Title:

Environment-dependent behavioral traits and experiential factors shape addiction vulnerability

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Abstract:

The transition from controlled drug use to drug addiction depends on an interaction between a vulnerable individual, their environment and a drug. However, the determining factors of this interaction are not well understood. We show in rats that the environment influences the acquisition of drug intake through its effect on behavioral markers of resilience to addiction. In contrast, the development of both compulsive cocaine and alcohol intake is facilitated by the experiential factors associated with the initiation of drug taking in a negative, deprived state occasioned by the contrast between enriched housing conditions and a relatively impoverished drug taking setting. Similarly, the acquisition of alcohol drinking as a coping strategy is associated with the development of compulsive intake. These data demonstrate that addiction vulnerability depends significantly on environmentally determined experiential factors.

keywords:

cocaine, alcohol, compulsivity, environmental enrichment, schedule-induced polydipsia

Short title:

There is more to addiction than a drug

One Sentence Summary:

Environmental control of the behavioral and experiential factors of vulnerability to acquire, and lose control over, drug use

Main Text:

Introduction

Ten to thirty-five percent of individuals recreationally using drugs are estimated eventually to develop the compulsive drug seeking and taking (*1*) that characterise drug addiction (*2*). It has long been considered that this individual vulnerability to transition from controlled to compulsive drug taking stems from the interaction between genetically determined behavioral traits, the nature of the neurodevelopmental environment and the context in which the drug is taken (*3*). However, the evident limitations of longitudinal studies in humans, which cannot readily control environmental and experiential factors (*4, 5*), and the inherent limitations of preclinical animal models (*6*) have made understanding the nature of these interactions and their underlying mechanisms extremely difficult.

The discovery of individual differences in the vulnerability to develop addiction-like behavior (*7, 8*), measured by operationalising behaviorally in rats three key features of the diagnosis of the disorder in the DSM (*9*), has helped to identify factors that mediate the effects of gene x drug interactions on the tendency to take drugs and the subsequent vulnerability or resilience to addiction (*10*). For example, high locomotor reactivity to novelty, suggested to operationalize the human trait of sensation seeking, has been shown to predict an increased tendency to self-administer stimulants (*11*), and also to be to be a marker of resilience to the transition to addiction (*7, 12*). This is consistent with evidence that sensation seeking in humans is related to recreational drug use, but not to stimulant addiction (*13*). Other behavioral traits (*10*), such as anxiety, impulsivity and novelty preference, have been shown specifically to predict loss of control over cocaine intake (*14*) or the transition to cocaine addiction in rats (*7, 12*) and in humans (*13*).

It is not yet understood how these behavioral factors of vulnerability are influenced by, or interact with, the living or drug use environment to shape the propensity to engage in drug taking. Similarly, it has not been established whether experiential determinants of drug taking, such as seeking sensory or affective enhancement ,or coping with distress (*4, 15*), independently mediate the vulnerability to transition from controlled to compulsive drug taking. The impact of an individual's environment on the vulnerability to addiction has to date been considered to be both unidimensional and unidirectional: impoverished environmental conditions such as those experienced by individuals in low socio-economic groups, or, inferentially, by rodents kept in impoverished housing conditions, are considered to promote addiction (*16*). However, experimental evidence

to support the latter claim is lacking and recent epidemiological data bring the former into question (*17*). Experimentally, rats raised in an enriched environment (EE) seem less likely to self-administer amphetamine (*18*), yet are more sensitive to the reinforcing effects of drugs than rats raised in a standard environment (SE). EE rats show lower self-administration titration rates than SE rats under fixed ratio schedules of reinforcement (*16, 18*), and also freely drink more alcohol under two-bottle choice conditions than SE rats (*19*). These observations are consistent with the epidemiological evidence of more premature drug-related deaths among individuals from high socio-economic populations (*17*), who also drink more often and consume higher quantities of alcohol than those from lower socio-economic backgrounds, even though the latter seem to experience more negative consequences of drug use (*20*). It may therefore reasonably be hypothesized that experiential factors related to drug use, rather than living conditions per se, are important determinants of the vulnerability to addiction across environmental conditions, as also indicated by the rise in drug-related deaths observed in very wealthy individuals (*21, 22*).

Here we tested the hypothesis that housing conditions and experiential factors operating in the drug taking environment interact with behavioral vulnerability traits to determine the likelihood of making the transition from controlled to compulsive drug use.

Results

Environmental enrichment decreases the propensity to acquire cocaine self-administration in addiction-resilient rats.

We first causally tested the influence of different housing conditions, i.e. EE vs SE (n=24 each) on behavioral traits that are related to personality factors relevant to addiction in humans, namely anxiety (*14*), sensation seeking (*7, 11, 23*), boredom susceptibility (*12*), reward sensitivity (*24*) and sign-tracking (*25*) (**Fig. S1**), that we propose to reflect a multidimensional, pseudo-personality model in the rat (**see SOM**). EE abolished the drug use proneness/addiction-resilience trait of high locomotor reactivity to novelty (HR), decreased the anxiety-associated behaviors of head dipping on the elevated plus-maze (EPM); EE also disrupted the asymmetric conditioned approach behavior usually displayed by sign-trackers towards a food CS, in line with previous evidence that EE impairs the attribution of incentive salience to food-paired cues (*26*) (**Fig. 1 and Fig. S2, S3**). EE did not influence each trait independently, but it shaped their relative multidimensional configuration in three distinct pseudo-personality models identified at the population level by cluster analysis (**Fig. 1B**). In particular EE increased the probability of displaying a pseudo-personality driven by sweetness and novelty preference (model 3), as opposed to that driven by stress reactivity (*11, 27-29*) (model 1) that is preferentially displayed by SE rats (**Fig. 1B**).

We then tested whether the influence of housing conditions on these behavioral traits mediated the well-established effect of EE on the tendency to self-administer cocaine. As previously described (*30, 31*), rats living under EE conditions were significantly less likely to acquire cocaine self-administration (SA) (**Fig. 2A**) than SE rats. However, the data also revealed that this EE effect was driven by a further decrease in the propensity to acquire cocaine SA in non-vulnerable populations, i.e. rats with a low locomotor

response to novelty (low responders, LR), low novelty (LNP) and low saccharine preference (LSP) (**Fig. 2B, 2C** and **Fig. S4-5**).

The increased weight of sweetness and novelty preference to the pseudo-personality shaped by EE, at the expense of locomotor reactivity to novelty which confers resistance to addiction (*7, 12*) (**Fig. 2C**), suggests that while decreasing the tendency to self-administer cocaine, EE may make the switch from controlled to compulsive drug use more likely in vulnerable individuals. We therefore investigated the influence of housing conditions on the individual vulnerability to develop addiction-like behavior following prolonged exposure to cocaine SA (*8, 12, 32*).

Environmental enrichment promotes the development of cocaine addiction-like behavior.

A new cohort of 48 rats (EE vs SE n=24 each) self-administered cocaine for ~50 days and was then tested for their addiction-like behavior, measured as the 3 criteria of high motivation for the drug, the persistence of drug taking despite adverse consequences and an inability to refrain from seeking the drug when signaled as unavailable (see (*7, 32*) and **Fig. S6**).

Regardless of their housing conditions, rats were identified as having 0, 1, 2 or 3 addiction-like criteria (**Fig. 3A** and S6, see **SOM**). As previously described (*7, 32*), the overall population was linearly distributed alongside an addiction severity index (*8*) in which only those rats displaying the three addiction criteria (3-criteria or 3crit) had a score that was beyond the standard deviation of the cohort, while 0crit, addiction-resilient rats had a negative addiction severity score. As previously described (*7, 12, 33, 34*), the linear addiction severity index provides a criterion-dependent difference in the manifestation of each of the addiction-like behaviors so that 3crit addiction-vulnerable rats had scores significantly higher than those of 0crit addiction-resilient rats in each of the addiction-like behaviors (**Fig. S6A**).

The individual vulnerability to develop addiction-like behavior was significantly influenced by housing conditions. A retrospective analysis revealed that EE rats had much higher addiction scores than SE rats (**Fig. 3B** and **S6B**) and that the entire 3crit population was, against expectation, comprised exclusively of EE rats (**Fig. 3C**). The influence of housing conditions on addiction vulnerability was primarily driven by a greater tendency to show compulsivity and the persistence of drug seeking, and less so by an increased motivation for the drug (**Fig. 3D** and **S6B**). Importantly it was not due to any overall differences in drug intake (**Fig. 3D** and **S6C)** nor to any differences in pain sensitivity (*35*) (**Fig. 3D**). This inter-dimensional approach provides compelling evidence that EE, while decreasing the likelihood that an individual will initially engage in drug use, it instead increases the likelihood of developing addiction-like behavior.

Acquiring alcohol drinking in a negative experiential state is a gateway for the transition to compulsive alcohol drinking.

We further investigated whether the facilitatory effect of EE on the development of compulsive cocaine intake also extended to alcohol. In a third experiment, the tendency of rats housed in EE vs SE (n = 12 each) to develop compulsive alcohol intake was assessed after several months of intermittent access to alcohol in a two bottle-choice

procedure (*36, 37*). EE rats did not differ from SE rats in their alcohol intake over 47 daily sessions of intermittent or unpredictable access to a choice between alcohol and water (**Fig. S7A**). However, as compared to SE rats, EE rats showed an increased tendency to relapse to alcohol drinking following several weeks of imposed abstinence (**Fig. 4A**) and did so compulsively as assessed by the persistence in drinking alcohol despite adulteration by quinine (*38*) (**Fig. 4B** and **S7**). Ultimately, EE rats were shown to be more vulnerable than SE rats in their development of two key behavioral features of alcohol use disorder (*2, 39*).

Together, these results reveal a bidirectional effect of housing conditions on the initial tendency to self-administer drugs and on the vulnerability compulsively to take drugs (*2*). While these results may at first glance appear counter-intuitive, they highlight the importance of the experiential factors inherent in the contrast between a rat's living conditions and the drug-taking setting (**Fig. S8**), shown previously to influence the relative preference for cocaine or heroin (*40*). It should be noted that the present study differs significantly from the 'Rat Park' procedure (which does not provide a measure of compulsive drug taking) by providing access to cocaine or alcohol in a relatively impoverished drug setting, rather than in the enriched housing environment itself (*41*). Therefore, EE rats, unlike SE rats, first encountered the drug in a state of relative social, sensory and cognitive deprivation because of the marked contrast between their enriched housing conditions and the drug SA context (**Fig. S8**). This negative environmental contrast likely biases individuals to self-administer drug in the experiential setting to ameliorate an internal deficit/distress state (*5, 42*). In humans, this negative motivational state is frequently associated with the initiation of drug use and increases the vulnerability to addiction (*4, 43*) by moderating or exacerbating the behavioral traits that we have here shown to be influenced by EE, namely sensation seeking, boredom susceptibility/novelty preference and anxiety (*43-45*) .

According to this framework, rather than solely an animal's housing conditions, it is the experiential factors in the drug setting, such as those related in humans to high boredom susceptibility or novelty preference in rats (*12, 46, 47*), that influence the vulnerability to subsequently compulsively take drugs. We therefore causally tested the hypothesis that the initial exposure to alcohol in a negative experiential setting drives persistent alcohol use to cope with distress, and thereby facilitates the transition to compulsive alcohol intake in rats living under similar housing conditions. In a fourth longitudinal experiment, alcohol was provided to a cohort of 48 SE rats (*48*) that had been trained to cope with distress in a schedule-induced polydipsia procedure (SIP) (*49, 50*). In this procedure, intermittent food delivery triggers a state of internal distress that rats learn to cope with by developing adjunctive behaviors, such as excessive intake of freely available water (*51*). Most rats readily acquire adjunctive drinking of large volumes of water (*52*) (**Fig. 4C**), but we hypothesized that those that do not would instead learn to cope with distress by drinking alcohol and that they would go on to develop compulsive (quinine-resistant) alcohol intake (see **SOM**).

The results show that a sub-population of rats that learned to cope with distress triggered by the SIP procedure by drinking water also maintained their established coping strategy after alcohol was introduced in place of water (Water Copers, WC). However, another sub-population of rats only developed a coping strategy by drinking

alcohol (Alcohol Copers, AC) (**Fig. 4C**). AC rats, which did not differ from WC rats in terms of total alcohol intake over the course of the 20 days of exposure (**Fig. S9** and **supplementary online material**), persisted in drinking alcohol when it had been adulterated with quinine (**Fig. 4D** and **S9**). This differential vulnerability to develop compulsive alcohol drinking was not due to differences in blood alcohol levels since they were similar in both groups (**Fig. S9**) and it was therefore specific to the experiential nature of the SIP context; only those rats that drank alcohol to cope with distress went on to drink alcohol compulsively.

Together these results show that a negative experiential context, whether inherent to the drug taking context or triggered by a negative affective contrast from housing/living conditions, at the onset of alcohol use is a gateway to the development of compulsive drinking in individuals that had been unable to learn to cope with negative states by alternative means (**Fig. 4D** and **S9**).

Discussion

Together the present findings provide clear evidence for the role of environmental and experiential factors in shaping an individual's vulnerability to make the transition from controlled to compulsive drug taking, which has previously been more restrictively understood in terms of behavioral/personality traits such as novelty preference, impulsivity and anxiety. The results show that considering the drug setting or the living environment alone when trying to decipher the nature of the gene x drug x environment interactions that predispose vulnerable individuals to become addicted does not fully capture the importance of experiential factors associated with initial exposure to the drug. These factors themselves depend on the interaction between housing/living conditions and the drug taking setting. While providing further evidence for a previously under-estimated role of non-pharmacological factors, highlighted by Ahmed and colleagues (*53*), in the vulnerability to progress from controlled to compulsive drug taking, the present results further suggest that initiating drug use through negative reinforcement-based self-medication (*5*) (from the "dark side" (*54*)), provides the conditions that are more likely to result in the development of addiction in vulnerable individuals.

Materials and Methods

Subjects

One hundred and ninety-two male Sprague Dawley rats (*Charles River laboratories, France or UK*), 6/7 weeks old, weighing approximately 200g upon arrival were housed 2 per cage for a week of habituation in a standard environment (40.5x35.5x23cm ventilated cages). Rats had ad libitum access to food and water and were maintained under a reversed 12-hour light/dark cycle (light ON between 7.00pm and 7.00am). Rats were then food restricted (15-20g chow daily) and tested in the behavioral procedures detailed below (6-7 days/week). Procedures were conducted in accordance with the European Community Council Directives (86/609/EEC) and with the United Kingdom Animals (Scientific Procedures) Act 1986, amendment regulations 2012 following ethical

review by the University of Cambridge Animal Welfare and Ethical Review Body (AWERB) PPL70/8072.

Environmental enrichment

Following a week of habituation, rats (see “specific experimental timelines”) either remained housed 2 per cage in standard cages, referred to as standard environment (SE) throughout the manuscript or were housed in groups of 4 in an enriched environment (EE) consisting of a large cage (80x50x80cm, *Ferplast*) equipped with toys (tubes, blocks), a running wheel, platforms, houses and hammocks. The toys and the configuration of the different elements within each cage were changed weekly (*55*).

Characterisation of behavioral traits

Anxiety-related behavior, novelty-induced place preference and locomotor reactivity to novelty were measured using a video tracking system (*ViewPoint Behavior Technology®*, *Lyon, France*) on an Elevated Plus Maze (EPM) (*56*), four Novelty Place Preference (NPP) boxes (*12*) and four Open Fields (OP) (*23*), respectively. Individual differences in incentive salience attribution to conditioned stimuli were assessed in operant chambers in an AutoShaping task (*57*) and sensitivity to natural rewards was conducted in a two-bottle choice procedure, water vs saccharine (*58*).

Anxiety-related behavior

High anxiety trait measured on an EPM has been shown to predict the escalation of cocaine SA (*56*).

The EPM is elevated 50cm above the floor and comprised two open arms (45x10cm), two closed arms (45x10x45cm) and a central platform (10x10cm). The luminosity was measured in each portion of the EPM and the light adjusted to reach an intensity of 40 Lux in the central platform, 50 Lux in the open arms and 30 Lux in the closed arms (*56*). The tests were conducted as previously described (*23, 56*) during the dark phase of the dark/light cycle between 8am and 11am.

At the beginning of a test session, rats were placed on the central platform with their head pointing towards an open arm (always the same arm for all subjects). They were then allowed to explore the maze for 5 minutes while their movements were monitored. In addition to entries into, and time spent in, each arm, exploratory behaviors, i.e. head dipping in the distal part (last 10cm) of open arms, were recorded as a measure of anxiety-related behavior (*59*).

An anxiety score, used in the pseudo-personality model, was calculated for each subject as a percentage of time spent in the open arms over the time spent in all arms. Individuals belonging to the upper and lower quartiles (25%) of the population were considered Low Anxious (LA) and High Anxious (HA), respectively (*56*).

Novelty-induced place preference

Novelty-induced preference has been shown to identify rats that are vulnerable (high novelty preference, HNP) or resistant (low novelty preference; NLP) to developing compulsive cocaine SA (*12, 60*).

The novelty-induced place preference (NPP) test was performed in four conditioned place preference boxes consisting of one narrow central corridor (10 x 50 x 50cm) surrounded by two large compartments (20 x 50 x 50cm), as previously described (*12*). Two guillotine doors located at both extremities of the central corridor gave access to the two large compartments protected by Plexiglas lids in order to decrease the luminosity to 0-0.2 Lux as compared to 10 Lux in the central corridor. The configuration of the boxes was adjustable such that the walls and floors could either be black or white and their texture smooth or rough. The different configurations were randomized between individuals (*12*).

The single test session for each rat, conducted during the dark phase of the dark/light cycle (7.00am-7.00pm), consisted of three successive steps as previously described (*12*). The animal was first placed in the central corridor with the two doors closed for a short habituation period of 5 min after which it was placed in one of the two compartments, considered the "familiar compartment" (randomized between individuals) for 25 min. In the final step, the animal was placed in the central corridor 5 seconds before the two doors were opened, giving free access to all the compartments of the box for 15 min.

The time spent in each of the 2 compartments (familiar and novel) was recorded and a score of NPP was calculated for each subject as the percentage of time spent in the novel compartment over the time spent in the other two compartments. Individuals in the upper and lower quartiles (25%) of the population were considered as HNP and LNP rats, respectively (*12*).

Locomotor reactivity to novelty

High locomotor reactivity to novelty has been shown to reflect individual differences in the activation of the stress system in response to a new inescapable environment (*61*) and has long been suggested to represent a subdimension of the sensation seeking trait (*28*). Additionally, the High Responder phenotype (HR) has been shown to be a behavioral marker of the increased propensity to acquired drug SA as well as a marker of resistance to addiction-like behavior to cocaine (*11, 12*).

The locomotor reactivity to novelty test was conducted as previously described (*23*) in four open fields (50 x 50 x 50cm) during the light phase of the dark/light cycle (7.00pm-7.00am) under a light intensity similar to that of the holding rooms (~ 550 Lux at the centre of the open field). Rats were placed in the open fields for two hours and their locomotor activity recorded throughout. Individuals whose total distance travelled was in the upper and lower quartiles (25%) of the population were considered High Responders (HR) and Low Responders (LR) rats, respectively.

Pavlovian conditioned approach: Sign tracking and Goal Tracking

Two days prior to the beginning of autoshaping training for food (i.e. 45 mg food pellets, *Bio-Serv, USA*), food-restricted rats received 20 pellets in their home cage in order to minimise any food neophobia.

The experimental sessions were conducted for 6 consecutive days in 12 operant chambers (31.8 cm x 25.4 cm x 26.7 cm each, *Med associates, St. Albans, USA*) located within a ventilated sound-attenuating cubicle. Front and back panels of the test

chambers were aluminium, while right and left walls and the roof were transparent acrylic plastic with stainless grid floor. A pellet dispenser was installed behind the front wall of each chamber, supplying food pellets to a food magazine located 2 cm above the grid floor. Each test chamber was illuminated by one 3-watt light bulb (house light) during the experimental session. The first day of training was a habituation session in which rats were placed in the operant chamber (house light and fan turned on) for 30min. The second day of training was a single session of “magazine training” for the rats to learn where food pellets were delivered in the experimental chambers. In this session, 60 pellets were delivered according to a 30s-variable time (VT) schedule. Rats were then trained to associate the presentation of a compound conditioned stimulus (CS) comprising a cue-light and a lever, deemed active in that it enabled the measurement of Pavlovian approach and contacts with the compound CS. Responding on that lever had no programmed consequences to the subsequent delivery of a food pellet (unconditioned stimulus: US); i.e. there was no instrumental contingency.

CS-US presentations were delivered according to a VT a Variable time (VT) 90 seconds schedule (VT-90s) such that upon termination of each variable time, the Cue light was illuminated and the active lever was inserted for a 8 seconds period, after which the CS was turned off, the active lever was retracted, and one pellet was delivered in the food magazine. During the 8 seconds interval, contacts with the compound CS measured as active lever depressions, were recorded as sign-tracking events while head entries into the food magazine were recorded as goal-tracking events (*57*). Inactive lever presses (uncoupled from the CS) were also recorded as an indicator of general activity. Individuals within the upper and lower quartiles of the population stratified on their level of sign tracking (lever presses) over the last three sessions, were considered sign-trackers (ST) and goal trackers (GT), respectively.

Saccharine preference

The saccharine preference test was carried out over 12 daily sessions, as previously described (*58*). Prior to testing, rats were water deprived for 2h in order to avoid any influence on performance of individual differences in thirst. Rats were weighed immediately before the beginning of each session in order to be able to compute a fluid intake/body weight ratio. The two-bottle choice sessions were conducted every day, in cages similar to that used for SE housing with the exception that no bedding was present.

After four habituation sessions during which rats were given access to two bottles of water, they were then given the choice between one bottle containing water and one bottle containing 0.2% saccharine (*Sigma*) for eight 2-hour sessions. Bottles were weighed immediately before and after each session to measure the intake of both solutions. One hour following the beginning of each test session, bottles were swapped for the remaining 1-hour period to control for the development of a side preference bias. The ratio between saccharine intake over the total fluid intake was calculated, and individuals belonging to the upper and lower quartiles of the population stratified on saccharine preference over the sessions were considered as High saccharin Preference (HP) and Low saccharin Preference (LP) rats, respectively (*58*).

Surgeries and drugs

Rats were anesthetised by intraperitoneal injection of a ketamine/xylazine mixture (*Ktamine1000, 90 mg/kg, Virbac, France; Rompun 2%, 6.7 mg/kg, Bayer, France*) and a silastic catheter (*CamCaths, UK*) was implanted into the right jugular vein as previously described (*62*). Rats were treated for one week with daily subcutaneous injection of an antibiotic (*Baytril, Bayer*) and catheters were flushed daily with 100µl of sterile physiological saline (0.9% sodium chloride) supplemented with heparin (50 IU/ml).

Cocaine hydrochloride (*Coopération Pharmaceutique Française*) was dissolved in sterile physiological saline (0.9% sodium chloride) at a final concentration of 2.5g/L and stored at 4°C.

Cocaine self-administration

Cocaine self-administration (SA) was conducted as previously described (*62*) in operant chambers made of aluminium and transparent acrylic plastic with stainless grid floor (31.8 cm x 25.4 cm x 26.7 cm, *Med associates, St. Albans, USA*) located within a ventilated sound-attenuating cubicle. Each chamber contained two retractable levers (4cm wide), located on the front panel such that rats had access to both an active and an inactive lever, the location of which was randomised between individuals. Each test chamber was illuminated by one 3W light bulb (house light) during the experimental session and cue lights (2.5W) located above each lever. An additional cue light (central cue light, 2.5W) was located above and between the lever-associated cue lights.

Flexible Tygon tubing, protected by a metal spring anchored to a swivel secured on a pivoting arm, linked a perfusion pump located outside the chamber at one end to the external port of the catheter on the other side. The stainless steel grid floor was linked to a scrambler (*Med associates, St. Albans, USA*) for the delivery of mild electric foot-shocks as previously described (*63*). All the SA experiments were performed 6/7 days a week during the dark phase of the light/dark cycle.

Intravenous cocaine self-administration training

SA training was performed as previously described (*7, 12, 33, 34*). Chiefly, daily SA sessions comprised three 40 min periods during which the drug was signalled as available by a discriminative stimulus (house light ON) separated by two 15-min drug free periods (called no-drug periods) during which the house light was turned off. During the "no-drug" periods, lever presses were without scheduled consequences. During the "drug" periods, active lever presses under a Fixed Ratio (FR) schedule of reinforcement resulted in the illumination of the drug-paired cue light (CS) for 5s and the activation of the infusion pump. Inactive lever presses had no scheduled consequences. Each infusion (0.25mg/100µL/5.7s) was followed by a 40sec time-out period. Rats acquired SA over the first 5-6 days under a FR1 schedule of reinforcement after which the FR was incrementally increased to 3 and then 5 over three days (*33*).

Progressive-ratio schedule

During the progressive-ratio schedule of reinforcement, drug availability was signalled by the illumination of the house 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), as previously described (*7, 12, 33, 34*). The maximal number of responses made by a rat to obtain one infusion determined the last ratio completed, which is referred to as the breaking point. The session ceased after either 5 hours or when a period of 1 hour had elapsed since the previously earned infusion.

Punishment sessions

The propensity of rats to persist in responding for cocaine despite punishment was tested for the duration of the first drug period, i.e. 40 min, as previously described (*7, 12, 33, 34*). The house light signalled that the drug was availability. The schedule was the following: FR1 led to the illumination of the central cue light signalling the presence of the shock. On completion of an additional 3 lever presses, thereby reaching FR4, a mild electric shock was delivered (0.45 mA, 2 sec). On completion of a fifth lever press, thereby completing the ratio requirement of 5 (FR5), rats received an electric shock (0.45 mA, 2 sec) followed by a cocaine infusion associated with the presentation of the CS for 5s. Then the central cue light was turned off.

If, within a minute following the first lever press (FR1), rats did not complete an FR4 or, after having received the shock following the fourth lever press, did not complete the FR5 sequence, the central cue light turned off and the sequence was reinitiated (*33*).

Pain sensitivity measurement

To ensure that potential differences in compulsivity were not attributable to a differential sensitivity to pain, rats were tested on a hot plate prior to the punishment sessions. Six hours following the self-administration session, rats were placed on a hot plate (*Ugo Basile, Gemonio, Italy*) set-up to remain at a stable temperature of 52ºC (*64*). The time elapsed before the appearance of pain-associated behaviors, including paw-licking and jumping, was measured and considered an indication of pain threshold (*64*). Rats were then immediately removed from the hot plate and returned to their home cage.

Two-bottle alcohol-water choice procedure

Rats and bottles were weighed prior to, and following, each session in order to compute a fluid intake/body weight ratio. The two-bottle choice sessions (alcohol versus water) were adapted from Simms et al. 2008 (*65*) and were conducted in cages similar to that of the standard environment (40.5 x 35.5 x 23cm ventilated cages).

Intermittent access to alcohol

To maximise alcohol consumption, rats were exposed to an intermittent two-bottle choice procedure adapted from Simms et al. (*65*). Rats had free access to two bottles containing either alcohol or water for twenty-four hours followed by twenty-four hours of abstinence (rats were returned to their home cages). The location of the alcohol bottle was changed over the course of each session to control for potential development of a side preference. The first four sessions consisted of habituation to alcohol during which the ethanol concentration was increased from 3% to 20% (3, 6, 10 and 20%). Rats then underwent a specific number of sessions (see specific experimental timelines) during which they had free access to water or 20% alcohol.

Unpredictable access to alcohol

In an attempt to elicit binge-like alcohol drinking, unpredictable access to alcohol was introduced in which the duration of each session was variable and unpredictable (from 30 minutes to 12 hours). The two-bottle choice sessions were separated by twenty-four hours of abstinence.

Alcohol adulteration

During the alcohol adulteration sessions, rats were given free access to a single bottle of 20% alcohol containing 0.1 g/L quinine (Sigma-Aldrich) (*66*) for four hours. In order to control for the specificity of the effect of quinine on alcohol intake, rats were also exposed to two 4-hour water adulteration sessions during which they had access to a single bottle of water containing quinine (0.1g/L).

Schedule-induced polydipsia

The Schedule-induced polydipsia (SIP) procedure was carried-out as previously described (*52, 67*). Rats were trained daily in operant chambers made of aluminium and transparent acrylic plastic with a stainless-steel grid floor (24 cm x 25.4 cm x 26.7 cm, *Med associates, St. Albans, USA*) located within a ventilated sound-attenuating cubicle. Chambers were equipped with a house light (3-W), a food tray (magazine) installed at the center of the front wall, and a bottle from which a stainless-steel sipper tube delivered water into a receptacle placed in a magazine on the wall opposite the food magazine. Water or alcohol were freely available throughout the 42 1hour sessions.

Habituation / baseline water intake

During the habituation session, rats were given access to water and 60 food pellets (45mg, *TestDiet, USA*) that were placed in the magazine. The volume of water consumed by each rat over this 60-min session was measured in order to determine the amount of water each individual drank while eating 60 45 mg pellets.

Rats were then tested in one 60-min magazine training session during which 60 food pellets were delivered under a random time schedule (RT-60 seconds) over 60 min. This magazine training provided the baseline level of homeostatic water intake over one hour.

SIP water

The SIP procedure was based on a fixed-time 60-second schedule of food delivery, previously shown to induce adjunctive drinking behavior with robust and persistent inter-individual differences in its magnitude (*67-69*).

Twenty-four hours after the baseline session, rats underwent 21 of these FT-60s SIP sessions (*67, 70*). 300ml bottles filled with fresh water were weighed and inserted into the operant box immediately prior to the initiation of each session. House lights were switched on at the beginning and switched off at the end of each session. The total amount of water consumed (ml) was calculated daily as the difference between the weights of the bottle before and after the session. Individuals whose average water consumption over the last three days of training was in the upper and lower quartiles of the population were considered as high (HD, n=12) and low drinkers (LD, n=12), respectively (see *52, 67*).

SIP alcohol

Twenty-four hours after the last SIP session for water, the water was replaced by 10% ethanol (*Sigma-Aldrich*). As predicted, some individuals only acquired SIP when the adjunctive response was directed to alcohol, and we therefore stratified rats based on their average alcohol consumption over the last three sessions of SIP with alcohol as "high drinker water-high drinker alcohol" (HDw-HDa, n=6) or "high drinker water-low drinker alcohol" (HDw-LDa, n=6), "low drinker water-high drinker alcohol (LDw-HDa, n=6) or "low drinker water-low drinker alcohol" (LDw-LDa, n=6). For clarification purposes, rats from the groups HDw-LDa and LDw-HDa were labelled Water Copers (WC) and Alcohol Copers (AC) rats respectively in the main manuscript.

Resistance to adulteration

Rats underwent a session of SIP with alcohol as described above, but the 10% ethanol solution was supplemented with 0.1g/L quinine (*Sigma-Aldrich*) (*71*). The resistance to adulteration was calculated as the ratio between alcohol+quinine intake over the average alcohol consumption over the last three sessions of SIP alcohol.

Blood alcohol level quantification

Blood samples were collected immediately after the last session of SIP with alcohol. Rats were anaesthetised under isoflurane and blood (0.5-1mL) was collected from the sublingual vein in heparinised tubes. After centrifugation (3000rpm, 5min, 4ºC), the plasma was collected and the blood alcohol levels (BAL) were determined in triplicate in mg/ml using Analox AM1 (*72*).

Specific Experimental Procedures

This study consisted of 4 independent experiments. Experiment 1 was conducted to test the hypothesis that the effect of housing conditions (EE vs SE) on the propensity to acquire cocaine SA was mediated by their influence on the behavioral traits of vulnerability or resilience to addiction and the associated propensity to acquire cocaine SA. Having reproduced the well-established observation that EE decreased the propensity to acquire cocaine SA, we further demonstrated that this effect was mostly driven by resilient individuals, e.g. rats previously shown to be less likely to develop compulsive cocaine self-administration such as HR or low novelty-preference rats. We were then able to investigate the influence of EE on the transition from controlled to compulsive drug use in vulnerable individuals. Thus, we tested the influence of environmental conditions on the development of addiction-like behaviors by exposing rats to ~ 50 days of cocaine SA and assessing their score in the 3-criteria model of cocaine addiction (*33*). Because this experiment demonstrated that EE promotes the development of addiction-like behavior, we tested the hypothesis that this effect is not restricted to cocaine. Thus, we demonstrated that this facilitatory effect of EE on the development of compulsive drug intake was also seen in rats drinking alcohol. We hypothesised that the facilitation of the transition to addiction by EE was dependent on the negative contrast between EE housing conditions and the relatively impoverished drug taking setting, thereby biasing the acquisition of drug taking as 'self-medication' of a deprived state. The final experiment was conducted to test the hypothesis that if individuals were to initiate alcohol use as a coping strategy in response to a distress

independent of a contrast between housing and testing conditions, as measured under schedule-induced polydipsia conditions (*73*), they should also be more vulnerable to develop compulsive alcohol drinking than individuals that had not have needed to drink alcohol to learn to cope with distress.

Experiment 1

Timeline

Following a week of habituation to the colony, forty-eight 6-7 week old male Sprague Dawley rats were housed in enriched (EE) (n=24) or standard (SE) (n=24) environment (day 7) until the end of the experiment. Subsequently, their sensitivity to a natural reward was assessed in a two-bottle choice procedure with water and saccharine as alternative reinforcers carried out from day 23 to day 46. Rats were then screened for their anxiety-related behavior and their locomotor reactivity to novelty at days 48-49 and 51-52 respectively. Two rats from the SE group and one rat from the EE group were excluded from the results of the EPM task after falling from the maze. The individual propensity to ascribe incentive value to a CS was assessed in an autoshaping (sign-tracking) task between day 59 and day 67 and the tendency to prefer novelty over a familiar environment was tested at days 87-89 in an NPP test. One rat from the EE group escaped from its NPP box and was therefore excluded from the analyses. Due to a software failure, the data of an entire NPP session (4 EE rats) could not be acquired, thus these rats were excluded from the analysis.

At day 96, rats underwent surgical implantation of an indwelling catheter in the right jugular vein for SA training. Following five days of recovery individual differences in the acquisition of cocaine SA was assessed, starting with 6 sessions of FR1 schedule of reinforcement followed by 1 session of FR3 and 3 session of FR5.

Data analysis and pseudo-personality assessment

A normalized score for each individual for each trait (% time spent in the OA, saccharine preference (%), time spent in novel compartment (%), total distance travelled in the OF and contacts made with the CS-associated lever) was calculated by subtracting the mean of the population to each individual value and dividing the result by the standard deviation of the population (z-score) as previously described (*33*).

The z-score of each rat was used within a multi-dimensional model (represented as radar plots) within which the contribution of each behavioral trait to the multidimensional behavioral characterisation of each individual (referred to as rat pseudo-personality) was calculated as the percentage of the normalized score of all the behavioral traits.

These pseudo-personality patterns were subjected to a k-means cluster analysis (*74, 75*) performed on the value (percentage) of each of the 5 behavioral traits with the following parameters: maximisation of distances between clusters, maximum 20 iterations, maximum of 3 clusters.

Experiment 2

Timeline

Following a week of habituation to the colony, forty-eight male Sprague Dawley rats were housed in EE (n=24) or SE (n=24) conditions (day 7) until the end of the

experiment. At day 39, rats underwent surgical implantation of an indwelling catheter in the right jugular vein for SA training. Following five days of recovery (from day 45), rats were trained to self-administer cocaine for 5 sessions under an FR1 schedule of reinforcement followed by 2 sessions of FR3 and 40 sessions under a FR5 schedule of reinforcement.

The addiction-like behaviors were assessed as follows: the motivation to self-administer cocaine was assessed during a single session of cocaine SA underwent 4 baseline sessions prior to being tested for their propensity to compulsively self-administer cocaine during a single 40 min punishment session (at day 99). Persistence of drug seeking measured over basal training sessions (days 92-93) were analysed.

Data analysis and identification of 0crit "resilient" and 3crit "addicted" rats

Twelve rats (EE group: n=5; SE group: n=7) were excluded from the final analysis due to loss of catheter patency.

Animals were scored for each addiction-like behavior independently. If the score of the individual was in the 33% highest percentile of the distribution, that 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). An addiction score was calculated for each animal as the sum of the normalized scores (Z-scores) for each of the three addiction-like criteria, as previously described (*12, 33*).

Experiment 3

Timeline

Following a week of habituation to the colony, twenty-four male Sprague Dawley rats were housed in EE (n=12) or SE (n=12) conditions (day 7) until the end of the experiment. Rats were first exposed to 4 alcohol habituation sessions (from day 98) followed by 17 two-bottle choice sessions under intermittent access. Rats then underwent 20 two-bottle choice sessions under unpredictable access (starting at day 140) to promote binge-like drinking behavior followed by 6 sessions under intermittent access. Access to alcohol was then withdrawn for 20 days during which rats stayed in their home cage with access only to water. Rats were subsequently challenged with a relapse test which consisted of a first 4-hour two-bottle choice session (at Day 222, identified as the relapse session) followed by a 24-hour two-bottle choice session.

EE and SE rats were then (day 225) divided into two subpopulations (n=6) each) displaying the same drinking behavior at every stage of the experiment (training pre-abstinence and during relapse). One subpopulation of EE and SE rats was exposed to two alcohol adulteration sessions while the other subpopulation was exposed to non-adulterated alcohol. This between-subject approach was carried out to control for any potential confounding influence of a habituation to the bitter taste of quinine (latent inhibition) from which a within-subject design may have suffered. Finally, to control for the specificity of the effect of quinine on alcohol intake, rats underwent two 4-hour water adulteration sessions during which they had access to a single bottle of water supplemented with quinine (0.1g/L).

Data analysis

The individual propensity to relapse was calculated as the ratio between alcohol intake at relapse and alcohol intake in the last 4-hour two-bottle choice session prior to abstinence (day 176).

Experiment 4

Timeline

Following a week of habituation to the colony, forty-eight male Sprague-Dawley rats were all housed in a SE. Rats were food restricted to 80% free-feeding body weight (*52, 67*) for a week before being trained in a SIP procedure with water for 20 sessions, as previously described (*52, 67*). Then, water was substituted with 10% alcohol and rats were trained in 20 additional SIP sessions with alcohol. The day following the last SIP session with alcohol, the individual propensity to persist in drinking despite alcohol adulteration was assessed as described above.

Rats then underwent five baseline SIP with alcohol sessions until they recovered their pre-adulteration alcohol intake level. Blood samples were collected immediately after the last session to measure their BAL. One blood sample from an individual of the LDw-HDa group was discard due to technical issue.

We then sought to verify that the persistence of drinking alcohol despite adulteration shown by rats trained under a SIP procedure was indeed attributable to their acquisition of alcohol drinking in the self-medication context of SIP. Thus, another cohort of twenty-four male Sprague-Dawley rats was housed in a similar SE under food deprivation. Rats were exposed to twelve 24-hour 2 two-bottle choice procedure sessions (alcohol 10% versus water) under intermittent access followed by twelve 1-hour 2 bottle choice sessions. Rats were then exposed to an intermittent access two-bottle choice procedure for 8 24-hour sessions so as to match the overall exposure to and intake of, alcohol displayed by rats in the four groups identified under SIP, namely HDw-HDa, HDw-LDa, LDw-HDa and LDw-LDa.

Rats were finally exposed to a single 1-hour session during which alcohol was adulterated with quinine as described above.

Data analysis

The individual propensity to persist in drinking alcohol despite adulteration was calculated as the ratio between alcohol+quinine intake over the average alcohol intake of the first hour during the last three two-bottle choice procedure sessions under intermittent access.

Data and statistical analyses

Data are presented as number of individuals, mean (for some radar plots) or mean +/- standard error of mean (SEM).

Assumptions for parametric analyses, namely homogeneity of variance, sphericity and normality of distribution were verified prior to each analysis with Cochran, Mauchly and Shapiro-Wilk's tests respectively.

Behavioral data were subjected to repeated measure, one-, two- factorial analyses of variance (ANOVAs) or non-parametric analyses, i.e. Kruskal-Wallis or Mann-Whitney U tests when the datasets did not meet assumptions for parametric analyses.

The experimental groups (LR/HR, HA/LA, GT/ST, LSP/HSP, LNP/HNP, SE/EE, number of positive addiction-like criteria…) were used as between-subject factor and, depending on the analysis, the arm (open/closed arms) and the time (number of sessions or session blocks) were used as within-subject factors.

Chi-squared test (maximum likelihood) was used to test the assumption of independent normally distributed data wherever necessary.

The confirmation of significant main effects and differences among individual means were further analysed using the Newman-Keuls post-hoc test and planned comparisons wherever appropriate.

The significance level was set at $p \leq 0.05$ and for all significant analyses, the effect size was reported by the partial eta-squared value (ηp^2).

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Author contributions:

DB, JLH, NV, LMP and ED designed the experiments. NV, ABR and MF performed the experiment and/or the data analysis of experiment 1. NV, SA and MF performed the experiment and/or the data analysis of experiment 2. MP, ED and DB performed the experiment and/or the data analysis of experiment 3. LMP, LZ and CG performed the experiment and/or the data analysis of experiment 4. MF, MP, BJE & DB wrote the MS.

Competing interests:

The authors have no financial disclosure or conflict of interest to report.

Supplementary Materials:

Supplementary Statistics

Supplementary Figures S1-S9

Supplementary References

Figures and figure legends.

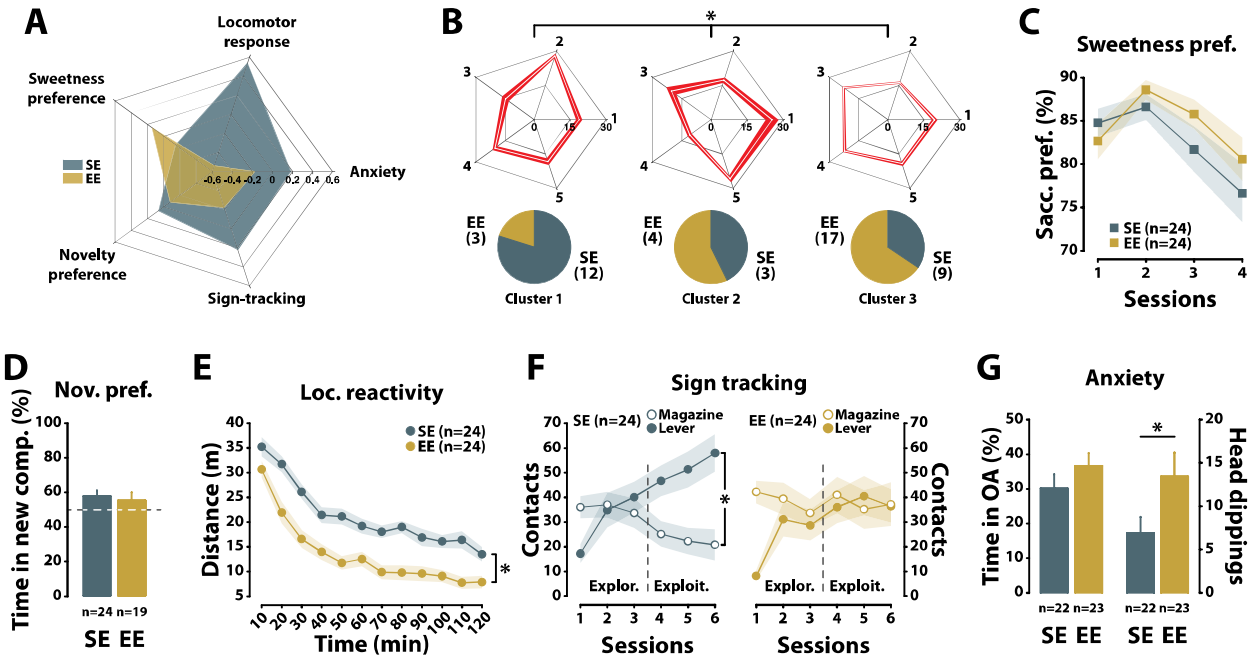

Figure 1. Environmental enrichment differentially shapes the behavioral traits of vulnerability and resilience to addiction in rats.

Enrichment of the housing environment (EE) exacerbates the contribution of sweetness preference to a multidimensional behavioral model of personality diminishing the contribution of anxiety, sign-tracking and reactivity to novelty **(A and B)**. These behavioral traits differed in their contribution to the three distinct, environment-dependent [EE vs SE: Chi2=8.43, $p<0.05$], pseudo-personality patterns identified from a cluster analysis [cluster x trait interaction: $F_{8,180}=14.373$, $p<0.0001$, $\eta p^2=0.39$], thereby suggesting a complex multidimensional interaction between the environmental conditions and behavioral traits of vulnerability versus resilience to addiction. Quantitatively, EE did not influence the sweetness preference of rats [main effect of group: $F_{1,46}<1$; session: $F_{3,138}=11.538$, $p<0.0001$, $\eta p^2=0.20$ and group x session interaction: $F_{3,138}=1.7583$, $p>0.05$] **(C)** nor their preference for novelty [main effect of group: $F_{1,41}=2.3124$, $p>0.05$] **(D)**. However, EE abolished the behavioral trait of resilience to addiction, namely high locomotor response to novelty (HR), in that EE rats showed a marked decrease in their locomotor reactivity to novelty as compared to SE rats [main effect of group: $F_{1,46}=19.274$, $p<0.0001$, $\eta p^2=0.30$; time: $F_{11,506}=102.16$, $p<0.0001$, $\eta p^2=0.69$; group x time interaction: $F(11,506)=1.5757$, $p>0.05$] **(E)**. Similarly, EE prevented the development of asymmetrical approach behavior, biased towards the CS, characteristically displayed by sign tracker rats raised in a SE during the exploitation phase of an AutoShaping task [SE, left panel: phase x approach response: $F_{1,23}=24.459$, $p<0.0001$, $\eta p^2=0.52$; EE, right panel: phase x approach response: $F_{1,23}=3.8233$, $p>0.05$] **(F).** EE influenced behavioral manifestations of anxiety on the elevated plus maze (EPM) **(G)** such that, despite spending a similar percentage of time in the open arms of an EPM [main effect of group: $F_{1,43}=1.472$, $p>0.05$], EE rats made

more head dippings while on the open arms than did SE rats [main effect of group: $F_{1,43}$=3.8447, p<0.05, ηp^2=0.08]. *: p≤0.05.

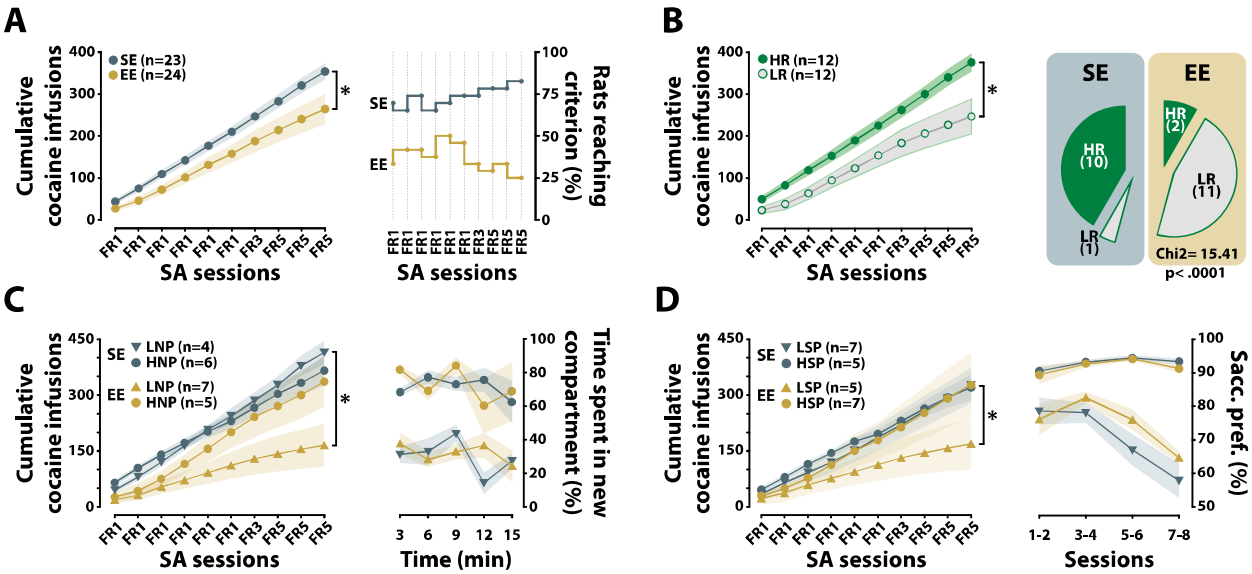

Figure 2: Environmental enrichment decreases the propensity to acquire cocaine self-administration in addiction-resilient rats.

EE rats showed a lower rate of acquisition of cocaine self-administration (SA) than SE rats [main effect of group: $F_{1,46}$=4.8168, p<0.05, ηp^2=0.10; session: $F_{9,405}$=200.63, p<0.0001, ηp^2=0.82 and group x session interaction: $F_{9,405}$=3.0058, p<0.01, ηp^2=0.06] (**A**, left panel). This effect was not simply driven by a small number of the EE population that failed to acquire SA. In fact, more than 50% of rats in the EE group stayed below the SA acquisition criterion (median number of cocaine infusions set at each session) (**A**, right panel). The high responder (HR) phenotype, which was almost non-existent in the EE population, violating the expected distribution typically observed in SE rats [Chi^2=15.41, p<0.0001] (**B**, right panel), was the behavioral trait that best recapitulated the differential tendency to self-administer cocaine shown by EE rats [main effect of group: $F_{1,22}$=7.1607, p<0.05, ηp^2=0.25; session: $F_{9,198}$=138.78, p<0.0001, ηp^2=0.86 and group x session interaction: $F_{9,198}$=3.7631, p<0.001, ηp^2=0.15] (**B**, left panel). EE also decreased the propensity of addiction-resilient, LNP rats, and rats with low reward sensitivity, LSP rats, to acquire cocaine SA [environment x phenotype x session interaction: $F_{9,162}$=5.2896, p<0.0001, ηp^2=0.23 and $F_{9,180}$=2.1008, p<0.05, ηp^2=0.10, respectively]. Thus, LNP and LSP rats from the EE population received far fewer cocaine infusions than their SE counterparts [planned comparison: p<0.01 and p<0.05 respectively] (**C** and **D**, left panels), even though the qualitative nature of these traits was not influenced by housing condition [environment x phenotype interaction: $F_{1,18}$<1; environment x phenotype x time interaction: $F_{4,72}$=1.9406, p>0.05, **C**, right panel and environment x phenotype interaction: $F_{1,20}$=1.7919, p>0.05; environment x phenotype x time interaction: $F_{3,60}$<1, **D**, right panel]. *: p≤0.05.

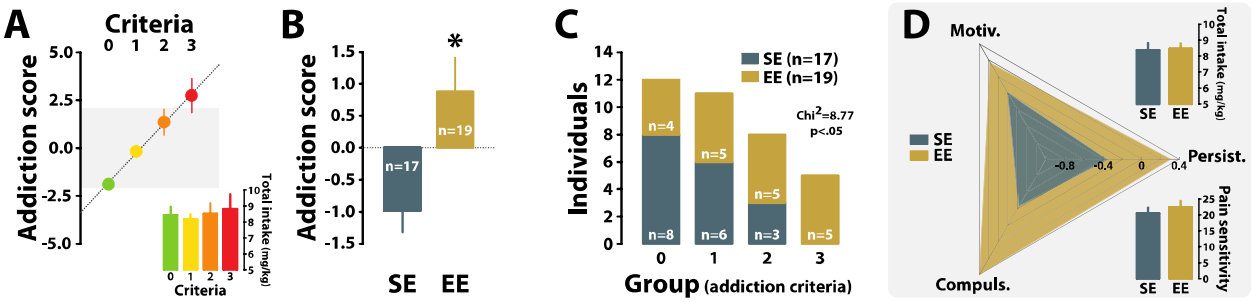

Figure 3: A negative contrast between housing conditions (EE or SE) and drug taking setting promotes the development of cocaine addiction-like behavior.

After 47 days of cocaine SA under a FR-5 schedule of reinforcement, rats from a heterogenous cohort comprising similar numbers of individuals housed in SE or EE were tested for the three behavioral criteria of addiction-like behavior: high motivation for the drug measured under progressive ratio; the maladaptive engagement in drug seeking, measured as the inability to refrain from responding when the drug is signaled as unavailable; the persistence of drug seeking despite negative consequences, measured as resistance to punishment. Rats were stratified on the number of criteria they displayed, i.e. 0, 1, 2 or 3 criteria (**A**). The 3crit group (n=5, 14% of the entire population) was the only one having an average addiction score that was outside the standard deviation of the overall population (grey bar). In contrast, the 0crit group (n=12, 33% of the overall population) was the only one displaying highly negative scores confirming their resilient phenotype [main effect of crit: $F_{3,32}$=17.955, p<0.0001, ηp^2=0.63] **(A)**. The difference between these groups was not attributable to a differential exposure to cocaine throughout their SA history [main effect of crit: $F_{3,32}$<1]. However, retrospectively factoring housing conditions revealed that EE rats had significantly higher addiction scores than SE rats, the latter actually having negative scores [main effect of environment: $F_{1,34}$=8.8255, p<0.01, ηp^2=0.21] **(B)**. All of the 3crit rats and the majority of 2crit rats were from the EE population, in clear contrast to the predominance of SE rats in the resilient, 0crit, population **(C)**. The higher addiction scores of EE rats as compared to SE rats were attributable to each of the addiction-like behaviours [main effect of environment: $F_{1,34}$=8.82, p<0.01, ηp2=0.21, behavior: $F_{1,2}$<1 and environment x behavior interaction: $F_{1,68}$<1], even if more pronounced differences were observed for compulsivity and persistence of drug seeking than for the motivation for the drug. The facilitation of the transition to addiction by EE was not attributable to a differential exposure to cocaine or to a differential sensitivity to pain [main effect of environment: $Fs_{1,34}$<1] **(D)**. *: p≤0.05.

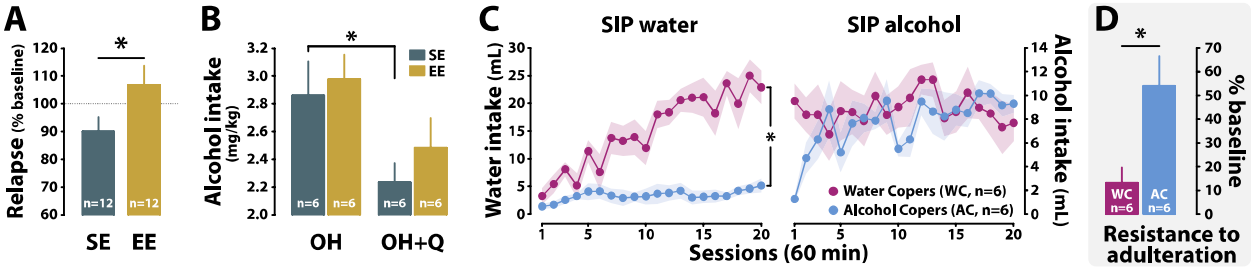

Figure 4: Alcohol drinking in a negative experiential state induced either by negative environmental contrast or by the aversive nature of the drug setting promotes the development of compulsivity.

After 20 days of abstinence following a 3-month history of exposure to alcohol in an intermittent two-bottle choice procedure, EE rats were more prone to relapse to alcohol drinking than SE rats [Mann-Whitney: U=37.00, $p<0.05$] **(A)**. EE rats were also more prone to persist in drinking alcohol despite adulteration with quinine, thereby displaying compulsive drinking behavior [Kruskal-Wallis EE: $H_{1,12}=2.08$, $p>0.05$; SE: $H_{1,12}=4.33$, $p<0.05$] **(B)**. In another cohort of outbred SE rats, introduction of the opportunity to drink alcohol as a means of coping with distress in a schedule-induced polydipsia procedure resulted in a specific subpopulation of individuals (Alcohol Copers, AC) that developed adjunctive alcohol drinking behavior [main effect of group: $F_{1,10}=1.2771$, $p>0.05$; session: $F_{19,190}=1.2348$, $p>0.05$; group x session interaction: $F_{19,190}=1.4429$, $p>0.05$; **C**, right panel] that they had failed to acquire when water was available, in marked contrast to rats that had acquired high levels of water intake (Water Copers, WC) [main effect of group: $F_{1,10}=33.619$, $p<0.001$, $\eta p^2= 0.77$; session: $F_{19,190}=9.8985$, $p<0.0001$, $\eta p^2=0.50$ and group x session interaction: $F_{19,190}=7.0845$, $p<0.0001$, $\eta p^2= 0.41$; **C**, left panel]. Although both WC rats and AC rats consumed the same amount of alcohol overall [main effect of group: $F_{1,10}=1.2771$, $p>0.05$], only the latter, i.e. those that had acquired a coping strategy by drinking alcohol, subsequently displayed compulsive alcohol drinking, being resistant to adulteration of alcohol with quinine [main effect of group: $F_{1,10}=8.3820$, $p<0.05$, $\eta p^2=0.46$] **(D)**. *: $p\leq0.05$.