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ORIGINAL INVESTIGATION

Differential effects of dopaminergic manipulations on risky choice

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Abstract
Rationale Evaluation of risks and rewards associated with different options is facilitated by components of the mesocorticolimbic dopamine (DA) system. Augmenting or reducing DA activity increases or decreases preference for larger, uncertain rewards when reward probabilities decrease within a session. However, manipulations of DA activity may differentially alter risky choice when shifts in the relative value of probabilistic rewards are greater or lesser than those experienced previously.
Objectives We investigated the effects of amphetamine and the DA antagonist flupenthixol on risk discounting, whereby we altered the manner in which reward probabilities changed.
Methods Rats chose between a "Small/Certain" (one pellet) and a "Large/Risky" lever that delivered four pellets in a probabilistic manner that changed during a session. Separate groups of rats were trained with a descending (100%, 50%, 25%, 12.5%), ascending (12.5–100%) or mixed (100%, 12.5%, 25%, 50%) order of probabilities associated with the large/risky option.
Results Flupenthixol consistently decreased preference for the large/risky option. In contrast, amphetamine increased preference for the large/risky lever when the probabilities decreased over a session, but reduced preference in the ascending condition.
Conclusions Reductions in normal DA tone consistently biases choice away larger, probabilistic rewards. In contrast, increases in DA release may disrupt adjustments in behavior in response to changes in the relative value of certain versus uncertain rewards. These findings further clarify the role of DA in mediating risk/reward judgments and how perturbations in DA signaling may interfere with the ability to adjust decision making in response to changes in reward contingencies.

Keywords Decision making · Amphetamine · Dopamine · Probabilistic discounting · Rats

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A growing body of evidence suggests that decision making, entailing evaluation of the relative risks and rewards associated with different options to determine appropriate courses of action is dependent on different components of the mesocorticolimbic dopamine (DA) system. Impaired decision making has been observed in patients with disorders linked to pathophysiology of the DA system. These include schizophrenia (Hutton et al. 2002), Parkinson's disease (Pagonabarraga et al. 2007) and stimulant addiction (Rogers et al. 1999). Thus, converging evidence suggests that alterations in DA transmission in different terminal regions may hamper cognitive operations related to cost/benefit evaluations about risks and rewards. The contribution of DA transmission to these processes may be related to the fact that neural activity of midbrain DA neurons encodes the reward uncertainty of stimuli associated with rewards delivered in a probabilistic manner (Fiorillo et al. 2003).

Further insight into the contribution of the DA system to decision making has been obtained from animal studies employing discounting tasks, whereby rats choose between small rewards associated with a nominal cost, or larger

rewards that come with a greater cost (e.g., increasing delays, effort requirements, or risk). Pharmacological manipulations of the DA system have been shown to alter choice behavior on these types of decision making tasks. For example, blockade of DA receptors reduces the preference for rats to wait longer or work harder to obtain a larger reward (Cardinal et al. 2000; Denk et al. 2005; Salamone et al. 1994; van Gaalen et al. 2006), whereas increasing DA transmission (e.g., amphetamine) can exert differential effects on effort- or delay-based decision making, either increasing or decreasing preference for larger rewards that come with a greater cost (Bardgett et al., 2009; Floresco et al. 2008a, b). More recently, there has been an interest in how DA activity may modulate probabilistic or "risk" discounting, whereby choice of a large reward option carries with it an inherent "risk" of not obtaining any reward on a given trial. Lesions or inactivations of DA terminal regions, such as the nucleus accumbens (Cardinal and Howes 2005) or basolateral amygdala (Ghods-Sharifi et al. 2009) shift choice preference towards smaller, certain rewards over large, uncertain ones. Recent studies in our laboratory have directly assessed the contribution of multiple DA receptor subtypes in these processes (St. Onge and Floresco 2009a). In that study, rats chose between a Small/Certain option (one food pellet), or a larger four-pellet option. However, the odds of receiving a large reward decreased over the course of a daily session in a systematic manner across four discrete trial blocks (100%, 50%, 25%, and 12.5%). In well-trained rats, increasing DA activity via systemic administration of amphetamine or D_1 or D_2 receptor agonists increased preference for the large/risky option. Conversely, blockade of D_1 or D_2 receptors significantly decreased choice of the risky option. These findings indicate that DA activity serves as a critical mediator of choices between certain versus uncertain rewards of different magnitudes.

In our previous study (St. Onge and Floresco 2009a), the relative value of the large/risky option decreased over the course of a session. However, there has been some indication that fluctuations in DA release associated with changes in reward value may differ if shifts in the relative value of rewards are greater or lesser than those experienced previously (Genn et al. 2004; Phillips et al. 2008). These findings suggest that DA transmission is not only linked to the absolute value of a reward, but also may encode increases or decreases in the *relative* value of rewards. In light of this notion, the possibility remains that DA manipulations may exert differential effects on risk discounting under conditions where the relative value of the risky option increases or is presented in a mixed order, rather than decreases over time.

The present study was designed to obtain a more comprehensive understanding of how manipulations of DA transmission affect risk discounting. Our particular interest was in assessing whether increases or decreases in DA activity always promote choice of large, risky or small, certain choices, respectively, or if these treatments induce differential effects depending on the manner in which reward probabilities change. In doing so, we tested the effects of the DA releaser amphetamine and the broad-spectrum DA antagonist flupenthixol on risk discounting, using a variety of procedures where we altered the manner in which changes in the probability of obtaining the large/risky reward were presented.

Materials and methods

Animals

Male Long-Evans rats (Charles River Laboratories, Montreal, Canada) weighing 275–300 g at the beginning of training were used. Upon arrival, rats were given 1 week to acclimatize to the colony and food restricted to 85–90% of their free-feeding weight for an additional 1 week before behavioral training. Rats were given ad libitum access to water for the duration of the experiment. Feeding occurred in the rats' home cages at the end of the experimental day and body weights were monitored daily to ensure a steady weight loss and maintenance. All testing was in accordance with the policies of the Canadian Council of Animal Care and the Animal Care Committee of the University of British Columbia.

Apparatus

Behavioral testing for all experiments described here was conducted in 12 operant chambers (30.5×24×21 cm; Med-Associates, St. Albans, VT, USA) enclosed in sound-attenuating boxes. The boxes were equipped with a fan to provide ventilation and to mask extraneous noise. Each chamber was fitted with two retractable levers, one located on each side of a central food receptacle where sweetened food reward pellets (45 mg; Bioserv, Frenchtown, NJ, USA) were delivered via a dispenser. The chambers were illuminated by a single 100-mA house light located in the top center of the wall opposite the levers. All experimental data were recorded by an IBM personal computer connected to the chambers via an interface.

Lever press training

Our initial training protocols have been described previously (Floresco et al. 2008a; St. Onge and Floresco 2009a). On the day prior to their first exposure to the chambers, rats were given approximately 25 reward pellets in their home

cage. On the first day of training, two to three pellets were delivered into the food cup and crushed pellets were placed on a lever before the animal was placed in the chamber. Rats were first trained under a fixed ratio; one schedule to a criterion of 60 presses in 30 min, first for one lever, and then repeated for the other lever (counterbalanced left/right between subjects).They were then trained on 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 houselight and the insertion of one of the two levers into the chamber (randomized in pairs). If the rat responded within 10 s, the lever retracted and a single pellet was delivered; failure to do so caused lever retraction and the chamber to darken until the next trial (omission). Rats were trained for 3–5 days to a criterion of 80 or more successful trials (i.e., ≤10 omissions).

Risk discounting task

The task was modified from Cardinal and Howes' (2005) procedure which we have previously used to assess the role of dopamine, the basolateral amygdala, and the prefrontal cortex in risk-based decision making (Ghods-Sharifi et al. 2009; St. Onge and Floresco 2009a, b). Rats received daily sessions consisting of 72 trials, separated into four blocks of 18 trials. A session took 48 min to complete, and rats were trained 6–7 days per week. A session began in darkness with both levers retracted (the intertrial state). A trial began every 40 s with illumination of the houselight and, 3 s later, insertion of one or both levers (the format of a trial is shown in Fig. 1). One lever was designated the large/risky lever, the other the small/certain lever, which remained consistent throughout training (counterbalanced left/right). If the rat did not press a lever within 10 s of its insertion, the chamber was reset to the intertrial state until the next trial (omission). When a lever was chosen, both levers retracted. Choice of the small/certain lever always delivered one pellet; choice of the large/risky lever delivered four pellets but with a particular probability (see below). When food was delivered, the houselight remained on for another 4 s after which the chamber reverted to the intertrial state. Multiple pellets were delivered 0.5 s apart. The 4 blocks were comprised of eight forced-choice trials where only one lever was presented (four trials for each, randomized in pairs). This was followed by ten free-choice trials, where both levers were presented. Latencies to initiate a choice were also recorded.

In our initial experiment, separate groups of rats were trained on one of three similar risk-discounting tasks, the key difference being the manner in which the probabilities associated with the large/risky lever varied over the course of a daily session. (1) *Descending condition:* the probability of obtaining four pellets after pressing the large/risky lever systematically decreased across the four blocks: it was initially 100%, then 50%, 25%, and 12.5%, respectively, as was used in our previous study (St. Onge and Floresco 2009a); (2) *ascending condition:* the probabilities increased across the four blocks: 12.5%, 25%, 50%, and 100%; and (3) *mixed condition:* probabilities changed in the following order across the four blocks: 100%, 12.5%, 25%, and 50%. For rats in each condition, the order the reward probabilities changed over a session remained constant for the duration of the experiment. Using these probabilities, selection of the large/risky lever would be advantageous in the 100% and 50% trial blocks, and disadvantageous in the 12.5% block, whereas rats could obtain an equivalent number of food pellets after responding on either lever during the 25% block. Therefore, in the 50%, 25%, and 12.5% probability blocks of this task, selection of the larger reward option

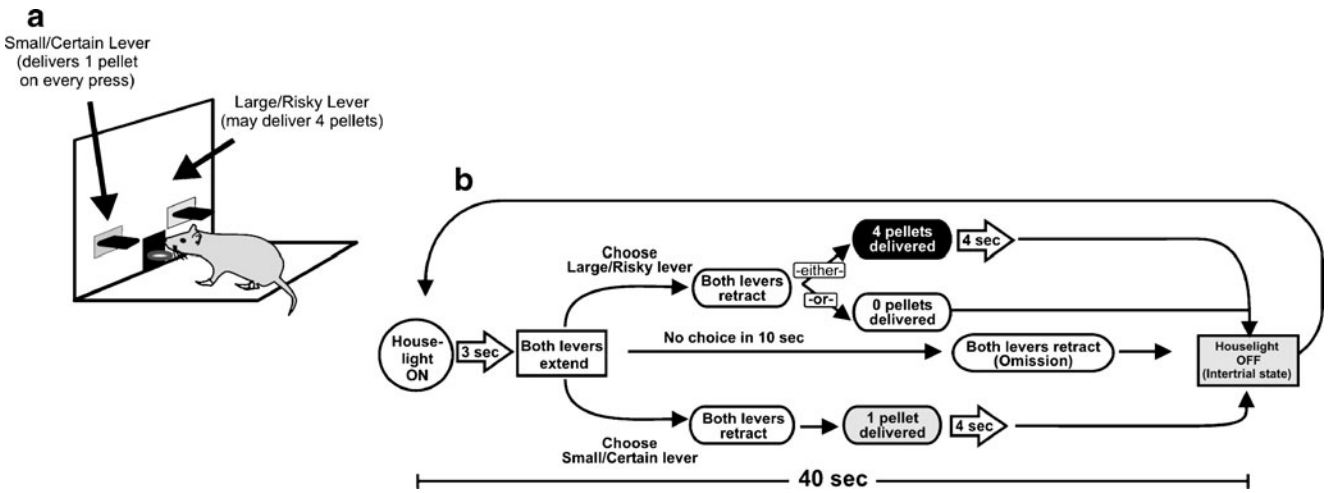

Fig. 1 Task design. Cost/benefit contingencies associated with responding on either lever (**a**) and format of a single free-choice trial (**b**) of the risk discounting task

carried with it an inherent "risk" of not obtaining any reward on a given trial.

Rats were trained on their respective task until as a group, they (1) chose the large/risky lever during the 100% probability block on at least 80% of successful trials, and (2) demonstrated stable baseline levels of choice. Drug tests were administered once a group of rats displayed stable patterns of choice for three consecutive days, assessed using a procedure similar to that described by Ghods-Sharifi et al. (2009) and St. Onge and Floresco (2009a, b). In brief, data from three consecutive sessions were analyzed with repeated-measures analyses of variance (ANOVA) with two within-subjects factors (day and trial block). If the effect of block was significant at the $P<0.05$ level but there was no main effect of day or day×block interaction (at $P>0.1$ level), animals were judged to have achieved stable baseline levels of choice behavior.

Drug tests

A within-subjects design for all drug tests was used. The following drugs were used: d-amphetamine (Sigma-Aldrich, Oakville, Ontario, Canada) and the mixed D_1/D_2 antagonist flupenthixol (Sigma-Aldrich). Flupenthixol was chosen because it blocks both D_1 and D_2 receptors, and our previous studies revealed that selective blockade of either of these receptors induced a similar decrease in risky choice (St. Onge and Floresco 2009a).

For our initial experiments using the three variations of the discounting task, all rats received injections of flupenthixol, d-amphetamine, and saline on separate test days. The order of drug or vehicle tests was counterbalanced for each animal using a modified Latin square design. Rats in this experiment also received a second saline injection at the end of their allocated drug tests to accommodate for any potential drift in baseline levels of choice. The data for the two saline tests were averaged. d-Amphetamine (0.5 mg/kg) and flupenthixol (0.4 mg/kg) were dissolved in physiological 0.9% saline. All drug doses were calculated as salt weights. The dose of amphetamine was chosen based on our previous studies that have shown this dose to induce a reliable and robust increase in choice of the large/risky lever (Floresco and Whelan 2009; St. Onge and Floresco 2009a). Likewise, the 0.4 mg/kg dose of flupenthixol was within the range of dosages of this drug that have been shown to affect other forms of cost/benefit decision making (Floresco et al. 2008a). The drugs were injected intraperitoneally in a volume of 1.0 ml/kg. Amphetamine, saline, flupenthixol were administered 10, 15, and 20 min, prior to a daily training session, respectively. Following an injection test day, rats were retrained until they again displayed stable patterns of choice (approximately another 3–5 days of training), after which subsequent drug tests were administered. This procedure was repeated until rats in a group had received each of their designated treatments.

Risk discounting with only uncertain probabilities

In a subsequent experiment, a separate group of rats was trained on a variation of the standard risk discounting task, where only three probabilities were used (50%, 25%, 12.5%) in order to ascertain whether starting with uncertain, rather than certain, probabilities would alter the effects of amphetamine on choice. In this experiment, the odds of obtaining the larger reward decreased over the course of a session. This task was identical in all other respects to the other discounting tasks, except there were only 54 trials (eight forced and ten free-choice per block over 36 min).

Reward magnitude discrimination

To determine whether DA manipulations induce a fundamental deficit in discriminating between large and small rewards, we trained a subset of rats from each of the descending, ascending and mixed groups on a reward magnitude discrimination task following completion of all other drug tests (see Ghods-Sharifi et al. 2009). In brief, this procedure consisted of four blocks of 12 trials (two forced choice, ten free choice). Here, a single response on one lever immediately delivered four pellets with 100% probability, whereas one press of the other lever always delivered one pellet immediately. After 8 days of training, rats received counterbalanced injections of saline or flupenthixol (0.4 mg/kg), after which they were retrained for 3 days and then received a second counterbalanced injection.

Data analysis

The primary dependent measure of interest was the percentage of choices directed towards the large/risky lever for each block of free-choice trials factoring in trial omissions. For each block, this was calculated by dividing the number of choices of the large/risky lever by the total number of successful trials. The choice data were analyzed using two-way within subjects ANOVAs, with drug and trial block as within-subject factors. The main effect of block for the choice data was significant in all experiments ($P<0.05$) indicating that rats discounted choice of the large/risky lever as the probability of the large reward changed across the four blocks. Response latencies were analyzed in a similar manner to the choice data. The number of trial omissions was analyzed with one-way within subjects ANOVAs.

Results

Task acquisition

Our initial analyses examined whether there were differences in the levels of discounting by rats in the three training conditions. We did observe some variation in the number of days of training required to achieve stable levels of discounting across the three tasks. Animals trained using descending probabilities (n=16) required an average of 35 days, those trained with ascending probabilities (n=16) required an average of 29 days, and those trained with mixed probabilities (n=12) required 38 days. Figure 2 shows baseline choice displayed by rats in each of the three training conditions, using data from five consecutive days prior to the first drug test, plotted as a function of probability block (presented in a descending order for comparison). Rats trained in the descending or ascending probability conditions displayed comparable discounting of the large/risky lever across the four blocks. However, we found that despite extensive training, rats in the mixed condition showed substantially less discounting of the large/risky option, suggesting that rats were not able to perform the task as effectively when the probabilities were presented in a mixed order. We analyzed these data using a mixed two-way ANOVA with task as a between subjects factor and probability block as a within subjects factor. The analysis revealed a significant main effect of task (F(2, 41)=3.26, P<0.05) and a significant task×block interaction (F(6, 123)=57.42, P<0.05, Tukey's, P<0.05). Rats in all groups showed significant discounting of the large/risky during the 25% and 12.5% blocks compared to their choice in the 100% and 50% block, when selection of this lever was advantageous. However, preference for the large/risky lever was significantly greater for the group with mixed probabilities (P<0.05) compared to both the ascending group (25% and 12.5% blocks) and the descending group (all blocks). Importantly, there were no differences in the levels of discounting between the descending and the ascending groups.

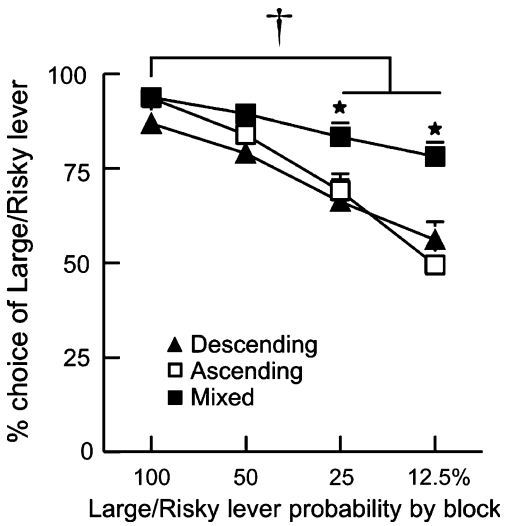

Fig. 2 Baseline performance by rats trained on the descending, ascending, and mixed versions of the risk discounting task. Percentage choice for the large/risky lever (y-axis) is plotted as a function of the large/risky lever probability by block (x-axis). Data are presented in a descending order for comparative purposes, and were obtained from the average of the last 5 days of training for each group prior to the first drug administration. Symbols represent mean + SEM. Stars denote significant (P<0.05) differences versus ascending and descending conditions at a specific block, and dagger denotes P< 0.05 difference compared to first block (all groups). Animals trained on the mixed version showed less discounting of the large/risky lever in the 25% and 12.5% probability blocks when compared to those in the descending and ascending conditions

Risk discounting with descending probabilities

One animal in the descending condition had a compromised amphetamine test day and its data was excluded from the analyses. Five animals that received injections of flupenthixol made an unusually high number of trial omissions (range=42–58), particularly in the latter two blocks, making interpretation of their data problematic. The data from these rats were excluded from the analyses. This required us to conduct separate analyses on the data obtained from the two drug test days, resulting in a final number of 15 rats that received amphetamine treatment and 11 rats that received flupenthixol. Analysis of the choice data for the amphetamine test revealed a significant main effect of drug (F(1,14)=6.37, P<0.05) but no drug×block interaction (F(3,42)=1.55, n.s.). Amphetamine administration significantly increased choice of the large/risky lever relative to saline (Fig. 3a), with this effect being most prominent in the latter two blocks. Amphetamine caused a slight, but statistically significant decrease in response latencies (saline=0.62±0.1 s; amphetamine=0.53±0.1 s; F (1, 14)=7.78, P<0.05). However, there was no effect on trial omissions (F(1, 14)=3.18, n.s.).

Analysis of the choice data for the flupenthixol test also revealed a significant main effect of drug (F(1,10)= 10.05, P<0.05) but no drug×block interaction (F(3,30)= 0.88, n.s.). Blockade of D_1/D_2 receptors significantly decreased preference for the Large/Risky lever compared to saline treatment (Fig. 3b). In this subset of rats, flupenthixol had no effect on trial omissions (F(1,10)= 3.72, n.s.). There was an increase in average response latencies following flupenthixol administration; however, the difference only approached statistical significance (saline=0.62±0.1 s; flupenthixol=0.93±0.2 s; F(1,10)= 2.78, P=0.06). Thus, in accordance with our previous findings (St. Onge and Floresco 2009a), pharmacological increases or decreases in DA activity induce corresponding

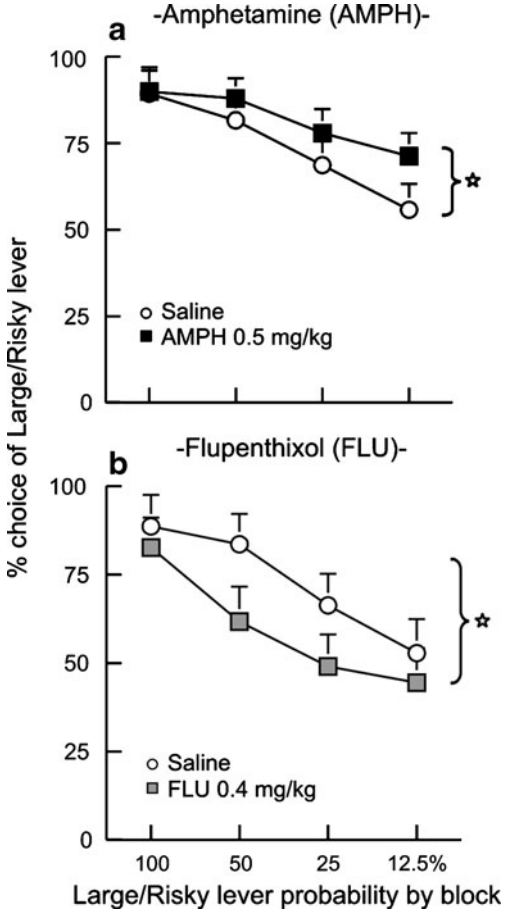

Fig. 3 The effects of **a** amphetamine (AMPH; 0.5 mg/kg) and **b** flupenthixol (0.4 mg/kg) on risk discounting with descending probabilities. All conventions are the same as Fig. 2. *Stars* denote significant ($P<0.05$) main effect of treatment. Amphetamine increased risky choice compared to saline treatment, whereas flupenthixol decreased choice of the large/risky lever

increases and decreases in risky choice when the probabilities of obtaining the larger reward decrease over a session.

Risk discounting with ascending probabilities

All 16 rats in the ascending condition received injections of amphetamine, flupenthixol and saline on separate days. Data from these test days were analyzed with a two-way ANOVA with drug and block as within-subject factors. Analysis of the choice data revealed a significant main effect of drug ($F(2,30)=4.63$, $P<0.05$) and drug×block interaction ($F(6,90)=3.18$, $P<0.05$, dunnett's, $P<0.05$; Fig. 4a). In contrast to what was observed from rats in the descending condition, simple main effects analyses revealed that amphetamine induced a significant ($P<0.05$) decrease in choice of the large/risky lever during the 12.5%, 25%, and 50% blocks. In addition, DA receptor blockade with flupenthixol also decreased risky choice during the 25%, 50%, and 100% blocks, compared to saline. Average response latencies were significantly increased by flupenthixol (saline=0.54±0.1 s; flupenthixol=1.57±0.2 s; $F(2,30)=13.18$, $P<0.05$), primarily in the first three blocks. In this experiment, amphetamine did not induce a significant change in latencies relative to saline (saline=0.55±0.06 s; amphetamine=0.57±0.04 s). Analysis of the omissions data also revealed a significant effect of drug ($F(2,30)=4.14$, $P<0.05$), with this effect being driven primarily by an increase following flupenthixol (4.3±2) when compared to amphetamine (0.06±0.06), or saline (0.21±0.1). Collectively, the key finding of this experiment is that the ability of amphetamine to modulate risk discounting is critically dependent on the manner in which reward probabilities change. In contrast, blockade of DA receptors induces similar effects on risky choice regardless of whether the odds of obtaining the larger reward increase or decrease over time.

Inspection of Fig. 4a reveals that as a group, rats in the ascending condition did not display a discernable bias

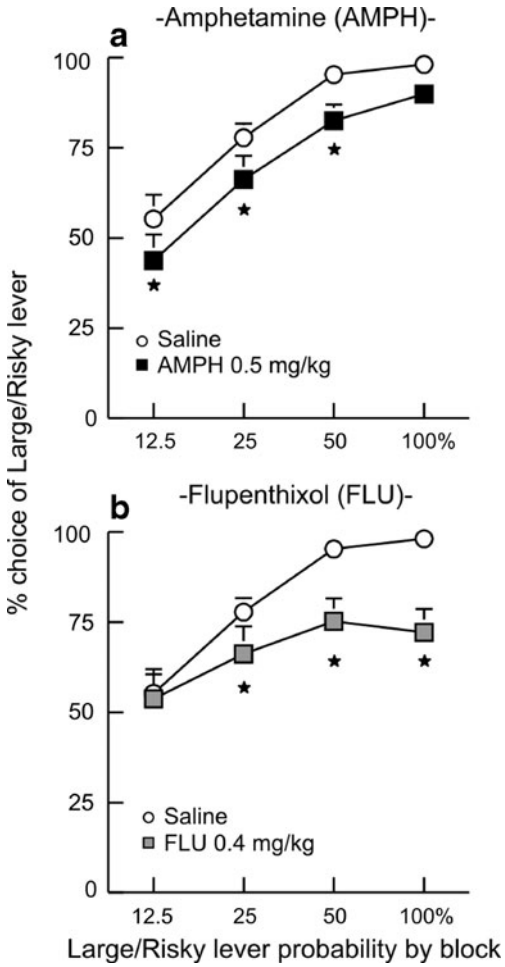

Fig. 4 The effects of amphetamine and flupenthixol on risk discounting with ascending probabilities. *Stars* denote significant ($P<0.05$) differences versus saline at a specific block. All other conventions are the same as Fig. 2. **a** Under these conditions, amphetamine decreased risky choice. **b** Flupenthixol also decreased risky choice compared to saline treatment (same saline curve as in **a** for clarity)

towards either the large/risky or small/certain lever during the first block after saline injections, even though selection of the latter would be more advantageous during this part of the session. However, of the 16 rats tested with amphetamine, six of the rats displayed a prominent preference for the small/certain lever (<30% choice of the large/risky lever) whereas the remaining ten rats showed a strong bias (>70%) for the risky option in the first block. When we analyzed the choice data from these two subsets of rats separately, we observed that amphetamine induced a significant decrease in risky choice in both groups (see Supplemental Figure 1). For rats that preferred the small, certain option initially, amphetamine did not alter choice during the first block, but did significantly reduce preference for the large/risky lever in the 25% and 50% blocks. For the large, risky option-preferring rats, amphetamine induced a pronounced decrease in choice during the first block that persisted for the duration of the session. This latter finding suggests that these effects are unlikely to reflect some form of response perseveration or development of a place preference directed towards the lever that rats initially displayed a bias for. Rather, increasing DA activity maintained a preference for the small, certain option when the odds of obtaining the larger reward were initially low and increased over the remainder of a session.

Risk discounting with mixed probabilities

The data from the 12 rats in the mixed condition were analyzed using a two-way ANOVA in the same manner as the ascending condition. Analysis of the choice data revealed a significant main effect of drug ($F(2,22)=4.41$, $P<0.05$) but no drug×block interaction ($F(6,66)=0.66$, n.s.; Fig. 5a). Amphetamine treatment induced a trend towards increased risky choice compared to saline, particularly in the second block (12.5% probability). However, post hoc analyses failed to confirm a significant difference between amphetamine and saline treatments. In contrast, flupenthixol was again effective at inducing a significant ($P<0.05$) decrease in risky choice compared to saline. Under these conditions, amphetamine again induced a slight, but significant reduction in response latencies (0.73±0.1 s), whereas flupenthixol tended to increase latencies (1.12±0.2 s) relative to saline treatment (0.89±0.2 s; $F(2, 22)=4.68$, $P<0.05$; Fig. 5b). In this experiment, neither drug had an effect on trial omissions ($F(2, 22)=1.44$, n.s.), although the trend was for flupenthixol to increase omissions (saline=0.63±0.3; flupenthixol=2.0±1.3).

Risk discounting with only uncertain probabilities

Amphetamine produced differential effects on risk discounting that were dependent on the manner in which reward probabilities change, increasing choice of the large/risky option in the descending condition but decreasing risky choice in the ascending condition. An obvious difference between these two tasks is that the long-term value of the large/risky option relative to the small/certain option either decreased or increased over the course of a session, respectively. However, another key difference is that during the initial block of the descending condition, the probability of obtaining the larger reward is certain (i.e., 100%), whereas in the ascending task, delivery of the larger reward is probabilistic (i.e., 12.5%). Thus, it was unclear whether the amphetamine-induced increase in preference for the large/risky lever observed with the descending task was due to an impaired ability to alter choice bias (1) specifically when reward probabilities shift from certain to uncertain, or (2) when the relative long-term value of the larger, probabilistic reward is initially greater than the small/certain option and subsequently decreases over time.

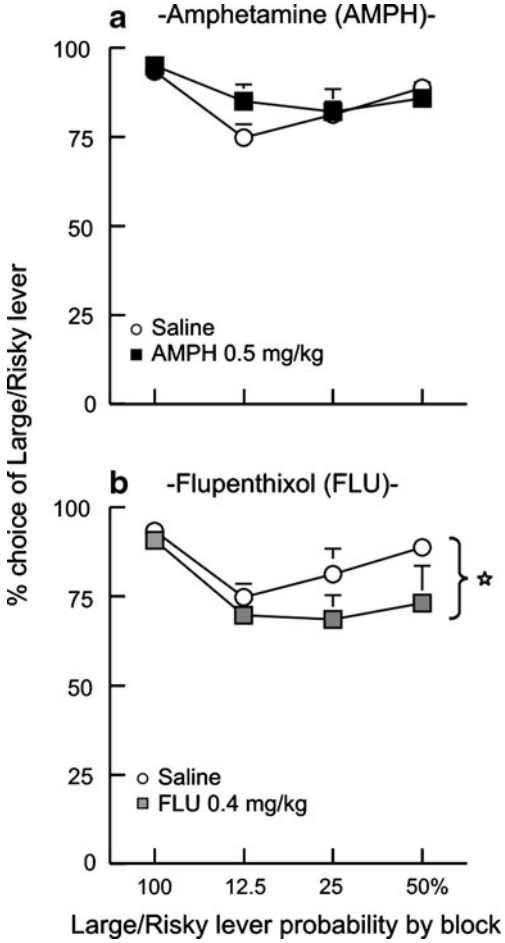

Fig. 5 The effects of amphetamine and flupenthixol on risk discounting with mixed probabilities. All conventions are the same as Fig. 3. **a** Amphetamine tended to increase risky choice during the second, 12.5% probability block, but this effect was not statistically significant. **b** Flupenthixol decreased choice of the large/risky lever relative to saline treatment (same saline curve as in **a**)

To address this question, we trained a separate group of animals on a similar discounting task, with the key exception that the probabilities associated with receiving the large reward were all uncertain throughout the session (i.e., 50%, 25%, and 12.5%).

A total of eight rats were used for this experiment and required an average of 32 days of training on the task prior to receiving counterbalanced injections of amphetamine or saline. The data from one animal was removed from the analysis due to an unusual increase in trial omissions after being tested with amphetamine. Analysis of the choice data for the remaining seven animals revealed a significant main effect of drug ($F(1,6)=6.21$, $P<0.05$) but no drug×block interaction ($F(2,12)=1.60$, n.s.; Fig. 6), indicating that amphetamine significantly increased risky choice across all three blocks. Amphetamine had no effect on average response latencies (saline=0.51±0.1 s; amphetamine=0.75±0.1 s; $F(1,6)=2.05$, n.s.) or trial omissions ($F(1,6)=0.49$, n.s.). Note that in the descending experiment, amphetamine also increased risky choice but significantly reduced response latencies. These results indicate that the ability of amphetamine to increase risky choice is primarily dependent on decreasing shifts in the relative long-term value of the large/risky option, regardless of whether or not reward probabilities are initially certain or uncertain. Furthermore, they suggest that the effects of amphetamine on choice do not appear to be related to its ability to modify response latencies.

Reward magnitude discrimination

A subsequent experiment was conducted to ascertain if the effect of DA receptor blockade on risky choice was attributable to a general deficit in discrimination between small and large rewards. Upon completion of testing on the discounting tasks, three, four, and seven rats from the descending, ascending, and mixed tasks, respectively (total $n=14$), were subsequently trained on a reward magnitude discrimination task, where rats chose between one lever that delivered one pellet and another that delivered four pellets. Both the small and large rewards were delivered immediately after a single response with 100% probability. After 8 days of training, rats displayed a strong preference for the four-pellet lever. Subsequently, they received counterbalanced injections of saline and flupenthixol on separate days. As is displayed in Fig. 7a, administration of flupenthixol did not alter preference for the large reward lever during the first two trial blocks, but did induce a slight decrease in choice of the four-pellet option in the latter two blocks. These data were analyzed with a three-way ANOVA with drug treatment and trial block as two within-subjects factors, and task history (descending, ascending or mixed training) as a between subjects factor. There was no significant main effect of task history or interactions with the other variable (all Fs<1.0, n.s.). However, there was a significant drug×trial block interaction ($F(3,39)=3.02$, $P<0.05$). Simple main effects analyses confirmed that that there were no differences in the proportion of choices of the large reward lever during the first two trial blocks. During the latter two blocks, the reduced choice of the large reward lever induced by D_1/D_2 receptor blockade was statistically significant ($P<0.05$), even though as a group, rats still displayed a strong preference for the four-pellet lever compared to the one-pellet lever (>80%). Analysis of the response latency data revealed a similar drug×trial block interaction ($F(3,39)=4.86$, $P<0.01$). Again, flupenthixol did not alter response latencies during the first two trial blocks, but did increase the latency to make a choice during the last two blocks (Fig. 7b).

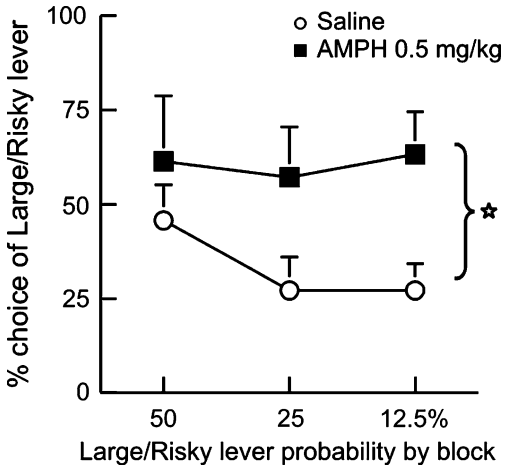

Fig. 6 The effects of amphetamine on risk discounting with only uncertain reward probabilities. All conventions are the same as Fig. 3. Amphetamine increased risky choice compared to saline treatment

Discussion

The main impetus of the present study was to obtain a more comprehensive understanding of how manipulations of DA transmission affect risk discounting and expand on our previous findings (St. Onge and Floresco 2009a). In our initial study using this discounting task, we reported that blockade of DA D_1 or D_2 receptors reduced preference for the large/risky option, whereas amphetamine induced a DA-dependent increase in risky choice when the odds of obtaining the larger reward decreased over a session. The present study provides important new insight into the manner in which increases or decreases in DA transmission may affect risk/reward judgments. Thus, blockade of DA receptors consistently decreased preference for the large/risky option that was independent of the manner in which

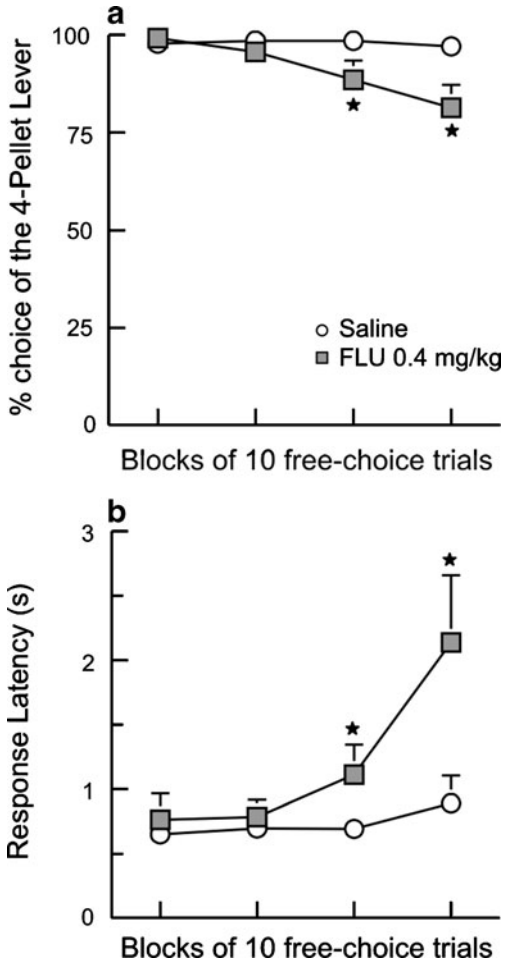

Fig. 7 The effects of flupenthixol on reward magnitude discrimination. Rats were trained to choose between two levers that delivered either a four or one-pellet reward immediately after a single press with 100% probability. **a** Percentage choice of the four-pellet reward (*y*-axis) is plotted as a function of four blocks of ten free-choice trials (*x*-axis). Flupenthixol decreased choice of the four-pellet lever only during the last two trial blocks. **b** Response latencies were also increased by flupenthixol, but only in the last two trial blocks

reward probabilities changed over a session. However, our experiments with amphetamine revealed that increasing DA activity can exert differential effects on risk discounting. Specifically, if the value of the large/risky option was initially greater than the small/certain option and then progressively decreased over a session, amphetamine treatment increased risky choice. However, when the odds of obtaining the large reward were initially low and then increased over subsequent blocks, amphetamine actually reduced preference for the large/risky lever.

Task comparison

With the tasks used in the present study, rats learn over training that changes in the probabilities associated with the large/risky reward are signaled by blocks of forced-choice trials that precede each set of free-choice trials. Thus, rats must remember the outcomes of previous trials and use this information to update their representation of the relative value associated with the large/risky lever as a session proceeds, adjusting their bias accordingly. When rats were trained on variants of the task where the large/risky reward probability either decreased or increased, rats in both groups displayed comparable patterns of choice across the four probability blocks, as we have observed previously (St. Onge and Floresco 2009b). However, rats trained on the mixed version showed substantially less discounting, even after an additional 3–11 days of training when compared to rats in the other conditions. This suggest that rats in the mixed condition may have had greater difficulty adjusting their choice in response to changes in reward probabilities relative to rats trained on tasks where probabilities changed in a systematic manner. Note that in the mixed condition, the large/risky option is initially advantageous (100% block) then disadvantageous (12.5%), then eventually advantageous again (50%), relative to the small/certain option. Thus, multiple shifts in relative value of the two options may have impeded the ability of rats to discount the larger reward in an effective manner, relative to conditions where the odds on the large/risky lever consistently improve or worsen over a session. In fact, six of the 12 animals in the mixed group showed minimal discounting during the 12.5% block, selecting the large reward option on at least 80% of free choice trials. Therefore, changes in reward probabilities may need to be presented in a systematic manner in order for rats to more effectively learn about changes in the long-term value of each option and display more prominent discounting of probabilistic rewards.

Effects of DA receptor blockade

The D_1/D_2 antagonist flupenthixol significantly decreased choice of the large/risky lever, regardless of the order in which probabilities of reward were presented. These data are consistent with the results of our previous study using selective D_1 and D_2 antagonists (St. Onge & Floresco 2009a). Recent neurochemical findings point to a role for phasic mesoaccumbens DA signaling in encoding the potential benefits associated with different actions (Gan et al. 2010). Thus, by blocking DA receptors, neural circuits that bias choice may be deprived of critical signal that provide information about the relative value of each response option. The effect of flupenthixol reported here complement the results of other studies investigating the role of DA in delay discounting (Cardinal et al. 2000; Floresco et al. 2008a) and effort discounting (Floresco et al. 2008a), where animals were also less likely to choose the larger, more costly reward. When placed in a broader

context, these findings further support the notion that normal DA tone may function to help an animal overcome a variety of different costs (uncertainty, delays, work) in order to obtain more beneficial rewards (Floresco et al. 2008b).

Across the three tasks, flupenthixol reduced choice of the large/risky option most consistently during the 50% and 25% probability blocks. These blocks provided maximal uncertainty about the most beneficial course of action in terms of obtaining the larger reward (50%) or the overall amount of food that could be obtained over the ten free-choice trials (four pellets at 25% versus one pellet at 100%). This observation resembles the effects of temporarily reducing DA synthesis in human patients recovering from depression, which reduced betting behavior on the Cambridge gambling task, particularly when the probability of receiving reward was more uncertain (Roiser et al. 2005). In this regard, it is interesting to note that the activity of midbrain DA neurons appear to encode the relative uncertainty about upcoming rewards. DA neurons show sustained increases in firing that are maximal when uncertainty about receiving reward is greatest (i.e., 50% probability) compared to stimuli that are highly predictive (i.e., 0% and 100%; Fiorillo et al. 2003). Thus, DA transmission may play a more prominent role in biasing choice towards certain versus uncertain rewards in situations where there is substantial ambiguity about the relative long-term value of different options.

To determine whether flupenthixol induced a generalized impairment in reward magnitude discrimination, we retrained a subgroup of animals to perform a simple task in which they chose between four pellets and one pellet, both delivered with 100% probability. Previous studies of how DA antagonism affects choice of rewards of different magnitudes have yielded mixed results. Using a T-maze procedure, Salamone and colleagues (1994) reported that the D_2 antagonist haloperidol did not alter preference for an arm baited with a higher reinforcement density. However, using a similar procedure, Denk et al. (2005) observed that haloperidol did induce a slight reduction in choice of a larger reward, but this effect was much more pronounced when access to the larger reward was either delayed or occluded by a scalable barrier. In the present study, flupenthixol did not alter preference for the larger reward in the early part of the test session, suggesting that this treatment does not induce a general reduction in preference for larger rewards. However, flupenthixol did cause a slight decrease in choice of this option in the last two blocks. It is important to highlight that this effect was relatively small in comparison to the more pronounced decrease in choice of the large reward option that occurred in both the descending and ascending versions of the risk discounting task, consistent with the findings of Denk and colleagues (2005). Moreover, the effects of flupenthixol on risky choice became apparent earlier during a test session on the ascending or descending tasks than on the reward magnitude task. It is notable that in the magnitude discrimination experiment, rats received a substantially greater amount of food (~170 pellets) compared to that typically received by the end of a risk discounting session (~120 pellets). It is possible, therefore, that flupenthixol may have exacerbated a satiety-induced reduction in motivation during these latter blocks. In support of this notion, flupenthixol also increased the latencies to make a choice, but only in the last two blocks. Thus, although, DA receptor blockade may slightly blunt the bias for larger versus smaller rewards under certain conditions, it is unlikely that this is a sufficient explanation for the substantial decrease in preference for the large/risky option induced by flupenthixol. Rather, we would propose that DA transmission exerts a greater influence over choice behavior in situations that require integration of multiple types of information (reward magnitude, response costs, motivational state, etc.) used to guide normal decision making.

Effects of amphetamine

In contrast to the consistent effects of DA receptor antagonism on risk discounting, amphetamine induced differential changes in choice behavior. When selection of the large/risky option was initially more advantageous (descending and uncertain experiments), amphetamine increased preference for this option. Conversely, amphetamine decreased risky choice in the ascending condition, where the likelihood of obtaining the larger reward increased over a session. Amphetamine also tended to increase risky choice in the mixed condition. The lack of a statistical significance in this experiment is likely attributable to a ceiling effect, as rats in this group displayed high baseline levels of choice of the large/risky lever. These differential effects of amphetamine on risk discounting are a key finding, as they show that increasing DA transmission with amphetamine does not uniformly make animals "risky" and increase their preference for uncertain rewards. Rather, it appears that amphetamine impairs the ability to shift preference away from or towards large/risky options upon changes in the relative value of probabilistic rewards.

There have been a number of reports that amphetamine can accelerate shifts in preference away from larger rewards as the relative costs associated with these rewards increases. For example, similar doses of amphetamine to those used here decrease choice of larger, delayed rewards when there is no cue to signal subsequent delivery of food (Cardinal et al. 2000; Evenden and Ryan 1996). Moreover, these effects on delay discounting are apparent regardless of whether the delay decreased or increased across a session (Slezak and

Anderson, 2009). Furthermore, we have observed that a 0.5 mg/kg dose of amphetamine reduced preference for larger rewards associated with a greater effort cost (Floresco et al. 2008). In a similar vein, Simon et al. (2009) found that amphetamine decreased preference for larger rewards associated with foot-shock punishment as the probability of receiving shock increased across a session. Despite these findings, we observed a somewhat opposite effect, in that rats treated with amphetamine were actually slower to adjust their choice behavior in response to changes in reward probability. Note that a key difference between the probabilistic discounting task used here and other tasks mentioned above is that in the latter, animals always obtain some reward after a choice. As such, the present data would suggest that amphetamine may exert differential effects on cost/benefit evaluations in situations where animals choose between different magnitudes of rewards that are always certain, compared to those requiring choice between certain and probabilistic rewards.

Superficially, the effects of amphetamine on risk discounting could be interpreted as an impairment in response flexibility, in that rats were merely perseverating towards the lever they displayed a preference for during the early part of a session. However, amphetamine typically enhances, rather than impairs, shifts in behavioral responding (Evenden and Robbins 1985; Weiner and Feldon, 1985; Weiner 1990). In addition, amphetamine has been shown to shift preference away from options associated with the largest long-term reward, towards others associated with smaller rewards, but smaller punishments, in a rodent model of the Iowa gambling task (Zeeb et al. 2009). Thus, amphetamine may not have caused animals to persist in choosing their initially preferred lever, but may have altered processes associated with determining the long-term value of each option when reward probabilities changed. In this regard, our supplemental analysis of the ascending group revealed that amphetamine decreased risky choice in animals that either showed a preference towards (>70%) or away (<30%) from the large/risky lever during the first block. Moreover, the fact that amphetamine decreased choice of the large/risky option in the subset of rats that showed a bias for that lever in the first, 12.5% probability block, suggests that this treatment may have amplified the effect of early non-rewarded trials on shifting preference towards the small/certain option. Viewed collectively, these findings suggest that it is unlikely that amphetamine caused response perseveration towards the *lever* that rats initially displayed a bias for. Rather, amphetamine may have caused rats to perseverate on the *perceived relative value* of a given option upon subsequent changes in the probability of obtaining the large/risky reward. When the value of probabilistic rewards was initially advantageous, amphetamine caused rats to persist in responding as if the large/risky option was more advantageous that it actually was during the latter parts of a session. Conversely, when the probabilistic option changed from being disadvantageous to advantageous, rats persisted to behave as if the small option was more beneficial as the session progressed.

Neurophysiological recordings in awake behaving animals have revealed that rewarded and non-rewarded events may be signaled in part by brief increases or decreases (i.e., phasic bursts or dips) in midbrain DA neuron firing (Schultz et al. 1997; Fiorillo et al. 2003). With respect to the present study, on trials when an animal chooses the large/risky lever and does not receive reward, phasic dips in DA activity may serve as a critical signal used by other forebrain systems (e.g., prefrontal cortex, nucleus accumbens; St. Onge and Floresco 2009b; Cardinal and Howes 2005) to evaluate changes in the likelihood of obtaining reinforcement. Under these conditions, large increases in DA release induced by amphetamine could effectively "wash out" these signals that may be used by prefrontal/ventral striatal circuits to update choice behavior in response to changing probabilities. Of course, amphetamine would also be expected to increase tonic DA levels, which has also been proposed to contribute to certain aspects of cost/benefit decision making (Niv et al. 2007). As such, the relative contribution of phasic versus tonic DA activity to risk-based decision making remains a topic for future research.

It is of interest to highlight that the effects of amphetamine reported here bear a striking resemblance to those induced by inactivation of the prelimbic region of the medial prefrontal cortex (PFC; St. Onge and Floresco 2009b). In that study, we observed that medial PFC inactivation using the descending version of the risk discounting task increased risky choice compared to saline treatment, but decreased risky choice in the ascending version. Similar inactivations did not disrupt performance of a within-session reversal or alter choice when reward probabilities remained constant over a session. We interpreted these findings to suggest that the medial PFC appears to play a specific role in updating behavior in response to changing reward probabilities. It is well established that pharmacological increases in mesocortical DA activity can perturb certain cognitive functions mediated by the PFC (Floresco and Phillips 2001; Floresco and Magyar 2006; Zahrt et al. 1997). Thus, the ability of amphetamine to alter patterns of risk discounting may be mediated in part by abnormal increases in PFC DA release. This in turn may disrupt patterns of neural activity in this region that normally serves to integrate multiple types of information (previous choices, likelihood of reward delivery, etc) that may bias the direction of choice behavior to maximize long-term payoffs.

In summary, the present findings provide novel insight into how alterations in DA transmission can interfere with

risk/reward judgments. Reducing DA activity uniformly shifts bias away from larger, probabilistic rewards. However, abnormal increase in DA release exert a more complex effect on these processes, retarding the ability to update response biases upon changes in the likelihood of obtaining larger rewards, which, depending on the circumstances, may promote either risky or risk-averse patterns of choice. This latter finding is of particular relevance to a number of neuropsychiatric disorders, including stimulant addiction, schizophrenia, and impulse control disorders observed in medicated Parkinsonian patients. Each of these disorders has been associated with impairments decision making, where subjects evaluate the relative risks and reward associated with different options (Cools et al. 2007; Hutton et al. 2002; Rogers et al. 1999). The present data would suggest that this aspect of cognitive dysfunction may be the result of aberrant increases in DA activity that are thought to contribute to the pathophysiology of these disorders.

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References

Bardgett ME, Depenbrock M, Downs N, Points M, Green L (2009) Dopamine modulates effort-based decision making in rats. Behav Neurosci 123:242–251

Cardinal RN, Howes NJ (2005) Effects of lesions of the nucleus accumbens core on choice between small certain rewards and large uncertain rewards in rats. BMC Neurosci 6:9

Cardinal RN, Robbins TW, Everitt BJ (2000) The effects of d-amphetamine, chlordiazepoxide, alpha-flupenthixol and behavioral manipulations on choice of signaled and unsignalled delayed reinforcement in rats. Psychopharmacology 152:362–375

Cools R, Lewis SJ, Clark L, Barker RA, Robbins TW (2007) L-DOPA disrupts activity in the nucleus accumbens during reversal learning in Parkinson's disease. Neuropsychopharmacology 32:180–189

Denk F, Walton ME, Jennings KA, Sharp T, Rushworth MFS, Bannerman DM (2005) Differential involvement of serotonin and dopamine systems in cost-benefit decisions about delay or effort. Psychopharmacology 179:587–596

Evenden JL, Robbins TW (1985) The effects of d-amphetamine, chlordiazepoxide and alpha-flupenthixol on food-reinforced tracking of a visual stimulus by rats. Psychopharmacology 85:361–366

Evenden JL, Ryan CN (1996) The pharmacology of impulsive behaviour in rats: the effects of drugs on response choice with varying delays of reinforcement. Psychopharmacology 128:161–170

Fiorillo CD, Tobler PN, Schultz W (2003) Discrete coding of reward probability and uncertainty by dopamine neurons. Science 299:1898–1902

Floresco SB, Phillips AG (2001) Delay-dependent modulation of memory retrieval by infusion of a dopamine D1 agonist into the rat medial prefrontal cortex. Behav Neurosci 115:934–939

Floresco SB, Magyar O (2006) Mesocortical dopamine modulation of executive functions: beyond working memory. Psychopharmacology 188:567–585

Floresco SB, Whelan JM (2009) Perturbations in different forms of cost/benefit decision making induced by repeated amphetamine exposure. Psychopharmacology 205:189–201

Floresco SB, Tse MT, Ghods-Sharifi S (2008a) Dopaminergic and glutamatergic regulation of effort- and delay-based decision making. Neuropsychopharmacology 33:1966–1979

Floresco SB, St. Onge JR, Ghods-Sharifi S, Winstanley CA (2008b) Cortico-limbic-striatal circuits subserving different forms of cost-benefit decision making. Cogn Affect Behav Neurosci 8:375–389

Gan JO, Walton ME, Phillips PE (2010) Dissociable cost and benefit encoding of future rewards by mesolimbic dopamine. Nat Neurosci 13:25–27

Genn RF, Ahn S, Phillips AG (2004) Attenuated dopamine efflux in the rat nucleus accumbens during successive negative contrast. Behav Neurosci 118:869–873

Ghods-Sharifi S, St. Onge JR, Floresco SB (2009) Fundamental contribution by the basolateral amygdala to different forms of decision making. J Neurosci 29:5251–5259

Hutton SB, Murphy FC, Joyce EM, Rogers RD, Cuthbert I, Barnes TR, McKenna PJ, Sahakian BJ, Robbins TW (2002) Decision making deficits in patients with first episode and chronic schizophrenia. Schizophr Res 55:249–257

Niv Y, Daw ND, Joel D, Dayan P (2007) Tonic dopamine: opportunity costs and the control of response vigor. Psychopharmacology (Berl) 191:507–520

Pagonabarraga J, García-Sánchez C, Llebaria G, Pascual-Sedano B, Gironell A, Kulisevsky J (2007) Controlled study of decision-making and cognitive impairment in Parkinson's disease. J Mov Disord 22:1430–1435

Phillips AG, Vacca G, Ahn S (2008) A top-down perspective on dopamine, motivation and memory. Pharmacol Biochem Behav 90:236–249

Rogers RD, Everitt BJ, Baldacchino A, Blackshaw AJ, Swainson R, Wynne K, Baker NB, Hunter J, Carthy T, Booker E et al (1999) Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology 20:322–339

Roiser JP, McLean A, Ogilvie AD, Blackwell AD, Bamber DJ, Goodyer I, Jones PB, Sahakian BJ (2005) The subjective and cognitive effects of acute phenylalanine and tyrosine depletion in patients recovered from depression. Neuropsychopharmacology 30:775–785

Salamone JD, Cousins MS, Bucher S (1994) Anhedonia or anergia? Effects of haloperidol and nucleus accumbens dopamine depletion on instrumental response selection in a T-maze cost/benefit procedure. Behav Brain Res 65:221–229

Schultz W, Dayan P, Montague PR (1997) A neural substrate of prediction and reward. Science 275:1593–1599

Simon NW, Gilbert RJ, Mayse JD, Bizon JL, Setlow B (2009) Balancing risk and reward: a rat model of risky decision making. Neuropsychopharmacology 34:2208–2217

Slezak JM, Anderson KG (2009) Effects of variable training, signaled and unsignaled delays, and d-amphetamine on delay-discounting functions. Behav Pharmacol 20:424–436

St. Onge JR, Floresco SB (2009a) Dopaminergic modulation of risk-based decision making. Neuropsychopharmacology 34:681–697

St. Onge JR, Floresco SB (2009b) Prefrontal cortical contribution to risk-based decision making. Cereb Cortex, published online Nov 5, doi:10.1093/cercor/bhp250

van Gaalen MM, van Koten R, Schoffelmeer ANM, Vanderschuren LJMJ (2006) Critical involvement of dopaminergic neurotransmission in impulsive decision making. Biol Psychiatry 60:66–73

Weiner I (1990) Neural substrates of latent inhibition: the switching model. Psychol Bull 108:443–461

Weiner I, Feldon J (1985) Reversal and nonreversal shifts under amphetamine. Psychopharmacology 89:355–359

Zahrt J, Taylor JR, Mathew RG, Arnsten AF (1997) Supranormal stimulation of D1 dopamine receptors in the rodent prefrontal cortex impairs working memory performance. J Neurosci 17:8528–8535

Zeeb FD, Robbins TW, Winstanley CA (2009) Serotonergic and dopaminergic modulation of gambling behavior as assessed using a novel rat gambling task. Neuropsychopharmacology 34:2329–2343