Itgb1* knockdown starting in adolescence and continuing into adulthood. CNO administration to silence striatal neurons occurs prior to the contingency degradation procedure. Mice are naïve during training.

The primary aim of this study was to determine how β1-integrin deficiency in the OFC starting in adolescence influences cortical input to the DMS necessary for goal-directed decision making. The disconnection technique we use here has been successful in studies involving OFC-striatum connections in general[10], providing evidence that it could be used to test whether β1-integrin mediates cortical control over striatal-dependent decision making. We hypothesized that OFC-selective knockdown of *Itgb1* would disrupt the function of the OFC-DMS connection necessary for goal-directed decision making, resulting in habit-biased instrumental behavior. Even though we reduced *Itgb1* starting in adolescence for maximal effect, our results revealed that disconnection did *not* result in a loss of goal-directed behavior. Instead, all groups showed flexible behaviors based on learned action-outcome contingencies. We have previously shown that bilateral *Itgb1* expression in the OFC is necessary for OFC-dependent decision-making, and that "disconnecting" other OFC circuits involved in OFC-dependent decision-making results in habit-biased behavior[38,18]. However, the results from this experiment indicate that goal-directed behavior may not be dependent on OFC β1-integrin-expressing neurons interacting with CaMKII-expressing MSNs in the DMS specifically. These results raise interesting questions about the specific roles of β1-integrins in the OFC, as well as the differing roles of specific populations of neurons in the DMS. More generally, these results help to elucidate specialties in the mechanisms by which the OFC regulates goal-oriented behavior.

Methods

Mice:

Subjects were transgenic mice expressing a 'floxed' *Itgb1* gene bred in-house and originally sourced from Jackson Labs. The 'floxed' *Itgb1* allowed for conditional knockdown of

Itgb1, and reduced expression of β1-integrin protein. Male mice were used, as previous data from the lab show that females are resilient to OFC-specific *Itgb1* knockdown, possibly due to higher density of estrogen receptors within the OFC which, when activated, stimulate β1-integrin-mediated neuroplasticity. Mice were provided food and water *ad libitum* until instrumental conditioning in early adulthood when body weight was reduced and maintained at ~90% of baseline.

Intracranial surgeries:

Intracranial surgery to deliver Cre± viral vectors for unilateral OFC *Itgb1* knockdown occurred at postnatal day (P) 24 in order to achieve peak *Itgb1* knockdown during a period of considerable dendritic spine pruning in adolescence. (Adolescence in mice is defined P28-56.) Infusions via Hamilton syringe were used to deliver adeno-associated viral vectors expressing either mCherry-tagged Cre-recombinase under the CamKII promotor (AAV8-CaMKIIa-Cre-mCherry) or a control vector (AAV8-CaMKIIa-mCherry). In affected animals, Cre was expected to excise exon 3 of *Itgb1,* reducing β1-integrin protein levels in infected neurons in the OFC. The viral vector used here has been previously used to reduce β1-integrin protein levels in the OFC by ~34%[38]. The mCherry tag allowed for visualization of virus-infected neurons, and enabled documentation of viral vector spread in both control and experimental groups. The CaMKII promoter allowed for restriction of Cre expression to CaMKII-expressing neurons within the target region.

Concurrently, mice received ipsilateral or contralateral (see experimental groups below) dorsal striatum-targeted infusions of AAV5-Gi-DREADD (AAV5-CaMkIIa-hM4Di-mCherry) or control viral vectors (AAV5-CaMKIIa-mCherry). The Gi-DREADDs enabled inducible

silencing of CaMKII-expressing neurons (in the striatum, overwhelmingly MSNs) upon administration of the DREADDs ligand, Clozapine N-oxide (CNO). Infusions targeted the DMS.

Because mouse OFC-DMS projections are largely organized ipsilaterally, ipsilateral infusions of AAV5-CaMkII-hM4Di-mCherry and AAV8-CaMKII-Cre-mCherry were expected to result in a disrupted OFC-DMS circuit in one hemisphere, leaving the opposite hemisphere largely intact. "Disconnection" via contralateral infusions resulted in β1-integrin-deficient OFC neurons projecting to intact striatal neurons in one hemisphere, while in the other hemisphere, intact OFC neurons interacted with inactivated striatal MSNs upon administration of CNO (Figure 3). Assuming OFC β1-integrin is critical to circuit function, this technique was expected to leave one intact region in both hemispheres, but result in dysfunctionality of both OFC-DMS connections and a loss of goal-directed behavior.

Experimental groups:

Intact control + CNO (n=9)

Control viral vectors (ipsilateral or contralateral OFC mCherry x DMS mCherry) + CNO (n=9)

Ipsilateral OFC Cre x DMS Gi-DREADD + CNO (n=10)

Contralateral OFC Cre x DMS Gi-DREADD + CNO* (n=15)

* "Disconnection" group

Instrumental conditioning and action-outcome contingency degradation with CNO injection:

Prior to instrumental conditioning, mice were food restricted to 90% of their baseline body weight to motivate food-reinforced instrumental response training. Mice were trained to

nose poke for food pellets within operant conditioning chambers containing two nose poke apertures (Figure 4, "training"). During training, a fixed ratio 1 (FR1) schedule of reinforcement (1 poke → 1 pellet reinforcer) was used. Training continued for 5-10 days (70 minutes per session, maximum 30 pellets/nose poke response) until mice responded equivalently on both nose poke apertures.

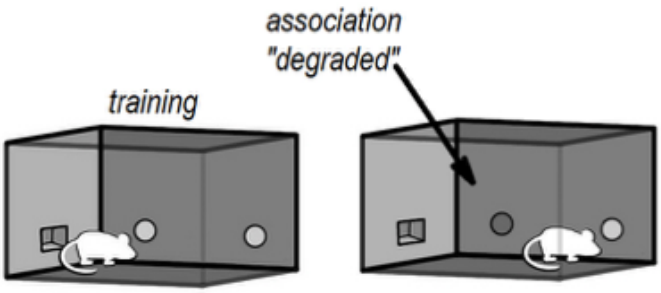

Figure 4: Instrumental response training and contingency degradation.
Mice were trained to nose poke using either nose poke aperture to trigger the delivery of a food pellet reward (left). During the contingency degradation session, the association between nose poke action using the available aperture and food pellet delivery was degraded.

Following response acquisition, the action-outcome contingency associated with one nose-poke aperture was reinforced in an additional session (25 minutes), during which the opposite aperture was occluded. Food reinforcers were delivered to the available aperture using a FR1 schedule to maintain the response-outcome association with that aperture. The following day, mice underwent a 25-minute contingency degradation session (Figure 4, right), in which case, the previously reinforced aperture was occluded and the action-outcome association with the other aperture was degraded by delivering food pellets non-contingently. Food pellets were delivered at a fixed interval (FI) rate matched to performance of the animal on the previous day: The total number of pellets delivered using the FI was consistent from the previous day, but the non-contingent delivery weakened the predictive relationship between that nose poke response and the associated food reinforcer, thus degrading the action-outcome relationship for that aperture. 30 minutes prior to contingency degradation, all mice received intraperitoneal (IP)

injections of CNO at a dose of 1mg/kg (dissolved in saline and 2% DMSO) in order to activate the hM4Di DREADD and silence the striatal neurons infected by the DREADD viral vector.

The following day, when the mice were drug-free, mice underwent a response preference probe test (15 minutes) during which both nose poke apertures were available and response rates were measured.

Histology

Following testing, mice were euthanized by rapid decapitation under isoflurane anesthetic. Brains were extracted, submerged in chilled 4% paraformaldehyde for 48 hours, then transferred to chilled 30% w/v sucrose for 72 hours. Brains were sectioned into 40 μm coronal sections using a microtome held at -20°C, then stored in cryoprotectant. Tissue was stained using Living Colors mouse anti-mCherry primary antibody, and Alexa Fluor 594 goat anti-mouse secondary antibody. Sections were mounted from the OFC to the striatum, and a fluorescent microscope was used to image viral vector spread. Representations of viral vector expression were superimposed onto images from the *The Mouse Brain in Stereotaxic Coordinates: Third Edition*[43] so that viral vector spread could be mapped.

Statistical Analysis

Multivariate Analysis of Variance (MANOVA) was performed using SPSS (IBM Corp., Armonk, New York). Significance was set at $p<0.05$.

Results

Histology:

Immunohistochemistry was used to verify the location of viral vector expression in the OFC and striatum. Viral vector expression was largely located in the lateral and ventral regions of the OFC (Figure 5, top). In some mice, expression spread to dorsomedial regions. Projections from the OFC appear in the central striatum (Figure 5, middle). In the striatum, viral vector expression was located primarily in the DMS, with some ventrolateral spread (Figure 5, bottom).

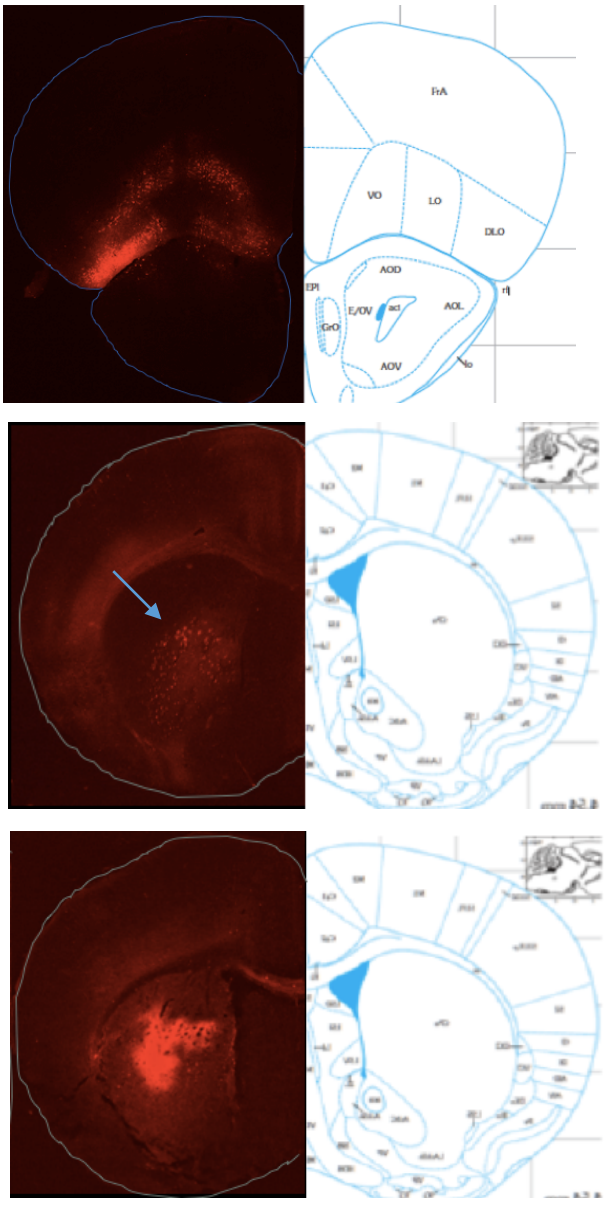

Figure 5: Representative images of viral vector expression in the OFC (top), and in OFC neurons projecting to the DMS (middle). Bottom image shows viral vector expression in the DMS.

Instrumental conditioning and contingency degradation:

Nose poke response rates were measured during training sessions in which mice learned to nose poke for food pellet reinforcers (Figure 6, top). The response rates during the last 5 training sessions are represented. There was no significant difference in response rate between

experimental groups (no effect of group x session F(12, 156)=2.65, p=0.125). A main effect of session (F(4,156)=35.29, p<0.05) indicated that all mice increased response rates across the last 5 training sessions. All mice were able to reach criteria within the 10-day training period.

Following training, mice underwent a contingency degradation session, during which the action-outcome relationship associated with one of the two nose-poke apertures was degraded by delivering food reinforcers non-contingently. Response rates were then measured during a 15-minute probe test the next day. During the probe test, all mice preferentially responded on the aperture associated with the intact response-outcome contingency (main effect of choice F(1,39)=26.906, p<0.001; no effect of group F(3,39)=1.156, p=0.339) (Figure 6, bottom). Preferential responding on the nose poke aperture for which response was reinforced over the aperture for which action-outcome contingency was degraded indicates that the mice were able to distinguish between the reward likelihoods and then respond in a goal-directed manner.

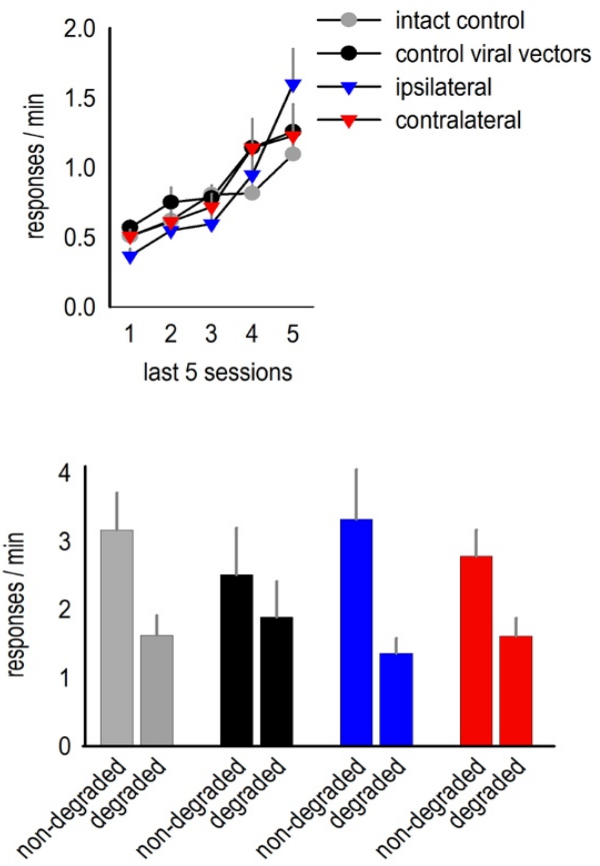

Figure 6: Instrumental response acquisition during the last five training sessions using a FR1 reinforcement schedule of reinforcement (top). All groups showed significant preference for the non-degraded contingency during a 15-minute probe test following instrumental contingency degradation (bottom). Means + SEMS.

Notably, two independent cohorts were used (Group 1: total n=24, Group 2: total n=19), and results were consistent between both cohorts. Values presented here reflect statistical analysis performed on combined data from both cohorts.

Discussion

We hypothesized that β1-integrin-expressing neurons in the OFC regulate goal-directed behavior via interactions with CaMKII-expressing medium spiny neurons (MSNs) in the DMS**.** We predicted that these connections would be necessary for response updating following degradation of a learned action-outcome contingency. Consequently, disrupting the connection between β1-integrin-expressing neurons in the OFC and MSNs in the DMS would be expected to disrupt this updating, therefore resulting in a loss of goal-directed behavior.

Contrary to our predictions, disrupting the connection between β1-integrin-expressing neurons in the OFC and CaMKII-expressing neurons the DMS did not result in a loss of goal-directed behavior. Following degradation of the action-outcome association for one nose-poke behavior, all mice neglected a "degraded" response in favor of a "non-degraded" response, regardless of disconnection status. This pattern demonstrates a sensitivity to the change in likelihood that one response would result in reward, and, consequentially, a change in action selection – a form of response updating typical of goal-directed behavior.

Previous studies have shown that β1-integrins in the OFC are necessary for goal-directed behavior. As such, bilateral *Itgb1* knockdown in the OFC, causing ~34% reduction in β1-integrin protein in the OFC, results in habit dominance[38], providing evidence that β1-integrins are indeed critical for OFC mediation of goal-directed action (typically conceptualized as countering habit-based behavior). An ongoing study in the Gourley lab used a similar technique relative to my

study to "disconnect" β1-integrin-expressing OFC neurons from the basolateral amygdala (BLA) and found that disconnection resulted in a loss of goal-directed behavior (Whyte and Gourley, unpublished). As here, this study used unilateral *Itgb1* knockdown in the OFC and contralateral BLA inactivation, so that β1-integrin deficient neurons in the OFC would interact with intact BLA neurons, while inactivated BLA neurons interact with the healthy OFC. Because the results in our present study indicate that outputs from the OFC to the DMS are not dependent on β1-integrins in the OFC, one conclusion might be that β1-integrins are potentially more critical for neurons in the OFC receiving inputs, for example from the BLA, rather than in mediating outputs, for example to the DMS.

Though previous studies have shown that *Itgb1* knockdown in the OFC results in ~34% reduction in β1-integrins in the OFC, it would be useful to measure the reduction of β1-integrins in the OFC of the mice used in this study in order to make further comparisons to other studies in the lab using the same viral vector knockdown technique. If β1-integrins were reduced to the same degree, then it could be that this level of reduction in the OFC is not sufficient to impact the functionality of OFC-DMS connections. Additionally, the expression of *Itgb1* in the OFC should be better identified in order to further investigate the role of β1-integrins in the OFC in mediating outputs to the DMS. For instance, *Itgb1* expression in OFC projection neurons that innervate the DMS could be measured using a fluorescence-expressing retrograde viral vector infusion into the DMS, along with a measure of early gene expression (for example, c-Fos) in the OFC, in order to label OFC-to-DMS-projecting neurons that are activated by instrumental contingency degradation. Then, these neurons could be evaluated for *Itgb1* expression. Overlap of c-Fos expression and the retrograde virus in *Itgb1*-expressing neurons would provide evidence that *Itgb1* expression is occurring in OFC neurons that project to the DMS.

While experiments using bilateral *Itgb1* knockdown revealed that β1-integrins are necessary for OFC regulation of goal-directed behavior[38], further manipulations of specific OFC circuitry may help to elucidate the precise role(s) of β1-integrins in encoding pertinent goal-related information. Schoenbaum et al. reported that encoding predicted outcomes in the OFC depends upon input from the BLA[44]. Specifically, following odor discrimination training, BLA-lesioned rats had fewer cue-selective neurons in the OFC than control rats; the cue-selective neurons did not include neurons that were responsive in anticipation of the predicted outcome; and the cue-selective neurons were bound to cue identity as opposed to association[44]. Unpublished studies from our lab suggest that inputs from the BLA are also required for action-outcome updating (Li and Gourley, unpublished), and that this updating is dependent on β1-integrins (Whyte and Gourley, unpublished).

Itgb1 knockdown in the OFC results in a loss of dendritic spines[38], linking β1-integrins to both behavioral phenotypes and dendritic spine stability. This conclusion is consistent with evidence that intracellular β1-mediated signaling is necessary for dendritic spine and synapse stabilization[45,22,33,34] and goal-directed behavioral phenotypes[22,39]. (For further discussion of dendritic spine stability mechanisms, see Kerrisk and Koleske 2013[46].) The proposed role for β1-integrins is also consistent with theories regarding dendritic spine dynamics as physical correlates of learning and cognition more broadly (For review, see Kasai 2010[17]). Altogether, we propose a model that β1-integrins in the OFC are necessary for OFC-dependent expectancy updating via inputs from the BLA synapsing onto stable, mature dendritic spines. \

While there is evidence that β1-integrins in the OFC are needed for response updating[18,38], our findings suggest that β1-integrins may not be involved in OFC regulation of action selection via output to the DMS (though this should be verified, as discussed previously).

In neurons, β1-integrins activate Arg kinase[33], which is highly expressed in the brain and is enriched in dendritic spines[47]. Dendritic spines in the cerebral cortex exist primarily on pyramidal neurons[17], and projections from layer V and layer III pyramidal neurons in the OFC terminate on dendritic spines of MSNs in the DMS[3]. These projections provide glutamatergic input to GABAergic MSNs, which innervate other MSNs and also cholinergic interneurons within the DMS[3]. The OFC also modulates DMS cholinergic interneurons via projections to the laterodorsal tegmental area, which provides cholinergic modulation to the DMS via the parafascicular thalamus[48,49,50]. If β1-integrin-mediated expectancy updating in the OFC was affecting direct OFC projections to the DMS, it would likely be via spiny pyramidal neurons in the OFC projecting to MSNs in the DMS. Thus, our results indicate that β1-integrins may not support response updating that is dependent on *direct* OFC-DMS connections.

Importantly, Klug et al. confirmed that CaMKII is not expressed in DMS cholinergic interneurons, even while it is abundant in MSNs[54]. The viral vectors used in our study expressed a CaMKII dependent promoter and therefore would be expected to favor MSNs and spare cholinergic interneurons. This would mean that "disconnection" targeted MSNs receiving input from β-integrin-expressing neurons in the OFC, but would have left connections between the OFC and cholinergic neurons intact the hemisphere without *Itgb1* knockdown in the OFC. The results presented here therefore would suggest that projections from β-integrin-expressing neurons in the OFC do not mediate goal-directed behavior via input to CaMKII-expressing MSNs, and that instead, OFC input to DMS cholinergic neurons could be sufficient for expectancy updating. Whether β1-integrins impact OFC inputs to DMS cholinergic interneurons remains unclear and could be tested in future investigations.

Sharpe et al. 2019 recently argued that OFC modulation of state representation in the DMS occurs through cholinergic interneurons instead of MSNs[14]. Cholinergic interneurons in the DMS are necessary for flexible behavior[16,51]. Further, disconnection of the parafascicular thalamus projections to cholinergic interneurons reduces the firing rate of these neurons, and caused a deficit in goal-directed action selection after changes in action-outcome contingencies[52]. DMS interneurons are also more responsive to cortical input than MSNs[53]. And, while MSN activity reflects current choice selection, cholinergic interneuron activity reflects encoding of associative information that is state-specific[16]. Specifically, Stalnaker et al. recorded putative cholinergic interneurons from the DMS while rats performed a series of tasks requiring application of contradictory associative information. They found that individual interneurons from the DMS represented the current state of each task, and that miscoding of states predicted inaccuracy in task performance[16]. Task encoding in cholinergic neurons was also dependent on input from the OFC – without OFC input, there was no acquisition of state-specific activity in the DMS[16]. With the caveat that these behavioral tests included Pavlovian (odor) cues to signal reinforcement, these studies suggest that DMS cholinergic interneurons are necessary for goal-directed action selection in varying states of action-outcome contingency. And, that encoding of outcome-related information in DMS cholinergic neurons is dependent on OFC input via the parafascicular thalamus.

In summary, "disconnection" of β1-integrin-expressing neurons in the OFC and MSNs in the DMS did not result in a loss of goal-directed behavior, even though β1-integrin-expressing neurons in the OFC are necessary for goal-directed action updating[38]. Given that our viral vector strategy targeted MSNs and not cholinergic interneurons, this study provides indirect support for emerging evidence that OFC modulation of action selection in the DMS occurs via indirect

projections to cholinergic interneurons in the DMS, which may play a critical role in state-dependent behavioral flexibility (reviewed by Sharpe et al 2019[14]). An open question is whether β1-integrins in the OFC are necessary for the indirect OFC modulation of the striatum via cholinergic interneurons. In order to test this possibility, a disconnection technique could be used that pairs ipsilateral *Itgb1* knockdown in the OFC with contralateral inhibition of DMS cholinergic interneurons, as opposed to DMS MSNs. Our experiments also raise questions regarding the specific role(s) that β1-integrin-mediated intracellular signaling might be playing in the OFC – for instance, might processing OFC inputs be dependent on β1-integrins (possibly due to the role of β1-integrin-mediated signaling in dendritic spine plasticity), while tuning outputs is not? Continued investigation along this line will further clarify the of the role of β1-integrins as mediators of synaptic and behavioral plasticity necessary for action-outcome association and flexible behavior, as well as the specific circuits necessary for OFC mediation of goal-directed behavior.

Conclusions

Addiction, OCD, and other behavioral disorders are characterized by compulsive, habitual behavior that can occur at the expense of goal-directed and flexible decision making. In both humans and rodents, the balance between goal-directed and habitual behavior is regulated by corticostriatal pathways that integrate context, outcome, and reward associations in order to inform future actions that maximize positive outcomes. Adolescence is a critical period for neuroanatomical development, specifically the maturation of dendritic spines, which mediate neuroplasticity and potentially play a role in instrumental learning. Cocaine exposure during adolescence interrupts normal OFC dendritic spine maturation, causing lasting neuroanatomical changes, as well as dominance of habit-based behavior. β1-integrin knockdown during adolescence also disrupts dendritic spine maturation and leads to habit dominance, indicating that β1-integrin plays a role in regulating the development of circuitry critical for the regulation of complex decision-making behavior. OFC projections to the striatum have been shown to be important for goal-directed behavior. However, the specific projections involved in distinct behaviors remain unclear. This study used 'floxed' *Itgb1* mice receiving OFC-targeted Cre infusions during adolescence and contralateral DMS-targeted Gi-DREADD infusions to conditionally "disconnect" specific OFC-DMS projections. Results suggest that OFC projections from β1-integrin-expressing cells to CamKII-expressing MSNs in the DMS are not directly involved in response updating following instrumental contingency degradation. OFC modulation of action selection regulated by the DMS may instead occur via indirect projections to cholinergic interneurons in the DMS. Further studies will continue to elucidate the functions of these projections, if any, within a complex web of circuits needed to regulate goal-directed behaviors.

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