Supporting Online Material

Material and Methods

Subjects.
Male Sprague-Dawley rats (n=102) weighing 280-300 g at the beginning of the experiments were used. A 12 hr dark/light cycle (on 20h00, off 8h00) was used in the animal house. Temperature (22 ± 1°C) and humidity (60 ± 5%) were also controlled.

Drugs.
Cocaine was dissolved in 0.9% NaCl.

Surgery.
A silastic catheter (internal diameter = 0.28 mm; external diameter = 0.61 mm; dead volume = 12µl) was implanted in the jugular vein (SJ) under ketamine (100 mg/kg) /xylacine (1 mg/kg) anesthesia. The proximal end was placed in the right atrium while the distal end was passed under the skin and fixed in the mid scapular region. Rats were allowed to recover for 5 to 7 days after surgery. During the first 4 days following surgery, rats received an antibiotic treatment (gentamicine 1 mg/kg i.p.). After surgery, catheters were flushed daily with a saline solution containing unfractionated heparin (100 IU/ml).

Intravenous self-administration (SA) apparatus.
The SA setup was constituted of 16 SA chambers made of plexiglas and metal. Each chamber (40 cm long x 30 cm width x 52 cm high) was located within a larger exterior opaque box equipped with exhaust fans that assured air renewal and masked background noise. Briefly, animals were placed daily in a SA chamber where their chronically implanted intra-cardiac catheter was connected to a pump-driven syringe (infusion speed: 20 µl/sec). Two holes, located in opposite
sides of the SA chamber at 5 cm from the grid floor, were used as devices to record responding. A white house light at the top of the chamber allowed its complete illumination. A white cue light (1.8 cm in diameter) was located 9.5 cm above the active hole. A green cue light (1.8 cm in diameter) was located 10 cm right to the white cue light. A blue cue light (1.8 cm in diameter) was located on the opposite wall at 33 cm of the floor on the left side. Experimental contingencies were controlled and data collected with a PC-windows-compatible software.

**Elevated plus-maze apparatus.**

The elevated plus-maze is a classical test for studying anxiety-like behavior that is largely used to screen benzodiazepines. According to the specifications of Pellow et al. (52), the apparatus (made of opaque PVC) consisted of two open arms (50 cm x 15 cm), alternating at right angles with two arms enclosed by 40 cm high walls. The four arms delimited a central area (15 cm x 15 cm). The whole maze was elevated 60 cm from the ground. Two video cameras mounted above the maze and connected to videotape recorders were used to observe and record the animal's behavior.

**Locomotor activity apparatus.**

The locomotor activity setup was constituted of 16 circular corridors. Each corridor (10 cm wide x 70 cm in diameter) was equipped with two perpendicular photocell beams automatically recording horizontal locomotor activity. Experimental contingencies were controlled and data collected with a PC-windows-compatible software.

**General Procedures.**

**Intravenous SA procedures.**

All the SA experiments were performed during the dark phase of the light/dark cycle. 

*Basal training protocol.* The daily SA session was composed of three drug components (40 min each) separated by 15 min drug free periods. "Drug" periods were signaled by the blue cue light,
while the "no-drug" periods were signaled by illumination of the entire SA box and extinction of the blue cue light. During the "no-drug" periods, nosepokes were without scheduled consequences. During the "drug" periods, introduction of the animal's nose into one hole (active device) 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. Nosepokes in the other hole (inactive device) had no scheduled consequences. The self-infusion volume was 40 µL (2 sec infusion) and contained 0.8 mg/kg of cocaine. Each infusion was followed by a 40 sec time-out period. During the first 5 days, an FR1 schedule of reinforcement (i.e. one nosepoke resulted in an infusion of 0.8 mg/kg of cocaine) was applied. Then, the FR was first increased to 3 (1 to 2 sessions) and finally to 5 for the rest of the experiment. Criterion for acquisition of cocaine SA was defined by a stable number of self-infusions over at least three consecutive SA sessions (± 10%).

*Progressive-ratio schedule.* During the progressive-ratio schedule of reinforcement, drug availability was signaled by the blue cue light. 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.

*Association of cocaine infusions with an electric shock.* During these sessions rats were placed for 40 min in the SA chamber. The blue cue light signaling drug availability was on. The schedule was the following. FR1 led to the illumination of the green cue light signaling the presence of the shock. When an FR4 was completed rats received an electric shock (0.8 mA, 2
sec). When FR5 was reached rats received both an electric shock (0.8 mA, 2 sec) and a cocaine infusion (0.8 mg/kg) associated with the corresponding conditioned stimulus (CS) (white cue light). Then the green cue light was turned off. The schedule could reinitiate at the end of the time-out period, i.e. 40 sec after the infusion. If, within a minute, animals did not complete an FR4 or an FR5 leading to shock and shock plus infusion respectively, the green cue light turned off and the sequence was reinitiated, i.e. the following FR1 turned on the green cue light.

**Extended access to cocaine:** During this session, rats were placed for 5 consecutive hr in the SA chamber. Cocaine (0.8 mg/kg/infusion) was continuously available according to the conditions applied during the "drug" periods of the basal training protocol, i.e. FR5, 40 sec time-out period.

**Extinction protocol:** Extinction conditions allow for the measurement of the persistence of responding for drugs or other reinforcers when they are no longer available. For all the experiments a 1 hr extinction session was performed. Rats were placed in the SA boxes, the blue cue light signaling drug availability was on but nosepokes were without scheduled consequences at any time. For each hole, responses were cumulated over 1 hr.

**Reinstatement protocols.**

**Cocaine-induced reinstatement:** Cocaine-induced reinstatement was performed over two consecutive sessions according to a protocol used previously (S3). On day 1, following a 90 min period of extinction, the intravenous infusions of four different unit volumes (20, 40, 80 and 160 µL in ascending order) of vehicle were successively triggered by the computer; one infusion every 30 min. On day 2, the same schedule was applied except that the vehicle solution was replaced by a cocaine solution (0.2 mg/kg/20µL). Consequently, four doses of cocaine (0.2, 0.4, 0.8 and 1.6 mg/kg) corresponding to the four unit volumes were tested. For each hole, responses were cumulated over the first 10 min following each vehicle or cocaine infusion.
CS-induced reinstatement: A cue light that had been associated with drug delivery during the SA training was used as the CS. CS-induced reinstatement was conducted over one session according to a protocol used previously (S4). Following 1 hr of extinction during which nosepokes were without scheduled consequences, the cocaine-associated CS (white cue light) was turned on for 2 sec signaling changes in the schedule. From this time-point, a 4 sec CS was contingently available for 1 hr. The first CS was obtained for an FR1, the following were obtained for an FR5. For each hole, responses were cumulated over the 1 hr of CS availability. This schedule is not a typical Pavlovian CS-induced procedure in which the CS would be presented non-contingently. However, this procedure is classically used to test CS-induced reinstatement and is considered a better test for reinstatement than the non-contingent presentation (S5).

Elevated plus-maze procedure.
The plus-maze test was conducted in the middle of the dark phase of the light-dark cycle, i.e. 5 to 8 hr after the beginning of the dark phase. Each rat was placed in the central area of the plus-maze facing a closed arm and was allowed to freely explore the maze for 5 min. The rat behavior was videotaped. Then, the time spent in the open and closed arms was measured.

Spontaneous locomotor activity procedure.
Spontaneous locomotor activity was tested during the light phase of the dark-light cycle. Each animal was placed for 2 hr in a circular corridor 10 hr and 30 min after the last SA session.

HRein and LRein classification.
This classification was based on the reinstatement (number of responses in the active hole) induced by the highest dose of cocaine (1.6 mg/kg). A 40% criterion was used. HRein and LRein animals contained animals that were in the 40% highest and lowest parts of the distribution
respectively. It is important to stress that group classifications in individual differences studies have principally a descriptive purpose. The real relationship between reinstatement and addiction-like behaviors is given by the regression analysis. This analysis shows that the largest part of the variance (multiple R=0.82) in reinstatement is explained by addiction-like behaviors.

**Addiction-like criteria classification.**

Animals were scored for each addiction-like behavior independently. If the score was in the 33% highest percentile of the distribution, the individual was considered positive for that addiction-like criterion. Animals were then separated in four groups depending on the number of positive criteria met (from 0 to 3). The 33% cutoff was set *a priori* as index of behavioral intensity. Importantly, the percentage of animals (17%) showing 3 positive criteria did not depend on the 33% cutoffs. Thus in a cutoff window between 25 and 40% no significant differences in the distribution were observed (from 14% to 19% of animals showing 3 criteria respectively).

**Specific Experimental Procedures**

**Experiment 1:**

Seventeen rats were tested for cocaine intravenous SA for a total of 76 sessions. Tests for addiction-like behaviors were performed on sessions 32 and 74 for the resistance to punishment (cocaine infusions associated with an electric shock), on sessions 35 and 52 for the progressive-ratio schedule and on session 60 for extinction. All other testing days animals received the SA basal training protocol and responding during the "drug" and "no-drug" periods were recorded. Finally, cocaine-induced reinstatement was tested after a 5 days withdrawal period.

**Experiment 2:**

Fifteen rats were trained for cocaine intravenous SA for a total of 73 sessions. Tests for
addiction-like behaviors were performed on the following sessions: 60 for the progressive-ratio schedule, 63 for extinction and 72 for the resistance to punishment. All other testing days animals received the SA basal training protocol and responding during the "drug" and "no-drug" periods were recorded. Finally, cocaine- and CS-induced reinstatements were tested after a 30 and 32 days withdrawal period respectively using a Latin square design.

Experiment 3:
Twenty-six rats were tested for cocaine intravenous SA for a total of 73 sessions. Tests for addiction-like behaviors were performed on the following sessions: 60 for the progressive-ratio schedule, 63 for extinction session and 72 for the resistance to punishment. All other testing days animals received the SA basal training protocol and responding during the "drug" and "no-drug" periods were recorded.

Experiment 4:
Forty-four rats were tested for cocaine intravenous SA for a total of 90 days. Tests for addiction-like behaviors were performed on the following sessions: 69 for the progressive-ratio schedule, 80 for extinction session and 85 for the resistance to punishment. Animals were also tested during an extended access to cocaine on session 78 and for drug seeking in a drug free state (a "no-drug" period was applied at the beginning of the session) between sessions 60 and 64. Finally, rats were submitted to the anxiety test (elevated plus-maze) on day 73 (rats were not tested for cocaine SA this day). Spontaneous locomotor activity (circular corridor) was measured during the light phase between sessions 76 and 77. All other testing days animals received the SA basal training protocol and responding during the "drug" and "no-drug" periods were recorded.

Data Analysis.
Three types of analyses were conducted: analysis of variance for repeated measures (ANOVA),
multiple regression and factor analysis. For ANOVAs, the group (LRein/HRein: 2 levels; number of positive addiction-like criteria: 2 or 4 levels) was used as between subjects factor. Depending on the analysis, the treatment (vehicle/cocaine, no-CS/CS: 2 levels), the volume or dose (vehicle or cocaine: 4 levels), the hole (active vs inactive: 2 levels), the arm (open/closed arms: 2 levels) and the time (number of sessions: 68 levels, number of time blocks: 10 levels) were used as within subjects factors. Posthoc analyses (Newmann-Keuls) were used to determine the locus of significant main effects and interactions. For the multiple regression, cocaine-induced reinstatement (i.e. responses during the first 10 min following the non contingent infusion of 1.6 mg/kg of cocaine) was used as the dependent variable, while breaking point during progressive-ratio schedule, responding when cocaine infusions were associated with an electric shock (% infusions of preceding baseline training session) and responses in the active hole during "no-drug" period were used as predictor variables. For the factor analysis, four variables were considered, responses in the active hole during the "no-drug" period, breaking point during the progressive-ratio schedule, responding when cocaine infusions were associated with an electric shock (% infusions of preceding baseline training session) and responses in the active hole during the extinction session. The principal factors were selected according to an eigenvalue > 1 (Table S1). A significant level of p < 0.05 was used for all statistical analyses.

Supporting text.

*Detailed analysis of the reinstatement results: figures 1D (experiment 1) and 2D, E (experiment 2) of the manuscript.*

Animals were classified in two groups containing individuals showing respectively the highest (HRein) and lowest (LRein) cocaine-induced reinstatement at the highest dose of drug. As shown in Fig. 1D and 2D of the manuscript, HRein rats showed a higher number of nosepokes in the
active hole in response to non contingent cocaine. The complete analysis revealed that cocaine reinstated SA behavior significantly more than vehicle infused on the previous test day at equivalent volume [experiment 1, Treatment effect, $F(1,12) = 17.63, P < 0.001$; experiment 2, Treatment effect, $F(1,10) = 4.93, P < 0.05$]. This effect was specific of the active hole [experiment 1, Treatment x Hole interaction, $F(1,12) = 15.02, P < 0.001$; experiment 2, Treatment x Hole interaction, $F(1,10) = 13.62, P < 0.005$]. Thus, both treatments similarly affected responding in the inactive hole. The effect of cocaine, as compared to vehicle, was significantly higher in HRein than in LRein groups [experiment 1, Treatment x Group interaction, $F(1,12) = 9.19, P < 0.01$; experiment 2, Treatment x Group interaction, $F(1,10) = 6.11, P < 0.05$] and was specific of the active hole [experiment 1, Treatment x Group x Hole interaction, $F(1,12) = 7.19, P < 0.01$; experiment 2, Treatment x Group x Hole interaction, $F(1,10) = 10.8, P < 0.01$]. Thus, HRein and LRein rats did not differ for the number of inactive nosepokes. Similarly, HRein and LRein rats did not differ for active and inactive responding during the extinction periods preceding the two tests (vehicle and cocaine) for reinstatement, although in both conditions the two groups showed a higher number of nosepokes in the active than in the inactive hole [experiment 1, first extinction: Hole effect, $F(1,12) = 48.46, P < 0.00001$; second extinction, Hole effect, $F(1,12) = 24.65, P < 0.0005$; Hole x Group interaction, ns in both cases; experiment 2, first extinction: Hole effect, $F(1,10) = 12.78, P < 0.005$; second extinction, Hole effect, $F(1,10) = 5.66, P < 0.05$, Hole x Group interaction, ns in both cases].

As shown in Fig. 2E, compared to LRein rats, HRein rats showed a higher number of nosepokes in the active hole in response to the contingent cocaine-associated stimulus (CS). On the contrary, the two groups did not differ for the number of inactive nosepokes. Furthermore, while HRein rats showed a significant discrimination between active and inactive holes [Hole effect, $F(1.5) = 8.08, P < 0.05$], LRein rats did not. Finally, HRein and LRein rats did not differ for active and
inactive responding during the extinction period preceding contingent CS presentations, although the two groups showed a higher number of nosepokes in the active than in the inactive hole [Hole effect, F(1,10) = 4.50, P < 0.05, Hole x Group interaction, ns].

**Detailed analysis of the "Persistence of drug seeking" results: figures 1A (experiment 1), 2A (experiment 2), 3A of the manuscript.**

As shown in Fig. 1A, HRein and LRein rats differed for the number of nosepokes in the active hole during the "no-drug" periods. However, they did not differ for the number of nosepokes in the inactive hole during the same component of the schedule. Furthermore, while for LRein rats, discrimination between active and inactive holes remained non significant at the three presented time-points, HRein rats showed a higher number of nosepokes in the active hole at the last time-point (P < 0.05). Similarly, in Fig. 2A, HRein rats showed a higher number of active nosepokes during the "no-drug" period [Group effect, F (1,10) = 13.73, P < 0.005]. However, HRein and LRein rats did not differ for the number of nosepokes in the inactive hole. As shown in Fig. 3A, the number of responses in the active hole during the "no-drug" period increased as a function of the number of positive addiction-like criteria. However, the number of responses in the inactive hole during the same period was not related to the number of positive addiction-like criteria. In contrast, discrimination between active and inactive holes was related to the number of positive addiction-like criteria [Hole effect, F(1,54) = 28.72, P < 0.0001; Hole x Number of positive criteria interaction, F(3,54)=13.83, P < 0.0001]. Thus, animals with 3 positive criteria showed discrimination (P < 0.0001), animals with 2 positive criteria only tended to show discrimination (P = 0.12) and the other two groups did not show discrimination between active and inactive holes during the "no-drug" period.
Detailed analysis of the relationships between impulsivity/disinhibition and addiction like-behaviors: figures 4A and 4B (experiment 4) of the manuscript.

The factorial analysis clearly shows that impulsivity/disinhibition do not account for addiction-like behavior. This idea is also supported by the comparison of the behavioral phenotype of animals showing addiction-like behaviors with the one of animals recently proposed as a model of impulsivity/disinhibition (S6). Impulsive/disinhibited individuals show: 1. resistance to extinction (S7, S8), 2. an increased responding in an inactive device (S9); 3. a lower performance in paradigms such as the progressive ratio schedule, in which the workload is increased (S9). 4. a lower response to conditioned stimuli (CS) (S9, S10); 5. a clear-cut motor hyperactivity (S6, S9).

Animals developing addiction-like behaviors do not share any of these behavioral features: 1. they do not have higher responding during extinction (Fig. 4A); 2. they do not respond more in the inactive device during SA testing (see supporting text), 3. they work harder in schedules in which the workload is increased (progressive-ratio schedule, Fig. 1C, 2C, 3C); 4. they are more sensitive to a cocaine-associated CS as shown by the higher CS-induced reinstatement (Fig. 2E); and 5. they do not show locomotor hyperactivity (Fig. 4B). This dissociation between addiction-like behaviors and impulsivity/disinhibition is in agreement with studies in humans. Thus, propensity to drug use has been repeatedly associated with different personality traits, including impulsivity/disinhibition, sensation seeking and cluster B antisocial personalities. However none of these traits alone is considered a sensitive or specific predictor of drug abuse (for review see S11).

Detailed analysis of the relationships between locomotor response to novelty and addiction-like behaviors: figure 4B (experiment 4) of the manuscript.
Animals positive for 0 or 3 addiction-like criteria (Fig. 4B) did not differ for the locomotor response induced by a 2 hr exposure to a novel environment. This result seems in disagreement with previous findings from our group showing that high and low responders to novelty (HR and LR respectively) differ for cocaine SA (S12, S13). In fact, the HR/LR status actually predicts the amount of drug that an individual will take during SA and its sensitivity to the initial reinforcing effects (S12, S13). In contrast, we show here that addiction-like behaviors can appear in individuals taking the same amount of drug and this only after prolonged exposure (Fig. 3E). It is then likely that an HR status (our previous work) could constitute a first facilitating stage of the addiction process. Thus, in naturalistic conditions HR-like subjects will be more prone to develop drug use and will use larger amounts of the drug. However, only the individuals that also have a HRein status (present work) will develop true addiction showing compulsive drug intake.

Detailed analysis of the relationships between anxiety and addiction-like behaviors: figures 4A and 4C (experiment 4) of the manuscript.

As shown in Fig. 4C, animals positive for 0 or 3 addiction-like criteria had a comparable anxiety-like behavior in the elevated plus-maze, i.e. they spent a similar amount of time in the open arms. The experimental conditions were undoubtedly anxiogenic since animals from both groups spent significantly less time in the open than in the closed arms [Arm effect, F(1,23) = 29.3, P < 0.0001]. These results indicate that differences in resistance to punishment, which imply a conflict situation, do not depend on differences in anxiety. This idea is also supported by the results of the factorial analysis (Fig. 4A, Table S2) showing that resistance to punishment loads on the same factor as responding during the "no-drug" periods and during the progressive-ratio -two behaviors that did not involve punishment. If differences in anxiety mediated differences in shock-induced suppression, this variable should load on an independent factor.
Detailed analysis of the relationships between sensitivity to the unconditioned effects of cocaine and addiction like-behaviors: figures 3F and 4D (experiment 4) of the manuscript.

As shown in Fig. 4D, animals showing 0 or 3 addiction-like criteria differed for drug seeking in a drug free state, i.e. for the number of nosepokes in the active hole during a "no-drug" period applied before the start of the SA session \(F(1,23) = 8.74, P < 0.005\). However, they did not differ for the number of nosepokes in the inactive hole during the same component of the schedule. Furthermore, as shown in Fig. 3F, animals profoundly differing for addiction-like behaviors (0 versus 3 criteria) did not differ for the unconditioned effects of the drug as measured by locomotor activity during SA. Locomotor activity during SA is a validated method for differentiating the unconditioned from the conditioned effects of cocaine (S9). In conclusion, these results clearly show that addiction-like behaviors do not depend on the unconditioned effects of the drug: 1. Persistence of drug seeking was also present when no drug was on board; 2. Animals differing for addiction-like behaviors did not differ for the unconditioned effects of cocaine as measured by locomotor activity during SA.

Preliminary Neurobiological differences.

Using a functional neuroanatomy approach, we studied the expression of the cocaine-induced immediate early gene EGR1, which is involved in learning and memory (S14) and brain plasticity (S15). These studies showed that animals differing in addiction-like behaviors also differed in expression of EGR1 in the cingulate cortex. In particular, animals positive for 3 addiction-like criteria had lower EGR1 mRNA levels than animals with 0 positive criteria. These findings are highly consistent with brain-imaging studies in humans suggesting that compulsive drug use is associated with a dysregulation of the activity of the frontal cortex (for review see: S16).
Supporting tables

**Table S1.** Characteristics of each factor for the factor analysis. Two factors were extracted (eigenvalue > 1). % tot var, percentage of total variation explained by each factor; Cumul %, cumulative percentage.

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<th>Eigenvalue</th>
<th>% tot var</th>
<th>Cumul %</th>
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**Table S2.** Score of factor loadings of each variable in the factor analysis. Variables correspond to the following parameters: Motivation for the drug = breaking point reached during the progressive-ratio schedule; Resistance to punishment = self-infusions (in percentage of baseline) when cocaine delivery was associated with an electric shock; Persistence in drug seeking = number of active nosepokes during a “no-drug” period. Extinction = number of active nosepokes during a 1 hr extinction session. The values computed here were collected during the last test for addiction-like behaviors of each experiment.

<table>
<thead>
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<th>Factor 2</th>
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<tr>
<td>Extinction</td>
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<td>0.94&lt;sup&gt;a&lt;/sup&gt;</td>
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<sup>a</sup> Loadings > 0.70.
Supporting references


