Behavioral/Cognitive

Prefrontal Dopamine D_1 and D_2 Receptors Regulate Dissociable Aspects of Decision Making via Distinct Ventral Striatal and Amygdalar Circuits

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Mesocortical dopamine (DA) regulates a variety of cognitive functions via actions on D_1 and/or D_2 receptors. For example, risk/reward decision making is modulated differentially by these two receptors within the prefrontal cortex (PFC), with D_2 receptors enabling flexible decision making and D_1 receptors promoting persistence in choice biases. However, it is unclear how DA mediates opposing patterns of behavior by acting on different receptors within the same terminal region. We explored the possibility that DA may act on separate networks of PFC neurons that are modulated by D_1 or D_2 receptors and in turn interface with divergent downstream structures such as the basolateral amygdala (BLA) or nucleus accumbens (NAc). Decision making was assessed using a probabilistic discounting task in which well trained male rats chose between small/certain or large/risky rewards, with the odds of obtaining the larger reward changing systematically within a session. Selective disruption of D_1 or D_2 modulation of separate PFC output pathways was achieved using unilateral intra-PFC infusions of DA antagonists combined with contralateral inactivation of the BLA or NAc. Disrupting D_2 (but not D_1) modulation of PFC→BLA circuitry impaired adjustments in decision biases in response to changes in reward probabilities. In contrast, disrupting D_1 modulation of PFC→NAc networks reduced risky choice, attenuating reward sensitivity and increasing sensitivity to reward omissions. These findings reveal that mesocortical DA can facilitate dissociable components of reward seeking and action selection by acting on different functional networks of PFC neurons that can be distinguished by the subcortical projection targets with which they interface.

Key words: amygdala; decision making; dopamine; nucleus accumbens; prefrontal cortex

Significance Statement

Prefrontal cortical dopamine regulates a variety of executive functions governed by the frontal lobes via actions on D_1 and D_2 receptors. These receptors can in some instances mediate different patterns of behavior, but the mechanisms underlying these dissociable actions are unclear. Using a selective disconnection approach, we reveal that D_1 and D_2 receptors can facilitate diverse aspects of decision making by acting on separate networks of prefrontal neurons that interface with distinct striatal or amygdalar targets. These findings reveal an additional level of complexity in how mesocortical DA regulates different forms of cognition via actions on different receptors, highlighting how it may act upon distinct cortical microcircuits to drive different patterns of behavior.

Introduction

Dopamine (DA) facilitates a variety of cognitive and executive functions governed by the prefrontal cortex (PFC), including selective attention, working memory, cognitive flexibility, and cost/benefit decision making (Robbins and Arnsten, 2009; Floresco and Jentsch, 2011; Floresco, 2013). DA acts on D_1 and D_2 receptors that reside on different populations of PFC pyramidal neurons that project to striatal and other subcortical targets (Gaspar et al., 1995), as well as on local GABAergic interneurons (Santana et al., 2009). In recent years, the contribution of these different receptors to various PFC functions has been clarified in

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part by psychopharmacological studies using selective DA receptor antagonists. An emerging picture is that mesocortical DA may regulate different cognitive functions via dissociable receptor mechanisms, some being relatively straightforward and others more complex. For example, attention and working memory both appear to be dependent on PFC D_1, but not D_2, receptor activity (Sawaguchi and Goldman-Rakic, 1994; Seamans et al., 1998; Granon et al., 2000), whereas cognitive flexibility entailing shifts between strategies is mediated by cooperative interactions between these two subtypes (Ragozzino, 2002; Floresco et al., 2006).

A more intricate interplay between PFC D_1 and D_2 receptor activity is involved in biasing action selection during certain forms of cost/benefit decision making. Previous work by our group used a probabilistic discounting task to investigate the neural basis of risk/reward decision making, revealing distinct cortical, limbic, and striatal circuits that regulate different aspects of choice behavior. A circuit linking the basolateral amygdala (BLA) to the nucleus accumbens (NAc) promotes preference of larger, uncertain rewards, yet these biases are tempered by the PFC, which exerts top-down control over the BLA to modify decision biases when the utility of options change (St. Onge et al., 2012a). Of particular interest, pharmacological manipulations of PFC D_1 and D_2 receptors unveiled how these receptors can modulate dissociable and somewhat opposing aspects of behavior. Blocking PFC D_1 receptors reduces risky choice by increasing sensitivity to nonrewarded actions, indicating that they aid in maintaining choice biases despite potential losses. In contrast, D_2 antagonism increases risky choice when odds shift from higher to lower probabilities, suggesting that these receptors facilitate adjustments in decision biases when reward probabilities are volatile (St. Onge et al., 2011). These findings are consistent with computational models of dopaminergic regulation of PFC network activity proposing that D_1 activity stabilizes representations, whereas D_2 receptor activation places networks in a more labile state that facilitates flexible patterns of behavior (Durstewitz et al., 2000; Seamans and Yang, 2004).

The above-mentioned findings pose an intriguing question: how can DA, acting in one brain region but on different receptors, mediate seemingly opposite patterns of behavior? A potential answer comes from neuroanatomical studies that have identified separate populations of PFC pyramidal neurons that only express D_1 or D_2 receptors, with relatively few neurons expressing both (Vincent et al., 1993; Gaspar et al., 1995; Santana et al., 2009). Moreover, D_1- versus D_2-expressing cells display distinct neurophysiological properties and, in some instances, different subcortical projection targets (Gaspar et al., 1995; Gee et al., 2012; Seong and Carter, 2012). This neuroanatomical framework leaves open the possibility that mesocortical DA may act on separate networks of neurons that are modulated by D_1 or D_2 receptor activity. Furthermore, some of the neurons within these PFC D_1/D_2 networks may project to different downstream targets, permitting their recruitment into broader PFC–subcortical networks that promote different patterns of behavior.

The present series of experiments sought to test this hypothesis. Specifically, we investigated whether D_1 and D_2 receptor activity differentially modulate separate PFC networks that interface with either the BLA or the NAc to guide different aspects of risk/reward decision making. Both of these regions receive projections from the PFC (Sesack et al., 1989; Vertes, 2004) and are part of broader cortical–subcortical networks that mediate different aspects of choice behavior (St. Onge et al., 2012a; Stopper et al., 2013; Orsini et al., 2015). We use a modified, asymmetrical disconnection procedure designed to disrupt selectively D_1 or D_2 modulation of either PFC→BLA or PFC→NAc circuitry (Floresco et al., 1997, St. Onge et al., 2012a; Tan et al., 2010; McGlinchey et al., 2016).

Materials and Methods

Subjects. Adult male Long–Evans rats (Charles River Laboratories) weighing 225–275 g at the start of the experiment were group housed and provided *ad libitum* access to water and food upon arrival. They were handled daily for 1 week and then food restricted to 85–90% of their free feeding weight, after which they were fed 14–18 g of food at the end of each experimental day. Their weights were monitored daily and their individual food intake was adjusted to maintain a steady but modest weight gain. The colony was maintained on a 12 h light/dark cycle with lights on at 7:00 A.M. Behavioral testing occurred between 8:00 A.M. and 12:00 P.M. each day. All experiments were conducted in accordance with the Canadian Council on Animal Care guidelines regarding appropriate and ethical treatment of animals and were approved by the Animal Care Centre at the University of British Columbia.

Apparatus. Behavioral testing was conducted in operant chambers ($31 \times 24 \times 21$ cm; Med Associates) enclosed in sound-attenuating boxes. The chambers were equipped with a fan that provided ventilation and masked extraneous noise. A single 100 mA house light illuminated the chambers and each chamber was fitted with two retractable levers located on each side of a central food receptacle in which 45 mg of sweetened food reward pellets (Bioserv) were delivered by a dispenser. All data were recorded by a computer connected to the chambers via an interface.

Lever-pressing training. The initial training protocols described below were identical to those described in our previous studies (St. Onge et al., 2012a; Larkin et al., 2016). The day before exposure to the operant chamber, each rat was given ~25 sugar reward pellets in their home cage to familiarize them with the reward. On the first day of training, two pellets were delivered into the food cup and crushed pellets were sprinkled on an extended lever before the rat was placed into the chamber. On consecutive days, rats were trained under a fixed-ratio 1 schedule to a criterion of 50 presses in 30 min on one lever and then the other side (counterbalanced). They then progressed to a simplified version of the full task. These 90 trial sessions began with the levers retracted and the operant chamber in darkness. Every 40 s, a trial was initiated with the illumination of the house light and the insertion of one of the two levers into the chamber (randomized in pairs). Failure to respond on the lever within 10 s caused it to be retracted and the chamber to darken and the trial was scored as an omission. A response within 10 s caused the lever to retract and a single pellet to be delivered with 50% probability. Rats were trained for ~3–4 d to a criterion of 80 or more successful trials (<10 omissions). Immediately after the final session of retractable lever training, rats were tested for their side bias toward a particular lever. Previous observations in our laboratory showed that we could reduce considerably the number of training days by accounting for rat's innate side bias when designating the risky lever compared with when we assigned the location of the risky lever randomly across subjects. This single session was made up of trials in which both levers were inserted into the chamber. On the first trial, a food pellet was delivered after a response made on either lever. After this choice, food was delivered only after the rat responded on the lever opposite to the one initially chosen. If the rat chose the same lever as in the previous response, then no food was delivered and the house light was extinguished. This would continue until the rat correctly chose the lever opposite to what it selected initially. After a response was made on each lever, a new trial would start so that each trial in this side bias task consisted of at least one response on each lever. Rats received seven such trials and would typically require 13–30 responses to complete the session. Their side bias was assigned based on the lever (left or right) selected most often during the initial choice of each trial. The only exception was if a rat happened to make a disproportionate number of responses on one lever over the entire session (i.e., a 2:1 ratio for the total number of presses). On the following day, rats started training on the probabilistic discounting task.

Probabilistic discounting task. Each daily session consisted of 72 trials separated into four blocks of 18 trials and took 48 min to complete. Rats

were trained 6–7 d/week. One lever was designated the large/risky lever and the other the small/certain lever and this designation remained consistent throughout training. For each rat, the lever that was designated as the large/risky option was the one opposite its side bias. Each session began in darkness with both levers retracted. Trials began every 40 s with the illumination of the house light; 3 s later, one or both levers was inserted into the chamber. Each of the four blocks consisted of eight forced choice trials (in which only one lever was presented, randomized in pairs), followed by 10 free choice trials (Fig. 1*A*, right). If no response was made within 10 s of lever presentation, the levers retracted and the chamber reverted to the intertrial state (omission). Selection of a lever caused its immediate retraction. A choice of the small/certain lever delivered one pellet with 100% probability. Choice of the large/risky lever delivered a four-pellet reward in a probabilistic manner that changed systematically across blocks of trials (100%, 50%, 25%, and 12.5%; Fig. 1*A*, left).

We tested the effects of our manipulations on rats trained on a variant of the task in which the probability of obtaining the large reward was initially 100% at the start of the session and then decreased over blocks (100→12.5%; called the descending variant). However, we determined a priori that, if a particular manipulation increased risky choice under these conditions, we would use the same manipulation in a separate group of rats trained on a variant during which the odds increased over blocks (12.5→100%; called the ascending variant). This was done to investigate whether increases in risky choice observed in the descending condition reflected either a general increase in preference for larger, uncertain rewards (as has been observed after medial orbitofrontal inactivation; Stopper et al., 2014) or an impairment in adjusting decision biases, as has been reported after systemic amphetamine treatment or inactivation of the prelimbic PFC (St. Onge and Floresco, 2010; St. Onge et al., 2010). In the latter case, one would expect to see reduced preference for the risky options in the ascending condition as blocks progressed. Conversely, manipulations that reduce risky choice on the descending variant on this task invariably induce the same effect on the ascending condition. These include those relevant to ones used in the present study, such as BLA manipulations, NAc lesions, or DA antagonism (Cardinal and Howes, 2005; St. Onge et al., 2010; Larkin et al., 2016). In light of this, if a particular manipulation reduced risky choice during the descending condition, then we opted not to test its effects on performance of the ascending variant.

Squads of rats were trained until they displayed stable choice behavior determined by analyzing data from three consecutive sessions with a two-way repeated-measures ANOVA with day and trial block as factors. If there was no main effect of day and no day × block interaction (at $p > 0.10$), then choice behavior of the group was deemed stable. Two to 3 d later, rats were subjected to surgery. Squads typically consisted of 16–24 animals with the intention of obtaining 12–16 rats per group with accurate surgical placements within the intended target regions. Our experience has shown this to be a sufficient number of subjects to observe statistically significant differences between treatment conditions using these types of within-subjects designs (Ghods-Sharifi et al., 2009; St. Onge et al., 2012a). Rats in the different groups required between 26 and 29 d of training before displaying stable patterns of choice.

Reward magnitude discrimination. As we have described previously, we determined a priori that, if a particular manipulation reduced risky choice on the discounting task, then we would test the effect of that same manipulation on a separate group of animals trained on a reward magnitude discrimination. This task consisted of 48 trials partitioned into four blocks of two forced-choice and 10 free choice trials (12 trials per block). The probabilistic nature of the task was removed so that a choice on the large reward lever delivered four pellets, whereas a choice on the other lever would deliver one pellet, both with 100% probability. After 8–10 d of training, rats displayed a strong bias toward the larger reward and then received microinfusion tests in the same fashion as the animals that tested on the probabilistic discounting task. Because this task requires considerably fewer training sessions to achieve stable choice behavior compared with the discounting task, these rats were implanted with guide cannulae before behavioral training.

Pharmacological disconnection design and rationale. Classical disconnection designs have traditionally used asymmetrical unilateral lesions or inactivations of interconnected brain regions to identify components of a functional neural circuit (Everitt et al., 1991; Gaffan et al., 1993; Floresco et al., 1997; Floresco and Ghods-Sharifi, 2007). These designs are based on the assumption that information is transferred serially from one region to an output structure downstream and these signals are transmitted in both sides of the brain in parallel (Fig. 1*B*). It further assumes that dysfunction results from blockade of neural activity at the origin of a pathway in one hemisphere and the termination of the efferent pathway in the contralateral hemisphere. The modified procedure used here was designed to address a more specific question, clarifying whether D_1 or D_2 receptor activity modulates the functional output of distinct networks of PFC neurons that may ultimately interface with either the BLA or the NAc. Specifically, we administered unilateral infusions of either a D_1 or D_2 antagonist in the PFC in combination with contralateral inactivations of the BLA or the NAc in separate groups of rats. Similar pharmacological disconnections have been used to investigate the neurochemical interactions between prefrontal, striatal, and amygdalar regions that mediate learning and reward-related behaviors (Tan et al., 2010; Lasseter et al., 2014; McGlinchey et al., 2016).

As an example of how these disconnections may disrupt communication in a circuit, a unilateral infusion of a D_1 antagonist would be expected to perturb the output of a population of PFC neurons with activity that is modulated by these receptors (a PFC:D_1 network; Fig. 1*Ci*) while leaving separate groups of PFC neurons modulated by D_2 receptors (a PFC:D_2 network) relatively undisturbed (Fig. 1*Cii*). In the opposite hemisphere, dopaminergic modulation of PFC activity would be unaffected (Fig. 1*Ciii*), yet inactivation of a region downstream (e.g.; the BLA) prevents this nucleus from processing incoming signals from the PFC (Fig. 1*Civ*). Accordingly, this asymmetrical manipulation would somewhat selectively hamper communication between PFC:D_1 networks and the BLA in both hemispheres. In comparison, D_1 modulation of other PFC networks that interface with other downstream targets would be relatively intact in one hemisphere (Fig. 1*Ciii*), as would be D_2 modulation of PFC networks that interface with the BLA and other regions (Fig. 1*Cii*). Therefore, in this example, alterations in certain patterns of behavior by this particular combination of infusions would suggest that said behavior is dependent on D_1 receptor modulation of a network of PFC neurons that interface with the BLA. Conversely, a lack of effect would suggest that mesocortical DA transmission influences this behavior either via D_2 receptor modulation of PFC→BLA circuits or D_1 modulation of other PFC output pathways. It should be emphasized that an alteration in behavior induced by this type of disconnection does not necessarily imply that D_1 or D_2 receptors act exclusively on PFC neurons that project to the BLA or the NAc. These PFC networks are likely composed of both pyramidal projection neurons and GABAergic interneurons and D_1 or D_2 receptors can modulate the activity of these networks via actions on both types of cells. The ability of these networks to interface with distinct subcortical targets is likely mediated by a subpopulation of neurons within a network that send direct axonal projections to the BLA or NAc. A preliminary report has shown that PFC projections to BLA and NAc arise from discrete, nonoverlapping populations of cells (Bloodgood et al., 2016).

An additional assumption of disconnection designs is that ipsilateral inactivation of one or both structures in a circuit should not have a disruptive effect on behavior because the intact structures in the opposite hemisphere should be able to at least partially compensate for the unilateral disruption in function. We controlled for this in two ways. First, each experiment included an ipsilateral infusion condition in which unilateral intra-PFC infusions of a DA antagonist and inactivation of a downstream nucleus were administered in the same hemisphere. Furthermore, a subset of rats in these studies also received single unilateral infusions of saline, as well as unilateral inactivation of the BLA/NAc or intra-PFC infusions of D_1/D_2 antagonists. Last, in order for these disconnections to be maximally effective, the contralateral projections between the two brain regions should be minimized. The PFC sends both ipsilateral and contralateral descending projections to the BLA (Vertes, 2004). To accommodate for this, our surgical procedures included a transection of the corpus callosum in a region just caudal to the PFC where the axons from the ipsilateral PFC cross over and descend toward the contralateral

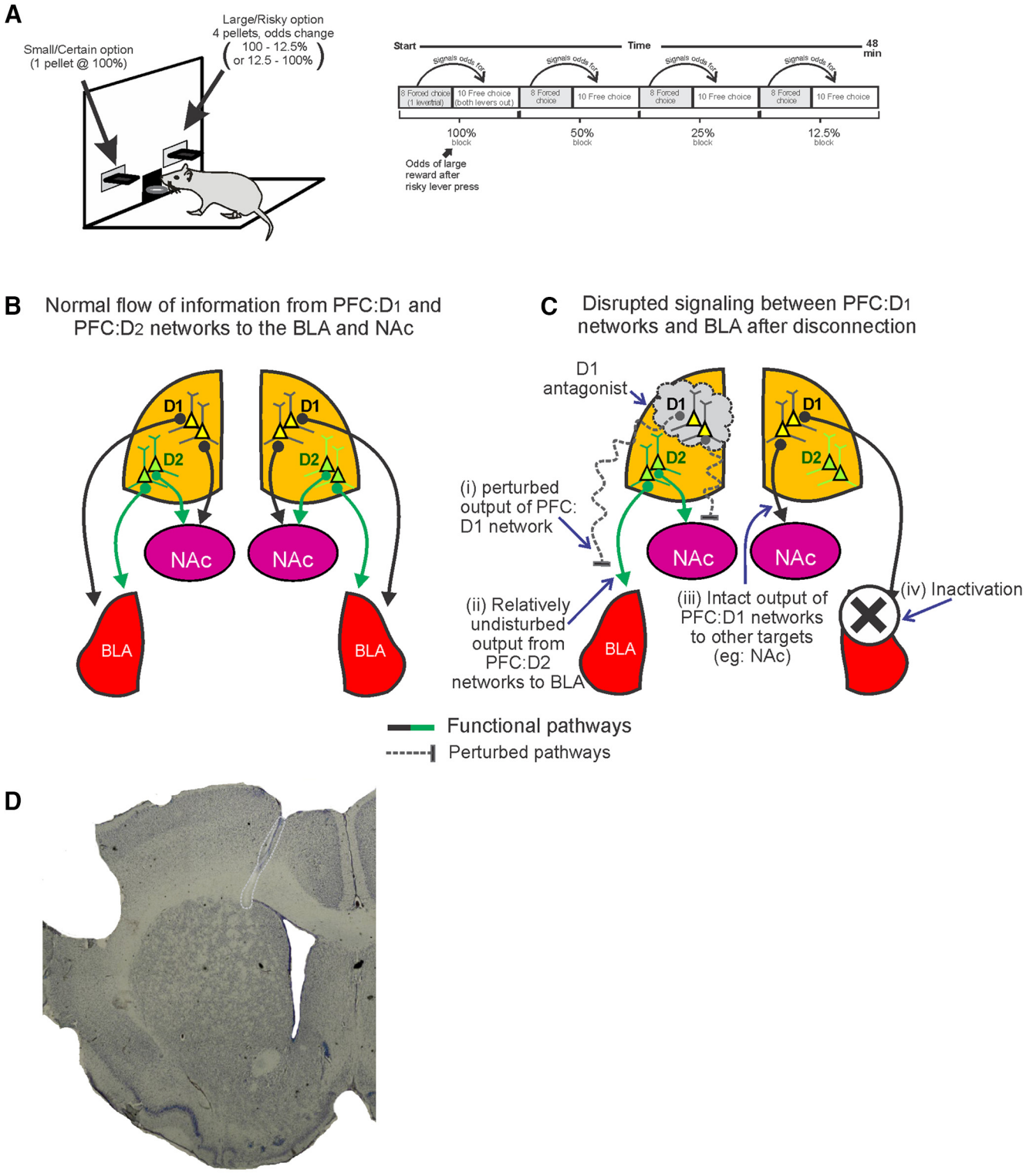

Figure 1. Task diagram, study design, and callosal transection. ***A***, Cost/benefit contingencies associated with responding on either lever on the probabilistic discounting task used in the present study. Right, Format of the sequence of forced and free choice trials within each probability block for the standard descending variant of the task in which the odds of obtaining the larger reward decreased from 100% to 12.5% across four blocks of trials. ***B***, Schematic representing normal information flow from PFC:D_1 and PFC:D_2 networks to the BLA and NAc. ***C***, Example of disrupted information flow after asymmetrical infusion of a DA receptor antagonist into the PFC combined with a contralateral inactivation of the BLA. Unilateral intra-PFC DA antagonism (a D_1 blocker in this example) is combined with a contralateral inactivation of an output nucleus (the BLA). This disrupts selectively D_1 modulation of networks of PFC neurons that ultimately interface with the BLA in both hemispheres, but leaves other pathways (e.g., D_2-expressing PFC networks interfacing with NAc/BLA, PFC-D_1⟶NAc) relatively intact. ***D***, Photomicrograph displaying representative callosal transection (highlighted region) for studies involving PFC⟶BLA disconnections.

BLA, as described in the subsequent section. An example of this type of transection is presented in Figure 1*D*.

Surgery. Rats were provided food *ad libitum for* 1–3 d before surgery, given a subanesthetic dose of ketamine and xylazine (50 and 4 mg/kg, respectively), and maintained on isoflurane for the duration of the procedure. They were then implanted stereotaxically with two sets of bilateral 23-gauge stainless steel guide cannula. Two different combinations of implantations were used (flat skull): (1) medial PFC: anteroposterior (AP) = +3.4 mm; medial-lateral (ML) = ±0.7 mm from bregma; dorsoventral (DV) = −2.8 mm from dura and NAc: AP = +1.5 mm; ML ±1.3 mm; DV = −6.3 mm; or (2) medial PFC (same coordinates as above) and BLA: AP = −3.0; ML = ± 5.3 mm; DV = −7.0 mm. Rats implanted with cannulae in the BLA also received a 1 mm transection of the corpus callosum (AP = +1.0 mm; ML= ± 0.7 mm; DV= −4.0 mm) to sever axonal projections from one hemisphere of the PFC to the contralateral BLA (St. Onge et al., 2012a). This procedure was not required for rats implanted with cannulae in the NAc because the corpus callosum was transected by the NAc guide cannula. Cannulae were held in place with stainless steel screws and dental acrylic. Thirty gauge obdurators were inserted into the guide cannula and remained in place until infusions were performed. The animals were given a minimum of 1 week to recover from surgery before being retrained on the probabilistic discounting task for a minimum of 5 d and until a group reestablished stable patterns of choice behavior.

Drugs, microinfusion procedure, and experimental design. Once stable choice behavior was reestablished, animals received a mock infusion to familiarize them with the procedures. Obdurators were removed and injectors were placed inside the guide cannula for 2 min, but no infusion was administered.

One or 2 d after the mock infusion, animals received their first microinfusion test day. Drugs or saline were infused at a volume of 0.5 μl. The DA antagonists used in this study were as follows: D_1 antagonist R-(+)-SCH23390 hyrdochloride (1 μg; Sigma-Aldrich) and D_2 antagonist eticlopride hydrochloride (1 μg; Sigma-Aldrich) dissolved in 0.9% saline. Inactivations were induced using a solution containing the $GABA_B$ agonist baclofen (125 ng for BLA experiments, 100 ng for NAc experiments; Sigma-Aldrich) and the $GABA_A$ agonist muscimol (125 ng for BLA experiments, 100 ng for NAc experiments; Sigma-Aldrich). These doses were chosen based on previous studies showing that they are maximally effective at altering risky choice when infused bilaterally into different target regions (Ghods-Sharifi et al., 2009; Stopper and Floresco, 2011; St. Onge et al., 2011). Previous studies have shown that infusion of these GABA agonists at similar concentrations and volumes can induce dissociable effects on behavior when administered into different subregions of the BLA or NAc that are separated by ~1 mm (McLaughlin and Floresco, 2007; Stopper and Floresco, 2011). A lower dose of baclofen/muscimol was infused into the NAc compared with the BLA because initial studies using the higher dose yielded a disproportional number of trial omissions. Infusions were administered via 30-gauge injection cannulae that protruded 0.8 mm past the end of the guide cannulae at a rate of 0.4 μl/min by a microsyringe pump so that the infusion lasted 75 s. The injection cannulae remained in place for 1 additional minute to allow for diffusion.

For the probabilistic discounting experiments, four separate groups of rats received three counterbalanced infusions 10 min before behavioral testing on separate days (a within-subjects design). The order of treatment and hemispheres that received the ipsilateral/contralateral infusions was counterbalanced across animals. The three primary treatment conditions were as follows: (1) asymmetrical unilateral saline infusions in the PFC and BLA/NAc (control), (2) infusions of a D_1 or a D_2 antagonist into one hemisphere of the PFC in combination with GABA agonists into the BLA or NAc in the same hemisphere (ipsilateral condition), and (3) intra-PFC infusion of a D_1 or D_2 antagonist in combination with GABA agonists into the contralateral BLA or NAc (disconnection). For disconnections involving the BLA, GABA agonists were infused into the hemisphere ipsilateral to the callosal transection. After the first infusion test, animals were retrained for 1–3 d until their choice behavior deviated by >10% from their preinfusion baseline, after which they received their second counterbalanced sequence of infusions, and this continued until each rat received the three primary treatments. In these experiments, three rats (all with PFC–NAc cannulae) either made no choices over 10 free choice trials in a particular block or made more than five omissions during free choice trials in two or more blocks after either ipsilateral or asymmetrical drug infusions. These rats were retrained after the first sequence of infusions was complete and then received an additional test day of same treatment. The data from both days were combined to ensure that there were sufficient choice data for each block of trials for the analyses.

After the initial series of infusion tests were complete, a subset of rats from each group were retrained for 3–5 d before receiving another sequence of counterbalanced single unilateral infusions. These included infusions of either the D_1 or D_2 antagonists into the PFC or GABA agonists into the BLA or NAc, as well as unilateral saline infusions in these regions as a control. Given the asymmetrical nature of the infusions administered, no rat received >3 infusions in a particular brain region.

The reward magnitude discrimination experiments were conducted in a separate group of rats. Here, animals received unilateral infusions of both D_1 and D_2 antagonists into the PFC, in combination with inactivation of the NAc on separate days. However, the infusions were performed in a manner such that each hemisphere of the PFC was infused with only the D_1 or D_2 antagonist for both the contralateral and ipsilateral treatments.

Histology. After completion of all test days, rats were killed in a carbon dioxide chamber. Brains were fixed in a 4% formalin solution. Each brain was frozen and sliced in 50 μm sections, mounted, and stained with Cresyl violet. Placements were located with reference to the neuroanatomical atlas of Paxinos and Watson (2005). When plotting these placements on the figures, each point represented the tip of the injector cannula. Data from rats with placements that resided outside of the borders of the mPFC, NAc, or BLA were removed from the analysis.

Data analysis. The primary dependent variable of interest was the proportion of choices of the large reward option, factoring out trial omissions. This was calculated in each block by dividing the number of choices of the large reward lever by the total number of trials in which the rats made a choice. Choice data were typically analyzed using two-way within-subjects ANOVA with treatment and trial block as the two within-subjects factors. However, for experiments in which we assessed the effects of a disconnection on both the descending and ascending variants of the discounting task, data were analyzed using three-way between-/within-subjects ANOVA, with treatment and block as two within-subject factors and task variant as a between-subjects factor. In this analysis, a treatment × task or three-way interaction indicates that these treatments altered discounting in different manners in animals tested on one variant of the task versus the other. All follow-up multiple comparisons were made using Dunnett's test where appropriate. In these analyses, the main effect of trial block was always significant ($p < 0.001$) and will not be discussed further.

If a treatment induced a significant alteration in choice behavior on the probabilistic discounting task, we conducted a supplementary analysis to clarify whether these effects were attributable to changes in reward sensitivity (win–stay behavior) and/or negative-feedback sensitivity (lose–shift behavior). Each choice was analyzed according to the outcome of the preceding free choice trial and expressed as a ratio. The win–stay score was calculated as a proportion of the number of risky choices made after receipt of the larger reward (a risky win) divided by the total number of larger rewards obtained. Lose–shift scores were calculated as the proportion of small/certain choices made after a nonrewarded risky choice (risky loss) over the total number of nonrewarded choice trials. These scores were analyzed together using a two-way ANOVA, with response type (win–stay or lose–shift) and treatment as the two within-subject factors. Changes in win–stay/lose–shift behavior indexed changes in reward and negative feedback sensitivity, respectively. In addition, response latencies and the number of trial omissions were analyzed with one-way repeated-measures ANOVAs.

Results

Dopaminergic D_1 and D_2 modulation of PFC→BLA circuits

Selective disruption of top-down signals from the PFC to the BLA increased risky choice on a probabilistic discounting task when reward probabilities were initially high and then decreased over a session (St. Onge et al., 2012a), suggesting that this pathway may facilitate adjustments in choice biases as reward probabilities change. In one set of experiments, we sought to determine whether the integrity of PFC neural networks that interact with the BLA to facilitate flexible decision making are dependent on modulation by D_1 or D_2 receptor activity.

PFC:D_1

One group of rats trained on the descending variant received counterbalanced infusions of a D_1 antagonist (SCH 23390) into one hemisphere of the PFC combined with an inactivation (baclofen/muscimol) of either the same (ipsilateral disconnection) or contralateral (functional disconnection) hemisphere of the BLA or asymmetrical saline infusions as a control. Data from 12 rats with acceptable placements in both regions were included in the analysis (Fig. 2*D*). Disrupting D_1 modulation of PFC→BLA outputs had no significant effect on choice behavior ($F_{(2,22)} = 0.96$, $p = 0.40$; Fig. 2*A*), choice latencies, or trial omissions (all $F < 0.96$, all $p > 0.39$; Table 1). These results suggest that D_1 receptor modulation of PFC networks that interface with the BLA do not play an integral role in refining this form of decision making.

PFC:D_2

A separate group received unilateral intra-PFC infusions of the D_2 antagonist eticlopride in combination with ipsilateral/contralateral inactivation of the BLA. In an initial experiment, one group was trained on the standard descending variant of the discounting task and a preliminary analysis of these data revealed that contralateral PFC:D_2 disconnections increased risky choice relative to control treatments (described in the subsequent section). This finding prompted us to investigate whether these effects reflected an impairment in adjusting choice biases in response to changes in reward probabilities or a general increase in preference for larger, uncertain rewards. Therefore, we tested the effects of these same manipulations in a separate group trained on the ascending variant of the task.

The overall analysis included data from 14 (descending variant) and eight (ascending variant) rats with acceptable placements in both regions. Choice data from both groups were analyzed together with a three-way, between-/within-subjects ANOVA, with task variant (descending or ascending) as a between-subjects factor. This main analysis produced a significant three-way, treatment × block × task interaction ($F_{(6,120)} = 2.40$, $p = 0.03$), as well as a significant task × treatment interaction ($F_{(2,40)} = 4.19$, $p = 0.02$). These statistical interactions reflected that PFC:D_2→BLA disconnection altered choice differentially in a manner dependent on the direction that the reward probabilities changed over a session. Subsequent partitioning of these interactions consisted of separate two-way ANOVAs of the data from animals trained on each task variant.

Choice data from rats trained on the descending variant are presented in Figure 2*B*. Unlike the lack of effect of PFC:D_1→BLA disconnections, disrupting D_2 modulation of PFC→BLA networks increased choice of the large/risky option. This was confirmed by a two-way ANOVA that revealed a significant main effect of treatment ($F_{(2,26)} = 4.55$, $p = 0.02$), although the treatment × block interaction did not achieve statistical significance ($F_{(6,78)} = 1.87$, $p = 0.09$). Under control conditions, rats initially displayed a strong bias for the large/risky option, but gradually shifted choice away from this option as reward probabilities decreased across blocks. However, PFC:D_2→BLA disconnections led to a greater proportion of risky choices that continued across the session. Notably, this effect was similar to that induced by either bilateral PFC D_2 receptor antagonism or disconnection of PFC→BLA circuitry (St. Onge et al., 2011, 2012a). Multiple comparisons with Dunnett's tests confirmed that contralateral disconnections induced a significant ($p < 0.05$) increase in choice of the large/risky option, whereas choice after ipsilateral disconnections did not differ from saline treatments.

In contrast, for rats trained on the ascending variant, PFC:D_2→BLA disconnections induced the opposite profile (Fig. 2*C*). Here, rats under control conditions did not display a strong preference for either option at the start of the session, but shifted choice bias toward the large/risky option as its profitability increased over blocks. Again, these shifts in choice were disrupted by PFC:D_2→BLA disconnections. The two-way ANOVA yielded a significant treatment × block interaction ($F_{(6,42)} = 2.31$, $p = 0.05$), but no significant main effect of treatment ($F_{(2,14)} = 1.49$, $p = 0.26$). Additional simple main-effects analysis revealed no differences in choice across treatments during the initial 12.5% block ($F_{(2,42)} = 0.44$, $p = 0.65$). However, in each of the subsequent blocks, rats selected the large/risky option less often after asymmetrical drug infusions compared with saline treatments (all $F_{(2,42)} > 4.1$, all $p < 0.05$ and Dunnett's $p < 0.05$). In comparison, ipsilateral disconnections only reduced risky choice during the 25% block (Dunnett's $p < 0.05$), but not the last two blocks, relative to saline. With respect to other performance measures, there were no significant differences across treatments in choice latencies or trial omissions (all $F > 2.34$, all $p > 0.07$; Table 1). Collectively, these findings suggest that D_2 receptor activity modulates networks of PFC neurons interfacing with the BLA to facilitate modification of decision biases in response to changes in likelihood of receiving larger rewards. After disruption of communication between PFC:D_2 networks and the BLA, rats trained on either task variant persisted with their initially more preferred option as the profitability of the risky option changed.

Analysis of win–stay and lose–shift behavior exposed additional differences in the effects of PFC:D_2→BLA disconnections on performance of the two task variants. The overall analysis of these data revealed a significant three-way treatment × response type (win–stay/lose–shift) × task interaction ($F_{(2,40)} = 4.22$, $p = 0.02$), suggesting that disrupting D_2 modulation of PFC→BLA communication affected reward versus negative feedback sensitivity differentially depending on the manner in which reward probabilities changed. Follow-up two-way ANOVAs also yielded treatment × task interactions for win–stay ($F_{(2,40)} = 3.93$, $p = 0.03$) and lose–shift ($F_{(2,40)} = 3.95$, $p = 0.03$) behavior. Subsequent simple main-effects analysis further revealed that, for the descending condition, the increase risky choice induced by asymmetrical disconnections was not associated with a change in win–stay behavior ($F_{(2,26)} = 0.35$, $p = 0.71$), but rather, this was driven by a reduction in sensitivity to losses after contralateral, but not ipsilateral, disconnections ($F_{(2,26)} = 5.25$, $p = 0.01$ and Dunnett's $p < 0.05$; Fig. 2*E*). This indicates that asymmetrical PFC:D_2→BLA disconnections made rats less likely to shift to the small/certain option after a nonrewarded risky choice. Conversely, for the ascending condition, the reduction in risky choice induced by contralateral PFC:D_2→BLA disconnection was accompanied by a diminished tendency to follow a rewarded risky choice with another risky choice (i.e.; reduced win–stay behavior; $F_{(2,14)} = 3.84$, $p = 0.05$ and Dunnett's $p < 0.05$; Fig. 2*F*). Lose–

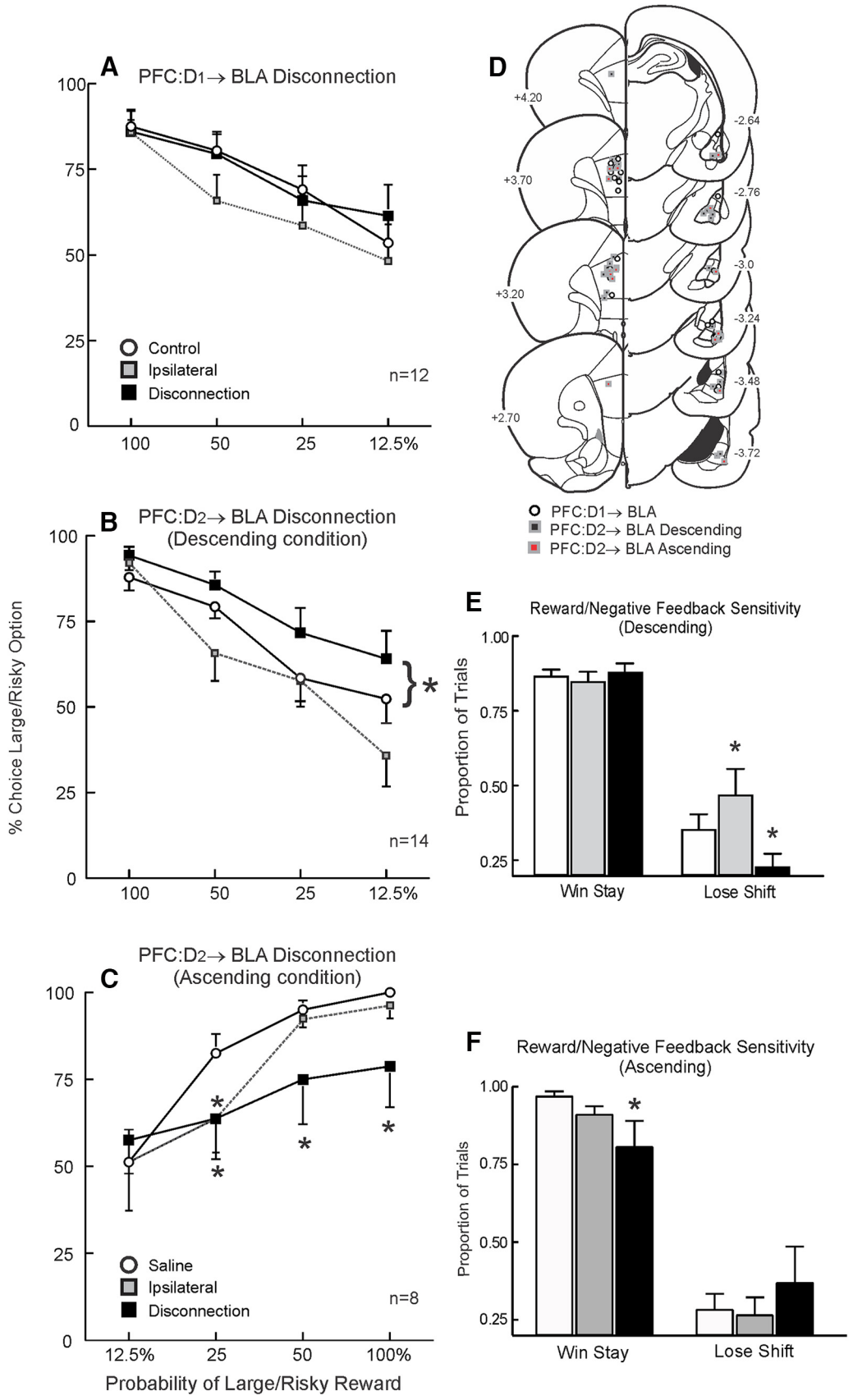

Figure 2. Disrupting D_2 (but not D_1) modulation of PFC⟶BLA communication impaired adjustments in choice biases. ***A***, Percentage choice of the large/risky option after disruption of D_1 modulation of PFC⟶BLA circuits and control treatments across four blocks of free choice trials. Symbols represent mean. Error bars indicate SEM. ***B***, Choice data after PFC:D_2⟶BLA disconnections from rats trained on the descending task variant in which reward probabilities decreased from 100% to 12.5% across blocks. These treatments increased risky choice in the latter trial blocks ***C***, Choice data from rats trained on the ascending (12.5% to 100%) variant of the task. In this instance, PFC:D_2⟶BLA disconnections led to fewer risky choices in the latter trial blocks. (*Figure legend continues.*)

Table 1. Response latencies and trial omissions

	Response latencies (s)	Trial omissions (no.)
PFC:D_1→BLA		
Control	1.44 ± 0.20	5.9 ± 0.2
Ipsilateral	1.47 ± 0.20	7.2 ± 0.2
Disconnection	1.48 ± 0.14	8.0 ± 2.5
PFC:D_2→BLA (descending)		
Control	0.69 ± 0.10	3.1 ± 1.0
Ipsilateral	1.08 ± 0.16	3.2 ± 1.4
Disconnection	1.45 ± 0.27	8.8 ± 2.7
PFC:D_2→BLA (ascending)		
Control	0.47 ± 0.06	0.0 ± 0.0
Ipsilateral	0.51 ± 0.06	0.1 ± 0.1
Disconnection	0.50 ± 0.06	0.4 ± 0.2
PFC:D_1→NAc		
Control	0.92 ± 0.12	2.0 ± 1.2
Ipsilateral	1.37 ± 0.20*	11.0 ± 4.2*
Disconnection	1.42 ± 0.18*	13.4 ± 3.5*
PFC:D_2→NAc		
Control	0.64 ± 0.05	0.4 ± 0.2
Ipsilateral	1.24 ± 0.21*	7.2 ± 2.4*
Disconnection	1.18 ± 0.13*	5.6 ± 2.2*
Unilateral $D_{1\ antagonist}$		
Saline	0.80 ± 0.14	0.4 ± 0.3
SCH 23390 1 μg	0.68 ± 0.12	0.8 ± 0.4
Unilateral $D_{2\ antagonist}$		
Saline	0.74 ± 0.12	1.0 ± 0.7
Eticlopride 1 μg	0.72 ± 0.08	0.6 ± 0.5
Unilateral BLA inactivation		
Saline	1.08 ± 0.18	4.4 ± 3.8
Inactivation	0.85 ± 0.25	2.0 ± 1.5
Unilateral NAc inactivation		
Saline	0.74 ± 0.09	0.3 ±0.2
Inactivation	0.90 ± 0.15	1.9 ±0.8
Magnitude discrimination		
PFC:D_1→NAc		
Control	0.89 ± 0.08	0.3 ± 0.2
Ipsilateral	1.37 ± 0.33	5.3 ± 2.3
Disconnection	1.17 ± 0.31	4.8 ± 3.1
PFC:D_2→NAc		
Control	0.89 ± 0.08	0.3 ± 0.2
Ipsilateral	0.88 ± 0.15	0.6 ± 0.3
Disconnection	1.54 ± 0.45	5.0 ± 1.6*

Data represent means ± SEM.

*$p < 0.05$ versus control treatments.

shift behavior did not differ across treatments ($F_{(2,14)} = 0.76, p = 0.49$). Therefore, when the odds of receiving larger rewards were initially high and then decreased, PFC:D_2→BLA networks appear to monitor changes in the frequency of nonrewarded actions. Conversely, when larger rewards are initially rare and then occur more frequently, these networks appears to attune more to variations in how often risky choices are rewarded.

←

(*Figure legend continued.*) ***D***, Schematic of sections of the rat brain showing location of acceptable infusions in the PFC and BLA for rats in these experiments. Numbers correspond to millimeters from bregma. Figure represents the asymmetrical disconnection procedure for clarity; hemispheres of infusions were counterbalanced across rats. ***E***, Win–stay/lose–shift ratios after saline infusions and ipsilateral and functional disconnections of the PFC:D_2→BLA pathway in animals trained on the descending variant. Under these conditions, PFC:D_2→BLA disconnections reduced sensitivity to "losses." ***F***, For rats tested on the ascending variant of the task, PFC:D_2→BLA disconnections reduced the tendency to follow a rewarded risky choice with another risky choice (i.e., win–stay behavior). *$p < 0.05$ versus control.

Dopaminergic D_1 and D_2 modulation of PFC→NAc circuits

Inactivation of the NAc reduces preference for large/risky rewards (Stopper and Floresco, 2011) but, somewhat surprisingly, complete disconnection of PFC→NAc circuitry entailing contralateral inactivations of both regions did not alter probabilistic discounting (St. Onge et al., 2012a). The possibility remains that a more selective disruption of a subset of PFC neurons (namely, those under modulatory control by D_1 or D_2 receptors) may reveal a contribution of this circuit to mediating certain components of risk/reward decision making. Separate groups of rats implanted with cannulae in the PFC and NAc underwent identical training to those in the PFC–BLA groups before receiving counterbalanced infusions of either D_1 or D_2 antagonists into one hemisphere of the PFC in combination with an ipsilateral or asymmetrical inactivation of the NAc.

PFC:D_1

Data from 16 rats with acceptable placements in the PFC and the NAc were included in the analysis (Fig. 3*E*). In stark contrast to the lack of effect from PFC:D_1→BLA disconnections, similar disconnections involving the NAc significantly reduced risky choice relative to saline, as indicated by a significant main effect of treatment ($F_{(2,30)} = 5.87, p = 0.007$), but no treatment × block interaction ($F_{(6,90)} = 1.02, p = 0.42$). Follow-up comparisons confirmed that disconnection treatments reduced risky choice relative to saline ($p < 0.05$). Choice behavior after ipsilateral disconnections was not statistically different from control treatments (Fig. 3*A*). As has been observed after conventional disconnections of this circuitry (St. Onge et al., 2012a), both ipsilateral and contralateral PFC:D_1→NAc disconnections increased choice latencies ($F_{(2,30)} = 4.31$, $p = 0.02$ and Dunnett's $p < 0.05$) and trial omissions ($F_{(2,30)} = 5.97, p = 0.007$ and Dunnett's $p < 0.05$; Table 1). These latter findings suggest that ipsilateral disruption of this pathway is sufficient to alter some aspects of behavior related to vigilance or motivation to approach reward-related stimuli.

Additional analysis of win–stay/lose–shift behavior revealed a significant treatment × response type interaction ($F_{(2,30)} = 6.44$, $p = 0.005$). This interaction was driven by a reduction in win–stay behavior ($F_{(2,30)} = 4.46, p = 0.02$) after the asymmetrical, but not ipsilateral, treatments (Dunnett's $p < 0.05$). These treatments also increased lose–shift tendencies in a manner similar to bilateral antagonism of PFC D_1 receptors (St. Onge et al., 2011), although the one-way ANOVA on these data only revealed a trend toward statistical significance ($F_{(2,30)} = 2.46$, $p = 0.10$; direct comparison of saline vs disconnection, $t_{(15)} = 1.98$, $p = 0.07$; Fig. 3*B*). These data indicate that D_1 receptor activity modulates PFC→NAc projections that in turn promote choice of larger, risky rewards. Furthermore, normal activity at D_1 receptors within this network appears to bias choice by increasing the tendency to repeat rewarded actions while potentially mitigating the tendency to shift choice direction after nonrewarded ones.

PFC:D_2

Nineteen rats that received unilateral intra-PFC infusions of the D_2 antagonist combined with inactivation treatments within the NAc had acceptable placements within both regions (Fig. 3*E*). Analysis of the choice data from these animals revealed a significant main effect of treatment ($F_{(2,36)} = 6.34$, $p = 0.004$), but no treatment × block interaction ($F_{(6,108)} = 1.09, p = 0.38$; Fig. 3*C*). Multiple comparisons revealed that this effect was driven primarily by the ipsilateral infusion condition, which induced a considerable reduction in risky choice relative to saline ($p < 0.05$).

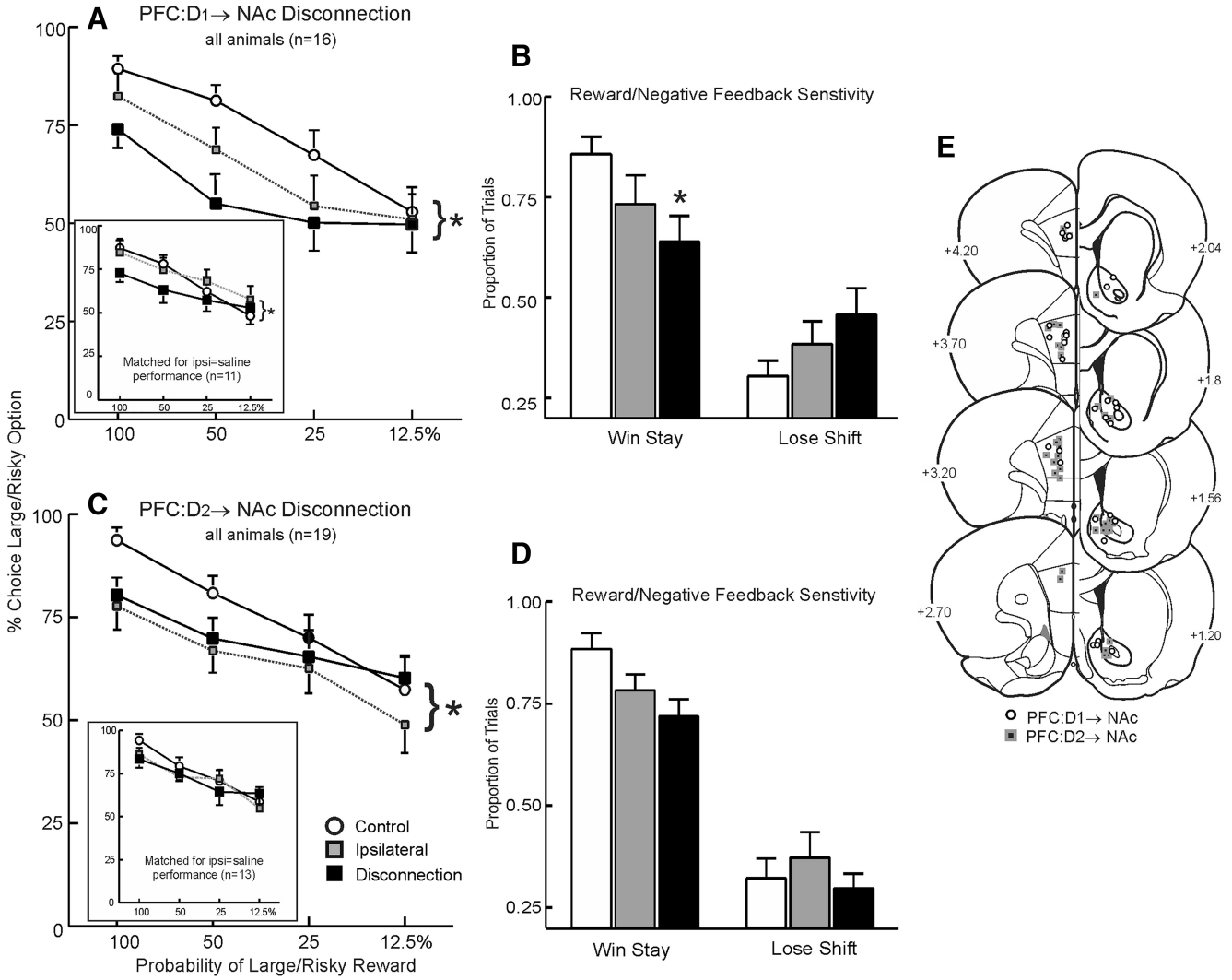

Figure 3. Effects of disrupting D_1 and D_2 modulation of PFC⟶NAc communication on risk/reward decision making. ***A***, PFC:D_1⟶NAc disconnection markedly reduced risky choice, whereas ipsilateral treatments did not differ significantly from control treatments. Inset, Data from a subset of rats that did not show a reduction in risky choice after ipsilateral inactivation ($n = 11$), yet still showed a reduction in risky choice after functional disconnection (*$p < 0.05$). ***B***, Win–stay/lose–shift data. PFC:D_1⟶NAc disconnection reduced reward sensitivity and increased sensitivity to reward omissions. ***C***, Choice for the large/risky option after PFC:D_2⟶NAc disconnection and control treatments. In this experiment, only ipsilateral drug infusions caused a significant reduction in risky choice relative to control treatments. Inset, Data for the subset of rats that did not show a decrease in risky choice after ipsilateral inactivation ($n = 13$). In these animals, ipsilateral and asymmetrical infusions did not alter choice relative to control treatments. ***D***, Win–stay/lose–shift behavior after PFC:D_2⟶NAc disconnections. ***E***, Acceptable location of infusions through the rostral-caudal extent of the medial PFC and NAc.

Conversely, asymmetrical PFC:D_2⟶NAc disconnections did not cause a statistically significant change in risky choice. Analysis of win–stay/lose shift behavior revealed a significant effect of treatment ($F_{(2,36)} = 3.73$, $p = 0.03$), but no significant interaction ($F_{(2,36)} = 1.67$, $p = 0.20$; Fig. 3*D*). This result reflects a reduction in both win–stay and lose–shift tendencies after the asymmetrical disconnection compared with saline, although inspection of Figure 3*D* indicates that this was driven primarily by a reduction in reward rather than negative feedback sensitivity. With respect to other performance measures, both ipsilateral and contralateral PFC:D_2⟶NAc disconnections increased choice latency ($F_{(2,36)} = 8.80$, $p = 0.001$ and Dunnett's $p < 0.05$) and trial omissions ($F_{(2,36)} = 5.50$, $p = 0.008$; Table 1).

There are other examples in the literature showing that certain forms of reward seeking are sensitive to disruption by both ipsilateral and contralateral disconnection of cortical or limbic inputs to the NAc such as reinstatement of drug-seeking behavior (Bossert et al., 2016) and probabilistic discounting (St. Onge et al., 2012a). However, closer inspection of the data from the PFC:D_2⟶NAc disconnection experiment revealed that the reduction in risky choice induced by ipsilateral treatments was driven primarily by six of the 19 animals, the behavior of which seemed to be much more sensitive to this manipulation compared with the rest of the group. These animals displayed a 20–40% reduction in choice of the large/risky lever over the session after ipsilateral disconnection, whereas the remaining 13 rats showed minimal change relative to saline treatments (on average, <5%). Comparison of the relative change in risky choice induced by ipsilateral disconnections versus saline treatment revealed a statistically significant difference between these two subgroups ($t_{(17)} = 7.24$, $p < 0.001$).

In light of this, we conducted a supplementary analysis on the choice data obtained from the 13 rats that showed similar patterns of choice behavior on their ipsilateral and saline infusion days, as we have done previously (St. Onge et al., 2012a). Notably, in this subset of rats, there were no differences across treatment

conditions ($F_{(2,24)} = 1.93$, $p = 0.17$; Fig. 3*C*, inset). We subsequently conducted a similar targeted analysis on data obtained in the PFC:D_1→NAc disconnection. In this case, ipsilateral disconnections induced a 20–40% reduction in risky choice relative to saline in 5 rats; the remaining 11 animals showed minimal change in choice (<3%; between-group comparison: ($t_{(14)} = 6.63$, $p < 0.001$). In contrast to the above-mentioned findings, when we analyzed choice data from these 11 animals, the reduction in risky choice induced by asymmetrical PFC:D_1→NAc disconnection was still apparent ($F_{(2,20)} = 6.14$, $p = 0.008$; Fig. 3*A*, inset). Together, the results of these two experiments suggest that dopaminergic modulation of PFC networks that interface with the NAc to refine risk/reward decision making is mediated primarily by actions on D_1 receptors, with D_2 receptor activity playing, at most, a secondary role in these processes.

Unilateral control infusions

After their first set of microinfusion test days, a subset of rats were retrained on the discounting task and then received three more unilateral infusions to ascertain whether unilateral inactivation of the NAc or BLA or disruptions of PFC DA activity had any disruptive effect on behavior. Different groups received either unilateral intra-PFC infusions of the D_1 ($n = 8$) or D_2 ($n = 11$) antagonist, unilateral inactivation of the BLA ($n = 6$) or NAc ($n = 14$), and each of these animals also received a corresponding saline infusion. The order of infusions and the hemisphere in which they were administered were counterbalanced across animals. Notably, none of these unilateral manipulations had any effects on choice behavior ($F < 0.26$, $p > 0.62$; Fig. 4*A–D*). These important null effects confirm a critical assumption of these disconnection designs, showing that one hemisphere was in fact able to compensate for a unilateral disruption of activity in a region from the other hemisphere. Furthermore, they indicate that alterations in choice behavior induced by the different disconnections used here are likely the result of disrupted communication between different populations of PFC neurons and their subcortical targets, rather than perturbations induced by any single unilateral manipulation.

Reward magnitude discrimination

Disruption of both D_1 and D_2 modulation of PFC→NAc pathways reduced preference of the large/risky reward. To assess whether this was driven by a general disruption in discriminating between different reward magnitudes, we conducted a follow-up experiment with a separate group of rats trained on a simpler task requiring them to choose between two levers that delivered either one or four reward pellets, both with 100% certainty. Nine rats with acceptable placements in the PFC and NAc were trained for ~10 d, after which they displayed a strong preference for the larger reward. They then received counterbalanced ipsilateral and contralateral PFC:D_1 and PFC:D_2→NAc disconnections, as well asymmetrical saline infusions. The data from each type of disconnection (D_1 vs D_2) were analyzed separately. PFC:D_1→NAc disconnections did not alter choice ($F_{(3,24)} = 0.857$, $p = 0.43$; Fig. 4*E*), nor did it affect choice latencies or trial omissions ($F < 1.81$, $p > 0.20$; Table 1). Similarly, PFC:D_2→NAc disconnections also did not affect preference for larger vs smaller rewards ($F_{(2,16)} = 1.09$, $p = 0.36$; Fig. 4*F*), or choice latencies ($F_{(2,16)} = 2.15$, $p = 0.15$), although these asymmetrical infusions did increase omissions ($F_{(2,16)} = 8.23$, $p = 0.003$ and Dunnett's $p < 0.05$; Table 1). The location of these infusions is displayed in Figure 4*G*. Collectively, these data suggest that the effects of PFC→NAc disconnections on risk/reward decision making are unlikely to be attributable to impairments in discriminating between smaller versus larger rewards or nonspecific disruptions in motivational or motor processes. The finding that disruption of PFC:D_1→NAc circuits impaired win–stay behavior but did not affect the ability to discriminate rewards of differing magnitudes highlights an important distinction in how mesocortical DA mediates different aspects of reward processing. Specifically, PFC DA appears to mediate reward sensitivity in situations in which likelihood of receiving larger rewards is uncertain but does not appear to contribute to more basic forms of reward sensitivity required to discriminate between rewards of different magnitudes delivered in a deterministic manner.

Discussion

The present findings provide novel insight into how mesocortical DA facilitates dissociable component processes of risk/reward decision making via actions on anatomically and functionally dissociable networks of PFC neurons. PFC D_1 or D_2 receptors appear to modulate separate networks of neurons that promote different aspects of reward seeking via communication with distinct subcortical projection targets. DA acting on D_2 (but not D_1) receptors aids in surveying changes in reinforcement contingencies to promote flexible patterns of reward seeking via actions on a network of PFC neurons that interfaces with the BLA. Conversely, PFC D_1 receptors modulate separate networks interfacing with the NAc to bias choice toward larger, uncertain rewards, promoting repetition of rewarded actions and attenuating the impact of nonrewarded ones. Note that these results do not necessarily imply that differential patterns of behavior are manifested by DA acting exclusively on PFC→BLA or PFC→NAc projection neurons, which may express D_1 or D_2 receptors. Rather, it is likely that these receptors modulate distinct PFC networks of both local circuit interneurons and pyramidal neurons, some of which project to the BLA or NAc.

Dopaminergic modulation of PFC→BLA circuitry

Perturbing D_2 modulation of PFC→BLA networks impaired adjustments in choice, retarding shifts in bias away or toward the risky option when the odds of obtaining larger rewards decreased or increased over a session. These findings highlight a critical role for PFC D_2 receptors in promoting flexible decision making via networks exerting top-down control of the BLA and complement other studies implicating PFC D_2 receptors in mediating different forms of behavioral flexibility, such as set shifting (Floresco et al., 2006; Puig and Miller, 2015). Of particular relevance to the present findings, extinction of conditioned fear, a simpler form of flexibility also mediated by PFC→BLA circuitry, is also disrupted by PFC D_2 receptor antagonism (Mueller et al., 2010; Sierra-Mercado et al., 2011).

The effects of disrupting D_2 modulation of PFC→BLA networks were not attributable to uniform changes in reward or negative feedback sensitivity. Rather, these manipulations blunted loss sensitivity in rats trained on the descending variant, but reduced sensitivity to rewarded actions in those trained on the ascending variant. These analyses provide additional insight into how PFC→BLA circuits process volatility in reward contingencies to modify choice biases under different conditions. When the odds of obtaining larger rewards are initially good but then diminish, these circuits appear to monitor changes in the frequency of unrewarded choices. Conversely, when odds are initially poor and then improve, PFC→BLA circuits instead track how often larger rewards are received. In both situations, the PFC facilitates adjustments in choice biases by tracking deviations in the

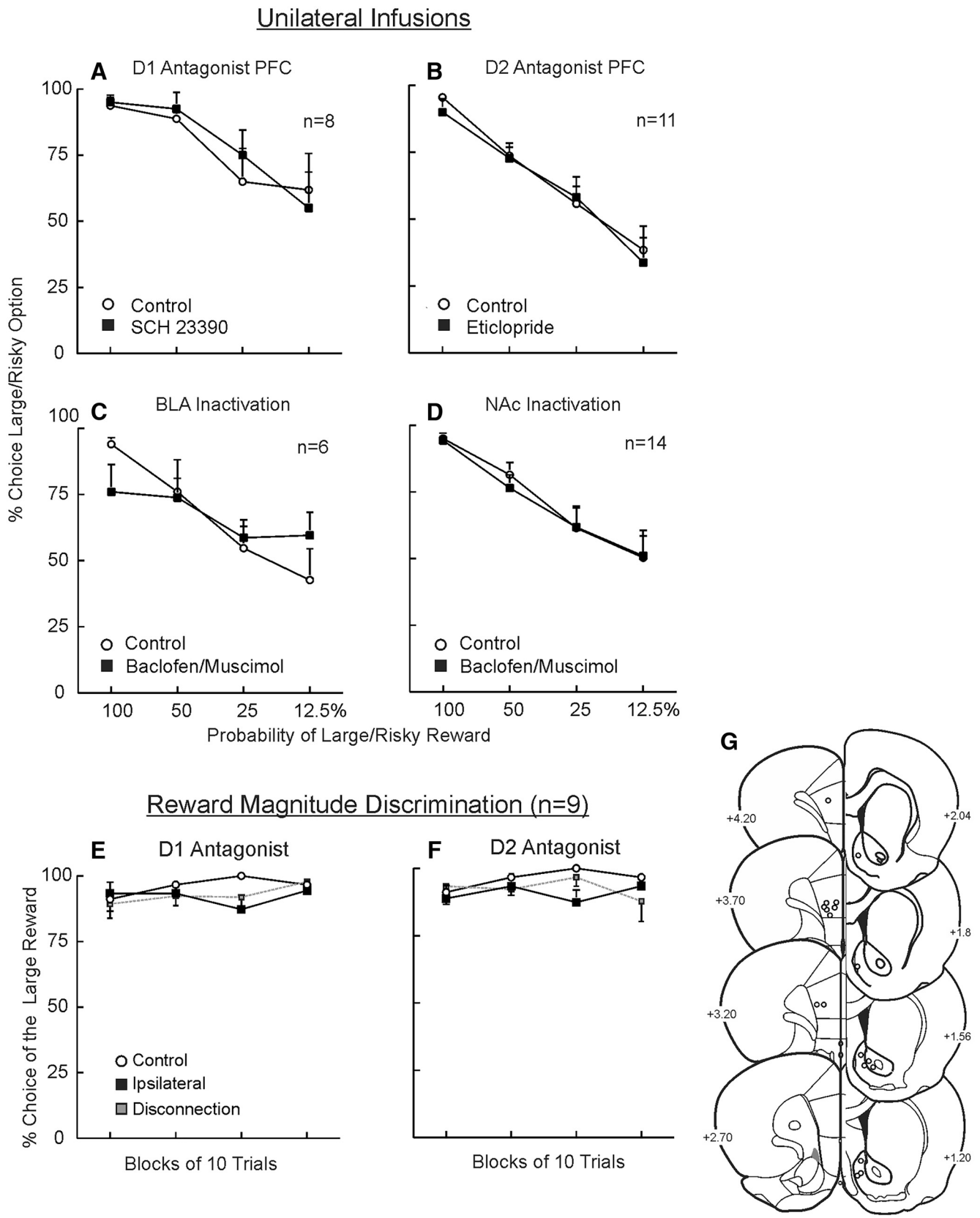

Figure 4. Control manipulations. ***A–D***, Choice behavior on the probabilistic discounting task was unaffected by unilateral infusions of a D_1 (***A***) or D_2 (***B***) antagonist in the PFC or a unilateral inactivation of the BLA (***C***) or NAc (***D***). ***E***, ***F***, Disrupting D_1 or D_2 modulation of PFC⟶NAc pathways had no effect on preference for larger versus smaller rewards on a simpler reward magnitude discrimination. ***G***, Acceptable location of infusions through the rostral-caudal extent of the medial PFC and NAc.

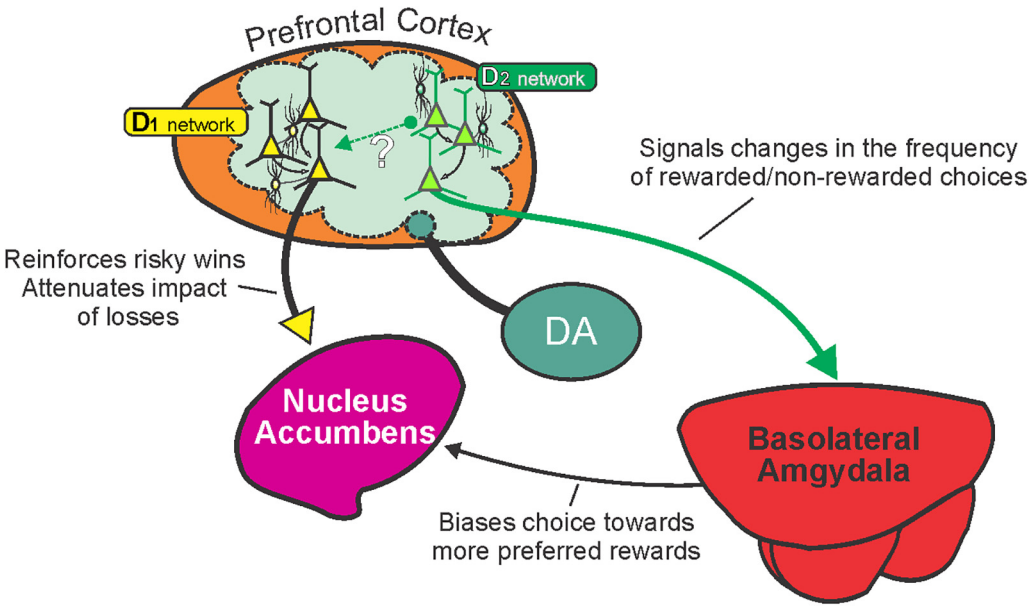

Figure 5. Diagram summarizing the implications of the present findings. DA may facilitate different aspects of action selection during risk/reward decision making through modulation of separate networks of PFC neurons that may be distinguished in terms of the DA receptor subtypes that modulate their functional output and the subcortical regions with which they interface. These networks are likely composed of both interneurons and pyramidal projection neurons, with a subpopulation of pyramidal neurons within a network sending direct projections to different subcortical targets (i.e., the BLA or NAc). D_2 modulation of PFC→BLA networks may identify changes in in the frequency of rewarded/nonrewarded actions to adjust decision biases. D_1 modulation of PFC interactions with the NAc reinforces rewarded actions and attenuates sensitivity of nonrewarded ones and these signals may be integrated with converging input from the BLA→NAc pathway to promote choice of more preferred rewards.

frequency of probabilistic outcomes that are initially more prevalent: "wins" in the descending variant and "losses" in the ascending one, with these processes being dependent on D_2 receptor modulation of PFC→BLA circuits

Conversely, disrupting PFC:D_1→BLA circuitry did not affect choice. It is interesting to compare this null result with the results of Land et al. (2014), which revealed that optogenetic silencing of D_1-expressing PFC→BLA neurons reduced food consumption. Note that optogenetic silencing determines whether neural firing of D_1-expressing neurons drives a particular behavior, but does not identify whether dopaminergic modulation of these cells plays a role in the behavior. This is in contrast to the approach used here, which more specifically assessed how D_1 modulation of certain PFC networks that interface with the BLA regulates patterns of behavior. Nevertheless, this combination of findings points to an intriguing dissociation of how PFC DA may modulate flexible reward seeking versus consumption differentially via recruitment of distinct PFC→BLA networks that are modulated preferentially by D_2 versus D_1 receptors.

Dopaminergic modulation of PFC→NAc circuitry

In contrast to the lack of effect of PFC:D_1→BLA disconnections, disrupting D_1 modulation of PFC→NAc networks reduced risky choice in a manner similar to PFC D_1 antagonism (St. Onge et al., 2011). Therefore, as opposed to PFC D_2 receptors, D_1 receptors aid in biasing choice toward larger/risky rewards that may have greater utility, acting on neurons that interface with the NAc, as opposed to the BLA. These effects were driven in part by reduced reward sensitivity, similar to what is seen after suppression of NAc neural activity (Stopper and Floresco, 2011; Dalton et al., 2014). However, bilateral antagonism of D_1 receptors within PFC or NAc also increased sensitivity to losses (St. Onge et al., 2011; Stopper et al., 2013). Together, these results indicate that D_1 modulation of PFC→NAc pathways refine action selection during reward seeking by both supporting the reinforcement of rewarded actions (Britt et al., 2012) and potentially suppressing shifts in choice direction after nonrewarded ones. Interestingly, previous work by our group has shown that complete disconnection of PFC→NAc communication using asymmetrical inactivations did not alter choice behavior reliably (St. Onge et al., 2012a). The present findings highlight that more selective targeting of certain corticostriatal circuits may sometimes be more effective at revealing their contributions to behavior compared with more global disruptions of communication between regions.

Unlike the clear alteration in decision making induced by PFC:D_1→NAc disconnections, a parallel experiment combining PFC D_2 receptor antagonism with NAc inactivation yielded more equivocal results. The overall analysis revealed a statistically significant difference between treatments, yet this was driven by a reduction in risky choice after ipsilateral infusions. Asymmetrical infusions yielded a comparatively smaller effect not significantly different from control treatments. A parsimonious conclusion from these findings may be that D_2 receptors also modulate PFC inputs to the NAc to promote choice of large/risky rewards and these processes are particularly sensitive to ipsilateral disruption of this circuitry. However, closer inspection of the individual data revealed the effects of the ipsilateral treatment were driven by only six of 19 animals, whereas the majority of the rats in this experiment were relatively unaffected. Notably, a targeted analysis on the subset of rats that were insensitive to the ipsilateral treatment revealed no differences across treatment conditions on any performance measures, although this effect was still apparent in the PFC:D_1→NAc group. It should also be emphasized that single, unilateral infusions of DA antagonists in the PFC or GABA agonists into the NAc did not affect choice. The possibility remains that some D_2-expressing neurons may influence PFC:D_1→NAc networks via local circuit connections. However, taking all of these observations into consideration, our impres-

sion is that D_2 receptors do not serve a particularly consequential function in modulating the activity of PFC networks that interface with the NAc to guide choice during this form of decision making. At most, D_2 receptor modulation of PFC→NAc network activity plays an ancillary, permissive role in mediating choice relative to the more prominent contribution of D_1 receptors. Here, it is important to emphasize that, if D_2 receptors do play some role in refining choice via actions on this corticostriatal pathway, then these effects are distinct from how it influences reward seeking via PFC→BLA circuits. This provides additional evidence that mesocortical DA can affect action selection in differing and sometimes opposing ways depending on the particular network and output pathway that it may act upon.

Proposed framework

These findings clarify how DA, acting on different receptors within a particular brain region, can facilitate dissociable aspects of behavior, identifying complementary yet distinct roles for mesocortical DA within distributed corticoamygdala–striatal circuitry that refine decision making. D_1/D_2 modulation may enhance or suppress patterns of activity of different neurons within each network that may in turn encode different types of information used to guide action selection, such as changes in frequency of rewarded actions or the relative utility of different options. These differential effects may also be mediated by the manner in which D_1 and D_2 networks register and respond to variations in DA extracellular levels, which display dynamic fluctuations within the PFC during this form of decision making (St. Onge et al., 2012b). Therefore, DA, acting on D_2 receptors, facilitates adjustments in decision biases via actions on PFC neural networks that interface with the BLA. Dopaminergic tone on D_1 receptors serves to reinforce actions yielding larger rewards and lessen the impact of nonrewarded choices via a distinct network that interfaces with the NAc. Signals in this corticostriatal pathway likely converge with those from the BLA to further refine how choice biases are transformed into actions. (Fig. 5). In this way, DA, acting on D_1 and D_2 receptors, may facilitate two key functions of the frontal lobes, promoting both flexibility (Kehagia et al., 2010; Puig et al., 2014) and persistence of behavior (Holroyd and Umemoto, 2016; Winstanley and Floresco, 2016) via actions on different networks and their output pathways.

The idea that D_1 and D_2 receptors mediate distinct patterns of behavior via modulation dissociable populations of projection neurons within a particular brain region is certainly not novel. It is well established that these receptors mediate different aspects of action selection via actions on neurons in the striatonigral and striatopallidal pathways of the dorsal striatum (Gerfen, 1992). The present findings suggest that similar principles of operation may underlie dopaminergic modulation of different cognitive and reward-related functions within the PFC, with D_1 and D_2 receptors modulating distinct neuronal networks that interface with regions in the NAc and temporal lobe. These findings have important implications for understanding how D_1 and D_2 receptors regulates other functions of the frontal lobes such as working memory (Wang et al., 2004; Vijayraghavan et al., 2007), timing (Parker et al., 2013, 2015), and cognitive flexibility (Floresco, 2013) and how abnormal mesocortical DA activity contributes to cognitive dysfunction in diseases such as schizophrenia and Parkinson's disease (Okubo et al., 1997; Abi-Dargham et al., 2002; Narayanan et al., 2013; Robbins and Cools, 2014). Therefore, a more fundamental implication of these findings is that future studies aiming to clarify how PFC DA regulates both normal and abnormal cognitive function will need to focus, not only on which receptors DA may act on to facilitate these functions, but also the particular PFC→subcortical networks that DA influences to drive these behaviors.

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