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Prefrontal cortical–striatal dopamine receptor mRNA expression predicts distinct forms of impulsivity

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Abstract

Variation in dopamine receptor levels has been associated with different facets of impulsivity. To further delineate the neural substrates underlying impulsive action (inability to withhold a prepotent motor response) and impulsive choice (delay aversion), we characterised rats in the Differential Reinforcement of Low Rates of Responding task and a delay discounting task. We also measured performance on an effort-based discounting task. We then assessed D1 and D2 dopamine receptor mRNA expression in subregions of the prefrontal cortex and nucleus accumbens using *in situ* hybridisation, and compared these data with behavioral performance. Expression of D1 and D2 receptor mRNA in distinct brain regions was predictive of impulsive action. A dissociation within the nucleus accumbens was observed between subregions and receptor subtypes; higher D1 mRNA expression in the shell predicted greater impulsive action, whereas lower D2 mRNA expression in the core predicted greater impulsive action. We also observed a negative correlation between impulsive action and D2 mRNA expression in the prelimbic cortex. Interestingly, a similar relationship was present between impulsive choice and prelimbic cortex D2 mRNA, despite the fact that behavioral indices of impulsive action and impulsive choice were uncorrelated. Finally, we found that both high D1 mRNA expression in the insular cortex and low D2 mRNA expression in the infralimbic cortex were associated with willingness to exert effort for rewards. Notably, dopamine receptor mRNA in these regions was not associated with either facet of impulsivity. The data presented here provide novel molecular and neuroanatomical distinctions between different forms of impulsivity, as well as effort-based decision-making.

Keywords

delay discounting; effort discounting; impulsive action; impulsive choice; rat

Introduction

Psychopathological disorders associated with abnormal dopaminergic transmission, including attention deficit hyperactivity disorder, schizophrenia, and addiction, are characterised by heightened impulsivity (Bechara *et al.*, 2001; Ahn *et al.*, 2011; Nolan *et al.*, 2011; Winstanley, 2011; Dalley & Roiser, 2012). It has been well-established that impulsivity is not a unitary trait, but instead can be divided into distinct components. These include impulsive action, the inability to withhold a prepotent motor response, and impulsive choice, the excessive discounting of delayed reinforcement (Mazur, 1987; Evenden, 1999; Winstanley *et al.*, 2006a; de Wit, 2009). Because both forms of impulsive behavior can lead to a range of deleterious outcomes, including exacerbation of the disorders with which they are associated, there is considerable interest in uncovering their neuropharmacological mechanisms.

Variation in dopamine receptor expression has been linked to individual differences in impulsive behavior. The DRD2 A1 allele, a polymorphism associated with D2 receptor hypofunction, is prevalent in attention deficit hyperactivity disorder and drug addiction, both of which are characterised by impulsivity (Blum *et al.*, 1995; Noble, 2003). Striatal D2/D3 receptor availability is negatively correlated with impulsive action in rodents (Dalley *et al.*, 2007; Laughlin *et al.*, 2011), and with impulsive choice and action in humans (Lee *et al.*, 2009; Ghahremani *et al.*, 2012), whereas D1 receptor expression in the medial prefrontal cortex is positively correlated with impulsive choice in rats (Loos *et al.*, 2010). Baseline levels of impulsivity can also predict responses to dopaminergic intervention. Administration of a D2/D3 receptor agonist in humans attenuates the high impulsive action observed in stimulant-dependent individuals, whereas the same drug does not affect performance in healthy individuals (Ersche *et al.*, 2011). Baseline levels of impulsive choice in rats predict locomotor responses to cocaine and sensitivity to the effects of both amphetamine administration and orbitofrontal cortex inactivation on impulsive choice (Stanis *et al.*, 2008; Zeeb *et al.*, 2010). Additionally, rats with a history of extended cocaine exposure, which has been shown to affect dopamine receptor expression (Briand *et al.*, 2008), are insensitive to the modulatory effects of dopaminergic drugs on impulsive choice (Winstanley *et al.*, 2007; Setlow *et al.*, 2009).

To further examine how different facets of impulsivity are related to dopamine receptor expression, we characterised rats in both a Differential Reinforcement of Low Rates of Responding task (DRL), used to measure impulsive action (Uslaner & Robinson, 2006), and a delay discounting task, used to measure impulsive choice (Evenden & Ryan, 1996). Rats were then trained in an effort-based discounting task, which is also modulated by dopamine signaling (Floresco *et al.*, 2008b; Salamone *et al.*, 2009; Mai *et al.*, 2012; Treadway *et al.*, 2012). This task is procedurally similar to delay discounting, but replaces delayed rewards with rewards that require extended effort. We then examined whether individual differences in performance across these tasks were related to D1 and D2 receptor mRNA expression in prefrontal cortical and striatal brain regions implicated in impulsivity and decision-making (Cardinal, 2006; Dalley *et al.*, 2008; Floresco *et al.*, 2008a; Hauber & Sommer, 2009; Simon *et al.*, 2011; Treadway *et al.*, 2012).

Materials and methods

Subjects

A group of male Long-Evans rats ($n = 18$; 4 months old, weighing 275–300 g upon arrival, Charles River Laboratories, Raleigh, NC, USA) was utilised for this experiment. Rats were individually housed and kept on a 12 h light/dark cycle (lights on at 08:00 h) with free access to food and water except as noted. All procedures were conducted in accordance with

the Texas A&M University Laboratory Animal Care and Use Committee and NIH guidelines. *In situ* hybridisation data from this cohort of rats were reported previously in Simon *et al.* (2011). Behavioral testing was initiated at 6 months of age, and rats were killed at 10 months.

Behavioral apparatus

Each test chamber was equipped with a recessed food pellet delivery trough fitted with a photobeam to detect head entries and a 1.12 W lamp to illuminate the food trough, which was located 2 cm above the floor in the center of the front wall. Grain-based food pellets (45 mg; PJA1, Test Diet, Richmond, IN, USA) could be delivered into the food trough. A 1.12 W house light was also mounted on the rear wall of the isolation cubicle. A single lever was located directly above the food trough for DRL training, and this lever was removed for the delay and effort-based discounting procedures. For use in these decision-making tasks, retractable levers were located to the left and right of the food trough, 11 cm above the floor. Test chambers were interfaced with a computer running Graphic State software (Coulbourn Instruments), which controlled programmed task events and data collection.

Behavioral procedures

Differential reinforcement of low rates of responding task—Performance on the DRL has been used as a measure of impulsive action, defined as the inability to withhold a prepotent motor response (Neill, 1976; Sokolowski & Salamone, 1994; Uslaner & Robinson, 2006). Prior to training, rats were food deprived to 85% of their free-feeding weight over the course of 1 week. Training began with a 64 min session of magazine training, during which a single food pellet was delivered into the food trough at 100 ± 40 s intervals. On the following day, rats were trained to press a fixed lever located above the food trough to receive a single food pellet. After achieving a criterion of 50 reinforced lever presses in a 30 min session, rats began DRL training. Each DRL session was 45 min in duration, with the house light illuminated throughout the session. Rats were initially trained on a DRL-5 s schedule for five sessions, during which a lever press only resulted in food pellet delivery if at least 5 s had elapsed since the previous press. If the rat performed a premature lever press, the 5 s time period was reset; thus, rats were only reinforced if they withheld a response for greater than 5 s. Rats were then trained for five sessions on a DRL-10 s schedule, during which the response had to be withheld for 10 s to obtain reinforcement. Finally, rats were given 10 sessions on a DRL-20 s schedule. Impulsive action was assessed on session 10 of the DRL-20 s schedule, and was defined as the ratio of rewarded responses (lever presses emitted at least 20 s after the previous, rewarded press) to total responses, with higher ratios reflecting more accurate performance, indicative of less impulsive action.

Delay discounting task—The delay discounting task was used to assess impulsive choice, defined as preference for small, immediate rewards over larger, delayed rewards. The task design was modified from Evenden & Ryan (1996) and Simon *et al.* (2007). As during DRL training, rats were maintained at 85% of their free-feeding weight. During this training, the fixed lever utilised for responding during DRL training was not present in the chamber. Rats were tested in the task for 20 sessions, each consisting of five blocks of 12 trials. Each 60 s trial began with a 10 s illumination of the food trough and house lights. A nosepoke into the food trough during this time window extinguished the food trough light and triggered extension of either a single lever (forced-choice trials) or of both levers simultaneously (free-choice trials) for 10 s. Trials on which rats failed to nosepoke during this time window were scored as omissions. Each block consisted of two forced-choice trials used to expose the rats to the delays in effect for that block, followed by 10 free-choice trials. A press on one lever (either left or right, balanced across rats) resulted in one food

pellet (the small reward) delivered immediately. A press on the other lever resulted in three food pellets (the large reward) delivered after a variable delay. Failures to press either lever were scored as omissions. Once either lever was pressed, both levers were retracted for the remainder of the trial. The delay duration increased between each block of trials (0, 4, 8, 16, 32 s), but remained constant within each block (Evenden & Ryan, 1996; Winstanley *et al.*, 2006b; Mitchell *et al.*, 2012).

Effort-based discounting task—This task was modified from Floresco *et al.* (2008b). Once more, rats were maintained at 85% free-feeding weight. The basic parameters were similar to the delay discounting task, with exceptions for reward size and the criterion required to obtain the large reward. A press on the small reward lever caused immediate delivery of a single food pellet as in the delay discounting task. A press on the large reward lever caused the small reward lever to retract but the large reward lever remained extended; at that point, multiple presses on the large reward lever were required to achieve fulfillment of an effort-based criterion. After the criterion was met, the large reward lever was retracted and two food pellets were delivered. The same lever that had been associated with the large reward during delay discounting was again associated with the large reward during effort-based discounting. The effort criteria for the five blocks were 1, 2, 5, 10, and 20 lever presses. Each session lasted 60 min.

Experimental timeline—Rats began the behavioral training included in this study at approximately 7 months of age. DRL training lasted for 25 consecutive daily sessions, and the final session of DRL-20 s performance was used for analyses. On the following day, rats began 30 consecutive sessions of delay discounting, and the final five sessions were averaged together for analyses. Finally, rats were trained for 10 consecutive sessions in effort-based discounting, and the final five sessions were averaged together for analyses. All subjects experienced the same duration of training and sequencing of tasks.

Dopamine receptor mRNA analyses

Tissue preparation—Following behavioral testing, rats were given an overdose of pentobarbital anesthesia and perfused intracardially with phosphate-buffered saline followed by phosphate-buffered saline/4% paraformaldehyde. Brains were removed and stored in 4% paraformaldehyde solution overnight, then postfixed in 4% paraformaldehyde/20% sucrose for 24 h followed by rapid freezing and storage at -80°C . Brains were sectioned in the coronal plane on a freezing microtome, and sections (30 μm) collected in a one-in-six series beginning at the anterior portion of the prefrontal cortex [5.2 mm from bregma, according to Paxinos & Watson (1998)], and ending posterior to the nucleus accumbens (NAC; -0.26 mm from bregma).

In situ hybridisation—For detailed methods of D1 and D2 receptor probe preparation and *in situ* hybridisation methodology, see Simon *et al.* (2011). Free-floating tissue sections were washed in 0.75% glycine in 0.1 M phosphate buffer, pH 7.2, and 0.1 M phosphate buffer alone to remove excess fixative. Sections were treated for 30 min at 37°C with proteinase K (1 mg/mL in 0.1 M Tris buffer containing 0.05% sodium dodecyl sulfate), acetylated in 0.25% acetic anhydride in 0.1 M triethanolamine, pH 8.0, and rinsed twice in $2 \times$ saline sodium citrate buffer (SSC; $1 \times \text{SSC} = 0.15 \text{ M}$ sodium chloride and 0.015 M sodium citrate, pH 7.0). Tissue was then hybridised for 42–44 h at 60°C in solution containing 50% formamide, $1 \times$ Denhardt's solution, 10% dextran sulfate, $4 \times \text{SSC}$, 0.25 mg/mL yeast tRNA, 0.3 mg/mL herring sperm DNA, 100 mM dithiothreitol and the ^{35}S -labeled cRNA at a final concentration of 1×10^7 CPM/mL. Following hybridisation, sections were washed at 30 min intervals, twice in $4 \times \text{SSC}$ and once in 50% formamide/ $2 \times \text{SSC}$ at 60°C and then treated with ribonuclease A (20 mg/mL in 10 mM Tris saline buffer

containing 1 mM ethylene-diaminetetracetic acid) for 30 min at 37 °C. Tissue sections were then washed further in descending concentrations of SSC buffer containing 100 µM dithiothreitol to a final wash of 0.1 × SSC and mounted onto gelatin-coated slides for film autoradiography. Air-dried sections were exposed along with ¹⁴C-standards to phosphoimage screens (Perkin Elmer, Waltham, MA, USA). Because dopamine receptor mRNA is less abundant in the prefrontal cortex than in the NAC (Meador-Woodruff *et al.*, 1991; Simon *et al.*, 2011), brain sections containing the prefrontal cortex were exposed for 72 h, whereas sections containing the striatum were exposed for 24 h. Screens were scanned at high resolution using a Typhoon Phosphoimager (Perkin Elmer).

Relative D1 and D2 mRNA expression was quantified by densito-metric analysis using Densita imaging software (MBF Biosciences, Williston, VT, USA). Hybridisation densities were linearised and calibrated relative to the ¹⁴C-labeled standards that were exposed to each phosphoscreen along with tissue sections. Multiple measures were obtained from four to six sections per brain region per rat. For each brain structure analysed, these values were averaged to provide an individual mean hybridisation density (µCi/g protein) per region in each rat. These means were used for correlations and group comparisons.

For regional analyses, the prefrontal cortex was divided into multiple subregions based on Paxinos & Watson (1998): the orbitofrontal, insular (INS), infralimbic (ILC), and prelimbic (PLC) cortex. NAC core and shell regions were also analysed separately. One rat was removed from prefrontal analyses due to tissue damage.

Data analysis—The ratio of correct to total responses was used as the performance index for the DRL (impulsivity ratio), and average choice of the large reward across all blocks was used for delay discounting. For effort-based discounting, only the final block was used as the performance index, as the greatest degree of individual variability was observed in this block.

Relationships among these behavioral variables were quantified in two different ways. First, we used Pearson correlations to search for linear relationships between behavioral indices and region-specific mRNA expression. Second, we analysed behavior in a non-continuous fashion by dividing rats into groups on the basis of performance in each task. This distinction was important because rats at the extreme ends of each behavioral spectrum (e.g. high vs. low impulsivity) are more representative of the behavioral profiles observed in psychiatric conditions. Because the variability differed between tasks, task-specific criteria were used for dividing rats into groups (if a consistent criterion were used for all three tasks, rats with similar scores would have been split into separate groups). For impulsive action, high impulsive rats were those with a DRL ratio < 0.1, whereas low impulsive rats had a DRL ratio > 0.2. For delay discounting, high impulsive choice rats performed < 50% choice of the delayed reward, and low impulsive choice rats had > 75% choice of delayed reward. Finally, for effort-based discounting, high effort rats selected the effortful reward on > 50% of trials, and low effort rats selected the effortful reward on < 25% of trials. Importantly, when forming these groups, subjects with scores between these criteria were eliminated from analyses as in Flagel *et al.* (2007) and Molander *et al.* (2011). Dopamine receptor expression was then compared for each of the high and low group divisions using unpaired *t*-tests.

Results

Relationships among behavioral variables

Individual performance and group means from all tasks are shown in Fig. 1. There were no correlations between impulsive action (ratio of rewarded to total lever presses on the

DRL-20 s), and impulsive choice (mean percent choice of the large reward on the delay discounting task; $r = 0.31$, $P = 0.22$; Fig. 2A), consistent with previous findings of no relationships between these two forms of impulsivity (Solanto *et al.*, 2001; Diergaarde *et al.*, 2008; Broos *et al.*, 2012). Impulsive choice was correlated with effort-based discounting, such that rats that preferred the large delayed reward also preferred the large effortful reward ($r = 0.60$, $P < 0.01$; Fig. 2B); however, there was no relationship between impulsive action and effort-based discounting ($r = 0.02$, $P = 0.92$; Fig. 2C).

Relationships between behavioral performance and dopamine receptor mRNA expression

Following behavioral characterisation, brains were collected and *in situ* hybridisation was performed to quantify D1 and D2 dopamine receptor mRNA expression. These mRNA expression values were then correlated with behavioral measures of impulsive action, impulsive choice, and effort-based discounting, and were also compared between groups of high vs. low responders for each behavioral assay. Representative brain sections depicting the regions analysed are displayed in Fig. 3.

Impulsive action and dopamine receptor expression

There was a negative linear correlation between D1 receptor mRNA expression in the NAC shell and the impulsivity ratio in the DRL, such that high D1 mRNA expression was predictive of high impulsive action (low DRL ratio; $r = -0.47$, $P < 0.05$; Fig. 4A). Accordingly, the high impulsive action group demonstrated greater D1 mRNA expression than the low group in the NAC shell ($t_{12} = 2.26$, $P < 0.05$). There were no relationships between D1 mRNA expression and impulsive action in any other brain region analysed (see Table 1 for full statistics).

There was no correlation between impulsive action and D2 receptor mRNA expression in the NAC core. However, there was a difference between high and low impulsive action responders, such that lower impulsive action was associated with higher D2 expression ($t_{12} = -2.16$, $P = 0.05$; Fig. 4B). We also uncovered a significant positive correlation between the impulsivity ratio and PLC ($r = 0.49$, $P < 0.05$; Fig. 4C), indicating that high D2 mRNA expression in this region predicted low impulsive action. Additionally, we observed a near-significant trend in which high impulsive action rats expressed more D2 mRNA than low impulsive choice rats in the PLC ($t_{11} = -2.06$, $P = 0.06$). There were no other correlations between impulsive action and dopamine receptor expression, nor were there any other differences in D2 mRNA expression between high and low responders in any other brain regions analysed (see Table 1).

Impulsive choice and dopamine receptor expression

The D2 receptor mRNA expression in the PLC was positively correlated with choice of the delayed reward, such that high PLC D2 mRNA expression predicted low impulsive choice/high preference for large, delayed rewards ($r = 0.55$, $P < 0.05$; Fig. 5). There was also a near-significant difference in PLC D2 mRNA expression between high and low impulsive choice rats ($t_{11} = -2.10$, $P = 0.06$). There were no other correlations between impulsive choice and dopamine receptor expression or differences between high and low impulsive choice groups in receptor expression in any other brain region analysed (see Table 1).

Effort-based discounting and dopamine receptor expression

There was a positive correlation between choice of the effortful reward and D1 mRNA expression in the INS ($r = 0.49$, $P < 0.05$; Fig. 6A), indicating that high D1 in the INS predicted willingness to exert effort for rewards. Conversely, there was a negative correlation between choice of the effortful reward and D2 expression in the ILC ($r = -0.50$,

$P < 0.05$; Fig. 6B). There were no other relationships between dopamine receptor mRNA expression and effort-based discounting, and no differences between high and low effort groups for any other brain regions (see Table 1).

Discussion

Data summary

Impulsive action and choice are proposed to be distinct traits (Evenden, 1999; Winstanley *et al.*, 2004; Broos *et al.*, 2012); therefore, we expected to observe no statistical relationship between performance in tasks that quantify these constructs. Indeed, we observed that performance on the DRL and delay discounting tasks was statistically independent. A correlation was observed between delay discounting and effort-based discounting, such that preference for delayed rewards predicted preference for effort-based rewards. This was not surprising, as choice of effortful rewards requires tolerance of delays prior to reward receipt. However, it is important to note that, during these delay periods, rats were required to continue to respond at the lever for reinforcement. In addition, unlike delay discounting, there was no delay to reward delivery following completion of the final response in the sequence. It should be noted that, in both the delay and effort-based discounting tasks, the spatial location of the large and small reward levers remained consistent; therefore, the correlation between tasks may have been a result of rats failing to modify their response preference following the task shift. However, rats have previously demonstrated the ability to shift lever preference quickly following similar shifts between other decision-making tasks with the lever identities held constant (Simon *et al.*, 2009), indicating that the correlation is likely not simply a result of perseverative responding. Finally, measures of delay and effort-based discounting correlated differently with dopamine receptor expression, suggesting that the two tasks assessed at least partially distinct cognitive constructs.

Dopamine transmission has been found to modulate multiple facets of impulsivity as well as effort-based discounting (Cardinal *et al.*, 2000; Robbins, 2002; Floresco *et al.*, 2008b). We quantified D1 and D2 dopamine receptor gene expression because such measures, particularly in the prefrontal cortex and striatum, have been demonstrated to be predictive of some aspects of cognition as well as vulnerability to drugs of abuse (Thanos *et al.*, 2001; Hitzemann *et al.*, 2003; Belin *et al.*, 2007; Briand *et al.*, 2008; Loos *et al.*, 2010; Kramer *et al.*, 2011). We found that expression of both D1 and D2 receptor mRNA in specific brain regions was predictive of impulsive action. In particular, a dissociation within the NAC was observed between subregions and receptor subtypes; higher levels of D1 mRNA in the shell predicted greater impulsive action, whereas lower levels of D2 mRNA in the core predicted greater impulsive action. We also observed a negative correlation between impulsive action and D2 mRNA expression in the PLC. Interestingly, this same relationship was present between impulsive choice and D2 mRNA in the PLC, despite the fact that behavioral indices of impulsive action and impulsive choice were not correlated. Finally, we found regional dopaminergic correlates of effort-based discounting, in that both high D1 mRNA expression in the INS and low D2 mRNA expression in the ILC were associated with willingness to exert effort for large rewards. Notably, these two regions were not associated with either facet of impulsivity.

Differential reinforcement of low rates of responding task and dopamine receptor expression

The DRL has frequently been used as a measure of impulsive action (Neill, 1976; Uslander & Robinson, 2006; Hankosky & Gulley, 2012), as it assesses rats' ability to withhold a prepotent behavioral response (Evenden, 1999). It has previously been demonstrated that DRL performance is modulated by dopamine transmission (Peterson *et al.*, 2003; Liao &

Cheng, 2005), and that enhancing dopamine neurotransmission in the NAC increases impulsive action in the DRL (Neill & Herndon, 1978). The negative relationship between impulsive action and D2 mRNA expression in the NAC core revealed here is consistent with previous work using a different assessment of impulsive action, the five-choice serial reaction time task. Dalley *et al.* (2007) utilised positron emission tomography to measure D2/D3 receptor availability in rats *in vivo*, whereas the current study measured D2 receptor mRNA after testing was complete. Taken together, these two studies demonstrate a consistency between the five-choice serial reaction time task and DRL as behavioral measures of impulsive action, and provide converging evidence that baseline levels of impulsivity are related to D2 receptor expression in the NAC. The current study expands on Dalley *et al.* (2007) by separating the NAC into core and shell subregions, and by analysing both D1 and D2 receptor mRNA expression. We found that the negative relationship between D2 and impulsive action is localised to the core, whereas the opposite relationship exists in the shell with D1 receptors. This second relationship was consistent with the finding that injecting dopamine in solution directly into the NAC enhanced impulsive action in the DRL (Neill & Herndon, 1978).

Previous work has demonstrated dissociations between these subregions of the NAC in modulation of impulsive action. Sesia *et al.* (2008) tested the effects of deep brain stimulation of the NAC core and shell on impulsive action using the five-choice serial reaction time task, and found that stimulating the core decreased impulsive action, whereas stimulating the shell increased impulsive action. Several other studies have used drug microinfusions to examine the modulatory effects of dopaminergic manipulations in these NAC subregions on impulsive action. Using the five-choice serial reaction time task, Besson *et al.* (2010) found that infusions of a D2/D3 antagonist into the NAC core decreased impulsive action, whereas infusions into the shell increased impulsive action. Pattij *et al.* (2007) found conflicting results, with D1 blockade in both the core and shell reducing impulsive action, and no effects of D2 blockade in either region. The findings of Pattij *et al.* (2007) in the NAC shell were similar to those observed here, in which less D1 expression predicted less impulsivity. The findings of Besson *et al.* (2010) are not as consistent with the current data; however, it is important to note that Besson *et al.* (2010) used an antagonist that affected both D2 and D3 receptors, whereas the *in situ* hybridisation findings in the present study were specific to D2 receptors. Additionally, Besson *et al.* (2010) only observed effects in rats previously characterised as high impulsive responders. These effects may be a function of differences in baseline D2 receptor availability in high impulsive vs. low impulsive rats, as found here and in Dalley *et al.* (2007).

It is important to note that inaccurate performance on the DRL (characterised here as impulsive action) could reflect an impairment in timing and/or working memory. Rats are required to wait a fixed interval between responses in the DRL, and early responses may reflect an inability to accurately represent that time interval in working memory. Indeed, it has been demonstrated that dopamine receptors modulate timing behavior (Coull *et al.*, 2011), and that the NAC plays a role in guiding a response based on the timing of rewards (Singh *et al.*, 2011). However, other groups have obtained conflicting data (Galtress & Kirkpatrick, 2010), and some reports indicate that dopaminergic manipulations of the NAC shell do not influence timing behavior (Meck, 2006; Kurti & Matell, 2011). Importantly, it would seem unlikely that a deficit in reward timing would account for impulsive-like performance in the DRL, as such a deficit would be expected to increase choice of immediate rewards in the delay discounting task. The fact that performance in the two tasks was not related suggests that at least somewhat independent mechanisms account for impulsive patterns of behavior across the two tasks.

Delay discounting and dopamine receptor expression

The only relationship observed between delay discounting and dopamine receptor expression was a linear relationship between PLC D2 mRNA and delay discounting, with greater D2 expression predicting greater preference for the large, delayed reward. Dopamine receptor expression in the orbitofrontal cortex and NAC, two brain regions commonly implicated in impulsive choice (Cardinal *et al.*, 2001, 2004; Roesch *et al.*, 2007a; Winstanley, 2007; Sellitto *et al.*, 2010; Zeeb *et al.*, 2010; Valencia-Torres *et al.*, 2012), showed no relationships with impulsive choice. Similar to the results found here, Loos *et al.* (2010) found that the medial prefrontal cortex was the only locus of a relationship between dopamine receptor mRNA expression and delay discounting. However, in contrast to the current result, they found a positive relationship between D1 expression and impulsive choice. This difference could result from multiple factors, including the fact that the current study separated the PLC from the ILC, and found a relationship with delay discounting in the PLC only, whereas Loos *et al.* (2010) combined the PLC and ILC for their analysis.

A lesion study has confirmed involvement of the PLC in delay discounting (Cardinal *et al.*, 2001); however, these lesions did not seem to influence impulsivity *per se*, but instead impaired rats' ability to shift preference from one option to the other during the task, causing what appeared to be 'indifference' between reward choices. This may be a result of an inability to update reward expectancies within a session and adjust behavior accordingly. St. Onge *et al.* (2011) found that D2 activation in the PLC caused rigid reward preference throughout a probabilistic discounting task, with rats demonstrating attenuated shifting of reward preference even as the large reward became less advantageous. Therefore, it is possible that the correlation between D2 mRNA and delay discounting performance does not reflect individual differences in impulsive choice, but instead in the ability to shift from one reward option to the other, which could promote persistent choice of the large reward even as the cost of that reward (delay to its delivery) increases.

Effort-based discounting and dopamine receptor expression

Choice of the large but effortful reward was associated with high D1 mRNA expression in the INS and low D2 mRNA expression in the ILC. Neural activity in the human anterior insula seems to represent the value of effortful rewards (Prevost *et al.*, 2010). Additionally, positron emission tomography imaging showed that D2/D3 dopamine receptor binding potential is inversely related to preference for effortful rewards in the anterior insula (Treadway *et al.*, 2012). There is some evidence that the ILC is involved with effort-based discounting, as a decrease in infralimbic metabolic activity was observed during effort-based decision-making in rats (Endepols *et al.*, 2010). However, lesions of this region do not appear to affect effort-based discounting (Walton *et al.*, 2003). The current experiment provides further evidence for the involvement of the INS and ILC in effortful decision-making, and expands upon it by revealing specific correlations between willingness to exert effort for rewards and region-specific D1 and D2 receptor mRNA.

Implications

The results of this experiment provided novel associations between dopamine receptor expression and different facets of impulsivity. Whereas impulsive action had multiple substrates (particularly in the NAC), both impulsive choice and impulsive action shared a relationship with D2 receptor expression in the PLC. This is particularly noteworthy because the prefrontal cortex is abnormal in individuals with attention deficit hyperactivity disorder and addictive disorders (Castellanos *et al.*, 1996; Castellanos & Tannock, 2002), which are characterised by both facets of impulsivity (Winstanley, 2011). This suggests that D2 receptor availability in the PLC may play a role in the manifestation of or vulnerability to these disorders. Indeed, the DRD2 A1 allele, which is associated with D2 receptor

hypofunction, is prevalent in patients with attention deficit hyperactivity disorder and addictive disorders (Blum *et al.*, 1995; Noble, 2003). Therefore, targeting PFC D2 receptors may be a potential therapeutic strategy for disorders characterised by both forms of impulsivity.

Addiction, in particular, has been associated with both impulsive choice and impulsive action (Bickel & Marsch, 2001; Bechara, 2005). It has been demonstrated in rat models that impulsive choice is enhanced following extended drug exposure (Dallery & Locey, 2005; Roesch *et al.*, 2007b; Simon *et al.*, 2007; Mendez *et al.*, 2010; Schippers *et al.*, 2012), and that both impulsive choice and impulsive action predict subsequent addiction-related behaviors (Dalley *et al.*, 2007; Belin *et al.*, 2008; Diergaarde *et al.*, 2008; Perry *et al.*, 2008; Economidou *et al.*, 2009). Additionally, chronic cocaine exposure has been shown to cause a reduction in D2 receptor mRNA in the medial prefrontal cortex (Briand *et al.*, 2008). We observed that reduced D2 mRNA in the PLC was associated with both impulsive choice and impulsive action; therefore, D2 receptors in the PLC are not only a common neural substrate of both facets of impulsivity, but might also play a mediating role in the relationship between impulsivity and drug (at least cocaine) consumption.

We also observed that D1 mRNA in the NAC shell was correlated with impulsive action. Previous work from our laboratory reported a similar relationship between D1 receptor expression in the NAC shell and risky decision-making behavior, using a task in which rats choose between a small, 'safe' reward and a large reward associated with risk of foot-shock punishment (Simon *et al.*, 2009, 2011). Together, these findings suggest that NAC shell D1 receptors may be a common neural substrate of both impulsive action and risky decision-making. This neuropharmacological profile may also have particular relevance for addiction, as both impulsivity and risky choice behavior are hallmarks of addiction (de Wit, 2009; Doremus-Fitzwater *et al.*, 2010; Schultz, 2011).

In conclusion, the data presented here provide novel molecular and neuroanatomical distinctions between different forms of impulsivity, as well as effort-based decision-making. Given the prevalence of impulsivity in psychiatric disorders, it will be important in future studies to further characterise the dopaminergic substrates of impulsive behavior, in order to better understand the etiological factors underlying these disorders, and ultimately for development of therapeutic interventions.

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Abbreviations

DRL	differential reinforcement of low rates of responding task
ILC	infralimbic cortex
INS	insular cortex
NAC	nucleus accumbens
PLC	prelimbic cortex
SSC	saline sodium citrate buffer

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Fig. 1.

(A) Distribution of DRL ratio scores. The DRL ratio consists of total correct lever presses/total lever presses, with higher scores indicative of lower impulsive action. Group means are displayed in the bar graph to the right. The criterion for high impulsive action rats was a DRL ratio < 0.1 , and for low impulsive action rats was a DRL ratio > 0.2 . (B) Distribution of performance in the delay discounting task, with higher scores indicative of less impulsivity. Groups means for high impulsive responders (criterion: $< 50\%$ average choice of delayed reward) and low impulsive responders (criterion: $> 75\%$) are displayed to the right. (C) Distribution of scores in effort-based discounting, with high scores indicative of willingness to exert effort. Group means for the low effort group (criterion: $< 25\%$ choice of effortful reward) and high effort group (criterion: 50%) are displayed to the right. All graphs to the right represent means $\pm$ SEM.

Fig. 2.

Scatterplots depicting relationships between behavioral tasks. (A) There was no relationship between impulsive action (lower scores indicative of higher impulsivity) and impulsive choice (lower scores indicative of higher impulsivity). (B) There was a positive correlation between effort-based discounting and impulsive choice, such that higher tolerance of effort predicted higher tolerance of delays (reduced impulsivity). (C) There was no correlation between effort-based discounting and impulsive action.

Fig. 3. Hybridisation of radiolabeled D1 and D2 mRNA in prefrontal cortical and striatal regions. Images from film autoradiograms display D1 (A and C) and D2 (B and D) dopamine receptor mRNA expression in sample coronal sections. PL, PLC; IL, ILC; OFC, orbitofrontal cortex; NC, NAC core; NS, NAC shell (Simon *et al.*, 2011).

Fig. 4. Significant relationships between D1 or D2 receptor mRNA expression and DRL ratio. (A) There was a negative correlation between D1 mRNA in the NAC shell and the DRL ratio such that high D1 predicted high impulsive action. There was also significantly greater expression of D1 mRNA in the high impulsive action group than in the low impulsive action group. (B) There was no significant correlation between the NAC core D2 mRNA and DRL ratio, but there was significantly less D2 mRNA in the core in the high impulsive action group than in the low impulsive action group. (C) There was a positive correlation between D2 receptor mRNA in the PLC and DRL ratio, such that high D2 mRNA predicted low impulsive action. All bar graphs represent means $\pm$ SEM. *significant difference between groups, $P < 0.05$

Fig. 5.

There was a significant positive correlation between D2 receptor mRNA in the PLC and choice of the large delayed reward during delay discounting, indicating that high D2 predicted low impulsive choice. The difference between high and low impulsive choice groups approached but did not achieve significance ($P = 0.06$). Bar graph represents means $\pm$ SEM.

Fig. 6. Significant relationships between effort-based discounting and dopamine receptor mRNA. (A) There was a significant positive correlation between D1 receptor mRNA in the INS and choice of effort-based reward, indicating that rats willing to expend effort for large rewards had higher levels of INS D1 mRNA. (B) The opposite relationship was apparent between ILC D2 mRNA and effort, such that rats willing to expend effort expressed lower levels of D2 mRNA.

Table 1

Statistical relationships (Pearson's correlations and independent *t*-tests) between mRNA expression and DRL performance (impulsive action), delay discounting (impulsive choice), and effort-based discounting

	DRL-20		Delay discounting		Effort-based discounting	
	D1	D2	D1	D2	D1	D2
NACs	$r = -0.47, P = 0.048^*, t = 2.26, P = 0.04^*$	$r = 0.05, P = 0.86, t = 0.52, P = -0.51$	$r = -0.22, P = 0.39, t = 0.72, P = 0.49$	$r = 0.08, P = 0.76, t = -0.72, P = 0.49$	$r = 0.04, P = 0.87, t = -0.29, P = 0.78$	$r = 0.45, P = 0.06, t = -1.23, P = 0.24$
NACc	$r = -0.21, P = 0.41, t = 0.90, P = 0.39$	$r = 0.25, P = 0.32, t = -2.16, P = 0.05^*$	$r = -0.16, P = 0.54, t = -0.22, P = 0.83$	$r = 0.28, P = 0.26, t = -1.43, P = 0.18$	$r = 0.02, P = 0.95, t = -0.60, P = 0.56$	$r = 0.22, P = 0.38, t = -1.20, P = 0.26$
PLC	$r = -0.23, P = 0.37, t = 0.83, P = 0.42$	$r = 0.49, P = 0.047^*, t = -2.06, P = 0.06$	$r = -0.24, P = 0.36, t = 0.70, P = 0.50$	$r = 0.55, P = 0.02^*, t = -2.10, P = 0.06$	$r = 0.05, P = 0.84, t = 0.44, P = 0.67$	$r = 0.36, P = 0.16, t = -1.15, P = 0.28$
ILC	$r = -0.12, P = 0.64, t = 0.91, P = 0.38$	$r = 0.30, P = 0.24, t = -1.44, P = 0.18$	$r = 0.00, P = 0.99, t = 0.22, P = 0.83$	$r = 0.10, P = 0.70, t = 0.22, P = 0.83$	$r = 0.42, P = 0.09, t = -1.05, P = 0.31$	$r = -0.50, P = 0.04^*, t = 0.89, P = 0.40$
OFC	$r = -0.16, P = 0.55, t = 0.32, P = 0.76$	$r = -0.03, P = 0.91, t = 0.05, P = 0.96$	$r = 0.22, P = 0.40, t = -0.62, P = 0.55$	$r = -0.29, P = 0.26, t = 0.70, P = 0.50$	$r = 0.31, P = 0.23, t = -1.01, P = 0.34$	$r = -0.07, P = 0.78, t = -0.26, P = 0.80$
INS	$r = -0.26, P = 0.54, t = 0.46, P = 0.65$	$r = -0.25, P = 0.33, t = 1.68, P = 0.12$	$r = 0.23, P = 0.38, t = -0.89, P = 0.39$	$r = -0.40, P = 0.11, t = 1.77, P = 0.10$	$r = 0.49, P = 0.04^*, t = -1.24, P = 0.24$	$r = 0.05, P = 0.86, t = -0.20, P = 0.85$

* $P < 0.05$. NACs, NAC shell; NACc, NAC core; OFC, orbitofrontal cortex.