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Adolescent Risk Taking, Cocaine Self-Administration, and Striatal Dopamine Signaling

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Poor decision making and elevated risk taking, particularly during adolescence, have been strongly linked to drug use; however the causal relationships among these factors are not well understood. To address these relationships, a rat model (the Risky Decision-making Task; RDT) was used to determine whether individual differences in risk taking during adolescence predict later propensity for cocaine self-administration and/or whether cocaine self-administration causes alterations in risk taking. In addition, the RDT was used to determine how risk taking is modulated by dopamine signaling, particularly in the striatum. Results from these experiments indicated that greater risk taking during adolescence predicted greater intake of cocaine during acquisition of self-administration in adulthood, and that adult cocaine self-administration in turn caused elevated risk taking that was present following 6 weeks of abstinence. Greater adolescent risk taking was associated with lower striatal D2 receptor mRNA expression, and pharmacological activation of D2/3 receptors in the ventral, but not dorsal, striatum induced a decrease in risk taking. These findings indicate that the relationship between elevated risk taking and cocaine self-administration is bi-directional, and that low striatal D2 receptor expression may represent a predisposing factor for both maladaptive decision making and cocaine use. Furthermore, these findings suggest that striatal D2 receptors represent a therapeutic target for attenuating maladaptive decision making when choices include risk of adverse consequences.
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INTRODUCTION

Chronic drug (particularly cocaine) use is associated with a range of cognitive and behavioral alterations, including high levels of risk taking and other maladaptive behaviors (Bornovalova *et al*, 2005; Coffey *et al*, 2003; Ersche *et al*, 2011). Such alterations are of concern not only because of reduced functioning and quality of life but also because of their potential to promote continued drug use and relapse. Associations between cognitive/behavioral alterations and drug use may be present as early as adolescence, which in itself is a period of heightened risk taking, impulsivity, and sensation seeking (Chambers *et al*, 2003; Spear, 2000; Stansfield *et al*, 2004; Vaidya *et al*, 2004); however, it has been difficult to disentangle causal relationships among cognitive/behavioral alterations and chronic drug use in humans. Animal models have provided considerable insight into the nature and direction of relationships between some maladaptive behaviors and drug use. For example, high levels of impulsive choice predict both acquisition and escalation of cocaine self-administration, which in turn can cause increases in impulsive choice (Anker *et al*, 2009; Mendez *et al*, 2010; Perry *et al*, 2008; Setlow *et al*, 2009). There is less known, however, regarding relationships between risk taking and drug use, particularly in the context of adolescence. The experiments described below were designed to address these relationships, and to begin to determine the underlying neural mechanisms.

To model risk-taking behavior, we used a task in which rats choose between two response levers, one that delivers a small, 'safe' food reward and the other that delivers a large, 'risky' food reward accompanied by a systematically increasing probability of a mild footshock. Rats performing this 'Risky Decision-making Task' (RDT) display a shift in their choices from the risky to the safe reward as risk of footshock increases within test sessions, and choice performance remains stable over long time periods (Mitchell *et al*, 2011; Simon *et al*, 2009). Importantly, as in humans, rats display considerable individual variability in choice preference in this task, such that some can be characterized as 'risk taking' (showing strong preference for the risky reward), whereas others are 'risk averse' (showing strong preference for the safe reward). This variability is not associated with individual differences in several factors that could contribute to choice performance, including

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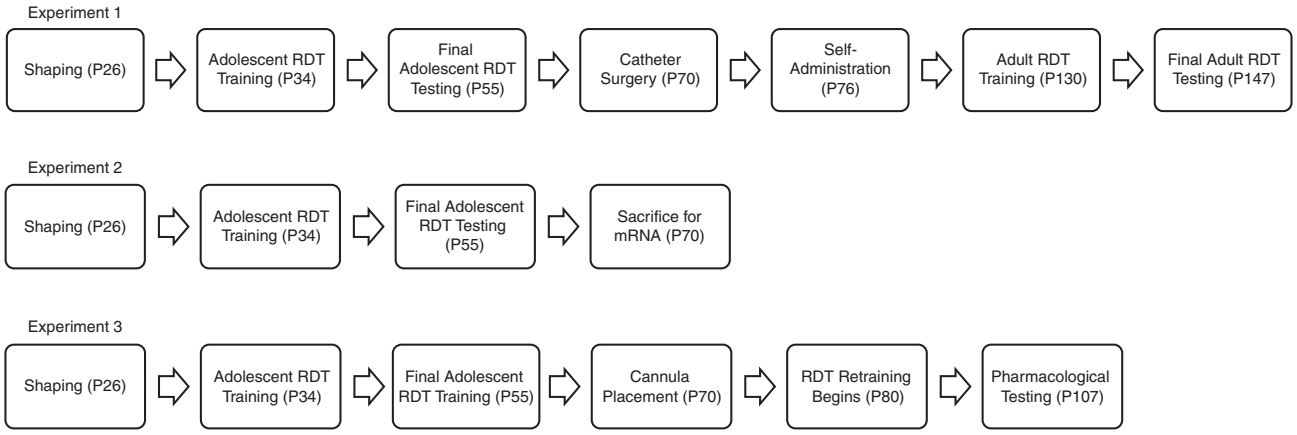

Figure 1 Experimental timeline of each of the three experiments and the respective ages of the rats at each stage in the experiments.

appetitive motivation, consummatory motivation, anxiety, and pain sensitivity (Simon *et al*, 2009; Simon *et al*, 2011).

Dopamine neurotransmission, and striatal D2/3 receptors in particular, are implicated in both decision-making processes and drug abuse. For example, striatal D2/3 receptor availability is linked to trait impulsivity and behavioral disinhibition (Dalley *et al*, 2007; Ghahremani *et al*, 2012), and low striatal D2/3 levels are associated with elevated cocaine self-administration (Caine *et al*, 2002; Morgan *et al*, 2002; Nader *et al*, 2006). Furthermore, recent data from our laboratory show that low levels of striatal D2 receptor mRNA expression are associated with elevated risk taking in adult rats (Simon *et al*, 2011).

To begin to determine relationships between risk taking and drug use, the RDT was used to test whether adolescent risk taking predicts vulnerability to cocaine self-administration (SA), and whether cocaine SA in turn causes further alterations in risk taking. To investigate dopaminergic mechanisms supporting relationships between risk taking and cocaine SA, regionally specific dopamine receptor mRNA expression was assessed in rats characterized in the RDT, and RDT performance was evaluated following intracerebral administration of the D2/3 agonist quinpirole.

MATERIALS AND METHODS

Subjects

Male Long-Evans rats (P26 at start of testing; Exp 1, $n=42$; Exp 2, $n=12$; Exp 3, $n=26$; Charles River Laboratories, Raleigh, NC) were individually housed and kept on a 12-h light/dark cycle (lights on at 0800 hours) with free access to food and water except as noted. During testing, rats were maintained at 85% of their free-feeding weight, with allowances for growth (Supplementary Table S1). Animal procedures were approved by the University of Florida IACUC and followed NIH guidelines. Figure 1 shows the design for each experiment.

Experiment 1

The goals of Experiment 1 were first to determine the relationship between adolescent risk taking and adult cocaine SA, and second to determine how cocaine SA affects risk taking.

Risky Decision-Making Task

Approximately 20 daily sessions were needed for rats to acquire stable choice behavior in the RDT. Accordingly, shaping to perform the task began on P26, but stable behavior was not evident until ~P51–55 (Figure 1). The RDT was conducted in operant test chambers equipped with two retractable levers and a centrally located food trough. Before RDT testing, rats were shaped to perform the various tasks components (nose poking into the food trough and lever pressing for food delivery) as in Simon *et al* (2009) and Mitchell *et al* (2011) (see Supplementary Information for full description).

Each daily RDT test session consisted of five blocks of trials. On each trial, a nose poke into the food trough triggered extension of either a single lever (forced choice trials) or both levers simultaneously (free choice trials, eight per block). A press on one of the levers resulted in one food pellet (the small safe reward). A press on the other lever (counterbalanced across rats) resulted in three food pellets (the large, risky reward); however, selection of this lever was also accompanied by a possible 1-s footshock contingent on a preset probability specific to each trial block. The probability of footshock accompanying the large reward was set at 0% during the first block of trials. In subsequent blocks, the probability of footshock increased to 25, 50, 75, and 100%.

Self-Administration Procedures

Following initial RDT testing and maturation to adulthood, rats were divided into two groups (cocaine and sucrose SA, balanced for RDT performance). Rats in the cocaine SA group ($n=28$) underwent surgery at P70–75 to implant jugular catheters (as in Mendez *et al* (2010)—see Supplementary Information for details). Cocaine SA sessions took place in operant chambers located in a different room from those used for RDT testing. During these sessions, cocaine solution was delivered through a fluid line connected to the catheter on a fixed ratio 1 (FR1) schedule following a nose poke into an illuminated (active) nose poke hole in a volume of 0.16 ml over 6 s, followed by a 20-s timeout period. Responses at a non-illuminated (inactive) nose-poke hole were recorded but had no programmed

consequences. The left/right positions of the active and inactive nose-poke holes were counterbalanced in relation to the left/right positions of the large reward levers used in the RDT. Cocaine SA consisted of 5 2-h sessions at 0.5 mg/kg/infusion ('acquisition'), followed by 14 6 h sessions at 1.0 mg/kg/infusion ('maintenance'). Note that only two of the three cohorts of rats used for Experiment 1 were tested in maintenance and subsequent post-SA testing in the RDT. In addition, because of subsequent failures in catheter patency and infections, only $n = 9$ rats completed testing beyond acquisition.

The sucrose SA group ($n = 14$) did not undergo surgery and was trained in the same chambers to nose poke (FR1) in the active hole to obtain access to a 20% sucrose solution from a liquid dipper, on a schedule such that the number of reinforcers allowed to be earned by each rat was matched to the number earned by a partnered cocaine rat with similar RDT performance. This group controlled for elements of cocaine SA that were not cocaine-specific, such as experience with a novel context and learning of a new instrumental response, and equated the number of reinforced responses between the two groups. Following SA procedures (both cocaine and sucrose), rats were left undisturbed for 3 weeks before returning to the RDT for retesting for an additional 20 sessions.

Data Analysis

Statistical analyses were conducted in SPSS 20. Stable RDT performance was assessed with a two-factor repeated measures ANOVA (session × trial block) conducted on data collected over five consecutive sessions, and was defined by a significant main effect of trial block and the absence of a main effect or interaction involving session (Simon *et al*, 2009; Simon *et al*, 2010; Winstanley *et al*, 2006). To assess relationships between risk taking and cocaine SA, rats were median split into 'risk taking' and 'risk averse' subgroups on the basis of adolescent RDT performance (mean % choice of the large risky reward during stable performance, averaged across all five blocks of trials). Cocaine SA (intake) was compared between these two groups using repeated measures ANOVA. Pearson's correlations were also used to compare RDT performance (mean % choice of the large, risky reward averaged across all five blocks) with mean daily cocaine intake. Performance in the RDT pre- and post-SA was compared using repeated measures ANOVA. Relationships between SA variables and pre- and post-SA RDT performance were examined using Pearson's correlations. For all analyses, $p < 0.05$ was considered significant.

Experiment 2

Adolescent rats were trained in the RDT as in Experiment 1. At P70 (approximately the time of cocaine SA in Experiment 1), rats were killed and perfused, and brains dissected and frozen. Coronal sections were collected through the prefrontal cortex and striatum using a freezing microtome. Isotopic *in situ* hybridization was used to assess regionally specific D1 and D2 receptor mRNA expression. Both *t*-tests and Pearson's correlations were used to compare RDT performance with D1 and D2 hybridization signals as in Experiment 1 and Simon *et al* (2011) (see Supplementary Information for details).

Experiment 3

Adolescent rats were trained in the RDT as in Experiment 1. At P70, rats underwent surgery to implant chronic bilateral intracerebral guide cannulae targeting dorsal or ventral striatum (see Supplementary Information for details). This timepoint was chosen to most closely match the timepoints used in Experiments 1 and 2, given the time constraints of adolescent characterization in the RDT, surgery, and RDT re-training. On recovery, rats were retrained in the RDT until stable performance was established. Before RDT test sessions, rats received microinjections of the D2/3 agonist quinpirole (0.1, 1.0, and 10 μg in 0.5 μl per hemisphere (Haluk and Floresco, 2009)) or vehicle in a randomized, counterbalanced order such that each rat received each dose of the drug. Following testing, rats were killed and cannula placements verified and mapped onto standardized coronal sections (Supplementary Figure S5; Paxinos and Watson, 2008). Effects of quinpirole were assessed using repeated measures ANOVA (drug dose × trial block).

RESULTS

Experiment 1

Rats achieved stable performance in the RDT during adolescence (21–26 sessions, reaching stability at P51–55), with substantial individual variability in choice preference (Figure 2a). Before SA experience, there were no differences in RDT performance between rats assigned to the cocaine and sucrose groups (main effect of block, $F_{(4,84)} = 50.33$, $p < 0.0001$; main effect of group, $F_{(1,21)} = 0.33$, $p > 0.57$; block × group interaction, $F_{(4,84)} = 1.84$, $p > 0.13$) (Figure 3a; see also Supplementary Table S2).

Adolescent Risk Taking as a Predictor of Future Cocaine Self-Administration

To determine the relationship between adolescent risk taking and cocaine SA, rats were divided into risk taking and risk averse subgroups (median split based on mean % choice of the large risky reward during adolescence; Figure 2b). Comparison of these two groups revealed significantly greater cocaine intake in the risk taking *vs* the risk averse subgroup over the 5-day acquisition period (two-factor ANOVA (subgroup × day), $F_{(1,26)} = 4.79$, $p < 0.05$; Figure 2c). This relationship was also evident in a significant linear correlation across all rats, such that greater choice of the large risky reward was associated with greater mean daily cocaine intake ($r = 0.45$, $p < 0.05$; Supplementary Figure S1). In contrast, there was no relationship between risk taking and cocaine intake during the maintenance period (Supplementary Figure S2 and Table S5).

Long-Term Increase in Risk Taking Resulting from Cocaine Self-Administration

Following cocaine (or sucrose) SA as described above, rats remained abstinent for the remainder of the experiment.

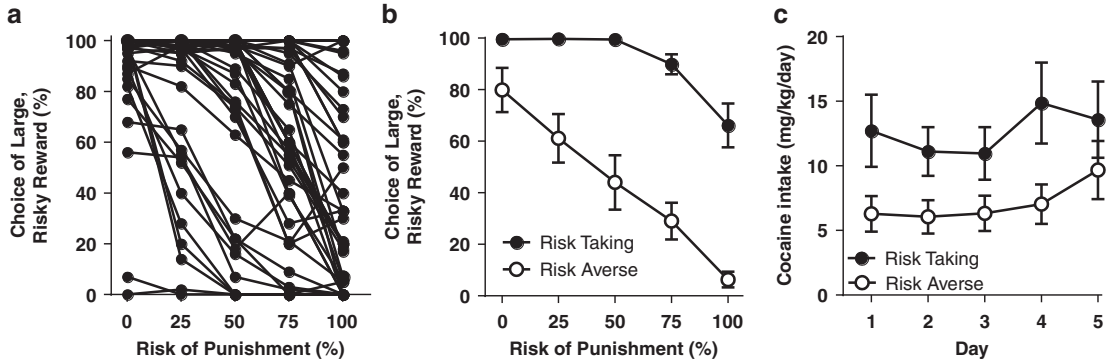

Figure 2 (a) Individual variability in Risky Decision-making Task (RDT) performance during adolescence (pre-SA). Each line represents performance of one rat in the RDT ($n=42$). Percent choice of the large, risky reward is shown on the *y*-axis; risk of footshock is shown on the *x*-axis. (b) Adolescent RDT performance in rats designated as 'risk taking' and 'risk averse', which then underwent cocaine SA ($n=28$). Note that this does not include the rats ($n=14$) characterized in adolescence that then underwent sucrose SA. (c) Adolescent risk taking predicts cocaine SA in adulthood. Over the course of the 5 days of acquisition of cocaine SA (*x*-axis), risk taking rats (closed circles) self-administered significantly more cocaine (*y*-axis) than risk averse rats (open circles).

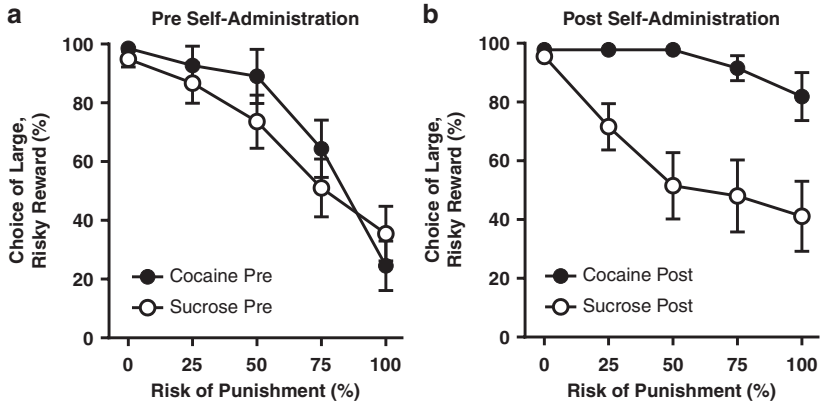

Figure 3 Cocaine self-administration causes long-lasting elevations in risky choice. (a) There were no differences in RDT performance between groups during adolescence (before cocaine or sucrose SA). (b) After 6 weeks of abstinence, cocaine SA rats ($n=9$) chose the large, risky reward significantly more than sucrose SA controls ($n=14$).

Three weeks after SA, they were retested in the RDT for 20 sessions. Final RDT performance was recorded during the last five of these sessions, at which point rats had regained stable performance. RDT data (percent choice of the large, risky reward) were analyzed using three-factor repeated measures ANOVA (time (pre- *vs* post-SA) × SA condition (cocaine *vs* sucrose) × trial block). This analysis did not reveal a main effect of time ($F_{(1,21)}=1.52$, $p>0.2$), but did reveal main effects of SA condition ($F_{(1,21)}=4.45$, $p<0.05$) and block ($F_{(4,84)}=39.23$, $p<0.0001$), as well as interactions between SA condition and block ($F_{(4,84)}=3.12$, $p<0.05$) and between time, SA condition, and block ($F_{(4,84)}=5.29$, $p<0.001$). Most importantly, there was a significant interaction between time and SA condition ($F_{(1,21)}=6.48$, $p<0.05$), such that cocaine and sucrose SA groups differed during the post- but not pre-SA period. Follow-up two-factor ANOVAs (SA condition × block) were used to compare rats in each SA condition during pre- and post-SA periods. Whereas there was no difference between SA conditions pre-SA (Figure 3a; see statistics above), there was a significant main effect of SA condition post-SA, such that cocaine SA rats chose the large, risky reward significantly more than sucrose SA rats (main effect of SA condition, $F_{(1,21)}=8.51$, $p<0.01$; interaction with block, $F_{(4,84)}=12.80$, $p<0.0001$; Figure 3b). Additional comparisons of pre- *vs* post-SA performance within each SA condition revealed that cocaine SA rats significantly increased their choice of the large, risky reward from pre- to post-SA ($F_{(1,8)}=7.19$, $p<0.05$), whereas sucrose SA rats did not ($F_{(1,13)}=0.99$, $p>0.3$; Supplementary Figure S3). Elevated choice of the large, risky reward following cocaine SA was not correlated with cocaine intake ($r=-0.20$, $p>0.6$; see also Supplementary Table S4). Finally, among sucrose SA rats, mean choice of the large reward was correlated at the adolescent and adult timepoints (Supplementary Table S5), indicating the stability of RDT performance across several months (Simon *et al*, 2009; Simon *et al*, 2011). No such correlation was present in cocaine SA rats, likely because all rats in this group shifted to almost complete preference for the large, risky reward, irrespective of adolescent choice preference.

Experiment 2

In situ hybridization was used to assess dopamine receptor mRNA expression in striatal and prefrontal cortical subregions of rats behaviorally characterized in the RDT during adolescence and killed at approximately the same timepoint at which rats began cocaine SA in Experiment 1. When rats were median split into risk averse and risk taking

subgroups, risk taking rats showed lower D2 mRNA expression than their risk averse cohorts in both dorsolateral striatum and nucleus accumbens shell ($t_{(10)} > 2.4$, $p < 0.05$; Figure 4a and c) but not nucleus accumbens core ($t_{(10)} = 0.78$, n.s.). Similar results were obtained using Pearson's correlations (dorsolateral striatum, $r = -0.75$, $p < 0.05$; nucleus accumbens shell, $r = -0.72$, $p < 0.05$; Figure 4b and d). These data indicate that less D2 mRNA expression in both striatal subregions was associated with greater preference for the large, risky reward. In addition, there were significant or near-significant relationships between adolescent RDT performance and striatal D1 mRNA expression (dorsomedial striatum: $r = -0.62$, $p < 0.05$; dorsolateral striatum: $r = -0.57$, $p = 0.053$; Supplementary Figure S4). There were no other significant relationships with risk taking for either receptor subtype (Supplementary Table S6).

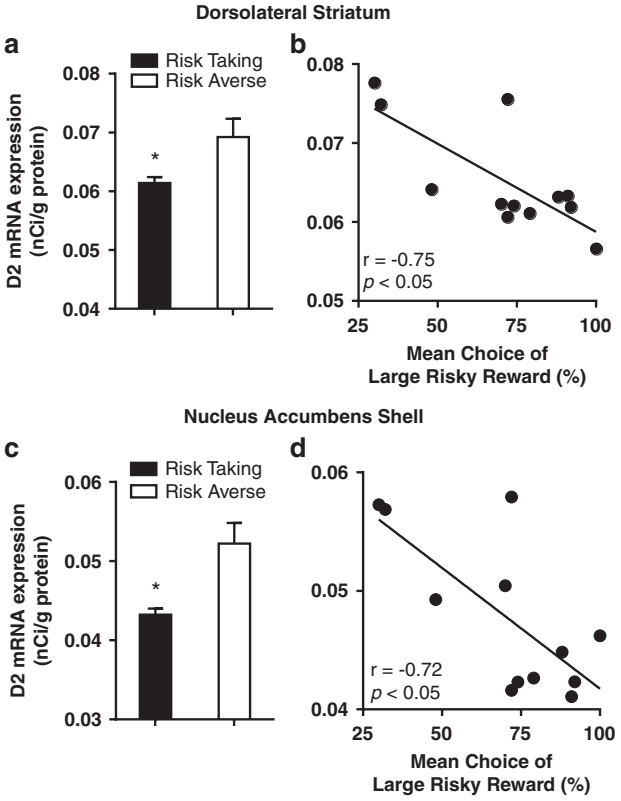

Figure 4 D2 mRNA expression is associated with adolescent Risky Decision-making Task (RDT) performance. Dividing rats into risk taking and risk averse subgroups ($n = 6$ each) on the basis of adolescent RDT performance revealed that in both dorsolateral striatum and nucleus accumbens shell, risk-taking rats had significantly lower D2 mRNA expression than risk averse rats (a and c). There were also significant negative correlations between these two variables in both subregions, such that lower D2 mRNA expression was associated with greater choice of the large, risky reward (b and d). *$p < 0.05$ compared to risk averse subgroup.

Experiment 3

The highest dose of quinpirole (10.0 μg) caused a substantial number of trial omissions (Supplementary Table S7). Hence, only data from the 0.1- and 1.0-μg doses were used for further analyses. Microinjections of quinpirole into dorsal striatum did not alter choice of the large, risky reward (main effect of drug: $F_{(2,16)} = 0.97$, n.s.; drug × block interaction: $F_{(8,64)} = 0.88$, n.s., Figure 5a). However, microinjections into ventral striatum resulted in a decrease in choice of the large, risky reward (main effect of drug: $F_{(2,22)} = 6.18$, $p < 0.01$; drug × block interaction: $F_{(8,88)} = 0.80$, n.s., Figure 5b), with *post-hoc* comparisons showing that the 1.0-μg dose differed from both vehicle and 0.1-μg conditions (Fs > 6.70; *ps* < 0.05).

DISCUSSION

Drug use in humans is associated with elevated risk taking, but causal relationships between drug use and risk taking have been difficult to disentangle. The results here suggest that elevated risk taking in adolescence may increase vulnerability to cocaine use later in life, and that cocaine use, in turn, can cause long-lasting elevations in risk taking, potentially creating a 'vicious cycle' of elevated risk taking and cocaine use. Hence, these data suggest that both pre-existing conditions and the drug itself could contribute to elevated risk taking in chronic cocaine users.

The finding that elevated risk taking in adolescence predicts future cocaine SA is consistent with both human

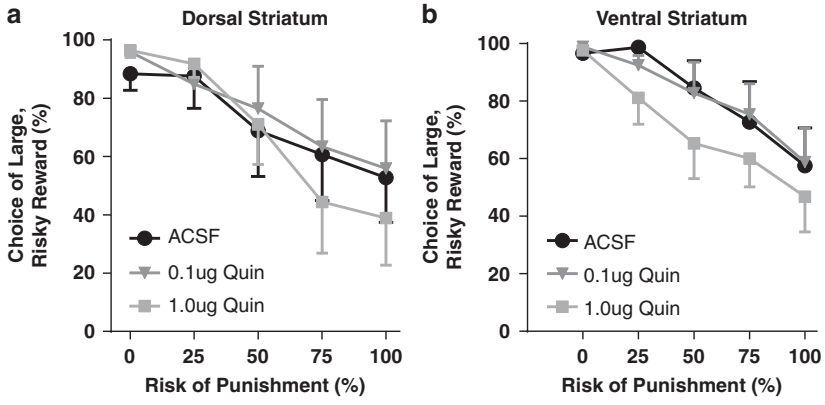

Figure 5 Effects of intra-striatal quinpirole administration on Risky Decision-making Task performance. Microinjections of the D2/3 agonist quinpirole did not alter choice behavior when administered into the dorsal striatum (a, $n = 10$) but dose-dependently decreased choice of the large, risky reward when administered into the ventral striatum (b, $n = 12$).

and animal literature assessing risk taking and related behaviors. Adolescents who display greater impulsivity, risk taking, and sensation seeking display concomitantly greater rates of substance use (Chambers *et al*, 2003; Spear, 2000), and studies in adult rats show that measures of both impulsive action and impulsive choice predict cocaine SA (Dalley *et al*, 2007; Perry *et al*, 2005; Perry *et al*, 2008). In Experiment 1, risk taking predicted cocaine SA only during the acquisition and not the maintenance period, whereas impulsivity measures are reported to predict acquisition as well as measures of compulsive-like cocaine SA that are more closely linked to addiction (Anker *et al*, 2009; Belin *et al*, 2008; Broos *et al*, 2012; Deroche-Gamonet *et al*, 2004; Perry *et al*, 2005; Perry *et al*, 2008; Vanderschuren and Everitt, 2004). The absence of relationships between risk taking and cocaine intake during maintenance could be due to the fact that SA under such conditions is reportedly not strongly tied to compulsive-like cocaine intake (Belin *et al,* 2008). Importantly, however, neither the difference in risk taking nor the difference in acquisition of cocaine SA between risk averse and risk taking subgroups was likely due to differential learning abilities, as rats in these two subgroups do not differ in performance on either a visual discrimination learning task or a delayed response working memory task conducted in conditions similar to those used here (Shimp *et al*, submitted).

In addition to risk taking predicting acquisition of cocaine SA, cocaine SA in turn caused a robust increase in risk taking in comparison with both pre-SA baseline and sucrose SA controls. This increase occurred irrespective of pre-SA baseline (ie, all rats showed nearly 100% choice of the large, risky reward following cocaine SA; Figure 3b), indicating that under these conditions, the effects of chronic cocaine on risk taking overwhelm pre-existing individual differences. This latter finding suggests that elevated risk taking observed in chronic cocaine users may be mediated to a greater degree by the consequences of drug exposure, rather than by pre-existing individual differences.

The finding of increased risk taking following cocaine SA is consistent with a large body of literature showing that cocaine exposure causes lasting alterations in other aspects of cognition and motivated behavior (Dandy and Gatch, 2009; Mendez *et al*, 2008; Mendez *et al*, 2010; Saddoris *et al*, 2011; Schoenbaum and Setlow, 2005; Simon *et al*, 2007). It is also consistent with findings of increased impulsivity and risk taking in cocaine users (Bechara *et al*, 2001; Bolla *et al*, 2003; Coffey *et al*, 2003). Importantly, in the current study, the increased choice of the large reward following cocaine SA was not likely due to perseverative choice behavior or increased motivation for large rewards *per se*, as similar cocaine SA regimens cause decreased choice of large, delayed rewards in a delay discounting task using a task design similar to that of the RDT (Mendez *et al*, 2010; Mitchell *et al*, submitted). Instead, cocaine-induced elevations in risk taking may result from reductions in sensitivity to punishing stimuli. Consistent with this interpretation, chronic cocaine causes a selective reduction in orbitofrontal cortex neural activity in response to aversive (but not rewarding) stimuli (Stalnaker *et al*, 2006), as well as insensitivity to the response-suppressive effects of aversive cues on cocaine SA (Vanderschuren and Everitt, 2004) see also Johnson and Kenny (2010; for similar findings with chronic consumption of palatable food). Finally, cocaine-induced increases in risk taking were likely not due to alterations in body weight, shock reactivity, or locomotion, as these factors were not associated with choice behavior (Supplementary Tables S3 and S5).

In Experiment 2, there was an inverse relationship between adolescent risk taking and D2 mRNA expression in the dorsolateral striatum and nucleus accumbens shell, suggesting a role for both striatal subregions in mediating risk taking. These results contrast somewhat with those of our previous work, in which only dorsal and not ventral striatal D2 receptor mRNA expression was negatively associated with risk taking in adult rats (Simon *et al*, 2011). This difference could reflect a developmental shift from adolescence to adulthood. Alternatively, this difference could represent a time-dependent reorganization of the neural substrates of dopaminergic modulation of risk taking, as considerably more time elapsed between RDT testing and killing for mRNA analysis in Simon *et al* (2011) than in the present study. Importantly, however, in the present study, the results of Experiment 3 suggest that only D2/3 receptors in ventral striatum have a significant functional role in risk taking, as quinpirole microinjections into dorsal striatum had no effect on RDT choice performance.

The results of Experiments 2 and 3 are consistent with evidence that individuals with lower D2/3 receptor availability in the striatum show higher rates of risk taking as measured by the Iowa Gambling Task (Linnet *et al*, 2011a, b), as well as with previous work from our laboratory showing that systemic administration of the D2/3 agonist bromocriptine reduces risk taking (Simon *et al*, 2011). Moreover, D2 mRNA is selectively lower in the accumbens shell of high impulsive compared with low impulsive rats (Besson *et al*, 2013), and blockade of D2/3 receptors in the accumbens shell increases impulsive action in the five-choice serial reaction time task (whereas blockade of D2/3 receptors in the accumbens core has the opposite effect; Besson *et al*, 2010). These data provide functional support for the association between low striatal D2 receptor expression and elevated risk taking, and implicate the accumbens shell as an important site of action for mediating the effects of D2/3 agonists on risk taking.

Interestingly, a recent report showed that ventral striatal quinpirole administration had no effect on choice behavior in a probability-discounting task, which is similar to the RDT but in which the 'cost' associated with the large reward is risk of reward omission (Stopper *et al*, 2013). The authors attributed the lack of quinpirole effects to a greater role for D3 than D2 receptors in this task. The different effects of quinpirole in the two tasks may be due to the relative importance of rewards *vs* reward 'costs' in guiding choice behavior in the probability-discounting task and RDT, respectively (Shimp *et al*, submitted; Simon *et al*, 2009; St Onge and Floresco, 2009), and it may be that D3 and D2 receptors are differentially involved in mediating their influences on behavior. It will be of interest in future studies to assess the effects of more selective D2- and D3-acting drugs in the RDT.

In addition to links with risk taking and impulsivity, low levels of D2/3 receptor availability in ventral striatum are

also directly associated with stimulant use. Stimulant users have reduced striatal D2/3 receptor availability (Lee *et al*, 2009; Volkow *et al*, 2001), and ventral striatal D2/3 receptor availability decreases after chronic cocaine SA in monkeys, suggesting that low D2/3 receptor availability in human cocaine users may be due in part to effects of cocaine itself (Nader *et al*, 2006). Moreover, low ventral striatal D2/3 availability in rats predicts both trait impulsivity and high levels of cocaine SA (Dalley *et al*, 2007). Together, these data suggest that low D2 receptor availability within the ventral striatum (possibly specific to the accumbens shell) of risk-preferring adolescent rats may underlie their increased propensity for acquisition of cocaine SA. It remains to be determined whether cocaine-induced decreases in D2 receptors are responsible for the increased risk taking observed in Experiment 1. Notably, however, a recent study showed that chronic cocaine SA causes decreased striatal D2 receptor mRNA expression in rats (Besson *et al*, 2013), supporting the hypothesis that either intrinsic or drug-induced decreases in striatal D2 receptors mediate high levels of risk taking.

The data derived from the RDT model suggest that elevated risk taking during adolescence (accompanied, and perhaps caused, by low levels of ventral striatal D2 receptor activity) is a predisposing factor for initiation of cocaine use. Although initiation does not necessarily lead to chronic use, in cases in which this does occur, cocaine use would be expected to cause further elevations in risk taking, which could in turn promote further drug use and other maladaptive behaviors. To our knowledge, this combination of adolescent development, bi-directional relationships between an addiction-associated phenotype and drug intake, and a signature neurobiological feature of addiction (reduced striatal D2 receptors) is unique among animal models linking addiction-associated traits and drug use (Belin *et al*, 2008; Besson *et al*, 2010; Dalley *et al*, 2007; Saunders and Robinson, 2010, 2011). In particular, because predispositions for drug use can be evident in human adolescence (Chambers *et al*, 2003; Chartier *et al*, 2010), the ability to capture a predictive phenotype at this stage of development is important for modeling vulnerability to drug use. However, it is also notable that several other adult rodent models of addiction-associated phenotypes, particularly those related to impulsive behavior, predict aspects of cocaine SA that are more closely linked to addiction than acquisition (eg, enhanced motivation to self-administer, resistance to extinction; Anker *et al*, 2009; Belin *et al*, 2008; Dalley *et al*, 2007; Perry *et al*, 2005). The extent to which elevated risk taking predicts such 'addiction-like' elements of SA behavior remains to be determined; however, the fact that low levels of ventral striatal D2/3 receptor availability are associated with compulsive cocaine intake in other models (Dalley *et al*, 2007) suggests that such predictive relationships may be evident with risk taking as well.

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Supplementary Information accompanies the paper on the Neuropsychopharmacology website (http://www.nature.com/npp)