Large-scale characterization of cocaine addiction-like behaviors reveals that escalation of intake, aversion-resistant responding, and breaking-points are highly correlated measures of the same construct.

Giordano de Guglielmo[1#]*, Lieselot LG Carrette[1#], Marsida Kallupi[1], Molly Brennan[1], Brent Boomhower[1], Lisa Maturin[1], Dana Conlisk[2], Sharona Sedighim[1], Lani Tieu[1], McKenzie J Fannon[1], Angelica R. Martinez[1], Nathan Velarde[1], Dyar Othman[1], Ben Sichel[1], Jarryd Ramborger[1], Justin Lau[1], Jenni Kononoff[2], Adam Kimbrough[1], Sierra Simpson[1], Lauren C Smith[1,2], Kokila Shankar[1,2], Selene Bonnet-Zahedi[1,3], Elizabeth A. Sneddon[1], Alicia Avelar[1,2], Caitlin Crook[1], Lucas Chun[1], Ashley Vang[1], Kristel K. Milan[1], Paul Schweitzer[1], Bonnie Lin[1], Beverly Peng[1], Apurva Chitre[1], Oksana Polleskaya[1,4], Leah C Solberg Woods[4], Abraham A. Palmer[1,5] and Olivier George[1]*

[1]Department of Psychiatry, University of California, San Diego, La Jolla CA 92093, USA.
[2]Department of Neuroscience, The Scripps Research Institute, La Jolla, CA 92037, USA.
[3] Institut de Neurosciences de la Timone, Aix-Marseille Université, Marseille, 13005, France
[4]Department of Internal Medicine, Section on Molecular Medicine, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA.
[5]Institute for Genomic Medicine, University of California, San Diego, La Jolla CA 92093, USA.

# These 2 authors contributed equally
*Corresponding authors

Dr. Giordano de Guglielmo and Dr. Olivier George, Department of Psychiatry, University of California San Diego, School of Medicine, La Jolla, CA 92093, USA. Tel: +18582465538. E-mail: gdeguglielmo@health.ucsd.edu olgeorge@health.ucsd.edu

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Abstract

Addiction is commonly defined as a chronic, relapsing disorder characterized by taking drugs in excess, compulsive drug seeking, and continued use despite harmful consequences. A key unanswered question for addiction research remains why moderate levels of drug consumption escalate to problematic patterns associated with high motivation and a compulsive-like pattern of drug use in some individuals, but not others, and how sex may affect this trajectory. However, most studies to date have had low statistical power due to low sample size and used animal models with limited genetic diversity and limited access to the drug that are often not associated with significant levels of intoxication or dependence, making it difficult to draw definitive conclusions and translate the results to human. Here we characterized addiction-like behaviors in >500 outbred heterogeneous stock (HS) rats using extended access to cocaine self-administration (6h/daily) and analyzed individual differences in escalation of intake, progressive-ratio (PR) responding, continued use despite adverse consequences (contingent foot shocks), and irritability-like behavior during withdrawal.

Principal component analysis showed that escalation of intake, progressive ratio responding, and continued use despite adverse consequence loaded onto a single factor that was orthogonal to irritability-like behaviors. Characterization of rats in four categories of resilient, mild, moderate, and severe addiction-like phenotypes showed that females showed higher addiction-like behaviors, particularly due to a lower number of resilient individuals. These results demonstrate that escalation of intake, continued use despite adverse consequence, and progressive ratio responding are highly correlated measures of the same psychological construct when tested in heterogeneous rats with a history of extended access to the drug, and suggest that a significant proportion of males, but not females may be resilient to addiction-like behaviors.

Introduction

Cocaine use disorder (CUD) remains a serious public health problem in the USA, with 2.2 million regular cocaine users in 2019 and over a million individuals with CUD in the past year (SAMHSA, 2019). The use of cocaine is associated with substantial morbidity and elevated rates of health care utilization (Butler et al., 2017), and over the last 10 years, the number of cocaine-related deaths has quadrupled (Hedegaard et al., 2018). A key unanswered question for addiction research remains why casual patterns of drug consumption escalate to problematic patterns associated with high motivation and a compulsive-like pattern of drug use in some individuals, but not others and how sex may affect this trajectory. Although addiction affects both males and females, most preclinical studies have focused on males. Even in studies that use males and females, low sample size often makes it difficult to draw reliable conclusions about the role of sex differences in addiction vulnerability. To investigate the critical questions about how sex and individual differences interact to produce the vulnerability to develop addiction-like large sample sizes are urgently needed.

Recently, the addiction neuroscience field has moved from recognizing that "compulsive drug seeking/use" and "continued seeking/use despite negative consequences" are two distinct aspects of addiction to defining the former nearly exclusively by the latter in animal models (Chen et al., 2013; Domi et al., 2021; Giuliano et al., 2019; Li et al., 2021; Siciliano et al., 2019; Timme et al., 2022). The three most prevalent behavioral measures used in animal models of addiction are escalation of drug intake, increased motivation under a progressive ratio (PR) schedule of reinforcement, and continued drug use despite adverse consequences, such as a footshock. While these three behaviors capture different aspects of addiction-like behaviors, a pervasive view in the field is that the only way to identify an individual with an addiction phenotype is to measure continued drug use despite adverse consequences, because it reflects the compulsive nature of drug use while escalation of drug intake or increased motivation under a progressive ratio schedule of reinforcement does not. A key argument in favor of this hypothesis is that responding despite adverse consequences is sometimes uncorrelated to drug taking/seeking, therefore suggesting that it is measuring a different psychological construct. Unfortunately, most of these studies have used low sample size and used animal models with very limited access to the drug that are often not associated with significant levels of intoxication or dependence, making it difficult to draw definitive conclusions.

To address these issues, we analyzed addiction-like behaviors in >500 male and female rats that were phenotyped for an ongoing gene-wide association study. We used heterogeneous stock (HS) rats because they are the most highly recombinant rat intercross available and exhibit remarkable individual differences in addiction-like behaviors (Carrette et al., 2021; Carrette et al., 2022; Chitre et al., 2020; Duttke et al., 2022; Kallupi et al., 2020; Sedighim et al., 2021; Solberg Woods and Palmer, 2019). HS rats were created by interbreeding eight inbred strains and maintaining them as an outbred population in a way that minimizes inbreeding, thereby maximizing genetic diversity within the colony (Solberg Woods and Palmer, 2019). We used an advanced model of intravenous self-administration with extended access to cocaine and characterization of escalation of cocaine intake, breaking point under a progressive ratio-schedule of reinforcement, continued use despite adverse consequences (footshock), and irritability-like behavior as a measure of negative affective state during withdrawal.

METHODS

Detailed procedures associated with the experimental timeline in Fig. 1A can be found in the George lab protocol repository on protocols.io (https://www.protocols.io/workspaces/george-lab).

Animals. HS rats (Rat Genome Database NMcwiWFsm:HS #13673907, sometimes referred to as N/NIH:HS) were created to encompass as much genetic diversity as possible by outbreeding eight inbred rat strains (ACI/N, BN/SsN, BUF/N, F344/N, M520/N, MR/N, WKY/N and WN/N) in 1984 (Hansen and Spuhler, 1984; Solberg Woods and Palmer, 2019). HS rats were bred at Wake Forest University School of Medicine by Dr. Leah Solberg Woods (n = 600). To minimize inbreeding and control genetic drift, each generation of the HS rat colony consists of at least 64 breeder pairs and is maintained using a breeding strategy that minimizes inbreeding, with each breeder pair contributing one male and one female to subsequent generations. Each rat received a chip with an RFID code that was used to track animals throughout the experiment. Rats were shipped at 3-4 weeks of age, kept in quarantine for 2 weeks and then housed two per cage on a 12 h/12 h reversed light/dark cycle in a temperature (20-22°C) and humidity (45-55%) controlled vivarium with ad libitum access to tap water and food pellets (PJ Noyes Company, Lancaster,

NH, USA). Animals were tested in 12 cohorts of 46-60 rats per cohort, 6 cohorts at The Scripps Research Institute and 6 cohorts at UC San Diego. All procedures were conducted in strict adherence to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Institutional Animal Care and Use Committees of The Scripps Research Institute and UC San Diego.

Drugs. Cocaine HCl (National Institute on Drug Abuse, Bethesda, MD) was dissolved in 0.9% sterile saline and administered intravenously at a dose of 0.5 mg/kg/infusion.

Intravenous Catheterization. Rats were anesthetized with vaporized Isoflurane (1-5%). Intravenous catheters were aseptically inserted into the right jugular vein using the procedure described previously (Kallupi et al., 2020). Catheters consisted of Micro-Renathane tubing (18 cm, 0.023-inch inner diameter, 0.037-inch outer diameter; Braintree Scientific, Braintree, MA, USA) attached to a 90 degree angle bend guide cannula (Plastics One, Roanoke, VA, USA), embedded in dental acrylic, and anchored with mesh (1 mm thick, 2 cm diameter). Tubing was inserted into the vein following a needle puncture (22G) and secured with a suture. The guide cannula was punctured through a small incision on the back. The outside part of the cannula was closed off with a plastic seal and metal cover cap, which allowed for sterility and protection of the catheter base. Flunixin (2.5 mg/kg, s.c.) was administered as analgesic, and Cefazolin (330 mg/kg, i.m.) as antibiotic. Rats were allowed three days for recovery prior to any self-administration. They were monitored and flushed daily with heparinized saline (10 U/ml of heparin sodium; American Pharmaceutical Partners, Schaumberg, IL, USA) in 0.9% bacteriostatic sodium chloride (Hospira, Lake Forest, IL, USA) that contained 52.4 mg/0.2 ml of Cefazolin. Catheter patency was tested throughout and at the end of LgA sessions using a short-acting anesthetic (Brevital), any animal that failed to react to the Brevital was excluded from the study.

Behavioral Testing

Operant self-administration. Self-administration (SA) was performed in operant conditioning chambers (29 cm × 24 cm × 19.5 cm; Med Associates, St. Albans, VT, USA) that were enclosed in lit, sound-attenuating, ventilated environmental cubicles. The front door and back wall of the

chambers were constructed of transparent plastic, and the other walls were opaque metal. Each chamber was equipped with two retractable levers that were located on the front panel. Each session was initiated by the extension of two retractable levers into the chamber. Cocaine (0.5 mg/kg per infusion) was delivered through plastic catheter tubing that was connected to an infusion pump. The infusion pump was activated by responses on the right (active) lever that were reinforced on fixed ratio (FR) 1 schedule, with the delivery of 0.1 mL of the drug per lever press over 6 s followed by a 20-s timeout period that was signaled by the illumination of a cue light above the active lever, during which active lever presses did not result in additional infusions. Responses on the left inactive lever were recorded but had no scheduled consequences. Fluid delivery and behavioral data recording was controlled by a computer with the MED-PC IV software installed. Initially, rats were trained to self-administer cocaine in 10 short access (ShA) sessions (2 h/day, 5 days/week). At the end of the ShA phase the animals then underwent 14 extended access (LgA) sessions (6 h/day, 5 days/week) in order to measure escalation of drug intake.

Progressive Ratio Testing. Rats were tested on a progressive ratio (PR) schedule of reinforcement at the end of each phase (twice after LgA, see Fig. 1 for detailed timeline), in which the response requirements for receiving a single reinforcement increased according to the following 1, 2, 4, 6, 9, 12, 15, 20, 25, 32, 40, 50, 62, 77, 95, 118, 145, 178, …. The breakpoint was defined as the last ratio attained by the rat prior to a 60-min period during which a ratio was not completed, which ended the experiment.

Compulsive-like responding using contingent foot shock. A 1-h footshock punishment testing was conducted between progressive ratio tests following the same parameters as the cocaine self-administration session. Punishment testing followed the same FR1 20-second timeout reinforcement schedule with contingent footshock (0.3 mA, 0.5 s) paired with 30% of the cocaine infusions. This was compared to a 1-h FR1 20-second timeout reinforcement schedule without footshock the day before, called preshock.

Irritability-like behavior (Bottle Brush Test). The bottle brush test consisted of ten 10 s trials with 10 s intertrial intervals in plastic cages (27 cm × 48 cm × 20 cm) with clean bedding. The

rats were placed in the back of the cage and a bottle brush was rotated rapidly toward the rat's whiskers. Both aggressive responses (smelling, biting, boxing, following, and exploring the bottle brush) and defensive responses (escaping, digging, jumping, climbing, defecation, vocalization, and grooming) were recorded by three trained observers in real-time. Total aggressive and defensive scores were calculated for each animal based on the average score of the observers. Both aggressive and defensive behaviors were summed to calculate the total irritability score. Irritability-like behavior reflects a composite measure of aggressive *vs.* defensive responses. (Kimbrough et al., 2017a). The test was performed after recovery from surgery (baseline) and in withdrawal 18 h after the last LgA session.

Statistical Analyses. Data were analyzed using Prism 9.0 software (GraphPad, San Diego, CA, USA) and R Studio. Self-administration data were analyzed using repeated-measures analysis of variance (ANOVA) followed by Bonferroni post-hoc tests when appropriate. For pairwise comparisons, data were analyzed using the Student's *t*-test. Correlations were calculated using Pearson *r* analysis. Missing data due to session failure was imputed as the average from the sessions before and after (<2% of data). The data are expressed as mean ± SEM unless otherwise specified. Values of $p < 0.05$ were considered statistically significant.

Results

Evaluation of Individual Differences in Addiction-Like Behaviors.

We assessed addiction-like behaviors in HS rats self-administering cocaine according to the standardized protocol shown in Figure 1A. Animals were tested in 12 cohorts of 46-60 rats per cohort. Thirty-four animals were excluded from the analysis because they lost their catheter patency (failed Brevital test) before the end of the behavioral protocol. After catheterization surgery and recovery, the rats were trained to self-administer cocaine in 10 daily 2h short access (ShA) sessions and then in 14 daily 6h long access (LgA) sessions. Over the course of the extended access of cocaine self-administration phase, the animals showed an escalation of intake, as measured by the significant increase of cocaine rewards taken per hour from day 3 of LgA onwards vs the first day of LgA (Fig. 1B, $F_{(566,7358)} = 124.4$; $p < 0.0001$ after one-way ANOVA, followed by Bonferroni post hoc comparisons). Evolution of total intake is given in the supporting material Fig S1. The number of cocaine infusions in the last three days of LgA were

averaged for each rat in order to compare the levels of cocaine intake at the end of the behavioral protocol in the whole population (Fig. 1C). The results showed a bimodal distribution with ~20% of rats maintaining very low levels of intake, defined as <50 infusions per session (that corresponded to ~8 infusions/h, levels normally achieved during ShA, see Fig 1B) and 80% of the rats escalating their cocaine intake over the course of the extended access protocol. Motivation for cocaine was tested both after ShA and LgA, using a progressive ratio (PR) test (Fig. 1D). The number of cocaine infusions significantly increased after extended access to cocaine self-administration, compared to the levels at the end of the short access phase ($F_{2,1690,} = 135.5$; $p < 0.0001$) with the population following a bimodal distribution. Compulsive-like behavior was tested by pairing the cocaine reward with a foot shock (0.3 mA, 30% contingency). HS rats showed high individual variability in compulsive-like cocaine intake with a general decrease in responding during the shock session compared to the preshock session ($t_{465}=17.88$, $p<0.0001$). Correlational Analysis showed that the lever pressing during the preshock session strongly predicted responding during the shock session. This result was true for the whole population ($R=0.58$ $p<2.2\ 10^{-16}$) as well as for each individual cohort (Fig. 1F). Cocaine-induced withdrawal was assessed by measuring irritability-like behavior using the bottle brush test (Kimbrough et al., 2017b). Irritability-like behavior was measured at the beginning and at the end of the behavioral paradigm (pre- and post- cocaine, Fig. 1G) and the differences from baseline were compared to data obtained from age-matched naïve animals. Irritability-like behavior was increased in cocaine rats during withdrawal compared to naïve animals as demonstrated by the increased total irritability score ($t_{427} = 7.57$; $p < 0.0001$ vs naïve after t-test). When breaking the total irritability score in defensive and aggressive responses we found that animals with a history of cocaine increased their aggressive ($t_{427} = 7.82$; $p < 0.0001$ vs naïve after t-test) and defensive behaviors ($t_{427} = 2.79$; $p = 0.0069$ vs naïve after t-test), compared to their naïve counterparts. Finally, to identify patterns in the multidimensional dataset of addiction-like behaviors, a principal component analysis (PCA) was used (Fig. 1H). This method allows for the identification of latent variables - principal components - that can account for most of the variance within the data, thus simplifying complex data structures. Three significant findings emerged from this analysis. First, the majority of the variance was accounted by the first two principal components, with PC1 and PC2 explaining 48.6% and 15.7% of the variance respectively. Second, measures related to short-term cocaine intake were orthogonal to those

collected during longer access periods, showing a consistent daily shift from primarily loading onto PC1 to gradually loading onto PC2. Third, metrics associated with escalating intake, motivation, and compulsive-like responses (calculated by normalizing the behavioral data into an indexes using *Z*-scores) consistently loaded onto PC2.

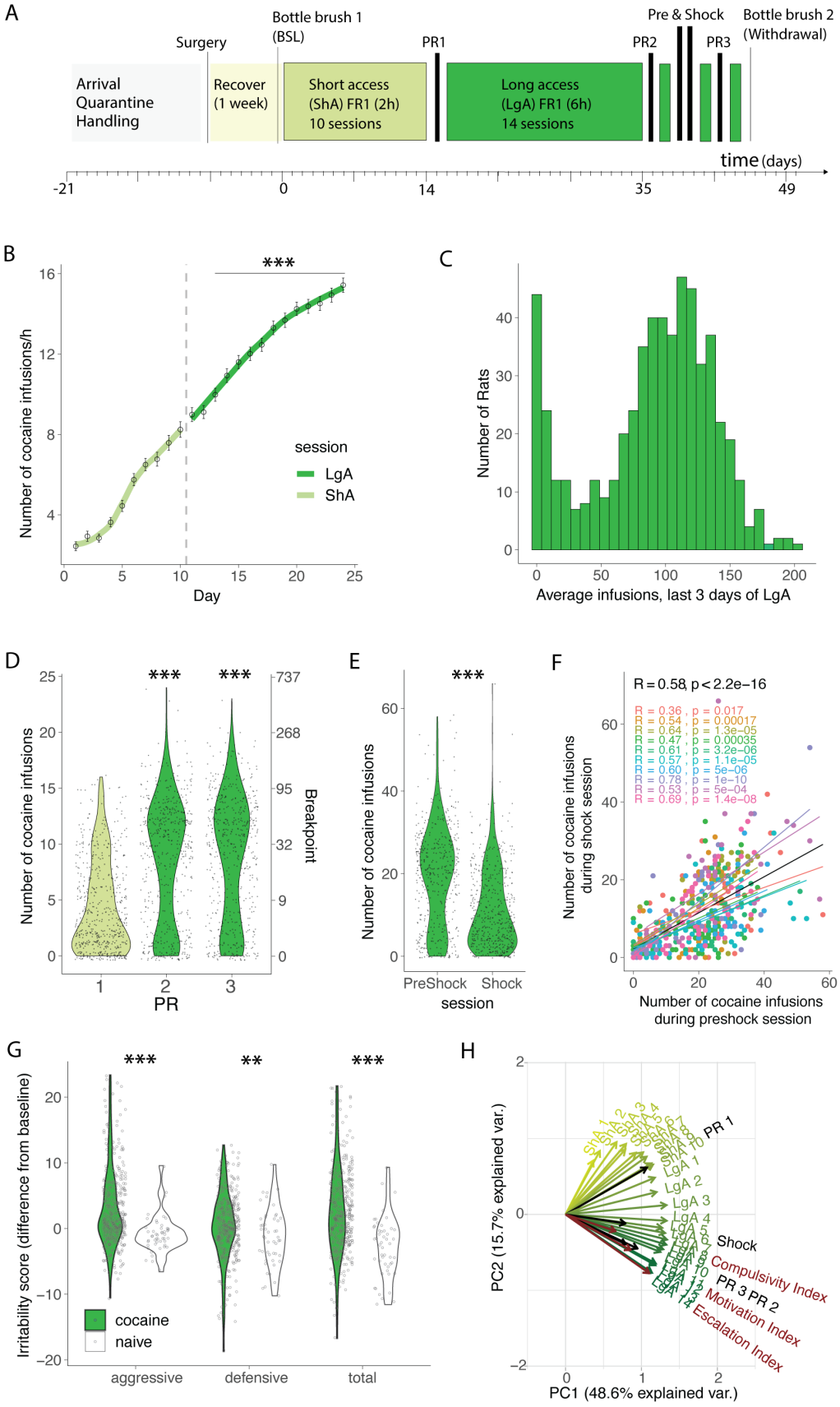
A
Arrival
Quarantine
Handling
Surgery
Recover
(1 week)
Bottle brush 1
(BSL)
Short access
(ShA) FR1 (2h)
10 sessions
PR1
Long access
(LgA) FR1 (6h)
14 sessions
PR2
Pre & Shock
PR3
Bottle brush 2
(Withdrawal)
time (days)
-21
0
14
35
49
B
Number of cocaine infusions/h
Day
session
LgA
ShA
***
C
Number of Rats
Average infusions, last 3 days of LgA
D
Number of cocaine infusions
Breakpoint
PR
***
***
E
Number of cocaine infusions
PreShock
Shock
session
***
F
R = 0.58, p < 2.2e−16
Number of cocaine infusions during shock session
Number of cocaine infusions during preshock session
G
Irritability score (difference from baseline)
aggressive
defensive
total
***
**
***
cocaine
naive
H
PC2 (15.7% explained var.)
PC1 (48.6% explained var.)
PR 1
Shock
Compulsivity Index
PR 3 PR 2
Motivation Index
Escalation Index

Figure 1. Individual differences in addiction-behaviors in HS rats following intravenous cocaine self-administration. *A) Timeline of the behavioral paradigms. B) Number of cocaine infusions in the first hour of cocaine self-administration during short (2h, ShA) and long (6h, LgA) access (N=567, *** p < 0.0001 vs the first LgA session). C) Average number of daily infusions for the last 3 days of LgA (N=567). D) Violin plot of number of cocaine infusions under progressive ratio (PR) test at the after ShA (1) and LgA, before (2) and after (3) the Shock session (N= 560, *** p < 0.0001). E) Number of infusions despite footshock after LgA compared to a 1 h preshock session (N=466, *** p < 0.0001). F) Correlation of responding during the shock and preschock session is reproduced in each of 10 color coded cohorts (N=466, p < 0.0001). G) Difference in irritability scores after LgA and at baseline (N=380 + 49 naive, behavior *** p < 0.0001, ** p < 0.001 vs naive) H) Principal component analysis of cocaine infusions over all sessions ShA1-10, LgA1-14, PR1-3, Shock.*

Sex differences in addiction-like behaviors

Major sex differences were observed with females acquiring cocaine self-administration faster and at higher levels than males under short access condition (2 h/day, Fig. 2A) and during the extended access (6 h/day, Fig. 2A). A two-way ANOVA with sex as between factor and sessions as within factor showed a significant effect of sex ($F_{(1, 565)} = 54.87$; $p < 0.001$), sessions ($F_{(23, 565)} = 345.3$; $p < 0.001$) and of the interaction sex*sessions ($F_{(23, 12836)} = 5.542$; $p < 0.001$). The Bonferroni post hoc test demonstrated that females self-administered more cocaine compared to males starting from day 4 onward ($p < 0.0001$). Figure 2B shows a bimodal distribution with some rats maintaining very low levels of intake. The group of rats with very low intake was composed mostly of male rats. In the progressive ratio test, the two-way ANOVA with sex as between subjects factor and time as within subjects factor showed a significant effect of sex ($F_{(1, 558)} = 23.57$; $p < 0.001$), time ($F_{(1, 558)} = 332.65$; $p < 0.001$) but not a sex*time interaction ($F_{(1, 558)} = 0.35$; p = NS), confirming that motivation for cocaine was increased in both sexes over time from ShA to LgA (time, $p < 0.001$) and that females had significantly higher motivation for cocaine compared to males (sex, $p < 0.001$) independently from the length of access to cocaine (Fig. 2C) . Similar results were found for compulsive-like behavior, with females obtaining more infusions despite adverse consequences ($t_{565} = 7.86$; $p < 0.0001$ after t-test, Fig. 2D). We did not detect any sex-differences in irritability-like behavior (Fig. 2E), as demonstrated by the non-significant sex factor in the two-way ANOVA ($F_{(1, 425)} = 0.78$; p = NS). Males and females showed similar increases in the total irritability scores, compared to naïve rats, as demonstrated by the significant effect of groups ($F_{(34, 64)} = 34.64$; $p < 0.0001$). Also when breaking the total irritability score in defensive and aggressive responses, we found similar (non-significant sex

factor in two-way ANOVA, $F_{(1,854)} = 0.005$; p = NS) increases in the aggressive and defensive irritability scores between both sexes, compared to naives.

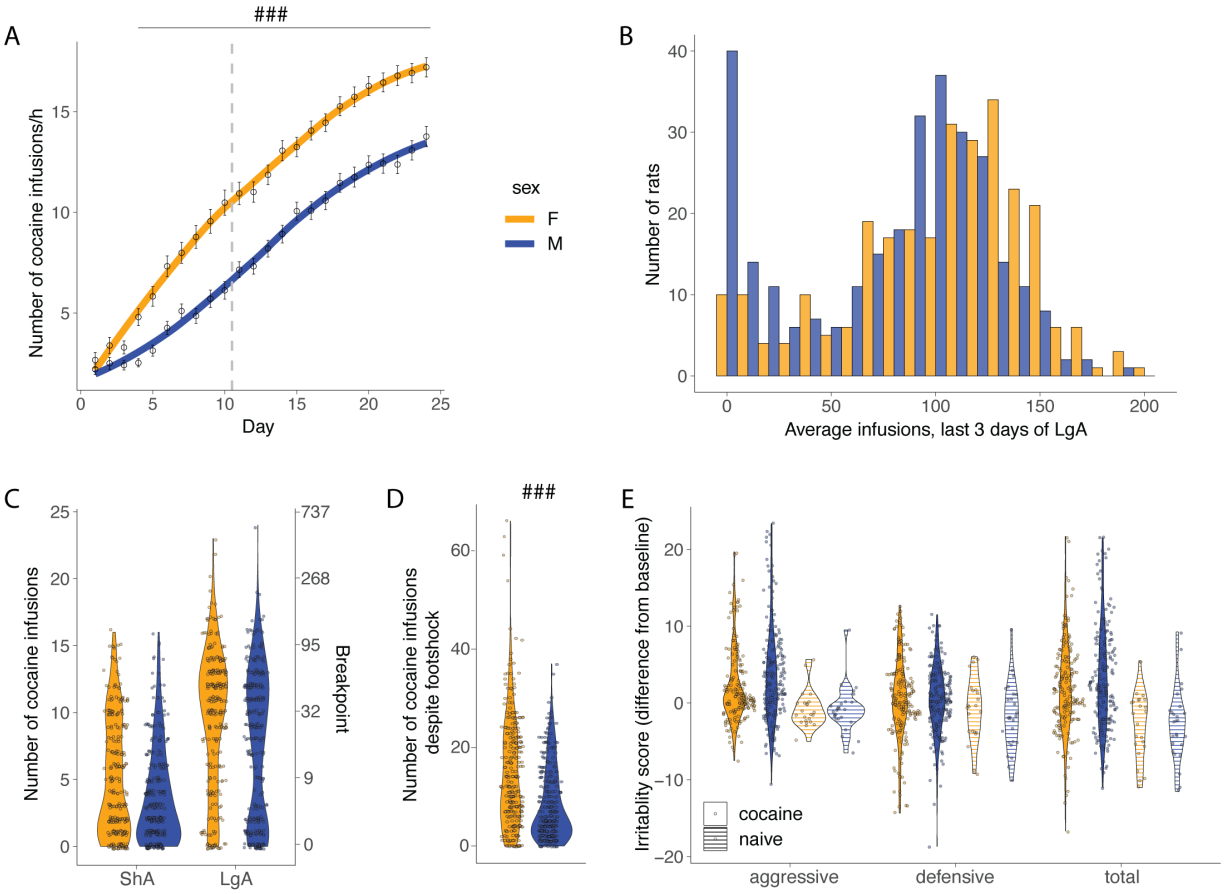

Figure 2. Sex differences in addiction-like behaviors. *A) Cocaine infusions during short (2h, ShA) and long (6h, LgA) access of cocaine self-administration (N = 275 F and 292 M, ### p <0.0001 vs males) B) Average number of daily infusions for the last 3 days of LgA in male and female rats. C) Violin plot of number of cocaine infusions under progressive ratio (PR) test after ShA and LgA (N = 273 F and 291 M). D) Number of infusions despite footshock after LgA (N= 275 F and 292 M, ### p < 0.0001). E) Increase in Irritability-like behavior in males and females after LgA compared to naive rats, no significant sex differences were detected (N = 186 F + 194 M + 23 naive F + 26 naive M).*

Individual differences in cocaine addiction-like behavior: characterization of 2 populations of rats

While all HS rats acquired cocaine self-administration during the ShA phase, we detected major individual differences in their escalation profile that resulted in a bimodal distribution of cocaine intake. We identified animals that were resilient (~20% of the entire population, they showed an

hourly intake of less than 8 infusions/h, similar to the intake during ShA, Fig 1B-C) or vulnerable (intake higher than 8 infusions/h) to cocaine addiction-like behaviors (Fig 3A-B). Vulnerable rats took more cocaine as demonstrated by the significant interaction on the two-way ANOVA with groups as between factor and time as within factor ($F_{(23, 12835)} = 85.02$; $p < 0.0001$). The Bonferroni post hoc showed higher levels of cocaine intake in the vulnerable population starting from ShA day 3. When looking at the escalation patterns in vulnerable and resilient rats, we found that the vulnerable group showed an escalation of cocaine intake (significant groups*day interaction, $F_{(13, 7344)} = 50.16$; $p < 0.0001$) starting from day 3 of extended access ($p < 0.001$ vs day 1, after two-way ANOVA followed by Bonferroni post hoc), while the rats in the resilient group did not show escalation of intake over the course of the behavioral paradigm, keeping the same level of cocaine infusions (Fig. 3A). Major differences between vulnerable and resilient rats were also observed in the motivation to obtain cocaine (PR, two-way ANOVA, significant groups* day interaction, $F_{(1, 558)} = 86.97$; $p < 0.0001$ followed by Bonferroni post hoc, Fig 3C) and in compulsive-like responding (foot shock, $t_{567} = 8.40$ $p < 0.001$, Fig 3D), but not in irritability-like behavior ($t_{378} = 1.17$ p = NS, Fig 3E). The difference in escalation of intake correlated with differences in the other addiction-like behaviors ($R = 0.6$, $p < 0.001$, for PR and $R = 0.37$, $p < 0.001$ for compulsivity), but did not correlate with irritability like behavior ($R = 0.048$, p = NS).

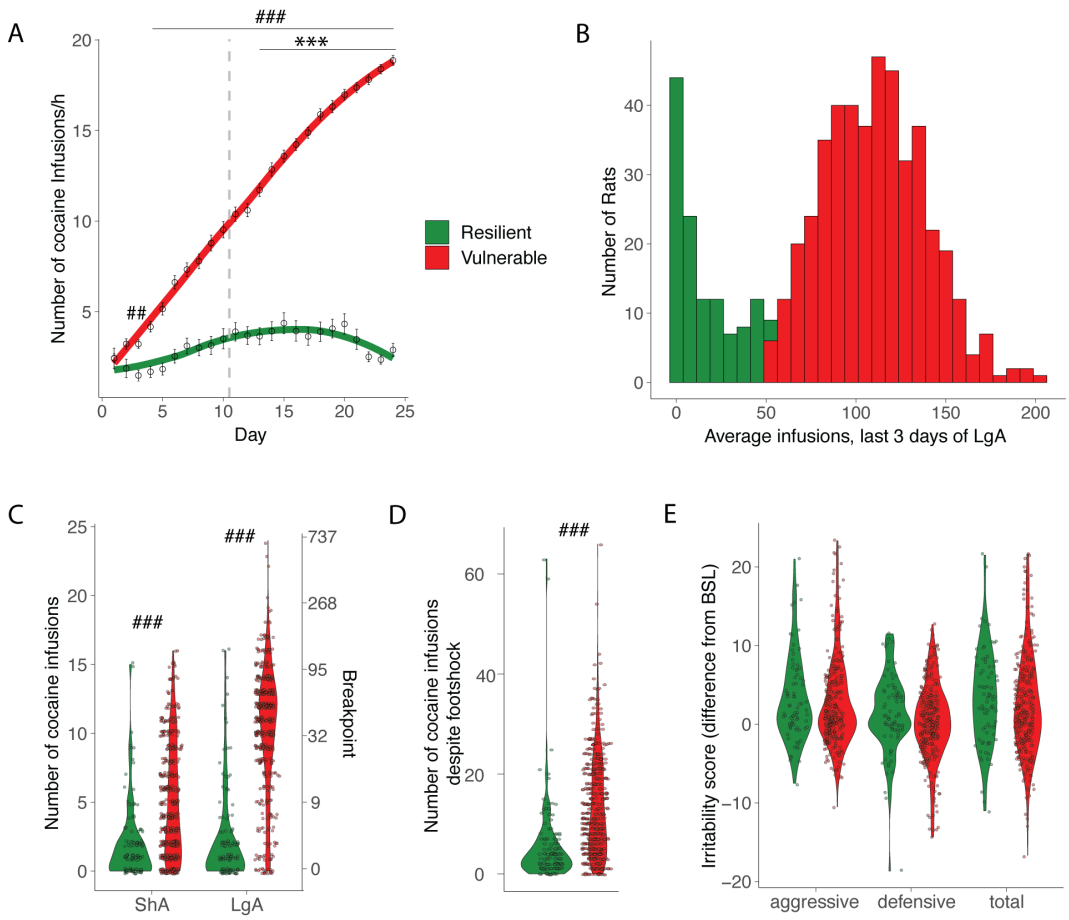

Figure 3. Correlation between the different addiction-like behaviors in resilient and vulnerable HS rats. *A) Number of cocaine infusions during short (2h, ShA) and long (6h, LgA) access of cocaine self-administration in resilient and vulnerable animals (N = 122 resilient and 445 vulnerable, *** $p < 0.001$ vs LgA day 1, ### $p < 0.0001$, ## $p < 0.001$ vs resilient). B) Average number of infusions over the last 3 days for the individual animals). C) Number of cocaine infusions under progressive ratio (PR) at the end of ShA and LgA (### $p < 0.0001$ vs resilient). D) Number of infusions despite footshock after LgA (### $p < 0.0001$ vs resilient). E) Irritability scores after LgA (N=79 resilient, 301 vulnerable).*

Addiction Index: evaluation of individual differences in addiction-like behaviors.

To take advantage of all the behaviors related to compulsive intake and withdrawal and further identify subjects that are consistently vulnerable *vs.* resilient to compulsive cocaine use, each measure was normalized into an index using its *Z*-score ($Z = \frac{x-\mu}{\sigma}$), where χ is the raw value, μ is the mean of the cohort, and σ is the standard deviation of the cohort. We thus obtained an FR Index, PR Index, and irritability Index. Figure 4A-D shows the z-scores for escalation, motivation, compulsive-like responding, and irritability-like behavior. A principal component

analysis incorporating the z-scores of the 4 behavioral measures showed that almost half of the variability (48%) can be explained by a single principal component (PC), which identified vulnerable and resilient rats and aligned with the escalation, motivation, and compulsivity z-scores (eigenvalue >1, explaining 48% of variance and to which all three behaviors contributed in a valuable way, $r = 0.52$ to 0.60, Fig. 4E). The irritability z-score was almost exactly perpendicular to it, indicating that there was little to no correlation with the other addiction-like behavioral measures ($r = 0.008$). For this reason, we calculated an Addiction Index, that provides a comprehensive evaluation of compulsive cocaine use, by averaging the Z-scores of the three dependent variables that explain almost 50% of the variance, escalation, motivation and compulsive-like behavior, leaving out irritability-like behavior (Fig. 4F).

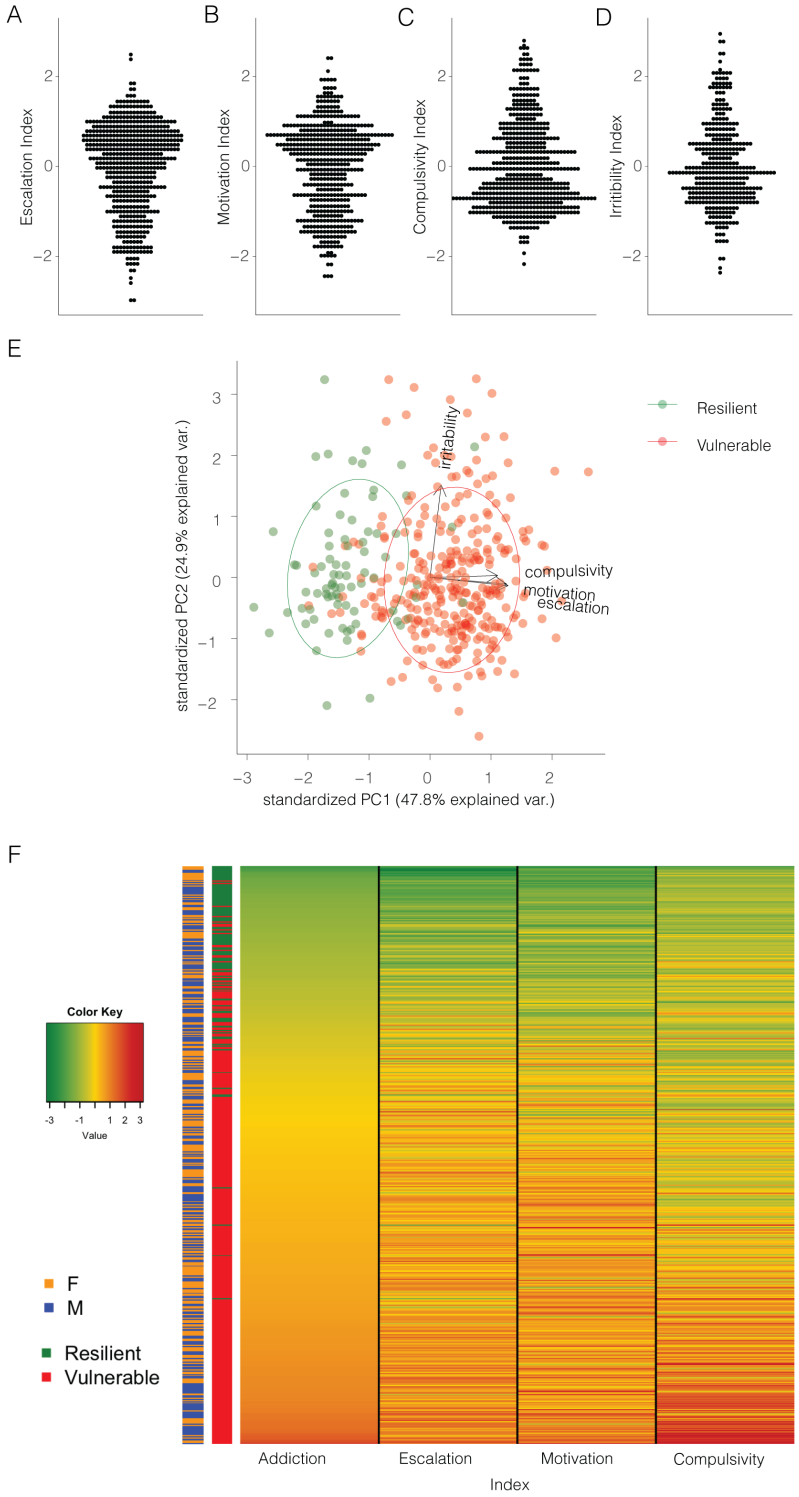

Figure 4. Normalizing and combining addiction like behaviors into an addiction index. *A) Z-score for escalation, B) motivation, C) compulsivity and D) irritability in the whole population (N=566). E) Representation of the individual rats, resilient (green) or vulnerable (red) along the two first principal components, based on escalation,*

motivation, compulsivity and irritability z-scores. F) Representation of the addiction index for the individual rats with the constituting individual z-scores and their identification as resilient or vulnerable and male or female.

Different degrees of addiction-like behavior

We used the addiction index to further differentiate between low, mild, moderate, and severe addiction-like behavior in the population, by dividing them into four quartiles. The intake in the different groups of rats, divided according to their Addiction Index over the last 3 days of LgA is shown in figures 5 A-B. Cocaine intake was increased from low over mild and moderate to severe groups. The two-way ANOVA with group as between factor and sessions as within factor showed a significant effect of group ($F_{(3, 511)} = 91.36$; $p < 0.0001$), sessions ($F_{(23, 511)} = 329.4$; $p < 0.0001$) and of the interaction group*sessions ($F_{(69, 11502)} = 18.48$; $p < 0.0001$). Pairwise comparisons for the significant main effects of groups, demonstrated that each of the subgroups (divided based on their Addiction Index) obtained more cocaine of their immediate lower subgroup (severe > moderate > mild > low, $p < 0.0001$; Fig 5A). In the progressive ratio test (Fig. 5C), the two-way ANOVA effect of group ($F_{(3, 504)} = 176.49$; $p < 0.0001$), time ($F_{(1, 504)} = 423.32$; $p < 0.0001$) and of the interaction group*time ($F_{(3, 504)} = 52.4$; $p < 0.0001$). Pairwise comparisons showed that all subgroups (divided based on their Addiction Index) except for the low addicted one, showed increased breakpoint after long access, compared to after short access ($p < 0.0001$ after Bonferroni post hoc). Within each timepoint, the mild, moderate, and severe subgroups showed increased motivation for cocaine to the low subgroup ($p < 0.0001$ after Bonferroni post hoc, Fig 5C). For the compulsivity test the one-way ANOVA showed a significant main effect ($F_{(3, 507)} = 76.6$; $p < 0.0001$) with each of the subgroups showing increased compulsivity compared to their immediate subgroup ($p < 0.001$ after the Bonferroni post hoc test, Fig. 5D).

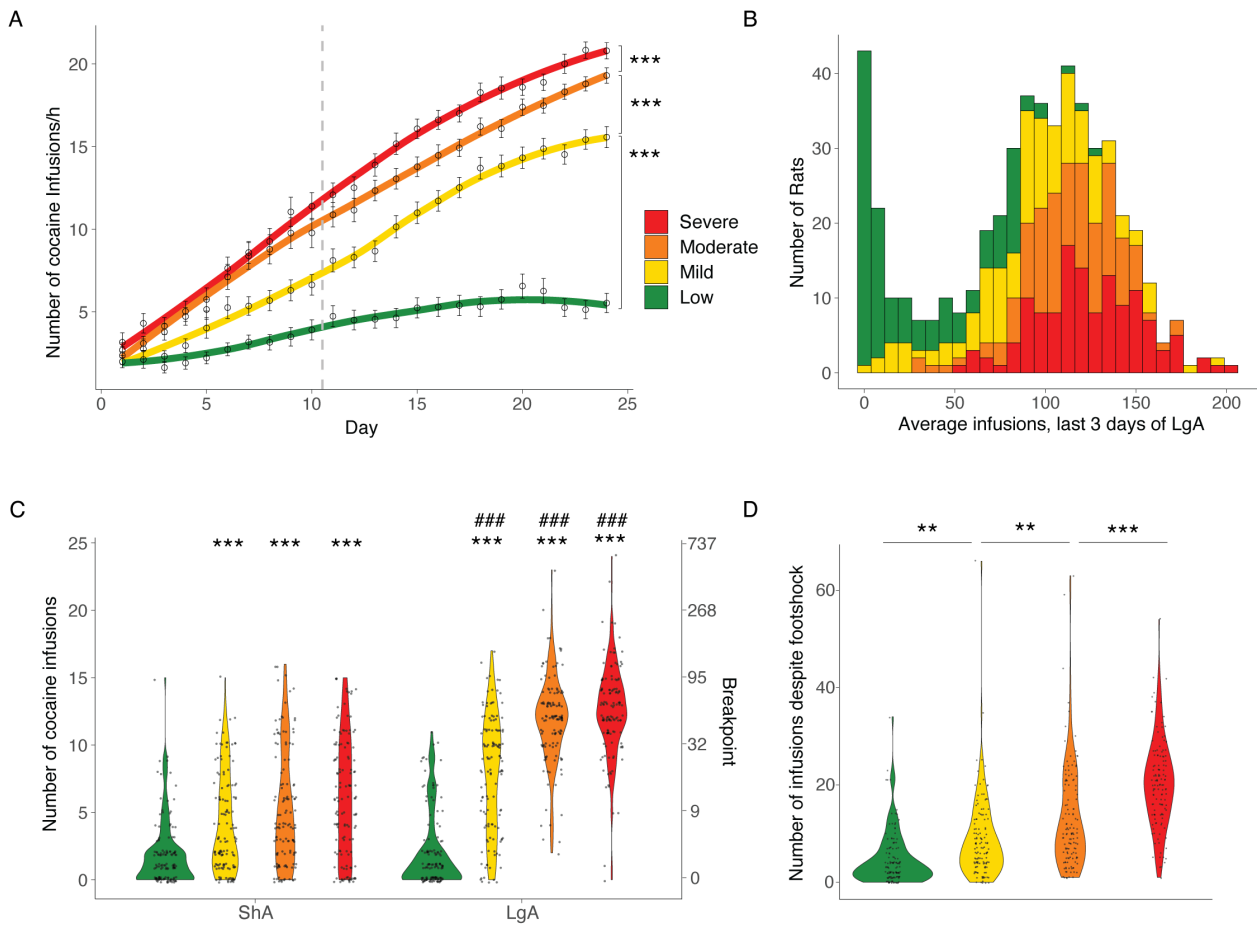

Figure 5. Different degrees of vulnerability to cocaine addictive behaviors. *A) Cocaine infusions during short (2h, ShA) and long (6h, LgA) access of cocaine self-administration (N = 128 Low + 128 Mild + 127 Moderate + 128 Severe; *** p <0.0001). B) Average number of cocaine infusions over the last 3 days for the individual animals in the low, mild moderate and severe vulnerable groups. C) Number of cocaine infusions under progressive ratio (PR) test at the end of the ShA and LgA phases (*** p < 0.0001 vs low, ### p <0.0001 vs ShA) D) Number of infusions despite footshock after LgA for the resilient and vulnerable groups (** p <0.001 and *** p <0.0001).*

Discussion

We characterized addiction-like behavior in 556 genetically diverse HS rats by establishing a large behavioral screening aimed at studying different cocaine-related behaviors (escalation, motivation, compulsive-like responding, and irritability-like behavior). A bimodal distribution was observed, with ~20% of rats maintaining low intake levels and ~80% escalating their cocaine intake when given long access. Females exhibited faster acquisition and higher levels of cocaine self-administration compared to males. Individual differences were found, with some rats categorized as resilient and others as vulnerable to addiction-like behaviors.

Vulnerable rats showed an increased escalation of cocaine intake, motivation, and compulsive-like responding. Correlational and principal component analysis revealed that a single construct explained 48% of the behavioral variability, separating vulnerable and resilient rats. This construct included the variables associated with escalation of intake, breaking point under a progressive ratio, and continued responding despite adverse consequences under long access, but not irritability-like measures or self-administration measures under short access. Moreover, responding before the shock session strongly predicted continued responding despite adverse consequences during the shock session. The use of an Addiction Index calculated based on the escalation of intake, breaking point under a progressive ratio, and continued responding despite adverse consequences allows to further identify different levels of severity of addiction-like behaviors from animals exhibiting resilience, low, moderate, and severe addiction-like behaviors.

We used a high throughput behavioral paradigm using a state-of-the-art model of extended access to intravenous self-administration combined with behavioral characterization of compulsivity using progressive ratio responding and responding despite adverse consequences (contingent foot shocks). To minimize cohort-specific effects, large cohorts (n = 46-60) were used and the behavior was normalized within cohorts using a Z-score. Intake after escalation was bimodally distributed. While ~80% of rats (vulnerable) escalated their cocaine intake after being given long access to cocaine, ~ 20% of rats (resilient) maintained a low and stable level of cocaine intake. The low and stable level of intake in resilient rats was not due to lack of catheter patency or failure to self-administer a reward (Sedighim et al., 2021). Withdrawal-induced irritability-like behavior was increased in rats with a history of cocaine self-administration compared to naïve aged-matched rats. This result is in line with previous evidence of a negative emotional states in the long-access model (Ahmed and Koob, 2005; Deroche-Gamonet et al., 2004; Mantsch et al., 2004), however, this increase in irritability-like behavior did not correlate with any self-administration measures and was orthogonal to them in the PCA, suggesting that the increased negative emotional state may be due to non-specific effects associated with the protocol including, surgery, chronic catheter implant, and daily testing for ~ 2 months. We found that females not only showed a low percentage of resilient animals but also showed higher levels of cocaine intake, motivation, and aversion resistance than males in rats with moderate and severe addiction-like behaviors. The increased motivation and compulsive-like responses in

females cannot simply be explained by the higher intake among females, as the significant difference remains when we compared only the vulnerable rats in both sexes (see Fig. S2). Previous studies have also shown significant sex differences in addiction. While cocaine use disorder is more prevalent in males than in females (SAMHSA, 2019), women may be more vulnerable to the reinforcing effects of cocaine, and acquire cocaine abuse faster and at higher levels than males (Cummings et al., 2011; Jackson et al., 2006). Sex differences were found in HS rats for cocaine cue preference (King et al., 2021). The current report was able to replicate and confirm these known effects and extend these findings by demonstrating that sex differences in cocaine self-administration may be not only quantitative (mild, moderate, severe addiction-like behaviors) but may also be qualitative (resilient, vulnerable) as over 2/3 of the resilient animals were male.

We choose the model of extended access to cocaine self-administration because it is highly relevant to cocaine use disorder (Edwards and Koob, 2013; George et al., 2014), and is associated with neuroadaptations that are also observed in humans with cocaine use disorder (Adinoff et al., 1990; Briand et al., 2008; George et al., 2008; George et al., 2012; Vendruscolo et al., 2012). There is now compelling evidence that escalation of cocaine intake using this protocol is associated with compulsive-like cocaine use as measured by an escalation of intake, increased progressive ratio responding, and increased resistance to adverse consequences (foot shocks). The escalation model has been shown to exhibit at least 7 of the 11 items in the DSM-V, including most of the criteria required for severe use disorder: 1) tolerance (Ben-Shahar et al., 2006), 2) withdrawal (Ahmed et al., 2002), 3) substance taken in larger amount than intended (Ahmed and Koob, 1998), 4) unsuccessful efforts to quit (Ahmed and Cador, 2006; Lenoir et al., 2007), 5) considerable time spent to obtain the drug (Wee et al., 2008), 6) important social, work or recreational activities given up because of use (George et al., 2008; Lenoir et al., 2013), 7) continued use despite adverse consequence (Seif et al., 2013; Vanderschuren and Everitt, 2004; Vendruscolo et al., 2012). Given these parallels, the extended access model could serve as a robust platform for understanding the neurobiological changes underpinning cocaine use disorder, thereby providing a valuable tool for further research into the complexities of this condition.

Addiction is commonly described as a chronic, recurring condition characterized by excessive drug consumption, compulsive drug seeking, and continued drug use despite negative consequences (SAMHSA, 2019). There is a prevailing notion in preclinical addiction research that the only way to identify addiction-like behaviors in individuals is by measuring compulsive drug use and seeking using continued responding despite adverse consequences. Continued drug use or seeking in the face of punishment, such as painful footshocks or aversive tastes, is widely considered the behavioral hallmark of compulsive-like drug-seeking behavior in preclinical models (Chen et al., 2013; Domi et al., 2021; Giuliano et al., 2019; Li et al., 2021; Siciliano et al., 2019; Timme et al., 2022). A key argument in favor of this hypothesis is that several studies have found that drug intake in the absence of an adverse consequence does not predict drug intake in the presence of an adverse consequence, suggesting that they are independent behavioral measures and that only measures of drug taking/seeking in the context of punishment can measure compulsive-like drug taking/seeking (Chen et al., 2013; Domi et al., 2021; Giuliano et al., 2019; Li et al., 2021; Siciliano et al., 2019; Timme et al., 2022). However, the results from the present report provide strong evidence that in heterogeneous outbred rats, continued responding despite adverse consequences is not an independent measure of compulsive-like responding that is orthogonal to other measures such as escalation of intake and motivation under a progressive ratio schedule of reinforcement. Both correlational and principal component analyses demonstrate that these three behaviors are highly correlated and load onto the same underlying construct. A possible explanation as to why previous studies failed to observe this correlation between escalation, motivation, and aversion-resistance is that most of the previous studies used small sample size that may not provide sufficient statistical power to observe this relationship between variables. Another explanation is that previous studies often used animal models with limited access to the drug where animals exhibit low levels of acute intoxication and very little if any signs of drug dependence (George et al., 2022). The present results suggest that while drug responding despite adverse consequences may be independent of drug intake in nondependent animals with limited access to the drug, escalation of drug intake highly predicts drug responding despite adverse consequences in an animal model with long access to the drug, high level of intoxication, and evidence of drug dependence.

This report also demonstrates the feasibility of characterizing a very large population of heterogeneous rats for addiction-like behavior using an advanced animal model of drug self-administration. Such a large-scale behavioral approach is required to unveil the genetic basis of addiction-like behavior. The evaluation of different addiction-like behaviors is important as multiple elements of addiction vulnerability were found to be independently heritable (Eid et al., 2019), and likely controlled by distinct genes that remain to be identified. Previous studies have clearly identified strain differences in the propensity for escalation of drug intake, which speaks to its heritability (Meyer et al., 2010). For instance, HR rats acquire cocaine self-administration more rapidly than LR rats (Davis et al., 2008). Lewis rats strongly prefer higher doses of cocaine than Fischer rats and show different level of (Cadoni and Di Chiara, 2007; Picetti et al., 2010; Valenza et al., 2016). The behavioral characterization of HS rats was the first step of characterizing and understanding individual differences in a genetically diverse population. Future studies will combine these differences with genetic data to perform a genome wide association studies (GWAS) aimed at identifying specific alleles and gene that contribute to vulnerability and resilience to cocaine addiction-like behavior (Chitre et al., 2020). Moreover, biological samples from animals that are resilient or have mild, moderate, or severe addiction-like behaviors are currently available through the Cocaine Biobank (www.cocainebiobank.org) to facilitate collaboration and investigation on the biological mechanisms of addiction-like behaviors (Carrette et al., 2021).

In summary, this study examined addiction-like behaviors in a large population of rats. We found that ~80% of the rats escalated their cocaine intake, while ~20% maintained low stable levels. Females had higher susceptibility to cocaine self-administration than males, both qualitatively and quantitively. Correlational and principal component analyses showed that escalation of intake, aversion-resistant responding, and breaking points are highly correlated measures of the same construct in animals with extended access to the drug. This study provides in-depth characterization of heterogeneous rats with a variety of addiction-like profiles from resilience to mild, moderate, and severe addiction-like behaviors that can be used to investigate the biological mechanisms of addiction.

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Supplementary Figure

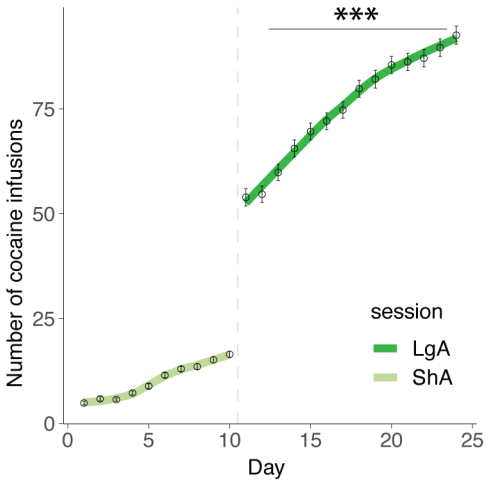

Figure S1. Total number of cocaine infusions during short (2h, ShA) and long (6h, LgA) access.

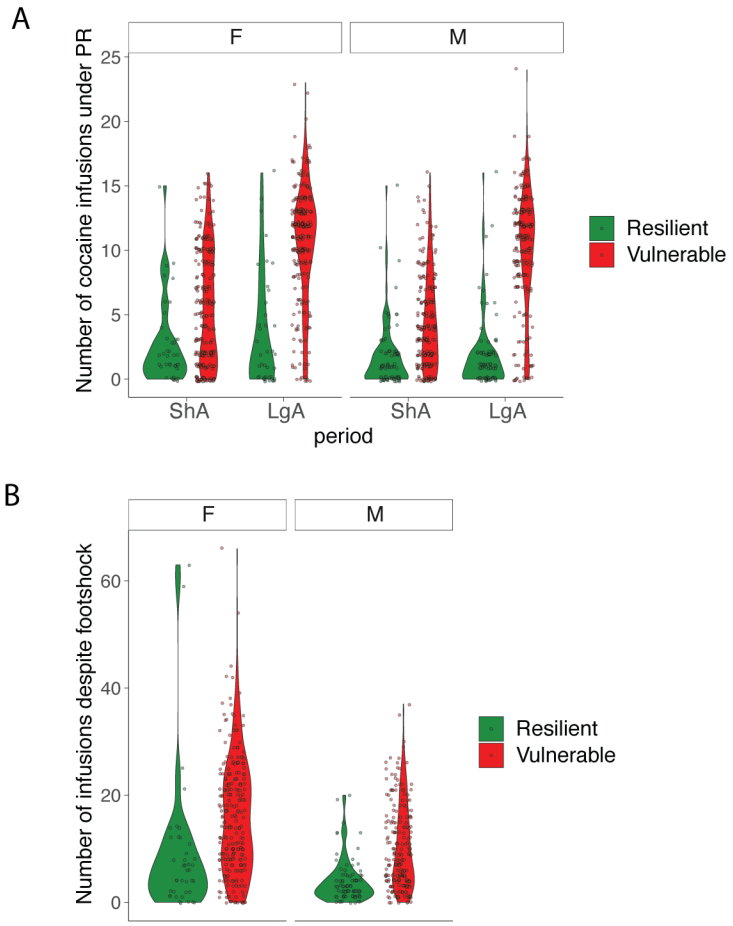

Figure S2. Motivation (A) and compulsivity (B) for the vulnerable and resilient populations split by sex