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Risk-preferring rats make worse decisions and show increased incubation of craving after cocaine self-administration

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ABSTRACT

Maladaptive decision-making may play an integral role in the development and maintenance of an addiction. Substance-dependent individuals make riskier choices on the Iowa Gambling Task, and these deficits persist during withdrawal and are predictive of relapse. However, it is unclear from clinical studies whether this cognitive impairment is a cause or consequence of drug use. We trained male Long-Evans rats on the rat Gambling Task, a rodent analogue of the Iowa Gambling Task, to determine how choice preference influenced, and was influenced by, cocaine self-administration, withdrawal and incubation of craving. Rats that exhibited a preference for the risky, disadvantageous options at baseline were uniquely and adversely affected by cocaine self-administration. Risky choice was exacerbated in these rats when decision-making was assessed during the same diurnal period as cocaine self-administration, whereas the choice pattern of optimal decision-makers was unaffected. This decision-making deficit was maintained during 30 days of withdrawal and correlated with greater cue-induced incubation of craving. Risk-preferring rats also made more drug-seeking responses during cocaine self-administration. These data demonstrate that poor decision-making prior to contact with addictive drugs is associated with a pro-addictive behavioural phenotype, characterized by further increased risky choice and heightened responding for drug both during cocaine self-administration and withdrawal. Such findings indicate that the elevated risky decision-making observed in substance-dependent populations is not merely circumstantial, but makes an important contribution to addiction vulnerability and severity that can now be effectively modelled in laboratory rats.

Keywords Addiction, decision-making, impulsivity, individual differences, rat gambling task, withdrawal.

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INTRODUCTION

At the moment of relapse, arguably the most clinically significant point in the addiction cycle, the value of drug use outweighs the benefits of sobriety. As such, maladaptive decision-making may be considered central to the phenomenology of substance use disorder (SUD) (Duka, Crombag, & Stephens 2011; Goldstein & Volkow 2002). Indeed, dependent populations perform poorly on laboratory-based cost/benefit decision-making tasks (Bechara 2005; Bolla *et al.* 2003; Rogers *et al.* 1999; Verdejo-Garcia & Bechara 2009). One of the most well established of such paradigms is the Iowa Gambling Task (IGT), purposefully designed to simulate 'real-world' decision-making, in which all choices can lead to both beneficial and detrimental outcomes (Bechara *et al.* 1994; Bechara *et al.* 1999). In this test, subjects pick cards from four decks to accumulate points. The optimal strategy is to choose cards from the two advantageous decks associated with small immediate gains but also low and infrequent penalties. Persistent selection from the two disadvantageous decks leads to large immediate gain but heavy losses in the long term and clearly represents a maladaptive, risky strategy. Such a suboptimal pattern of choice has been observed in numerous drug-dependent populations, including cocaine (Stevens *et al.* 2013; Verdejo-Garcia, Perales, & Perez-Garcia

2007), methamphetamine (Gonzalez, Bechara, & Martin 2007; Wang *et al.* 2013), heroin (Verdejo-Garcia *et al.* 2007), marijuana (Bolla *et al.* 2005), alcohol (Bechara *et al.* 2001; Goudriaan, Grekin, & Sher 2007) and polysubstance abusers (Grant, Contoreggi, & London 2000), as well as pathological gamblers (Goudriaan *et al.* 2005). Furthermore, poor choice behaviour persists during drug withdrawal and predicts treatment failure and addiction severity (Bechara *et al.* 2001; Stevens *et al.* 2013; Wang *et al.* 2012), implying that this cognitive deficit critically contributes to the maintenance of the addicted state.

Although multiple factors likely contribute to the manifestation of addiction, recognizing a prominent role for disordered decision-making may open up new treatment approaches (Bechara 2003; Bechara 2005). However, the nature of the relationship between poor decision-making and SUD is difficult to determine from clinical data, in which myriad environmental and circumstantial factors may predominate. Animal models can play a vital role in this regard (Potenza 2009). We previously developed a rat gambling task (rGT), based on the IGT, performance of which depends on similar neural systems across species (Paine *et al.* 2013; Zeeb, Robbins, & Winstanley 2009; Zeeb & Winstanley 2011, 2013). Just as in the IGT, favouring the tempting 'high risk–high reward' options results in significantly less reward over time; the advantageous strategy is to choose options associated with smaller per trial gain, but also lower punishments. Although most rats choose optimally, some instead exhibit a disadvantageous preference for the risky options. Here, we test the hypothesis that such risky choice represents a cognitive endophenotype for addiction vulnerability using the cocaine self-administration model of substance use, and the incubation of craving (IOC) assessment of cue-driven drug-seeking. Furthermore, concomitant rGT sessions also allowed us to determine whether the process of cocaine intake or withdrawal differentially affected decision-making in risk-preferring versus optimal decision-makers.

MATERIALS AND METHODS

Details as to experimental procedures can be found in the Supporting Information.

Subjects

Subjects were male Long-Evans rats (Charles River Laboratories, St. Constant, Canada). As risk-preferring rats make up only ~22 percent of the population, a total of four separate cohorts of 16 rats (64 in total) were trained on the rGT in succession, of which 28 were used in the current experiment (14 risk-preferring, 14 optimal choosers; refer to the *Statistical analyses* section). Optimal rats were included until a sufficient number of rats for behavioural analysis was obtained. The remaining 36 optimal rats were reassigned to other ongoing behavioural experiments. Animals weighed between 275 and 300 g upon arrival and were maintained at approximately 85 percent of their free-feeding weight by restricting their food to 14 g of rat chow per day. Water was available *ad libitum*. Animals were pair housed under a reversed 12-hour light/dark cycle (lights off at 8 am) in a temperature controlled colony room maintained at 21°C. Testing and housing were in accordance with the Canadian Council of Animal Care, and all experimental protocols were approved by the Animal Care Committee of the University of British Columbia.

Apparatus

Behavioural testing for the rGT was conducted in standard five-hole operant chambers enclosed within ventilated sound-attenuating cabinets (Med Associates, Inc., Vermont, USA). Cocaine self-administration was run in separate operant boxes fitted with infusion pumps located in a different room within the facility.

Jugular vein catheter implantation surgeries

Rats were anaesthetized using isoflurane and aseptically implanted with catheters constructed of Silastic silicone tubing attached to backmount cannulae into the right jugular vein. Catheters were passed subcutaneously with the backmount positioned to exit between the shoulder blades. Catheters were flushed daily with 0.1 ml of 50 percent heparinized saline to maintain patency. Animals were allowed 5–7 days of recovery prior to re-starting the rGT.

The rat gambling task

Animals were trained to perform the rGT as per previously published methods (Zeeb *et al.* 2009). A task schematic is provided in Fig. 1. In brief, animals had 30 minutes in which to earn reward by selecting from four options, signalled by illumination of four response apertures, that varied in the number of sugar pellets (1–4) that could be earned on win trials, but also the probability (0.1–0.6) and duration (5–40 seconds) of punishing time-out periods delivered on losses. Animals initiated each trial by nosepoking at the illuminated food tray. This triggered the start of a 5-second intertrial interval (ITI) before presentation of the stimulus lights in holes 1, 2, 4 and 5. A response at one of the illuminated holes resulted in either delivery of the associated reward or the start of the time-out penalty linked with that response. During these time-out periods, the stimulus light within the hole chosen flashed at a frequency of 0.5 Hz. Premature responses made at the array during the ITI voided the trial and were punished by

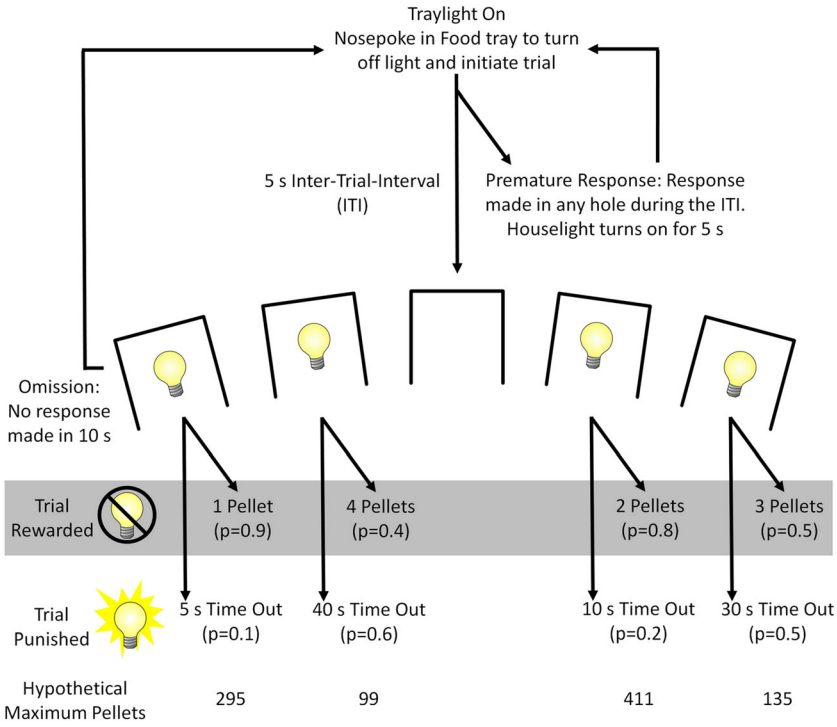

Figure 1 Schematic diagram showing the trial structure of the rGT. The task began with illumination of the tray light. A nose-poke response in the food tray extinguished the tray light and initiated a new trial. After an intertrial interval (ITI) of 5 seconds, four stimulus lights were turned on in holes 1, 2, 4 and 5, and the animal was required to respond in one of these holes within 10 s. This response was then rewarded or punished depending on the reinforcement schedule for that option (indicated by the probability of a win or loss in brackets for each option). If the animal was rewarded, the stimulus lights were extinguished and the animal received the corresponding number of pellets in the now-illuminated food tray. A response at the food tray then started a new trial. If the animal was punished, the stimulus light in the corresponding hole flashed at a frequency of 0.5 Hz for the duration of the punishing timeout and all other lights were extinguished. At the end of the punishment period, the tray light was turned on and the animal could initiate a new trial. Failure to respond at the illuminated holes resulted in an omission, whereas a response during the ITI was classified as a premature response and punished by a 5-second timeout during which the house light was turned on (schematic based upon Zeeb *et al.* 2009)

a 5-second time-out period. If the animal failed to make a response within 10 seconds of stimulus light illumination, the stimuli were turned off and the trial scored as an omission. Animals received 5–6 daily sessions per week until statistically stable patterns of behaviour across all measures were observed over three sessions. This took on average 35 sessions to achieve.

Rats were then divided into experimental groups (saline, $n = 16$; cocaine, $n = 12$) matched for rGT performance, and underwent jugular vein catheter implantation. In order to track any changes in cognitive function as a result of cocaine self-administration, animals were tested on the rGT during the morning and cocaine self-administration sessions occurred in the afternoon/evenings (Winstanley *et al.* 2009).

Cocaine self-administration

Animals were trained on a fixed ratio 1 schedule to lever press for 0.75 mg/kg/infusion cocaine hydrochloride or saline vehicle over 10 daily 3-hour sessions. Responses on the active lever resulted in a single 4-second infusion accompanied by a flashing cue-light and tone followed by a 40-second timeout (Calu *et al.* 2007). Responses on the active lever during infusions and timeouts were recorded. Inactive lever presses, while monitored, had no programmed consequences.

Incubation of craving

Twenty-four hours following the last self-administration session, rats were placed into the self-administration chamber for 1 hour to measure responding to cocaine-paired cues. Pressing the active lever resulted in presentation of the light-tone cues previously associated with cocaine administration, followed by a 1-second intertrial interval, but no drug was administered at any time. This test was repeated after 30 days (Grimm *et al.* 2001). rGT sessions continued in the morning during this withdrawal period, but the animals remained in their home cages at all other times.

Statistical analyses

All statistical analyses were completed using SYStat 12.0 software (Chicago, IL, USA). As per previous reports (Zeeb

& Winstanley 2011, 2013), the following rGT variables were analysed at baseline, during self-administration, and during withdrawal: score (P1 + P2; total number of advantageous options chosen minus P3 + P4; total number of disadvantageous options chosen); percent choice of each option, percent premature responses, omissions, trials completed and choice and reward collection latencies. A statistically stable baseline was determined by a repeated-measures analysis of variance across data from three consecutive sessions, in which the session and session × choice interactions were not significant. Animals with a mean positive score across this period were designated as 'optimal' (Figure S1; cocaine, $n = 6$; saline, $n = 8$), whereas rats with negative scores were classified as 'risk-preferring' (cocaine, $n = 6$; saline, $n = 8$). This between-subjects factor (group) was included in all analyses.

The total responses on each lever from cocaine self-administration and cue-induced drug-seeking test sessions were subject to repeated-measures analysis of variance with group and drug as between-subjects factors, and session and lever as within-subjects variables. The magnitude of IOC (#active lever presses on day 30 − #active lever presses on day 1 of withdrawal) was also analysed, and any correlation with the change in decision-making caused by cocaine self-administration (baseline score − post self-administration score) was determined in cocaine-exposed animals. For all analyses, results were deemed significant if *P*-values were less than or equal to an $\alpha = 0.05$.

RESULTS

Additional analyses are provided in the Supporting Information.

Baseline behaviour

The score variable was significantly lower in the risk-preferring group, indicative of an elevated preference for the risky, disadvantageous options. (Table S1; group: $F_{1,26} = 45.96$, $p = 0.00000$; session: $F_{2,52} = 0.09$, $p = 0.90$; session × group: $F_{2,52} = 0.55$, $p = 0.58$). In concordance with previous data, risk-preferring animals also made more premature responses (Table S1; $F_{1,26} = 15.90$ $p = 0.0005$). While risk-preferring rats completed fewer trials ($F_{1,26} = 8.45$, $p = 0.001$), this likely reflects the longer penalty periods incurred by these animals as a result of poor decision-making and higher levels of impulsive action, rather than reduced motivation for reward, as these animals also made fewer omissions ($F_{1,26} = 11.84$, $p = 0.002$), and were faster to choose between the options (group: $F_{1,26} = 10.55$, $p = 0.003$) consistent with previous results (Barrus *et al.* 2015). As all rats collected larger rewards more quickly, this resulted in an artificially lower average reward collection latency in risk-preferring rats that was simply an artefact of their choice bias; these rats were not actually any faster than optimal decision-makers to collect the larger or smaller rewards (Table S1; group: $F_{1,26} = 15.70$, $p = 0.0007$; choice: $F_{3,27} = 5.28$, $p = 0.005$; choice × group: $F_{3,27} = 0.33$, $p = 0.80$).

Self-administration

Responding on the active lever increased over the 10 self-administration sessions in animals responding for cocaine, but not saline (Fig. 2a; lever: $F_{1,24} = 27.19$, $p = 0.00002$; drug × lever × session: $F_{5,119} = 3.52$, $p = 0.005$). While this steady increase in response rates was similar across the cohort (session × group: $F_{6,58} = 0.27$, $p = 0.95$), risk-preferring animals pressed significantly more on the cocaine-paired lever overall (group: $F_{1,10} = 6.24$, $p = 0.03$). However, there was no significant difference in the number of cocaine infusions received across groups (Fig. 2b; group: $F_{1,10} = 3.21$, $p = 0.10$; session × group: $F_{6,58} = 0.39$, $p = 0.87$). In animal self-administering saline, active lever pressing decreased over sessions in all rats (group $F_{1,14} = 1.79$, $p = 0.20$; session: $F_{5,68} = 5.61$, $p = 0.0002$; session × group: $F_{5,68} = 1.07$, $p = 0.39$). Inactive lever pressing did not differ across groups in either drug condition (Figure S2; group: $F_{1,24} = 0.01$, $p = 0.92$, drug × group: $F_{1,24} = 1.32$, $p = 0.26$).

rGT performance

Risk-preferring rats' performance of the rGT became increasingly more maladaptive during the concurrent self-administration phase of the experiment, as indicated by a further decrease in the score (Fig. 3b; session × drug × group: $F_{5,113} = 2.73$, $p = 0.02$; cocaine-only session × group: $F_{5,47} = 3.71$, $p = 0.007$; session-risk-preferring: $F_{6,31} = 3.24$, $p = 0.01$). In contrast, choice preference did not change in the optimal group even though these rats were ingesting comparable amounts of cocaine (optimal, session: $F_{4,20} = 1.64$, $p = 0.20$). Self-administration of saline did not alter performance of the rGT in either group (Fig. 3a; $F_{4,20} < 1.64$, $p > 0.20$).

Although premature responding fluctuated during this phase of the experiment across the entire cohort, this variability was not isolated to any one group or drug condition (Table 1; session: $F_{7,159} = 2.66$, $p = 0.01$; session × group: $F_{7,159} = 1.58$, $p = 0.15$; session × drug: $F_{7,159} = 0.41$, $p = 0.88$), and analyses of each groups' performance revealed no differences across sessions (all $F_{8,40} < 1.76$, $p > 0.18$). This statistical anomaly therefore likely reflects a general increase in behavioural variation in this measure, perhaps caused by alterations in the rats' daily routine and environment. However, all other aspects of performance remained stable during this epoch (Table 1; session × group × drug: $F_{8,192} < 1.80$, $p > 0.18$).

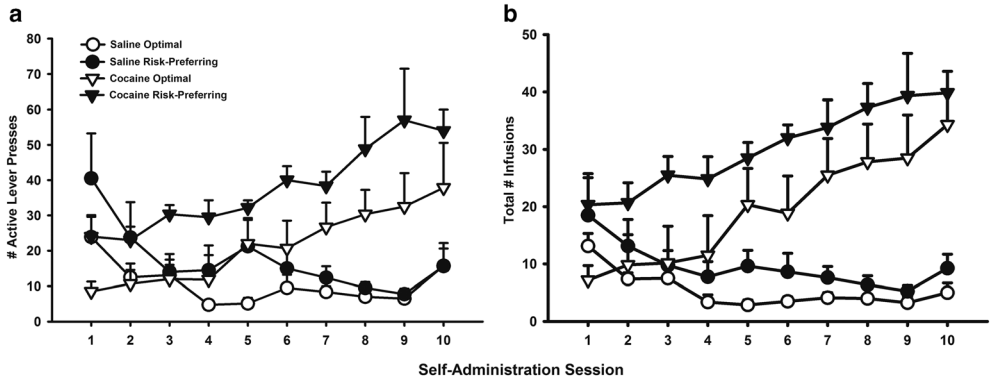

Figure 2 Responding during 3-hour drug self-administration sessions (a) Number of active lever presses across the subgroups over the self-administration period. Both groups self-administering cocaine developed a preference for the active lever over the 10 self-administration sessions, but risk-preferring rats pressed the active lever significantly more than optimal rats across all sessions. Animals self-administering saline decreased responding on the lever over the 10 sessions, with no significant difference observed between choice-preference groups. (b) Number of infusions received during self-administration sessions. As expected, cocaine animals received significantly more infusions than saline animals. However, there was no significant difference between cocaine optimal and risk-preferring animals' cocaine intake. Therefore, the changes in decision-making observed in risk-preferring rats cannot be attributed to elevated consumption. Data are presented as mean ± standard error of the mean

The decline in optimal decision-making was maintained during withdrawal, with neither an improvement nor further impairment observed in risk-preferring rats, or any other group (Fig. 4a and b; session: $F_{2,46} = 1.89$, $p = 0.16$; session × group: $F_{2,46} = 0.32$, $p = 0.72$; session × drug: $F_{2,46} = 0.12$, $p = 0.89$). Somewhat in keeping with previous reports that motor impulsivity is exacerbated during withdrawal, risk-preferring rats that had self-administered cocaine made significantly more premature responses towards the midpoint of withdrawal (Table 2; session × group × drug: $F_{2,48} = 7.03$, $p = 0.002$; session-risk-preferring: $F_{2,10} = 5.14$, $p = 0.03$, all other groups: all $F_s < 2.66$, $p > 0.10$), although this had normalized by the end of the 30 day period (24 hours versus 30 days withdrawal: session × group × drug: $F_{1,24} = 1.20$, $p = 0.28$).

Incubation of craving

As expected, active lever responding increased after 30 days of withdrawal from cocaine across groups (Fig. 5a; session × group × drug: $F_{1,24} = 5.15$, $p = 0.03$). Only a weak correlation was observed between the total number of active lever responses on day 30 and the total number of active lever presses during self-administration, suggesting that this IOC effect reflects a process at least partially distinct from the baseline tendency to respond on the active lever during self-administration sessions (Figure S3; $r = 0.51$, $p = 0.09$). Analysis of responding between the cocaine optimal and risk-preferring animals revealed that IOC was more pronounced in the risk-preferring group (session: $F_{1,\ 10} = 18.90$, $p = 0.001$; session × group: $F_{1,\ 10} = 5.86$, $p = 0.04$). Although the

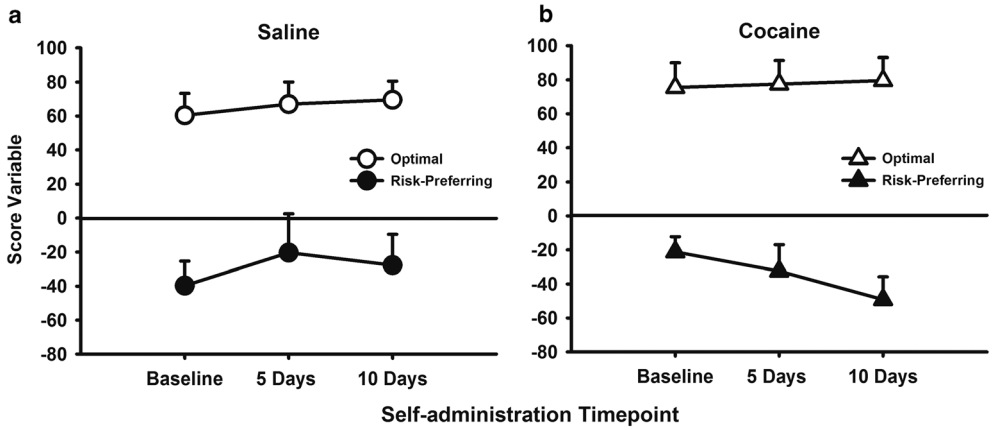

Figure 3 Decision-making as measured by the score variable during the self-administration period. (a) As expected, saline animals' decision-making remained unchanged. (B) However, while the advantageous choice pattern of optimal decision-makers self-administering cocaine did not alter, risk-preferring rats made significantly more risky and maladaptive choices during the self-administration epoch. Data are presented as mean ± standard error of the mean

Table 1 rGT performance over the 10 self-administration sessions.

Behavioural measure	*Saline optimal*	*Saline risk-preferring*	*Cocaine optimal*	*Cocaine risk-preferring*
Premature responding	11.99 ± 3.09	22.22 ± 5.07	4.54 ± 1.04	18.09 ± 4.08
Omitted responses	3.09 ± 0.91	1.01 ± 0.84	4.67 ± 1.39	1.39 ± 0.52
Trials completed	102.45 ± 6.62	71.76 ± 6.08	100.80 ± 9.91	61.65 ± 7.55
Choice latency	1.43 ± 0.19	0.99 ± 0.31	2.21 ± 0.28	1.12 ± 0.33
Collect latency	0.96 ± 0.09	0.55 ± 0.07	1.11 ± 0.13	0.83 ± 0.21

Values presented are averages ± standard error of the mean.

raw number of active lever presses was not significantly different between risk-preferring and optimal decision makers at the 30 day time point, we took advantage of the higher power made possible through this within-subjects design to determine the degree to which each individual rats' responding increased from the beginning to the end of withdrawal. This revealed a markedly elevated IOC in risk-preferring rats (Fig. 5c; group: $F_{1,24} = 8.22$, $p = 0.008$; drug: $F_{1,\ 24} = 11.97$, $p = 0.002$; group × drug: $F_{1,24} = 5.15$, $p = 0.03$; cocaine- group: $F_{1,10} = 5.86$, $p = 0.04$), suggesting enhanced susceptibility to the ability of drug-paired cues to promote relapse in these animals. Furthermore, the magnitude of this IOC correlated with the increase in risky decision-making observed during cocaine self-administration (Fig. 5d; $r = 0.61$, $p = 0.03$). Saline-experienced animals also demonstrated a much smaller but statistically significant increase in active lever presses, although this did not differ between optimal and risk-preferring animals (session: $F_{1,14} = 10.13$, $p = 0.007$; session × group: $F_{1,14} = 0.68$, $p = 0.42$). Inactive lever pressing also tended to increase across the two time points (Fig. 5b; session × group × drug: $F_{1,24} = 4.34$, $p = 0.05$), an effect that is largely attributed to a trend-level increase in lever-pressing in the cocaine-experienced risk-preferring rats (cocaine-only group: $F_{1,10} = 6.79$, $p = 0.03$; session × group: $F_{1,10} = 4.30$, $p = 0.07$; risk-preferring session: $F_{1,5} = 4.75$, $p = 0.08$; optimal $F_{1,10} = 1.96$, $p = 0.19$). However, responding was significantly lower on the inactive than active lever and may represent a general invigoration of behaviour caused by exposure to the drug cue in the risk-preferring animals, or even frustration in response to the lack of concomitant drug delivery. No change in responding on the inactive lever was seen in saline controls (session: $F_{1,14} = 5.77$, group: $F_{1,14} = 2.58$, $p = 0.13$; $p = 0.03$; session × group: $F_{1,14} = 0.001$, $p = 0.98$).

DISCUSSION

Here we show unequivocally, for the first time, that subjects making risky, maladaptive decisions at baseline are differentially and adversely affected by cocaine self-administration in a manner concordant with a pro-addictive phenotype. Animals identified as risk-preferring on the rGT made more responses on the drug-paired lever as compared with optimal decision-makers, and their decision-making became more biased towards the maladaptive, risky options during the diurnal periods, in which cocaine was self-administered. In contrast, the

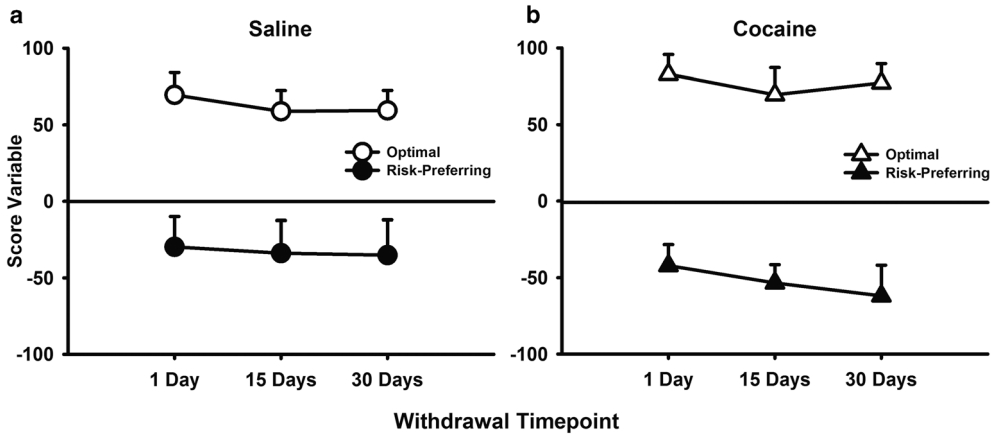

Figure 4 Decision-making as measured by the score variable during the withdrawal period. (a) As expected, saline animals' decision-making remained unchanged. (b) In keeping with the human literature, cocaine risk-preferring animals' score remained stable during this time and did not recover to pre-drug exposure levels. Data are presented as mean ± standard error of the mean

Table 2 rGT performance from the beginning, middle, and end of the 30 day withdrawal period.

Behavioural measure	*Withdrawal timepoint*	*Saline optimal*	*Saline risk-preferring*	*Cocaine optimal*	*Cocaine risk-preferring*
Premature responding	1 day	10.93 ± 3.78	17.89 ± 7.84	3.42 ± 0.62	**9.21 ± 3.66***
	15 days	18.01 ± 4.93	11.96 ± 5.36	4.22 ± 2.23	**19.85 ± 6.52***
	30 days	15.48 ± 3.34	13.24 ± 6.42	5.98 ± 2.56	**11.66 ± 2.74***
Omitted responses	1 day	4.00 ± 2.20	0.75 ± 0.49	9.16 ± 3.78	1.33 ± 0.84
	15 days	3.75 ± 2.20	0.87 ± 0.51	5.50 ± 1.33	0.50 ± 0.50
	30 days	0.86 ± 0.70	0.37 ± 0.70	1.67 ± 0.61	1.67 ± 0.95
Trials completed	1 day	112.38 ± 11.23	74.67 ± 7.60	**104.83 ± 13.50***	60.02 ± 9.55
	15 days	111.39 ± 10.00	83.02 ± 8.66	**126.83 ± 10.54***	56.37 ± 5.95
	30 days	112.16 ± 8.43	73.92 ± 9.56	**128.83 ± 12.55***	61.85 ± 8.00
Choice latency	1 day	1.38 ± 0.25	0.97 ± 0.23	1.93 ± 0.31	0.92 ± 0.26
	15 days	1.09 ± 0.27	1.14 ± 0.29	1.99 ± 0.27	0.72 ± 0.23
	30 days	0.93 ± 0.17	1.70 ± 0.29	1.70 ± 0.29	0.78 ± 0.15
Collect latency	1 day	0.97 ± 0.13	0.53 ± 0.08	1.12 ± 0.17	0.61 ± 0.10
	15 days	0.87 ± 0.13	0.62 ± 0.08	1.19 ± 0.14	0.51 ± 0.12
	30 days	0.86 ± 0.09	0.50 ± 0.05	1.09 ± 0.09	0.54 ± 0.08

Values presented are averages ± standard error of the mean. Values in bold denoted with an asterisk are deemed significant with a p-value < 0.05.

choice pattern of optimal rats remained consistent and advantageous, despite ingesting comparable amounts of cocaine. Risk-preferring rats also exhibited greater IOC, and the degree to which cocaine self-administration enhanced risky choice significantly correlated with this measure of cue-induced drug-seeking. Hence, risky decision-making may reflect a particularly important marker of addiction vulnerability.

As expected (Barrus *et al.* 2015), risk-preferring rats were also quicker to make decisions and made more premature responses at baseline. High levels of such motor impulsivity in rats is also associated with a behavioural pattern representative of addiction as opposed to simple drug-taking (Belin *et al.* 2008; Economidou *et al.* 2009). In the current study, premature responding did spike mid-way through the withdrawal from cocaine in risk-preferring rats, somewhat similar to a previous report (Winstanley *et al.* 2009). However, unlike the observed elevations in risky choice, this form of impulsivity was not exacerbated either during cocaine self-administration, or throughout withdrawal. Our results, although in general concordance with the view that high motor impulsivity reflects aspects of addiction vulnerability, therefore suggest that the exacerbation of poor decision-making in risk-preferring individuals uniquely reflects behavioural changes central to the addicted state, above and beyond the role played by behavioural disinhibition

Given that the rGT and self-administration sessions were run in completely distinct chambers using different manipulanda, it is unlikely that contextual conditioning caused by cocaine delivery could have contributed to the impairments in decision-making, particularly as these deficits were observed selectively in risk-preferring rats. High levels of impulsive choice as measured by greater-than-average preference for smaller-sooner than larger-later reward on delay-discounting tasks have also been identified as both a cause and consequence of cocaine self-administration (Mendez *et al.* 2010; Mitchell *et al.* 2014b; Perry *et al.* 2005). However, unlike in the current study, highly impulsive rats are not uniquely affected by cocaine—this form of impulsivity either increases across the cohorts tested, regardless of baseline choice patterns, or is not further exacerbated in highly impulsive rats. A similar pattern is observed in a probabilistic reward/punishment paradigm, with risky choice increasing universally in all rats after cocaine self-administration (Mitchell *et al.* 2014a). Furthermore, recent studies have dissociated changes in impulsive choice from cue-induced relapse, both at a phenomenological and pharmacological level; drugs that can reduce or enhance cue or context-induced reinstatement do not concurrently impact impulsive decision-making, and vice-versa (Broos *et al.* 2012; Broos *et al.* 2015). Risky decision-making, as assessed by the rGT, is again somewhat unique in that adverse consequences of cocaine intake are selectively observed in risk-preferring rats, and further impairments in choice are concordant with the manifestation of relapse vulnerability as measured by IOC. Such a conclusion is consistent with observations that substance abusers' risky decision-making on the IGT was maintained through withdrawal (Wang *et al.* 2013) and was a stronger predictor of relapse than impulsive choice (De Wilde *et al.* 2013). In terms of building the validity of this putative cognitive marker of addiction vulnerability, it will be important to evaluate the impact of long-access cocaine self-administration sessions, in which drug intake escalates and 'bingeing' can occur, thereby more closely modelling the pattern of drug intake seen in SUD.

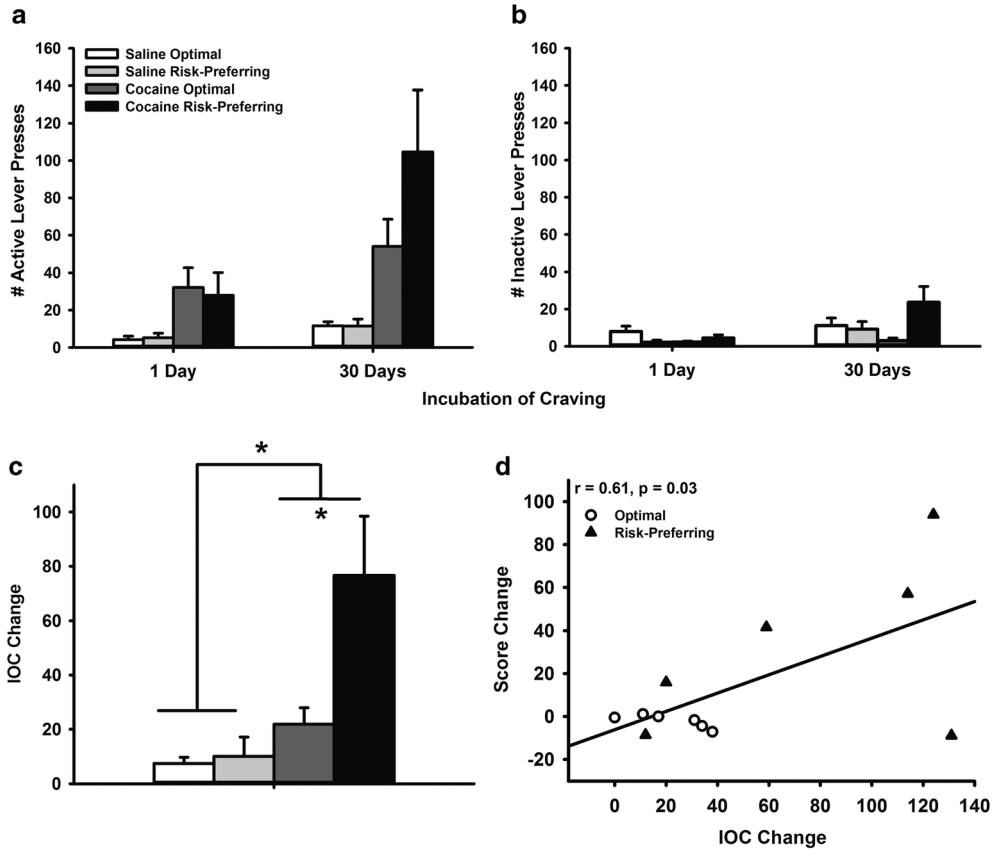

Figure 5 Number of responses on active and inactive levers during the incubation of craving (IOC) test sessions, comparing cue-induced drug-seeking behaviour at the start and end of the 30 day withdrawal period. (a) Compared with saline animals, cocaine-exposed rats pressed the active lever significantly at both time points. Within the cocaine group, although optimal decision-makers increased responding on the active lever after 30 days of withdrawal, this IOC effects was significantly greater in risk-preferring rats, indicative of greater relapse vulnerability in these animals. (B) While inactive lever responding was unchanged in the saline groups and cocaine optimal animals, cocaine risk-preferring rats did increase responding on the inactive lever during the IOC paradigm, potentially the result of general invigoration of behaviour caused by exposure to the drug cue. However, inactive lever presses were much lower than responses on the previously drug-paired lever. Data are presented as mean ± standard error of the mean. (c) While absolute scores did not demonstrate significant group differences between cocaine animals, the difference score (responses on day 30, responses on day 1 of withdrawal) reflected a significant increase in responding for cocaine-associated cues in cocaine risk-preferring rats. Data are presented as mean ± standard error of the mean. (d) The degree of IOC observed was significantly correlated with the magnitude of the decrease in score, indicating a relationship between the cocaine-induced deterioration in decision making and relapse vulnerability. Data presented are those of individual rats within the cocaine optimal and risk-preferring subgroups. Aterisk denotes $p < 0.05$

One factor that may have contributed to the comparatively robust nature of our findings is that unlike many other behavioural studies in which baseline differences are exploited (e.g. (Besson *et al.* 2010; Mitchell *et al.* 2014a; Perry, Nelson, & Carroll 2008; Robinson *et al.* 2009), we did not have to resort to arbitrary markers such as a median split or interquartile analysis to assign rats as either risk-preferring or optimal decision makers; defining risk preference as a negative score on the rGT is both objective and completely independent of the average behavioural output of a current or historical cohort. While this is a desirable feature of the current methodology, it inevitably requires the screening of large numbers of rats to obtain a sufficient sample size of risk-preferring animals. However, the proportion of individuals showing this behavioural pattern (22 percent) is comparable to the prevalence of addiction seen in humans, estimated as ~18 percent, further increasing the face validity of this model.

It is also worth emphasizing that as any rat with a negative score was designated risk-preferring, there was some variation in the degree of risk preference observed. It is also clear that the magnitude of the change in choice behaviour that resulted from cocaine self-administration varied, and this variation tracked that observed in the assessment of IOC. Why some rats are more affected than others is currently unknown, but may indicate the presence of factors capable of promoting either resilience or vulnerability to the deleterious effects of cocaine self-administration. Such a possibility should be considered

in future work aiming to elucidate the neurobiological basis underlying the behavioural impact of cocaine in risk-preferring rats. More specifically, such studies should be designed with sufficient power to detect variation in biochemical markers *within* this risk-preferring cohort, as well as between risk-preferring and optimally choosing animals, that can then be mapped to the individual differences in the behavioural phenotype.

Risk-preferring rats responded more times on the active lever than optimal rats, yet received statistically indistinguishable, albeit visibly higher, numbers of infusions. Although this null effect could simply reflect a lack of power, it may also offer insight into the mechanism underlying the higher drug-responsivity observed in risk-preferring rats. Theoretically, this behavioural effect represents a qualitatively similar response to that observed in the optimal group, yet of a larger magnitude, potentially indicative of a sensitized response to cocaine. As to the origins of such sensitization, recent data indicate that repeated exposure to conditioned stimuli associated with probabilistic reward delivery, or responding for probabilistic as opposed to guaranteed rewards, can enhance locomotor sensitization to amphetamine (Singer, Scott-Railton, & Vezina 2012; Zack *et al.* 2014). This raises the intriguing possibility that repeatedly engaging in risky decision-making actually contributes to a sensitized response to psychostimulants (Zack & Poulos 2009), rather than there being an innate difference between animals that go on to be risk-preferring versus optimal decision-makers. Such a hypothesis remains open to empirical verification, but could have implications for why conditions hallmarked by persistent, elevated risky choice, such as gambling and bipolar disorders, are highly comorbid with SUD.

Exacerbation of an already maladaptive choice strategy may reflect reduced cognitive flexibility or perseveration. Numerous studies have reported deficits in reversal learning and other indications of cognitive rigidity following chronic cocaine, largely attributed to a relatively insensitive and underactive prefrontal (PFC) network, mediated at least in part by deficient signalling of current cue-outcome associations in the basolateral amygdala (BLA) (Cervantes, Laughlin, & Jentsch 2013; Lucantonio *et al.* 2012; Stalnaker *et al.* 2007a; Stalnaker *et al.* 2007b; Stalnaker *et al.* 2009). Lesioning the BLA in optimal decision-makers increases choice of P3 and P4, whereas disconnection of the BLA from the orbitofrontal cortex (OFC) impairs task acquisition (Zeeb & Winstanley 2011, 2013). BLA lesions also attenuate the impact of losses on subsequent decisions in a rodent loss-chasing task, positing a specific role of the BLA in influencing decision-making through the representation of aversive consequences (Tremblay *et al.* 2014). Similarly, repeated administration of the psychostimulant amphetamine reduces the impact that conditioned aversive stimuli exert on instrumental behaviour (Tse, Cantor, & Floresco 2011), potentially because of impairments in the ability of BLA neurons to inhibit firing in the PFC via activation of local interneurons (Tse *et al.* 2011). One hypothesis, therefore, is that impairments in BLA-PFC/OFC signalling could be evident in risk-preferring rats, and exacerbated by cocaine self-administration, resulting in persistent selection of the risky options despite the ensuing negative consequences.

'Silent synapses', in which *N*-methyl-*D*-aspartate receptor expression at the synaptic membrane is upregulated in the absence of robust increases in AMPA receptors, have been detected within projections between the ventromedial prefrontal (infralimbic) cortex and nucleus accumbens (NAC) core, as well as within the BLA-NAC shell pathway, following cocaine self-administration and critically contribute to the expression of IOC (Lee *et al.* 2013; Ma *et al.* 2014). Given that the BLA and medial PFC influence risky choice on the rGT (Paine *et al.* 2013; Zeeb & Winstanley 2011), such a signalling pathway may likewise contribute to the maintenance of cocaine-induced increases in risky decision-making on the rGT, and its relationship to IOC. However, future studies investigating these mechanisms are required to discern the involvement of these circuits.

Understanding the neurobiological basis mediating the relationship between maladaptive decision-making and addiction may offer much-needed insight into the aetiology and trajectory of SUD. Future studies can now capitalize on this demonstration of a novel and readily quantifiable cognitive endophenotype for SUD. The fact that addicts must choose to continually engage in the addiction for use disorders to persist has led some to view addicts as deserving of retribution rather than treatment (Boyarsky *et al.* 2002). This demonstration of a robust interaction between poor choice on a gambling-like task and enhanced drug-seeking in an animal model of cocaine addiction suggests that this relationship arises not simply from environmental or circumstantial factors, but instead from physiological alterations in brain function within risk-preferring individuals that are uniquely and adversely affected by drug intake.

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Authors Contribution

JMNF and CAW were responsible for the study concept and design. JMNF collected animal data and performed data analyses. JMNF drafted the manuscript and assisted with interpretation of results. CAW provided critical revision of the manuscript for important intellectual content and interpretation of results. All authors critically reviewed content and approved final version for publication.

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