Psychopharmacology (2011) 215:721–731
DOI 10.1007/s00213-011-2167-x

ORIGINAL INVESTIGATION

High impulsivity predicting vulnerability to cocaine addiction in rats: some relationship with novelty preference but not novelty reactivity, anxiety or stress

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Received: 23 July 2010 / Accepted: 5 January 2011 / Published online: 28 January 2011

Abstract
Rationale Impulsivity is a vulnerability marker for drug addiction in which other behavioural traits such as anxiety and novelty seeking ('sensation seeking') are also widely present. However, inter-relationships between impulsivity, novelty seeking and anxiety traits are poorly understood.
Objective The objective of this paper was to investigate the contribution of novelty seeking and anxiety traits to the expression of behavioural impulsivity in rats.
Methods Rats were screened on the five-choice serial reaction time task (5-CSRTT) for spontaneously high impulsivity (SHI) and low impulsivity (SLI) and subsequently tested for novelty reactivity and preference, assessed by open-field locomotor activity (OF), novelty place preference (NPP), and novel object recognition (OR). Anxiety was assessed on the elevated plus maze (EPM) both prior to and following the administration of the anxiolytic drug diazepam, and by blood corticosterone levels following forced novelty exposure. Finally, the effects of diazepam on impulsivity and visual attention were assessed in SHI and SLI rats.
Results SHI rats were significantly faster to enter an open arm on the EPM and exhibited preference for novelty in the OR and NPP tests, unlike SLI rats. However, there was no dimensional relationship between impulsivity and either novelty-seeking behaviour, anxiety levels, OF activity or novelty-induced changes in blood corticosterone levels. By contrast, diazepam (0.3–3 mg/kg), whilst not significantly increasing or decreasing impulsivity in SHI and SLI rats, did reduce the contrast in impulsivity between these two groups of animals.
Conclusions This investigation indicates that behavioural impulsivity in rats on the 5-CSRTT, which predicts

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vulnerability for cocaine addiction, is distinct from anxiety, novelty reactivity and novelty-induced stress responses, and thus has relevance for the aetiology of drug addiction.

Keywords Endophenotype · Five-choice serial reaction time task · Novelty seeking · Elevated plus maze · Object recognition · Novelty place preference · Diazepam

Introduction

Impulsivity is a multi-dimensional behavioural construct involving thoughtless or risky behaviour with a tendency towards spur-of-the-moment actions (Evenden 1999). While it is part of normal human behaviour, pathological impulsivity is often associated with a number of psychiatric disorders such as attention-deficit hyperactivity disorder and substance-use disorders (Verdejo-Garcia et al. 2008; Winstanley et al. 2006). Although recent conceptualizations have categorized impulsivity in terms of deficiencies in decision-making, inhibitory response control and in bridging delays to future rewards (Dalley et al. 2008; Pattij and Vanderschuren 2008; Robinson et al. 2009; Winstanley et al. 2006), little is known of the relationship between impulsivity and other behavioural traits such as anxiety and novelty-seeking, which are also strongly linked with drug addiction (Koob and Le Moal 2008; Wills et al. 1994; Woicik et al. 2009; Zuckerman and Neeb 1979).

Previously, Gray (1981) suggested that 'high reactivity to behavioural approach' is a characteristic closely associated with increased impulsivity whereas 'high reactivity to behavioural inhibition' is a trait closely related to anxiety. Gray further suggested that impulsivity may be influenced by an underlying state of anxiety and that anxiety, under certain conditions, involves inhibitory response control mechanisms that potentially overlap with those mediating impulsive behaviour. Such mechanisms may also extend to novelty seeking behavioural traits, which are generally inversely related to anxiety-like behaviours in rats (Dellu et al. 1996b; Kabbaj et al. 2000). However, the extent to which anxiety and novelty-related behavioural traits co-exist and potentially interact with impulsivity has never formally been investigated.

In this study, we investigated in rats potential underlying contributions of novelty seeking and anxiety to the expression of naturally occurring high impulsivity on the five-choice serial reaction time task (5-CSRTT). The 5-CSRTT provides an automated behavioural assessment of sustained visual attention and impulsivity in rats and is analogous to continuous performance tests in humans (Robbins 2002). Previous research has established that spontaneously high impulsive (SHI) rats exhibit a marked escalation of intravenous cocaine and nicotine self-administration (Dalley et al. 2007; Diergaarde et al. 2008) and a greater propensity to develop compulsive cocaine self-administration than spontaneously low impulsive (SLI) rats (Belin et al. 2008). As well as showing high levels of impulsivity on the 5-CSRTT—measured by the number of anticipatory responses made before the presentation of a visual stimulus—SHI rats also show increased delay-discounting impulsivity for small immediate rewards versus larger delayed rewards (Robinson et al. 2009).

In the present study, we assessed anxiety levels in SHI versus SLI rats on the elevated plus maze (EPM), which exploits the natural aversion of rodents to heights and unprotected spaces (File et al. 2004). Novelty reactivity was assessed by locomotor activity in an open field (OF), novelty place preference (NPP) and novel object recognition (OR; Forwood et al. 2007). We also assessed hypothalamic–pituitary axis (HPA) functioning by assaying blood corticosterone before and after the exposure of SHI and SLI rats to a novel and inescapable environment (i.e. forced novelty). According to Gray's hypothesis, we predicted SHI rats to show an increased response to novel stimuli due to (1) a heightened propensity to elicit approach behaviour and (2) a reduced tendency to suppress behaviour in situations normally expected to elicit anxiety (e.g. novelty). Based on the latter prediction, we also investigated whether the anxiolytic drug diazepam could selectively increase impulsivity in SLI rats on the 5-CSRTT, possibly by decreasing anxiety in this group.

Methods and materials

Animals

Subjects were male outbred Lister Hooded rats purchased from Charles River (Margate, UK). SHI and SLI rats used for the NPP and OR studies were selected from one cohort of rats (n=48). SHI and SLI rats used for the EPM study and OF activity were selected from a second cohort of rats (n=48). Rats used for the blood corticosterone study were selected from a third cohort of rats (n=48). Animals weighed 250–275 g at the beginning of the study and were food-deprived to approximately 85% of their free-feeding weights with food made available at the end of each day's training or testing. Rats were approximately 22 weeks of age at the start of behavioural testing. They were housed under temperature- and humidity-controlled conditions on a reversed 12-h light/dark cycle (lights off at 7:00 am). All experimental procedures conformed to the UK (1986) Animal (Scientific Procedures) Act (PPL 80/2234).

5-CSRTT apparatus and training

Eight automated operant 5-CSRTT chambers (25×25×25 cm; Med Associates, Sandown Scientific Ltd, UK) were

used for this study. Chambers were controlled by Whisker Server and FiveChoice client software (Cardinal and Aitken 2001) and are described in detail elsewhere (Dalley et al. 2007; Robbins 2002). Each daily training session consisted of 100 discrete trials with stable performance being achieved after about 40 sessions. Rats initiated a trial by nose-poking into the magazine. After an inter-trial interval (ITI) of 5 s, a light at the rear of one of the apertures was presented randomly in one of the five apertures for a duration of 0.5 s. Responses in this aperture within a limited illumination period (the limited hold period) were recorded as correct responses and were rewarded by the delivery of a food pellet into the magazine (Noyes 45 mg dustless reward pellets, Sandown Scientific Ltd, UK). Responses in a non-illuminated hole (an 'incorrect' response), a failure to respond within the 5 s limited hold period (an 'omission'), and responses in one of the apertures during the ITI (a 'premature' response) were also recorded and signalled by a time-out period where the house-light was switched off for 5 s. The stimulus duration was 30 s in the initial training sessions and was progressively reduced to the final duration used for testing (0.5 s), depending on the rats individual performance. Rats reached the criterion of stable pre-operative performance when they achieved ≥80% accuracy with fewer than 20% omissions. An average of 40 daily sessions, each consisting of approximately 100 trials and lasting 30 min was required to reach this criterion. Rats were screened for impulsivity following the acquisition of the task over a 3-week period. SHI and LHI rats were selected on the number of premature responses elicited during three long-ITI challenges, each spaced 1 week apart and consisting of a fixed long ITI of 7 s. SHI rats made on average ≥60 premature responses across the three challenge sessions whereas SLI rats made ≤40 premature responses. There was no significant difference in the acquisition of the 5-CSRTT between SHI and SLI rats.

In addition to premature responses, a number of other behavioural variables were recorded: the number of correct and incorrect responses/session; accuracy (% correct responses/correct responses+incorrect responses); the number of omissions; correct and incorrect latencies (latency in msec to nose-poke in the correct and incorrect holes, respectively, after the onset of the stimulus); magazine latency (latency in seconds to collect the food pellet from the magazine after a correct response).

Spontaneous locomotor activity

SHI and SLI rats (each group $n=8$) were assessed for ambulatory locomotor activity over three consecutive days. Rats were run in a commercial locomotor activity system (San Diego Instruments, US) with activity measured by the number of photo-beam breaks over a 2-h period each day. Rats were not habituated to the activity chambers prior to this test.

Effects of diazepam on 5-CSRTT performance

Following 10 days of baseline training, including one long-ITI session, rats were injected with diazepam (0.3, 1, 3 or 5 mg/kg; 2 ml/kg IP) or its vehicle (45% 2-hydroxyprophyl-beta-cyclodextrin) according to a randomised Latin square design. Each injection was given 30 min prior to the start of the task and rats were tested under a fixed ITI of 5 s and a stimulus-duration of 0.5 s over 100 trials. The drug challenge session was followed by a wash out day where the rats were maintained in their home cage (day 2). On day 3, a baseline session was given prior to further drug testing the next day.

Elevated plus maze

The EPM apparatus was constructed from black Perspex and consisted of a central platform surrounded by two open arms and two enclosed arms in the shape of a plus sign. The apparatus was elevated 60 cm above the floor in a large room with an ambient light intensity of 70 lx. Rats were kept in their home cage and habituated to the experimental room for 40 min before testing started. Prior to the start of each session, the maze was cleaned with water and dried. Rats were then placed on the central platform facing an open arm. The effects of diazepam on the EPM were assessed 3 months after SHI and SLI rats were initially exposed to the EPM. Rats were injected via the intra-peritoneal route with vehicle (as above) or diazepam (2 mg/kg), 30 min prior to behavioural testing.

Exploratory behaviour during the first 5 min on the EPM was analysed, as previously recommended (Montgomery 1955), which was aided by a ceiling-mounted camera and DVD recording system. The following parameters were analysed: the time spent in open and closed arms; the number of entries in open and closed arms and the latency to first enter the open arm.

Novelty place preference

The NPP apparatus consisted of two rectangular compartments, equally sized, and separated by a removable plastic wall. The two compartments had distinct visual and tactile cues. One had white walls and a smooth floor. The other had black walls and a grid floor. An opening centred at the front lower part of the compartment allowed the rat free access into the other compartment during testing but was closed during training sessions. Each rat was habituated to one of the two compartments in the NPP apparatus, for two consecutive days. The initial compartment was counter-

balanced for both groups of rats and each rat was allowed to explore its compartment for 30 min on each day. During the test session (day 3) the rat was allowed to move freely in the NPP box for 15 min with the door removed. The time spent in each compartment, the number of crossings between compartments and latencies to enter each compartment were recorded.

Novel object recognition

Object recognition was conducted in a Y-shaped apparatus made with high, homogeneous white walls (Forwood et al. 2007). The start arm contained a guillotine door 18 cm from the rear of the arm. This provided a start box area within which the rat could be confined at the start of the sample and choice phases of a given trial. The floor and walls of the apparatus were wiped down with a dry paper towel between rats but otherwise were not cleaned during the experiment.

Each trial consisted of two phases. In the first, the sample phase, two identical objects (A1 and A2) were placed in the Y-shaped apparatus at the end of each exploration arm. The time spent exploring the two objects was scored by an experimenter viewing the rat on a video screen. The sample phase ended when the rat had explored the identical objects for a total of 3 min. After a delay of 3 h where rats were maintained in their home-cage, subjects were re-placed in the start box of the apparatus and released into the exploration area for the second choice phase. The Y-shaped apparatus now contained an identical copy of the sample (familiar) object in one arm and a novel object (B) in the other. The exploration arms in which the choice objects were placed were counterbalanced between rats and across trials. The rat was allowed to explore the objects for 2 min. Exploration of an object was defined as directing the nose to the object at a distance of <1 cm and/or directly touching the object.

Corticosterone sampling and analysis

Blood corticosterone levels were measured in SHI and SLI rats (n=9 and n=8, respectively) following exposure to an inescapable novel enclosure. Testing was carried out under a reversed light/dark cycle as described above. A chronically indwelling catheter was implanted in the external jugular vein as described previously (Belin et al. 2008). After a 1-week recovery period, baseline blood samples were collected every alternate day at either 07:00–09:00 or 19:00–21:00 (i.e. during the first 2 h of the dark or light period, respectively). No two samples were collected within 24 h of each another. Stress-induced corticosterone levels were determined by collecting samples after rats had been exposed to a new inescapable environment for 30 min between 7 and 9 am. A self-administration box measuring 25×25×25 cm and housed within a sound-attenuating chamber (Med Associate, Sandown Scientific Ltd, UK) was used as the novel enclosure. Post-stress samples were collected 30 min after rats had been returned to their home cage (i.e. 2 h after the first collection). A small aliquot of blood (~500 μl) was collected into 1.5-ml heparin-primed Eppendorf tubes and centrifuged at 3,000×g for 20 min. Plasma was stored at −80°C prior to the analysis of corticosterone by radioimmunoassay (Carter et al. 2004). One SHI and two SLI rats were excluded from the analysis due to a failure in collecting blood from the catheter.

Our rationale for measuring the HPA response to novelty was based on prior evidence that novelty-induced plasma corticosterone levels are sufficient to distinguish novelty-seeking high responder (HR) rats from low responder (LR) rats (Dellu et al. 1996b). Since this response is protracted, lasting considerably beyond the termination of forced exposure to novelty (i.e. up to 90 min; Dellu et al. 1996b), we took blood samples 30 min after rats had been removed from the novel environment. Our results indicate that this stressor was sufficient to elevate plasma corticosterone levels to peak values similar to those reported for other stressors such as tail-pinch (Rouge-Pont et al. 1998) and restraint stress (Dellu et al. 1996a; Kabbaj et al. 2000).

Drugs

Diazepam (Sigma-Aldrich, Steinheim, Germany) was suspended in 45% 2-hydroxyprophyl-beta-cyclodextrin and isotonic saline (pH 7.4; Sigma-Aldrich, UK). The doses of diazepam employed in the present study were based on previous studies showing doses of 1–2 mg/kg to produce anxiolytic effects on the EPM without inducing undue sedation (File et al. 2004; McDermott and Kelly 2008).

Statistical analysis

Statistical analyses were carried out using SPSS (version 12.0) and repeated-measures analysis of variance (ANOVA) with impulsivity phenotype (SHI or SLI) as the between-subjects factor and drug dose as the within-subjects factor. Significant main effects were further analysed using pair-wise comparisons and a SIDAK correction. Group-wise comparisons for the EPM and NPP studies were computed using univariate ANOVA followed by independent t tests. Corticosterone data were analysed by ANOVA and a Newman Keul's post-hoc test. Regression tests were computed using Pearson's product-moment correlation coefficient (r). A significance level of p=0.05 was used throughout the study. Data are presented as means ± SEM.

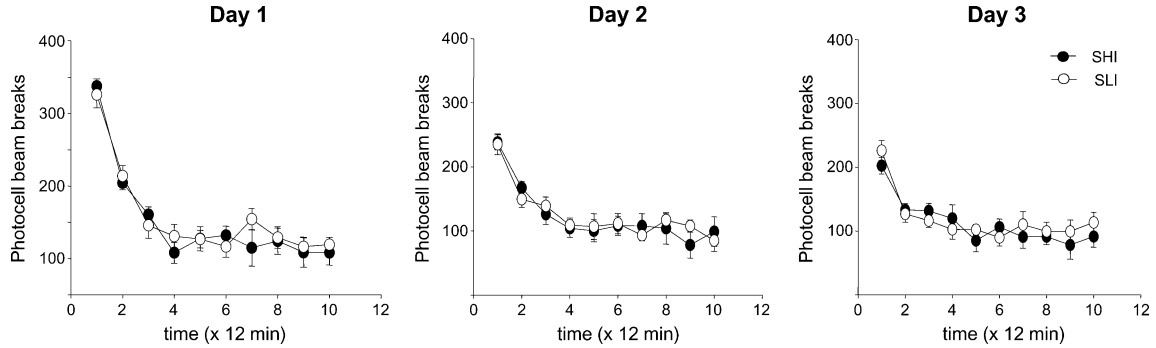

Fig. 1 Lack of effect of behavioural impulsivity in rats on open-field locomotor activity. Ambulatory activity was recorded on three consecutive days by photocell beam breaks collated over a 2-h period. Spontaneously high impulsive (SHI, $n=8$) and low impulsive (SLI, $n=8$) rats were selected on the 5-CSRTT prior to activity testing. Data are means±SEM

Results

Locomotor activity

SHI and SLI rats were initially assessed for open-field locomotor activity. The results shown in Fig. 1 demonstrate that novelty-induced locomotor activity was not significantly affected by inter-individual variation in impulsivity (day 1: $F_{1,14}=0.21$, $p=0.65$; day 2: $F_{1,14}=0.003$, $p=0.96$; day 3: $F_{1,14}=0.18$, $p=0.68$). A priori comparisons revealed no significant differences between SHI and SLI rats on day 1 ($F_{9,126}=0.93$, $p=0.50$), day 2 ($F_{9,126}=0.42$, $p=0.92$) or day 3 ($F_{9,126}=0.85$, $p=0.57$) indicating that impulsivity does not affect the habituation of locomotor activity in a novel environment.

Effects of diazepam on 5-CSRTT performance

Figure 2 shows the effects of diazepam on 5-CSRTT performance in SHI and SLI rats. Analysis of premature responding (Fig. 2a) revealed a significant group×dose interaction ($F_{4,52}=2.50$, $p=0.05$) and a significant main effect of group ($F_{1,13}=7.72$, $p=0.016$). Post hoc t tests revealed significant differences in premature responding between SHI and SLI rats following the administration of vehicle ($p=0.047$) and 5 mg/kg of diazepam ($p=0.025$) but not following the administration of the lower to intermediate doses of diazepam (0.3–3 mg/kg). Attentional accuracy was generally lower in SHI rats compared with SLI rats ($F_{1,13}=4.79$, $p=0.047$) but was not influenced by the diazepam treatment (Fig. 2b). Diazepam also had no significant effect

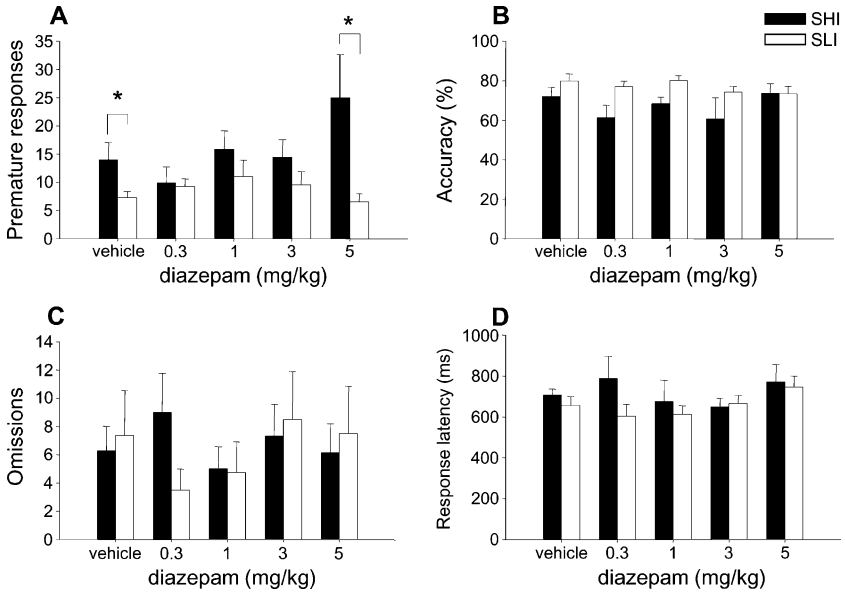

Fig. 2 Effects of systemically-administered diazepam on behavioural performance of SHI ($n=7$) and SLI ($n=8$) rats on the 5-CSRTT. Diazepam was administered by IP injection, 20 min prior to behavioural evaluation. Data are means±SEM. *$p<0.05$ (SHI vs. SLI rats)

Table 1 Summary of the effects of systemic diazepam administration on attentional performance of spontaneously high impulsive ('high', n=7) and low impulsive ('low', n=8) rats on the five-choice serial reaction time task

Behavioural variable	Impulsivity phenotype	Diazepam (mg/kg)				
		Vehicle	0.3	1.0	3.0	5.0
Correct responses	High	65.6±4.7	47.4±11.6	58.4±9.2	54.9±10.3	64.1±3.8
	Low	70.0±5.5	74.5±3.5	76.8±3.0	59.6±8.2	65.0±8.4
Incorrect responses	High	28.1±3.7	23.0±5.0	25.6±4.4	22.0±4.4	24.1±4.4
	Low	18.3±3.7	22.0±2.6	18.5±2.4	20.9±3.6	21.1±3.2
Perseverative responses	High	48.3±14.0	47.1±10.7	47.1±13.0	37.5±17.1	28.8±5.9
	Low	35.0±6.8	43.8±10.2	39.6±6.1	44.4±12.9	20.0±6.5
Incorrect response latency(s)	High	1.66±0.12	1.24±0.31	1.53±0.16	1.53±0.11	1.67±0.13
	Low	1.76±0.14	1.57±0.11	1.81±0.15	1.68±0.15	2.16±0.23
Magazine latency(s)	High	1.20±0.07	1.53±0.33	2.61±1.46	1.22±0.99	2.49±0.90
	Low	1.28±0.12	1.11±0.07	1.10±0.06	1.62±0.46	1.30±0.09

Data are means±SEM

on the number of omissions (Fig. 2c), latencies to respond correctly to the visual stimuli (Fig. 2d), or other behavioural variables on the 5-CSRTT (Table 1). These findings indicate that anxiolytic doses of diazepam reduce the contrast in impulsivity between SHI and SLI rats, an effect which is evidently lost following the administration of a higher dose (5 mg/kg).

Effect of diazepam on the elevated plus maze

Figure 3 and Table 2 summarise the effects of diazepam (2 mg/kg) on the EPM. Following diazepam administration both SHI and SLI rats significantly increased their time spent on the open arms compared with vehicle-treated rats (group effect $F_{1,19}$=15.9, p=0.0007, n=5–6; Fig. 3a). This anxiolytic effect was significant for both SHI (vehicle versus diazepam; p=0.031) and SLI (vehicle versus diazepam; p=0.009) rats. However, there was no significant difference in the percentage time spent on the open arm between SHI and SLI rats treated with vehicle or diazepam. Nevertheless, vehicle-treated SHI rats entered the open arm significantly more quickly than SLI rats (p=0.027), an effect that was diminished by diazepam treatment (Fig. 3b).

Novelty place preference

SHI rats explored the novel compartment of the NPP apparatus for a significantly longer period of time compared with the familiar compartment, a preference that was not observed in SLI rats (group×compartment interaction: $F_{1,24}$=6.53, p=0.017; post hoc t test p=0.031, Fig. 4). However, there was no significant difference between SHI and SLI rats in the total time spent in the novel compartment. SLI rats showed a trend increase in time spent in the familiar compartment compared with SHI rats (p=0.059).

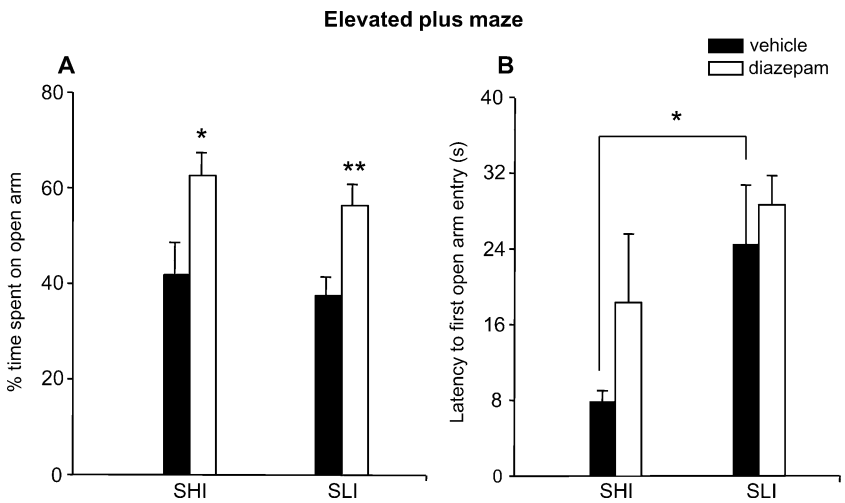

Fig. 3 Effect of diazepam (2 mg/kg) on the percentage of time of SHI (n=7) and SLI (n=8) rats spent on the open arms of the elevated plus maze (**a**) and latencies to first enter the open arms (**b**). Data are means±SEM. *p<0.05 (vehicle vs. diazepam or SHI vs. SLI), **p<0.01 (vehicle vs. diazepam)

Table 2 Effects in spontaneously high impulsive ('high', n=7) and low impulsive ('low', n=8) rats of pre-treatment with diazepam (2 mg/kg) or vehicle (2-hydroxyprophyl-beta-cyclodextrin) on the elevated plus maze

Behavioural variable	Impulsivity phenotype	Treatment	
		Vehicle	2 mg/kg
Open arm entries	High	6.2±0.9	6.7±1.1
	Low	5.1±0.5	6.2±0.8
Open arm time(s)	High	68.2±12.4	113.7±15.0
	Low	65.1±6.3	99.2±11.3
Closed arm entries	High	6.2±0.7	6.3±0.8
	Low	7.6±1.1	6.6±0.4
Closed arm time(s)	High	91.0±9.7	65.2±8.5
	Low	110.3±9.8	75.2±8.4
% closed arm time	High	30.3±3.2	21.7±2.8
	Low	36.8±3.3	25.1±2.8

Data are means±SEM

Novel object recognition

During the initial sampling phase, SHI rats spent significantly longer exploring the object than SLI rats (group effect: $F_{1,13}$= 12.092=0.004, Fig. 5). In addition, SHI rats explored the novel object for a significantly longer period of time than the familiar object during the choice phase (group×object interaction: $F_{1,26}$=5.135, p=0.032; post-hoc t test p= 0.041). This effect was evident despite the total exploration time being corrected for the latency to first encounter the object. There was no significant difference in the time spent exploring the novel and familiar objects in the SLI group, or between SHI and SLI rats during the choice phase.

Stress-induced changes in plasma corticosterone

We next compared HPA function in SHI and SLI rats by measuring plasma corticosterone levels at different time points of the circadian cycle, i.e., baseline 7:00 am and 7:00 pm, and in response to acute stress (time: $F_{3,48}$=22.99, p<0.001, Fig. 6). Exposure to inescapable novelty increased blood levels of corticosterone as compared to the control conditions, i.e., baseline am (p<0.05) and baseline pm (p<0.01). However, trait-like impulsivity had no significant effect on blood corticosterone levels either at baseline or following novelty stress (group: $F_{1,15}$=0.99, p= 0.34; group×session: $F_{3,45}$=1.16, p=0.33).

Regression analysis

Finally, we examined the relationship between impulsivity and the different behavioural measures of novelty seeking, anxiety and stress responsiveness. Impulsivity was not

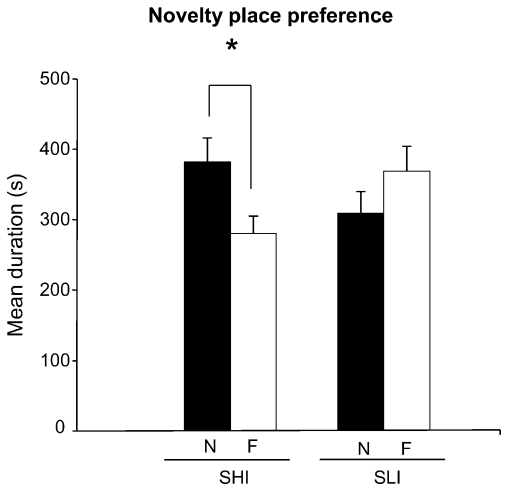

Fig. 4 Preference of SHI (n=7) and SLI (n=8) rats for the novel (N) and familiar (F) compartments of the NPP apparatus. SHI rats spent more time exploring the novel compartment compared with the familiar compartment, unlike SLI rats. Data are means±SEM. *p<0.05 (novel vs. familiar compartments)

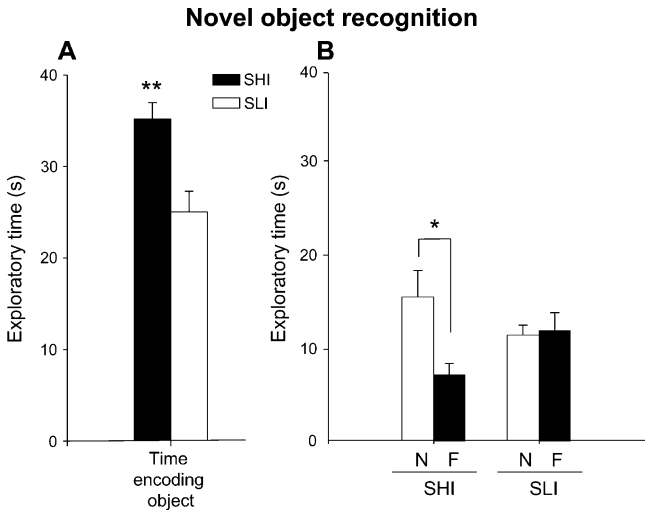

Fig. 5 Influence of behavioural impulsivity on novel object recognition in SHI (n=7) and SLI (n=8) rats. Shown are times spent sampling the objects in the first phase of the test (**a**) and the novel object in the second choice phase (**b**). SHI rats spent more time initially exploring the objects and more time in close proximity with the novel object compared with the familiar object in the choice phase. Data are means±SEM. **p<0.01 (SHI vs. SLI), *p<0.05 (novel N vs. familiar F)

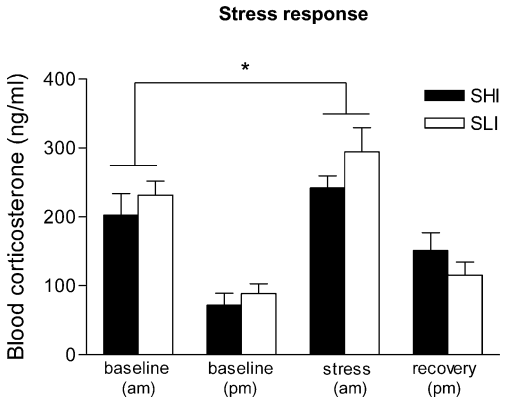

Fig. 6 Novelty-induced changes in plasma corticosterone levels in SHI (n=8) and SLI (n=8) rats. Novelty stress-induced effects on plasma corticosterone were compared with baseline samples collected during the first 2 h of the light and dark periods (am and pm, respectively). Data are mean plasma corticosterone levels (expressed as ng/ml) ± SEM

significantly related to the time spent in the novel compartment of the NPP apparatus (R^2=0.101, p=0.257), contact time with the novel object in the OR test (R^2= 0.182, p=0.113), time spent on the open arms of the EPM (R^2=0.021, p=0.65) or novelty-induced changes in blood corticosterone levels (R^2=0.04, p=0.237).

Discussion

The main findings of the present investigation indicate that high impulsive rats on the five-choice serial reaction time task are neither more anxious than low impulsive rats nor do they show differential behavioural and hormonal responses to mild novelty stress. The high impulsive phenotype is thus clearly distinct from the high responder phenotype described by Piazza and colleagues, which shows increased exploratory locomotion and a prolonged corticosterone response following exposure to the mild stress of a novel environment (Piazza et al. 1991). The results also indicate that novelty-seeking is not a significant dimensional component of behavioural impulsivity, again unlike HR rats (Dellu et al. 1996b). However, impulsive rats did show a general preference for novel as opposed to familiar objects and contexts compared with low impulsive rats and were generally faster to initiate exploratory behavior in novel settings. Since high impulsive rats are predisposed to relapse to cocaine seeking (Economidou et al. 2009) and show persistent responding for cocaine despite punishment (Belin et al. 2008), together with an increased propensity to escalate sucrose-seeking behaviour and intravenous cocaine and nicotine self-administration (Dalley et al. 2007; Diergaarde et al. 2008, 2009), these findings collectively indicate that impulsive rats may be more reactive to positive reinforcement than negative reinforcement and further highlight trait-like impulsivity as a core behavioural endophenotype underlying vulnerability for stimulant addiction.

Inter-individual differences in novelty-seeking or sensation-seeking behaviours are associated with a variety of psychiatric disorders including alcoholism and drug addiction (Wills et al. 1994; Woicik et al. 2009; Zuckerman 1990; Zuckerman and Neeb 1979). Diverse lines of evidence also indicate that impulsive behaviour is prevalent in drug abusing populations and is associated with a pre-disposition to drug use and addiction (Kirby and Petry 2004; Nigg et al. 2006; Verdejo-Garcia et al. 2008; Wong et al. 2006). Moreover, a recent study of human stimulant abusers and their non-drug using siblings has shown elevated ratings of impulsivity but not sensation-seeking in personality questionnaires administered to these biological siblings, suggesting that impulsivity but not sensation-seeking may be an endophenotype for stimulant addiction (Ersche et al. 2010). However, the role of anxiety disorders in the development of drug abuse and addiction is less clear, although these are common amongst adolescent and adult drug users and may play a major role in the persistence of drug use (Staiger et al. 2007). Based on Gray's (1981) personality taxonomy, which differentiates between impulsivity and anxiety in terms of behavioural activation and inhibition systems, we investigated whether SHI rats also show increased novelty seeking behaviour. Specifically, we investigated the hypothesis that impulsive rats would show greater behavioural activation to novel stimuli measured by increased approach behaviour and/or reduced behavioural suppression to the mild stress of a novel environment.

Our results clearly indicate that high impulsivity in rats is not associated with increased anxiety or a differential novelty-evoked hormonal stress response compared with low impulsive rats. Moreover, the anxiolytic drug diazepam did not selectively increase impulsivity in SLI rats, thereby discounting anxiety as a critical variable underlying the natural variation of behavioural impulsivity on the 5-CSRTT. However, the generality of this finding to other forms of impulsivity in rats such as delay-discounting impulsivity (Dalley et al. 2008; Winstanley et al. 2006) clearly warrants further investigation. Nevertheless, the present findings do show that these two behavioural traits can be dissociated in certain settings. Consistent with this view, Roman high-avoidance rats, which show *less* anxiety and reduced emotional reactivity to stressful stimuli compared with Roman low-avoidance rats (Escorihuela et al. 1999), are reported to be *more* impulsive on both the 5-CSRTT and the delay-discounting task (Moreno et al. 2010).

Nor could we demonstrate any obvious relationship between novelty seeking and impulsivity. Sensation/novelty seeking traits are generally modelled in rats by high locomotor reactivity in a novel inescapable environment (Blanchard et al. 2009; Dellu et al. 1996a; Piazza et al. 1989). They are also

studied by novelty preference paradigms (Bardo et al. 1996; Cain et al. 2004) with high novelty preferring (HNP) rats showing increased preference for novel as opposed to familiar environments. The distinction between HR and HNP phenotypes is important in this context since HR rats show a greater propensity to acquire drug self-administration (Piazza et al. 1989), whereas HNP rats, but not HR rats, show a greater vulnerability to develop compulsive cocaine self-administration that persists despite negative or adverse consequences (Belin et al. 2008, 2011). Thus, the HNP and SHI phenotypes apparently predict similar consequences for the switch from controlled to compulsive self-administration. However, it remains to be determined whether high novelty preference is associated with increased impulsiveness. Although our findings are not directly supportive of this association, further studies are warranted to investigate whether other forms of impulsive behaviour (e.g. delay-discounting impulsivity) are linked to novelty preference. In the present study, SHI rats, unlike their SLI counterparts, did show the expected preference for novel stimuli (Bardo et al. 1990), and were generally faster to initiate exploratory behaviour in a novel setting. However, in contrast to HR rats (Dellu et al. 1996b; Piazza et al. 1989), which are not impulsive on the 5-CSRTT (Belin et al. 2008), SHI rats were not hyperactive in a novel, inescapable environment indicating some relationship of high impulsivity with novelty preference but not novelty reactivity.

These data support the view that behavioural impulsivity on the 5-CSRTT is linked to an increased propensity for approach behaviour in novelty choice procedures by comparison to rats expressing the low impulsive phenotype. But this distinction does not extend to previously learned approach responses to classically conditioned appetitive stimuli for which neither SHI nor SLI rats have been shown to be impaired (Robinson et al. 2009). Taken together, therefore, these results indicate that the greater alacrity of SHI rats to approach the unprotected and open arms of the EPM likely reflects a generalized deficit in behavioural inhibition rather than an underlying influence of novelty seeking per se.

As a multidimensional behavioural construct, impulsivity arises though an inability to adequately suppress or inhibit inappropriate behaviour and by a general intolerance of delayed gratification (Dalley et al. 2008; Evenden 1999; Pattij and Vanderschuren 2008; Winstanley et al. 2006), another trait that in addition to novelty (sensation) seeking is also widely present in abstinent drug addicts (Kirby and Petry 2004; Wills et al. 1994; Woicik et al. 2009; Zuckerman and Neeb 1979). A key issue, therefore, is whether a particular form of impulsivity more strongly associates with novelty-seeking than other forms of impulsivity. In a recent study, rats selectively bred for high reactivity to a novel environment (i.e. novelty seeking HR rats) showed increased approach to both food- and cocaine-predictive cues compared with low responder rats, and were *less* impulsive on a delay discounting task (Flagel et al. 2010). Notably, however, these HR rats were *more* impulsive on a differential reinforcement of low rates of responding task—a measure of 'action' impulsivity (Pattij and Vanderschuren 2008; Winstanley et al. 2006). This distinction is potentially important because SHI rats not only show high impulsivity on the 5-CSRTT but are *also* impulsive on a delay-of-reward discounting task (Robinson et al. 2009). Thus, unlike HR rats, SHI rats show an intolerance of delayed rewards, a characteristic that also strongly predicts the escalation of stimulant self-administration (Anker et al. 2009; Dalley et al. 2007; Perry et al. 2005) and the later emergence of compulsive cocaine taking (Belin et al. 2008). By contrast, novelty seeking HR rats exhibit an increased propensity to *acquire* stimulant self-administration (Hooks et al. 1991; Mantsch et al. 2001; Piazza et al. 1989) but do not develop *compulsive* cocaine seeking behaviour defined by the persistence of drug-taking responses despite negative or adverse outcomes such as a mild foot shock (Belin et al. 2008; Deroche-Gamonet et al. 2004). These findings indicate multiple predisposing determinants of the propensity to self-administer cocaine, but emphasise that impulsivity is the main drive to compulsive drug seeking, or addictive behaviour.

The present results also have important implications for the neurobiology of behavioural endophenotypes underlying the risk for drug addiction. Novelty-seeking HR rats show greater elevations in nucleus accumbens dopamine release than LR rats (Flagel et al. 2010; Hooks et al. 1992) in addition to a prolonged corticosterone response to mild novelty stress (Dellu et al. 1996b). Compared with LR rats, HR rats also show a lower level of dopamine D2 mRNA in the nucleus accumbens (Hooks et al. 1994) and a higher proportion of dopamine D2 receptors in the functionally active state in the dorsal striatum (Flagel et al. 2010). By contrast, dopamine D2/3 receptors are reduced in number in the ventral, but not dorsal, striatum of SHI rats, despite dopamine release in the nucleus accumbens either being no different between SHI and SLI rats (Dalley et al. 2007) or significantly reduced (Diergaarde et al. 2008). The present results suggest that impulsivity and novelty-seeking are not only behaviourally distinct, but they may also be associated with different adaptations within the mesolimbic dopamine system.

An unexpected finding in the present study was that diazepam, at low to moderate doses, reduced significantly the contrast in impulsivity between SHI and SLI rats, but at the highest dose tested (5 mg/kg), this effect diminished as impulsive responding was again differentially expressed between SHI and SHI rats. Previous studies in normal healthy volunteers indicate that diazepam produces behavioural

disinhibition by reducing the threshold for a response without affecting delay-discounting or risk-taking (Acheson et al. 2006; Deakin et al. 2004). Consistent with these findings, diazepam significantly increased premature responding on the 5-CSRTT in mice (Oliver et al. 2009). Our failure to replicate this finding in rats presumably relates to species differences and the selection of extreme impulsivity phenotypes in the present study. Thus, ceiling effects may have precluded any further increase in impulsivity in SHI rats. However, this does not explain why diazepam failed to increase impulsivity in SLI rats. Clearly, further studies are needed to reconcile this apparent discrepancy.

The neural mechanisms mediating the normalising action of diazepam on 5-CSRTT impulsivity are unknown but speculatively may involve GABA dysfunction in SHI rats. In support of this assertion, we have recently discovered that glutamic acid decarboxylase, which catalyses the decarboxylation of glutamate to GABA, is significantly reduced in the nucleus accumbens of SHI rats (D Caprioli, E Merlo, S Sawiak, M Spoelder, DE Theobald, BJ Everitt, TW Robbins and JW Dalley, unpublished observations). We thus hypothesise that GABA neurotransmission in the nucleus accumbens of SHI rats may be restored by the systemic administration of diazepam leading, in turn, to a decrease in behavioural impulsivity relative to low impulsive rats. Indeed, it is relevant to note that GABA-ergic mechanisms have recently been linked to impulsivity rather than novelty-seeking in mice (Lafenetre et al. 2009).

In conclusion, the main findings of this study indicate that behavioural impulsivity in rats on the 5-CSRTT is not related to anxiety, novelty-induced stress responses or novelty reactivity but may have some relationship with novelty preference. Thus, impulsivity appears to be a reliable endophenotype for stimulant abuse in rodents, consistent with recent findings in human drug abusers (Ersche et al. 2010).

Acknowledgements This study was funded by the Wellcome Trust (089589/z/09/z) and MRC (G0401068, G0701500) within the Behavioural and Clinical Neuroscience Institute at Cambridge University and by the European Community's Sixth Framework Programme ('Imagen' LSHM-CT-2007-037286). This paper reflects only the authors' views and the Community is not liable for any use that may be made of the information contained therein. M.M was supported by a Jose Castillejo Fellowship and a grant from the Ministerio de Ciencia e Innovaión of Spain (PSI2009-08626).

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