Supporting Online Material for

High Impulsivity Predicts the Switch to Compulsive Cocaine-Taking

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Supporting online Material

Drugs.

Cocaine hydrochloride was dissolved in sterile 0.9% saline. The dose of cocaine was calculated as the salt.

Animals.

Forty adult male outbred Lister Hooded rats weighing ~280 g at the beginning of the experiment were housed 4 per cage under a reversed 12 h light/dark cycle (lights on at 7:00 P.M.). One week before the start of testing, rats were placed on a restricted diet of 50 g/d lab chow per cage, sufficient to maintain growth throughout the experiment. Water was available ad libitum, and food was given within every 1h after daily testing. After surgery the animals were housed individually and fed 20g/day. They were habituated to these new conditions for 15 days before the beginning of any new testing. Experiments were performed between 8:00 A.M. and 8:00 P.M., 5–7 d/week. Experiments were conducted in accordance with the United Kingdom 1986 Animals (Scientific Procedures) Act (Project License PPL 80/1767).

5-CSRTT

The apparatus has been described elsewhere (*1*). It consisted of eight 5-choice chambers (25x25x25 cm) each housed within a ventilated wooden sound-attenuating box. The rear wall of the chamber was curved with nine contiguous 2.5x2.5 cm apertures, 4-cm deep and set 2 cm above a wire grid floor. A metal insert blocked every alternate hole (i.e., holes 1, 3, 5, 7, and 9 were left open). A photocell beam was located at the entrance of each aperture to detect nose-poke responses. A 3W stimulus light was located at the rear of each the five apertures. On the front of the chamber, a magazine connected to a food dispenser allowed the automatic delivery of 45-mg food pellets. Subjects gained access to the food magazine by pushing a hinged Perspex panel monitored by a micro-switch. The apparatus was controlled by software written in Arachnid. Subjects were trained on the 5-CSRTT over approximately 40 days, with 5–7 daily 30-min sessions each week. In the initial training stage, 5–10 pellets were placed in the magazine and in each of the open apertures to encourage the subjects to enter these locations. In subsequent sessions, subjects were trained to detect the presence of a brief light stimulus (0.5s in duration) presented at the rear of each aperture. Training was facilitated in the early stages by lengthening the duration of both the visual stimulus and the limited hold period (see *2 for further details*). The limited-hold period marks the interval from the onset of the stimulus to the time available for the subject to respond. A failure to respond within the limited-hold period was deemed an 'omission' and was punished by the house light being extinguished for 5 s and no delivery of food reward. Each test session began with the illumination of the chamber by the house light and the delivery of a food pellet in the magazine. The collection of this pellet by pushing open the magazine panel started the first trial. After a fixed intertrial interval (ITI) of 5s, a light at the rear of one of the response apertures was briefly illuminated. Responses in this aperture within a limited hold period (5s) were reinforced by the delivery of a food pellet in the magazine. Responses in a non-illuminated hole were recorded as incorrect responses and were punished by a 5-s time-out period. During this time, the house light was extinguished and no food pellet was delivered. The 'accuracy' of target detection was computed as the percentage of correct responses to the total number of correct and incorrect responses. Additional responses in any aperture prior to food collection ('perseverative responses') were recorded but not punished. Responses made in any aperture before the onset of the target stimulus (i.e., ITI responses) were deemed 'premature' and were punished by a 5s time-out period. Subjects were considered to have acquired the basic version of the task (i.e., stimulus duration of 0.5s and an ITI of 5s) when their accuracy

was greater than 75% and omissions were fewer than 20%. After a week of stable responding under this schedule, subjects were submitted to 3 long 60min challenge sessions, during which the inter trial interval (ITI) was increased to 7s, separated by two baseline days.

Subjects were ranked upon their mean score during the last two long ITI sessions and the lower and upper quartiles of the population were selected as low and high impulsive rats, respectively (n=10 in each group). Such Inter-individual differences in premature responding have been reported using different strains of outbred rats and different housing conditions (*3*) and therefore are not restricted to the Lister-hooded the strain used here.

Surgery

Subjects were anaesthetized with ketamine (Ketalar, 90 mg/ kg i.p.) and xylazine (Rompun, 6.7 mg/kg, i.p.) and implanted with chronic i.v. jugular catheters. The catheter was inserted in the right jugular vein and exited dorsally between the scapulae. Subjects were given 7 days to recover from surgery before starting behavioral testings. During this 7-days period, animals received daily antibiotic treatment (baytril, subcutaneously) and catheters were flushed with 0.1–0.2 ml heparanized saline (50 U/ml 0.9% sterile saline).

Spontaneous locomotor activity

The analysis of locomotor reactivity to a novel environment was carried out during the first two hours of the dark phase. Subjects were placed for 120 min in activity chambers (29.5 x 32.5 x 23.5 cm) equipped with two photocell beams and connected to a Windows compatible PC-software. Horizontal locomotor activity was measured as photocell beams breaks. Animals were placed in the box for a first period of 30 min, at the end of which blood samples were collected to assess stress-induced corticoserone levels. After this manipulation which did not exceed 5 min, animals were placed again in the chambers for the remaining 90 min. The 10 most reactive subjects over the 110 min of the recorded session (or the upper quartile of the population) were selected as high responders while the 10 less reactive subjects (or the lower quartile) were selected as low responders. This strategy enables the discrimination of homogeneous HR and LR populations (*4-7*). Such Inter-individual differences in locomotor reactivity to a novel inescapable environment have been reported using different strains of outbred rats and different housing conditions (*6, 8-10*) and therefore are not restricted to the Lister-hooded the strain used here.

Self-administration

Rats were tested in operant chambers (29.5 x 32.5 x 23.5 cm) equipped with two 4-cm-wide retractable levers. The two levers were 12 cm apart and 8 cm from the grid floor. Above each lever was a cue light (2.5 W, 24 V), and a white house light (2.5 W, 24 V) was located on the top of the opposite wall. The floor of the chamber was covered with a metal grid with bars separated by 1 cm and connected to a shock generator and scrambler, which delivered 0.3-mA foot shocks for 1s. The grid was located 8 cm above an empty tray. The testing chamber was placed within a sound- and light-attenuating box, equipped with a ventilation fan that also screened external noise. SILASTIC tubing shielded with a metal spring extended from each animal's intravenous catheter to a liquid swivel mounted on an arm fixed outside of the operant chamber. Tygon tubing extended from the swivel to a Razel infusion pump located, adjacent to the external chamber. The operant chambers were controlled by software written by Rudolf Cardinal and Mike Aitken using the Whisker control system.

During the first 7 days of training, animals were placed 2 hours a day in the operant boxes where they were allowed to self-administer increasing doses of cocaine (0, 31.25, 62.5, 125, 250, 375 and 500 µg/injection) under a FR1 schedule of reinforcement, such that every lever press on the active lever delivered a 5s infusion of 100µl of either vehicle or cocaine solutions. The schedule was then switched to the daily session schedule adapted from Deroche-

Gamonet et al. (*11*). Active and inactive levers were counterbalanced between left and right sides for individual animals. “Priming” injections of cocaine were never given. Daily SA sessions were composed of three drug access periods (40 min each) separated by 15 min drug free periods. "Drug" periods were signaled by the cue light on the panel opposite to the levers, while the "no-drug" periods were signaled by no illumination of box. During the "no-drug" periods (data presented Fig. S1 and Fig. S3 were acquired between sessions 35 and 39 and represent the total number of responses on the active lever during the two ‘no-drug’ periods), lever presses were without scheduled consequences. During the "drug" periods, 5 active lever presses (FR5) turned on the white cue light located above it and then, 1 sec later, switched on the infusion pump. The cue light remained on for a total of 4 sec. Inactive lever presses had no scheduled consequences. The self-infusion volume was 100 µL (5 sec infusion) and contained 0.25 mg of cocaine. Each infusion was followed by a 40 sec time-out period. Criterion for acquisition of cocaine SA was defined by a stable number of self-infusions over at least three consecutive SA sessions (± 10%). During the progressive-ratio schedule of reinforcement carried out on SA day 32, cocaine availability was signaled by the discriminative stimulus on the panel opposite to the levers. The ratio of responses per infusion was increased after each infusion according to the following progression (10, 20, 30, 45, 65, 85, 115, 145, 185,225, 275, 325, 385, 445, 515, 585, 665, 745, 835, 925, 1025, 1125, 1235, 1345, 1465, 1585). The maximal number of responses that a rat performed to obtain one infusion (the last ratio completed) is referred to as the breaking point. The session ceased after either 5 hr or when a period of 1 hr elapsed since the previously earned infusion. The punishment session, carried out on SA day 40 lasted 40 min. Cocaine availability was signaled by the discriminative stimulus. During these sessions a first active lever press led to the illumination/activation of the shock-paired cue light, located 25 cm from the grid, between the two levers. When an FR4 was completed within 5 min, rats received an electric shock (0.4 mA, 2s), if not the sequence was reinitialized. When FR5 was reached within a min, rats received both an electric shock (0.8 mA, 2s) and cocaine infusion associated with its Cs. Then shock-paired stimulus turned off. The schedule could reinitiate at the end of the time-out period, i.e. 40s after the infusion. If, within a minute, animals did not complete the fifth lever press of the FR5 sequence, leading to shock- cocaine presentations, the shock-associated stimulus turned off and the sequence was reinitiated.

Progressive-ratio schedule of reinforcement and punishment sessions were also carried out at early stages of drug self-administration (SA d 15 and d18, respectively) (*11*). At this time point, no effect of group was observed after between-subject comparisons for resistance to punishment, for either 0 criterion vs 3 criteria rats, LI vs HI rats or LR vs HR rats as between subject factors, confirming that all the experimental populations similarly suppressed their responses when punished by electric foot-shocks at that time point. These comparisons for breaking points revealed that only 3 criteria rats differed from 0 criterion rats ($p<0.05$). Because in real world cocaine is not necessary available any single day, the subjects were submitted to a sub-chronic regimen of cocaine exposure, i.e. they had access to cocaine SA 4-7 days a week with randomly interspaced sessions.

Data and statistical analyses.

Establishment of Addiction-like criteria and subpopulations of rats.

Addiction-like behaviour has been previously established in singly-housed outbred Sprague-Dawley rats nose-poking for cocaine (*11*). Here we applied the same selection criteria to outbred Lister-hooded rats lever-pressing for cocaine infusions. For this, animals were ranked for each addiction-like behavior independently. Active lever presses measured during the first ‘no-drug’ period of sessions 28-31, break-point measured under a progressive ratio schedule of reinforcement on day 32, and percentage of infusions measured during the punishment session on day 40 relative to baseline (measured during the first ‘drug-periods’ of days 37-39) were used as

behavioural measures. If a rat's score was included in the 40% highest percentile of the distribution, this rat was considered positive for that addiction-like criterion and was given an arbitrary criterion score of 1. Then the arbitrary criteria scores were added so that four different groups were identified depending on the number of positive criteria they met (from 0 to 3) (see Table S2).

Addiction score

The addiction score (AS) is the algebraic sum of the normalized values of each of the addiction-like behaviours: $AS = \sum [(Vi - mean_{pop}) / SD_{pop}]$ [i.e. sum of individual scores (Vi) for each behavioral measure which have had the mean of the distribution subtracted ($mean_{dis}$) and which have then been divided by the standard deviation of the distribution (SD_{dis}) to yield scores which have a mean of 0 and a standard deviation of 1]. The addiction score was calculated from break point measured on session 32, percentage of self-administered cocaine infusions obtained during the punishment session on day 40 relative to baseline (first drug-periods of days 37-39) and active lever presses measured during the first 'no-drug' period of sessions 35-39). Two independent measures of the inability to refrain from cocaine-seeking were used for the establishment of addiction-like criteria and the addiction-score in order to avoid any direct influence of the addiction-like criteria selection on the addiction score. Nevertheless, the addiction score is highly representative of each of the addiction-like criteria (Spearman R=0.77 for motivation for cocaine, R=0.54 for resistance to punishment, and R= 0.71 / R=0.69 for inability to refrain from cocaine-seeking (measured on sessions 37-39 or 28-31, respectively), $p<0.01$.

Data analysis

Graphs show group means ± SEM. Data for each dependent variable were subjected to analysis of variance (ANOVA). Tests of significance were performed at $\alpha = 0.05$.

For all analysis, upon confirmation of significant main effects, differences among individual means were analyzed with appropriate post-hoc tests, i.e. either Newman-Keuls, Dunnett or Fisher LSD. The population was not normally distributed for each of the addiction-like criteria, we therefore carried-out a non parametric Spearman correlation analysis to address the relationships between putative predictive factors (novelty-induced locomotor activity, as measured by photocell beam breaks and impulsivity, as measured as the mean of the percentage of premature responses observed during the last two IITI sessions) and compulsive cocaine SA (resistance to punishment). Pearson correlation was used for the analysis of the relationships between (i) impulsivity and novelty-seeking as well as (ii) impulsivity or locomotor reactivity and the propensity to acquire cocaine self-administration, as measured by the number of infusions obtained during the first 2 doses of cocaine. For the factor analysis, three variables were considered: the addiction score, impulsivity and novelty-induced locomotor activity levels. The principal factors were selected according to an eigenvalue > 1.

Appendix 1: The populations tested for self-administration were highly representative of the initial populations

Because of the length of this experiment, rats' catheter patency was progressively lost, so that the population subjected to the entire self-administration experiment (n = 23) was smaller than the initial population (n = 40). However, the different remaining subpopulations were highly representative of the initial ones. The LI (n = 6), HI (n = 5), LR (n = 5) and HR (n = 5) rats that were tested for protracted cocaine self-administration were highly representative of the initial populations [initial vs sub population: F values < 1 for LI, HI and HR rats, $F_{1.13} = 1.51$, $p = 0.24$ for LR rats]. HI rats still display much more premature responses (52.64 ± 5.41 and 57.86 ± 11.7 for the last two lITI sessions, respectively) than LI (20.61 ± 1.5 and 16.58 ± 1.9 for the last two lITI sessions, respectively) during lITI sessions [Group: $F_{1,18} = 35.47$, $p < 0.001$, Group x Schedule: $F_{8,144} = 18.56$, $p < 0.001$], and HR (mean total locomotor activity = 1351 ± 94) are still more reactive than LR (624 ± 37) when exposed to a novel environment [Group: $F_{1,8} = 51.37$, $p < 0.001$].

Fig. 1

Pearson correlational analysis confirmed that impulsivity and novelty-induced locomotor activity are unrelated behavioral phenotypes (n = 40, Rs = -0.09 to -0.11, $p > 0.5$).

Fig. 2

Additionally, there was no correlation between impulsivity and the number of cocaine infusions obtained for the first two doses [Rs = 0.11 and 0.15, respectively, $p > 0.33$].

Fig. S1:

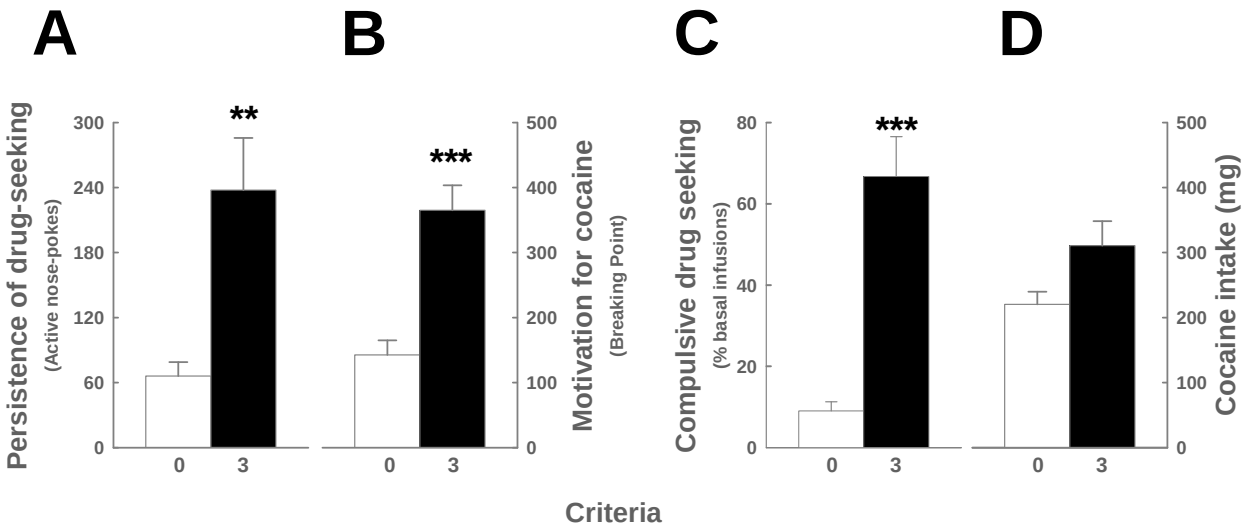
A
B
C
D
Persistence of drug-seeking
(Active nose-pokes)
Motivation for cocaine
(Breaking Point)
Compulsive drug seeking
(% basal infusions)
Cocaine intake (mg)
**
***
***
300
240
180
120
60
0
500
400
300
200
100
0
80
60
40
20
0
0
3
Criteria

Fig. S2:

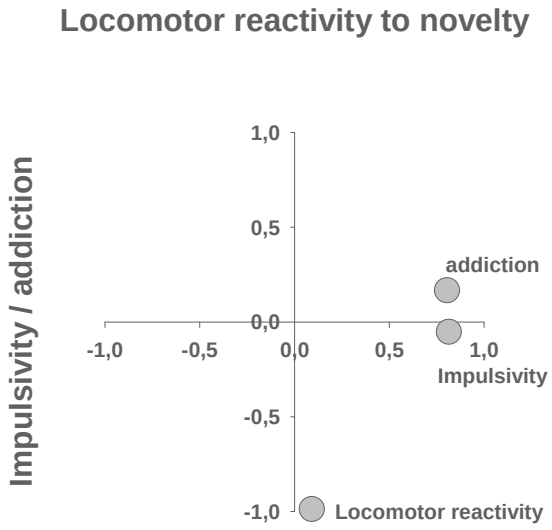
Locomotor reactivity to novelty
Impulsivity / addiction
1,0
0,5
0,0
-0,5
-1,0
-1,0
-0,5
0,0
0,5
1,0
Impulsivity
addiction
Locomotor reactivity

Fig. S3:

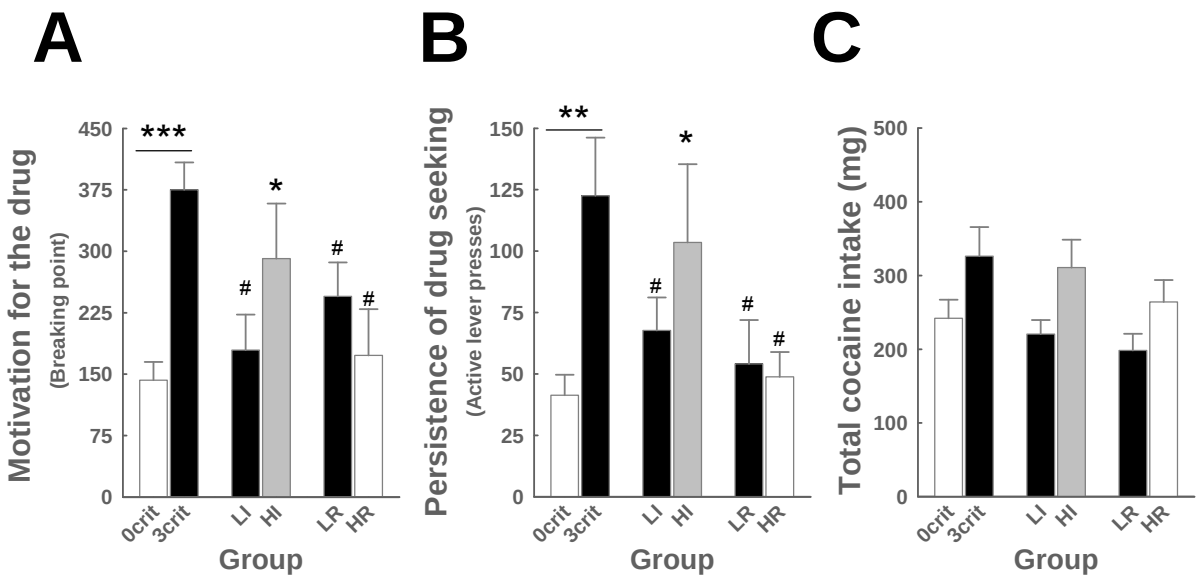
A
B
C
Motivation for the drug
(Breaking point)
Persistence of drug seeking
(Active lever presses)
Total cocaine intake (mg)
Group
0crit
3crit
LI
HI
LR
HR
***
**
*
#

Fig. S4:

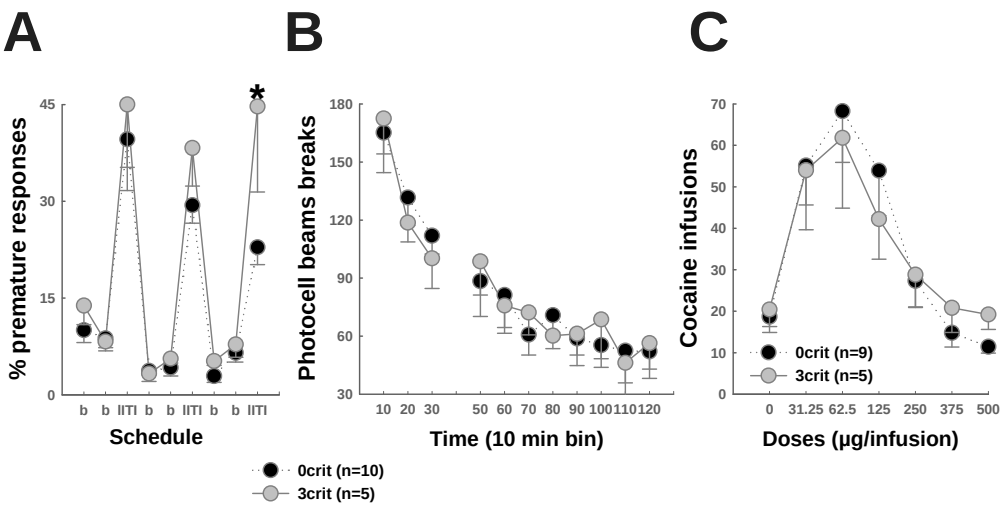
A
B
C
% premature responses
45
30
15
0
b b IITI b b IITI b b IITI
*
Schedule
Photocell beams breaks
180
150
120
90
60
30
10 20 30 50 60 70 80 90 100 110 120
Time (10 min bin)
Cocaine infusions
70
60
50
40
30
20
10
0
0 31.25 62.5 125 250 375 500
Doses (µg/infusion)
0crit (n=9)
3crit (n=5)
0crit (n=10)
3crit (n=5)

Fig. S1

Addiction-like behavior. Compared to 0 criteria rats (n = 10), 3 criteria rats (n = 5) responded more during 'no drug' periods [$F_{1,13}$= 20.81, $p < 0.01$] **A**, were more motivated for the drug, as shown by a higher breaking point during a progressive ratio session [$F_{1,13}$ = 34.72, $p < 0.01$] **B**, and resisted to punishment when cocaine infusions were associated with electric foot-shocks [$F_{1,13}$ = 61.37, $p < 0.01$] **C**. However, the two groups did not differ with respect to their total cocaine intake (**D**).

Fig. S2

Impulsivity but not novelty-induced locomotor activity predicts addiction-like behavior. When included into a factor analysis, impulsivity and addiction were explained by the same factor called here impulsivity/addiction, explaining 43% of the total variance, with loading scores of 0.81 and 0.80, respectively. On the other hand, novelty-induced locomotor activity loaded at 98% on an orthogonal independent factor that explained 33% of the total variance.

Fig. S3

HI rats developed addiction-like behavior. Neither LI (n = 6) and HI rats (n = 5) nor LR (n = 5) and HR rats (n = 5) differed in their motivation for cocaine (A) [$F_{1,9}$ = 2.1, ns, and $F_{1,8}$ = 1, ns, respectively], their persistence of cocaine-seeking (B) [$F_{1,9} < 1$, and $F_{1,8} < 1$, respectively] or their total amount of cocaine infused during the 34 sessions preceding the assessment of addiction-like behavior (C) [$F_{1,9}$ = 4.6, ns and $F_{1,8}$ = 3.1, ns, respectively]. When compared to 0 criteria (n = 10) and 3 criteria rats (n = 5) for their break point under a progressive ratio schedule [$F_{5,30}$ = 3.97, $p < 0.01$] or their persistence of drug seeking [$F_{5,30}$ = 3.61, $p < 0.05$], HI rats displayed an addiction-like behavior very similar to 3 criteria rats. HI rats did not differ from 3 criteria rats in their motivation for cocaine (**A**) or for their persistence of drug-seeking (**B**), in both cases, HI rats differed from HR ($p < 0.05$) but neither from LI nor LR rats. LI, LR and HR rats differed neither from 0 criteria rats not from each other. **C.** No difference was observed between the different populations tested [$F_{5,30}$ = 2.47, ns], *vs 0 criteria rats, $p < 0.05$, **$p < 0.01$, ***$p < 0.001$), # versus 3 criteria rats, $p < 0.05$.

Fig. S4

3 criteria rats (n = 5) were more impulsive than 0 criteria rats (n = 10) before any exposure to the drug but did not differ neither for their novelty-induced locomotor activity nor their propensity to acquire cocaine SA. A. 3 criteria rats displayed more premature responses than 0 criteria rats during the challenge sessions [Group x Schedule: $F_{8,104}$ = 2.17, $p < 0.05$], with a marked difference during the last long ITI session ($p < 0.05$). However, 3 criteria rats did not differ from 0 criteria rats during the test of novelty-induced locomotor activity (**B**) or for their propensity to acquire cocaine SA (**C**) (F values < 1).*versus 0 criteria rats, $p < 0.05$.

Table S1:

	LI (n=6)	HI (n=5)	LR (n=5)	HR (n=5)
0criterion (n=10)	4	0	2	3
1criterion (n=5)	2	2	1	2
2criteria (n=3)	0	1	1	0
3criteria (n=5)	0	2	1	0
3crit/(3crit+0crit) ratio	0	100	33	0

Table S2:

Addiction-like phenotype	Compulsive cocaine self-administration		Motivation for cocaine		Inability to refrain from cocaine-seeking	
number of criteria	score	criterion fulfilled = 1	score	criterion fulfilled = 1	score	criterion fulfilled = 1
0	0,0	0	10	0	67,6	0
1	0,0	0	385	1	69,6	0
0	0,0	0	225	0	32,8	0
1	0,0	0	145	0	120,6	1
2	0,0	0	445	1	124,4	1
0	3,5	0	45	0	76,8	0
1	5,9	0	325	1	66	0
0	6,1	0	185	0	17,4	0
2	6,6	0	385	1	155,2	1
0	7,7	0	145	0	28,4	0
0	10,0	0	145	0	26,2	0
0	12,5	0	145	0	34,4	0
0	13,3	0	225	0	84,6	0
0	16,0	0	115	0	5	0
0	21,4	0	185	0	40,6	0
3	**46,4**	**1**	**445**	**1**	**93**	**1**
3	**54,5**	**1**	**445**	**1**	**215,4**	**1**
3	**55,3**	**1**	**325**	**1**	**113,8**	**1**
3	**77,4**	**1**	**385**	**1**	**102,4**	**1**
1	90,3	1	145	0	34,8	0
3	**100,0**	**1**	**275**	**1**	**88**	**1**
1	111,1	1	145	0	55	0
2	153,8	1	275	1	45	0

Table S1

Distribution of the 4 different behavioral traits into the addiction-like categories. Analysis of the representation of high impulsivity and the high locomotor response to novelty in the different populations showing the addiction-like criteria revealed that LI, HR and LR rats were represented mainly in the 0 and 1 criteria populations, whereas HI rats were largely represented in the 2-3 criteria populations. Additionally, only highly impulsive rats were more frequently represented in the 3 criteria group than in the 0 criteria group. The ratio was calculated as n(3 criteria)/n(sum(3 criteria, 0 criteria))*100. Only HI rats displayed a ratio above 50%, illustrating that it is the only group which subjects are more represented by the 3 criteria than the 0 criterion population.

Table S2

Establishment of addiction-like subpopulations of rats.

Animals were ranked for each addiction-like behavior independently. Active lever presses measured during the first 'no-drug' period of sessions 28-31, break-point measured under a progressive ratio schedule of reinforcement on day 32, and percentage of infusions measured during the punishment session on day 40 relative to baseline (measured during the first 'drug-periods' of days 37-39) were used as behavioural measures. In this table, animals are ranked according to their resistance to punishment, the behavioural measure of compulsive cocaine self-administration. The selection criterion for the three behavioral measures was initially set to 40% to maximize the identification of 3 criteria rats. If a rat's score was included in the 40% highest percentile of the distribution (n = 9), this rat was considered positive for that addiction-like criterion. However, break-point scores being discrete variables, one rat's score was equal to the 9^{th} highest and was also selected. We thus set the selection criterion to 34.7% (n=8) for the other two behavioral measures so that our average selection cut-off (36%) was within the 33-40% range we have previously demonstrated to be optimal for the identification of the different addiction-like subpopulations (9). Then the arbitrary criteria scores were added so that four different groups were identified depending on the number of positive criteria each rat met (from 0 to 3).

References

1. J. W. Dalley *et al.*, *Neuropsychopharmacology* **30**, 525 (2005).
2. J. W. Dalley, D. E. Theobald, E. A. Pereira, P. M. Li, T. W. Robbins, *Psychopharmacology (Berl)* **164**, 329 (2002).
3. L. Diergaarde *et al.*, *Biol Psychiatry* (2007).
4. T. A. Kosten, M. J. Miserendino, *Pharmacol Biochem Behav* **60**, 785 (1998).
5. V. I. Chefer, I. Zakharova, T. S. Shippenberg, *J Neurosci* **23**, 3076 (2003).
6. M. Kabbaj, S. Evans, S. J. Watson, H. Akil, *Neuropharmacology* **47 Suppl 1**, 111 (2004).
7. M. E. Cain, D. A. Saucier, M. T. Bardo, *Exp Clin Psychopharmacol* **13**, 367 (2005).
8. F. Rouge-Pont, V. Deroche, M. Le Moal, P. V. Piazza, *Eur J Neurosci* **10**, 3903 (1998).
9. P. V. Piazza, V. Deroche-Gamonent, F. Rouge-Pont, M. Le Moal, *J. Neurosci.* **20**, 4226 (2000).
10. T. Saigusa, T. Tuinstra, N. Koshikawa, A. R. Cools, *Neuroscience* **88**, 1153 (1999).
11. V. Deroche-Gamonet, D. Belin, P. V. Piazza, *Science* **305**, 1014 (2004).