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Susceptibility to traumatic stress sensitizes the dopaminergic response to cocaine and increases motivation for cocaine

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Abstract

Patients with post-traumatic stress disorder have a heightened vulnerability to developing substance use disorders; however, the biological underpinnings of this vulnerability remain unresolved. We used the predator odor stress model of post-traumatic stress disorder with segregation of subjects as susceptible or resilient based on elevated plus maze behavior and context avoidance. We then determined behavioral and neurochemical differences across susceptible, resilient, and control populations using a panel of behavioral and neurochemical assays. Susceptible subjects showed a significant increase in the motoric and dopaminergic effects of cocaine, and this corresponded with heightened motivation to self-administer cocaine. Resilient subjects did not show differences in the motoric effects of cocaine, in dopamine signaling *vivo*, or in any measure of cocaine self-administration. Nonetheless, we found that these animals displayed elevations in both the dopamine release-promoting effects of cocaine and dopamine autoreceptor sensitivity *ex vivo*. Our results suggest that the experience of traumatic stress may produce alterations in dopamine systems that drive elevations in cocaine self-administration behavior in susceptible subjects, but may also produce both active and passive forms of resilience that function to prevent gross changes in cocaine's reinforcing efficacy in resilient subjects.

Keywords

PTSD; DAT; dopamine; resilient; susceptible; voltammetry

1.0 Introduction

Post-traumatic stress disorder (PTSD) and substance use disorder are highly co-morbid psychiatric conditions (Jacobsen et al., 2001; Kessler et al., 1995; Pietrzak et al., 2011), with PTSD onset generally occurring prior to the development of substance use disorders (Jacobsen et al., 2001). These observations suggest that the experience of traumatic stress may confer a vulnerability to developing substance use disorders. Interestingly, only 20–

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30% of individuals who experience traumatic stress develop PTSD symptoms (Cohen et al., 2012), and individuals that undergo traumatic stress without developing PTSD do not show an increased vulnerability to developing substance use disorders (Chilcoat and Breslau, 1998). This evidence indicates that the development of PTSD, rather than the experience of traumatic stress *per se*, is tied to the development of substance use disorder vulnerability in humans.

Animal models of PTSD and addiction may be useful for discerning some of the behavioral and biological disruptions that predispose an individual to developing substance use disorders following traumatic stress. Indeed, increases in motoric sensitization to cocaine (Garcia-Keller et al., 2013; Prasad et al., 1998) as well as increases in various aspects of cocaine self-administration have been observed following multiple stress protocols (Boyson et al., 2014; Garcia-Keller et al., 2015; Goeders, 2002; Miczek et al., 2011; Piazza and Le Moal, 1998; Tidey and Miczek, 1997). While these studies have generally examined stressed subjects as a homogenous population, it has recently become evident that, like in humans, stress exposure often produces a heterogeneous population in rodents (Cao et al., 2010; Cohen et al., 2012; Edwards et al., 2013; Friedman et al., 2014; Koresh et al., 2016; Krishnan et al., 2007; Levkovitz et al., 2015). This heterogeneity in response to stress may mask some of the behavioral and physiological factors that predispose individuals to developing cocaine self-administration behaviors when stressed populations are viewed as a homogenous group (Holly and Miczek, 2015). To date, the relationship between the variable expression of behavioral aberrations following traumatic stress and cocaine self-administration has not been directly tested (Holly and Miczek, 2015).

Emerging evidence suggests that susceptibility may be tied to neuroadaptations that underlie the propensity for cocaine addiction. Specifically, physiological changes to mesolimbic dopamine (DA) neurons determine susceptibility vs resilience to stress (Friedman et al., 2014; Krishnan et al., 2007), and extensive evidence indicates that the mesolimbic DA system is critically involved in the acute reinforcing effects of cocaine (Koob and Volkow, 2010; Roberts et al., 2013). Consistent with these observations, susceptible and resilient rodents express differential conditioned place preference for cocaine (Krishnan et al., 2007), and thus it is feasible that susceptible and resilient subjects may also express differences in the dopaminergic response to cocaine.

We sought to determine if the neurochemical and behavioral effects of cocaine varied with the appearance of maladaptive behaviors following traumatic stress. To this end, we used the predator odor stress model of PTSD, which has repeatedly been shown to produce prolonged behavioral changes representative of PTSD symptoms (Cohen et al., 2012; Cohen and Zohar, 2004; Edwards et al., 2013). We first characterized the heterogeneous response to predator scent stress in our laboratory by measuring a panel of PTSD-like phenotypic indicants. We then used this data to define behavioral cutoffs which we then used to segregate subjects based on anxiety behavior in the elevated plus maze (Cohen et al., 2012) and context avoidance (Edwards et al., 2013). Following behavioral segregation we used *in vivo* microdialysis or *ex vivo* fast scan cyclic voltammetry (FSCV) to query differences in the mesolimbic DA system of cocaine-naïve rats following traumatic stress. Finally, we tested how the variable response to stress corresponds to changes in the behavioral economics of

cocaine self-administration. Our results provide the first evidence that susceptible, but not resilient, rats express increases in the motivation to self-administer cocaine, and provide a putative mechanism by which enhanced dopaminergic sensitivity to cocaine drives elevations in the reinforcing effects of cocaine.

2.0 Methods

2.1 Animals

Male Sprague–Dawley rats (300–350 g, Harlan, Frederick, MD) were given ad libitum access to food and water and kept on a reverse 12:12 h light:dark cycle (lights on at 15:00 h). All protocols and animal care procedures were maintained in accordance with the National Research Council's Guide for the Care and Use of Laboratory Animals: Eighth Edition (The National Academies Press, Washington, DC, 2011) and approved by the Institutional Animal Care and Use Committee at Drexel University College of Medicine.

2.2 Chemicals

Cocaine hydrochloride was obtained from the National Institute on Drug Abuse. 2,4,5-trimethyl-3-thiazoline (TMT), butyric acid, sucrose, and all reagents used to make Dulbecco's phosphate buffered saline and artificial cerebrospinal fluid were obtained from Sigma–Aldrich (St. Louis, MO).

2.3 Predator odor stress, context avoidance, and elevated plus maze testing

Treatment of control and stressed rats differed only in the odor of exposure, otherwise all rats underwent the same series of procedures. Stressed rats were exposed to TMT, a compound isolated from fox feces that produces a reliable fear response (Endres and Fendt, 2009) and increases PTSD-like behaviors (Endres and Fendt, 2009; Hacquemand et al., 2013). Control rats were exposed to butyric acid, an unpleasant but not fear-inducing odor (Endres and Fendt, 2009).

Odor exposure and context avoidance testing were performed in a three-chamber place conditioning box (Med Associates, St. Albans, VT) held within a custom cabinet fitted with a ventilation system, bright lighting, and an overhead camera. Rats were exposed within one of two context chambers (11" × 8.5" × 8.5") that differed in both visual (white with vertical black stripes vs. black with horizontal white stripes) and tactile (grid vs. bar floor) features. On the first day of testing, rats were placed in the gray center chamber (6" × 8.5" × 8.5") and were allowed to freely explore all chambers of the apparatus in a recorded 5-min preference test. Time spent in each context chamber was recorded as baseline preference. All rats were single-housed following initial testing. On the following day, rats were confined to one of the two context chambers and 10 µl of either TMT or butyric acid was pipetted onto tissue paper placed below the chamber floor. Odor exposures were performed at the beginning of the light phase (ZT 0:00), and lasted for 15 min.

Elevated plus maze and context avoidance tests were performed 7 days after odor exposure since it has been repeatedly shown that maladaptive behaviors apparent at 7 days after predator odor stress generally persist over extended periods (Cohen et al., 2012; Cohen et

al., 2004). Rats were first tested in a recorded 5-min, free exploration of an elevated plus maze (File et al., 2004). Elevated plus maze tests were performed under dim red light, and were recorded with an overhead camera. To quantify behavior in the elevated plus maze, we measured time spent in the open arms of the maze. Animals were scored as being in an open or closed arm only when both forepaws passed over the open/closed dividing line. All subjects that fell off of the maze were excluded from further testing.

To test for context avoidance, rats were placed in the center chamber of the place conditioning box and were allowed to freely explore all three chambers of the apparatus in a recorded 5-min avoidance test. Time spent in the predator odor-paired and the unpaired chambers was scored. To quantify context avoidance behavior we calculated the change in time spent in the odor-paired chamber (Paired), which was defined as the difference in time spent in the odor-paired chamber during the avoidance and preference tests. Likewise, we calculated nonspecific avoidance (Unpaired) as the difference in time spent in the unpaired chamber during the avoidance and preference tests. All animals included in these studies underwent this series of procedures and tests, and we refer to this process as “stress and segregation testing” (Fig. 1A).

2.4 Acoustic startle response and circulating corticosterone testing

A cohort of rats was tested for acoustic startle response and late-dark phase corticosterone levels on the day after elevated plus maze and context avoidance testing. Acoustic startle response was measured in sound-attenuated startle chambers (SR-LAB system, San Diego Instruments, San Diego, CA) consisting of a Plexiglas cylinder resting on a movement-sensitive platform. Sound levels inside the chambers were calibrated with a sound level meter (Radio Shack, Fort Worth, Texas), and platform sensitivity was calibrated daily. Rats were tested in pairs and underwent a 5-min acclimation period with background noise of 68 dB before the onset of acoustic startle trials. The experiment consisted of 6 blocks of 5 trials, for a total of 30 trials, each containing a 120 dB burst of noise that lasted for 40 ms. Inter-trial intervals varied from 12 to 30 sec with an average interval of 15 sec. Acoustic startle response was calculated as the average startle amplitude across 30 trials. Percent habituation was calculated as: [(Average amplitude of Block 1 – Average amplitude of Block 6)/Average amplitude of Block 1] * 100%.

Rats were allowed to rest for 4 hr between acoustic startle response testing and the blood collection for testing corticosterone levels. Whole blood was drawn via saphenous vein puncture and collected in polypropylene serum collection tubes containing a clotting activator (Sarstedt, Kommanditgesellschaft, Germany). Blood was then allowed to rest at room temperature for 30 min before Samples were then centrifuged at $2000 \times g$ for 10 min at 4°C. Serum was frozen at -80°C prior to processing and analysis.

Corticosterone was measured by liquid chromatography-mass spectrometry using adaptation of previously published method for keto-steroids (Frey et al., 2016). Briefly, 20 µL of serum was extracted by addition of 20 µL of (100 pg/µL in methanol as an internal standard) $^2\text{H}_4$ -corticosterone, 15 µL saturated NaCl, acidification with 5 µL 1N HCl, 0.5 mL methyl-tert-butyl-ether, and 80 µL water. After mixing by vortexing for 10 min, phases were separated by centrifugation at $2000 \times g$ for 5 min at 4°C, then the upper organic layer was removed by

freezing the bottom layer at -80°C and decanting to a new container. The sample was then dried under nitrogen and derivatized by adding 200 μL 10% acetic acid in methanol, adding 20 μL Girard P reagent (1 mg/mL in water), vortexing, then incubating at 60°C for 10 min, followed by drying to completeness under nitrogen. The sample was resuspended in 100 μL 80:20 (v/v) methanol:water and 10 μL was injected for analysis on a Ultimate 3000 UHPLC coupled to a Q Exactive Plus operating in positive ion mode. A standard curve was prepared and analyzed in parallel to the serum samples from 500 pg/ μL and was linear across the range analyzed. Instrument control was through Xcalibur (ThermoFisher Scientific, Waltham, MA) and peak detection and integration were through Tracefinder (ThermoFisher Scientific) with quantitation using the exact parent ion mass (± 2.5 ppm) with a qualitative confirming product ion specific to the Girard P derivative.

2.5 Sucrose preference and locomotor activity testing

A second cohort of rats was tested for sucrose preference and locomotor activity following stress and segregation testing. For sucrose preference tests we used a two-bottle choice procedure similar to previous reports (Eagle et al., 2016). Rats were acclimated to two drip resistant water bottles for a total of 3 days prior to segregation testing. Following segregation, the water in one of the bottles was replaced with a 0.25% sucrose solution for the first 48 hr and then a 1% sucrose solution for the next 48 hr in order to prevent a negative contrast effect (Grigson et al., 1993). Rats were allowed equal access to the sucrose and water bottles for the entirety of the testing period. The side on which the sucrose bottle was initially placed was counterbalanced across rats. Bottles were weighed every 24 hr and then replaced on the opposite side of the wire cage lid in order to ensure that no side preferences developed. Sucrose and water intake were calculated as the difference between the bottle weight immediately following filling and the bottle weight following each 48 hr testing period. Sucrose preference was expressed as the percent of total fluid intake and was calculated individually for each sucrose concentration.

Following sucrose preference testing, we tested locomotor activity in an open field on the day after the completion of sucrose preference testing. On the day of testing, rats were acclimated to the lit room for 30 min before being placed in the center of the *SmartFrame* Open Field System (Kinder Scientific, Poway, CA). The distance traveled, as recorded by the number of beam breaks, was measured by MotorMonitor software (Kinder Scientific). Locomotor testing consisted of three consecutive 60-min sessions. In the first session, locomotor activity in response to the novel environment was measured. Immediately following novel environment testing, rats received an intraperitoneal (i.p.) injection of saline and were monitored for an additional 60 min. In the third session, which immediately followed the saline testing session, rats received an i.p. injection of 10 mg/kg cocaine and a final 60 min of locomotor activity was monitored.

2.6 Microdialysis

A third cohort of rats was tested for basal DA tone and the dopaminergic response to cocaine with microdialysis on the day following stress and segregation. Microdialysis surgery, in which a guide cannula was stereotactically implanted above the lateral NAc (A/P: +1.2, M/L: +1.4, D/V: -6.0), was conducted 6–10 days prior to stress and segregation. Rats received

post-surgical antibiotic (Neo-Predef, Pharmacia & Upjohn Company, New York, NY) and analgesic (5 mg/kg; Ketoprofen, Patterson Veterinary, Devens, MA). Following behavioral segregation, a concentric microdialysis probe (PAN. 30 kDa, MWCO, 320- μ m OD, 2-mm active membrane; Bioanalytical Systems, West Lafayette, Indiana) was implanted under isoflurane anesthesia via the guide cannula. Probes were flushed with Dulbecco's phosphate buffered saline (in milliequivalents: 137 NaCl, 2.7 KCl, 0.5 MgCl₂, 1.5 KH₂PO₄, 8.1 Na₂HPO₄, 1.2 CaCl₂, and 5 % glucose at pH 7.4) overnight at 0.2 μ l/min. Microdialysis experiments began on the following morning, and were completed as previously described (Brodnik et al., 2012; Brodnik et al., 2017a).

On the day of experimentation, probes were perfused at 1.0 μ l/min for at least 2.5 hr before beginning the collection of baseline samples at 20 min intervals. Following baseline collections, rats received an i.p. injection of saline vehicle, and 1 hr later received an i.p. injection of 10 mg/kg cocaine as previously described (Miczek et al., 2011).

Samples were analyzed by HPLC coupled with electrochemical detection as previously described (Brodnik and Jaskiw, 2014). Separation was achieved with a 100 $\times$ 3-mm reversed-phase C18 column with 3- μ m particles (Phenomenex Luna C-18(2), CA, USA) column and a mobile phase containing (in mM) 12.5 citrate, 20.0 acetate, and 0.1 EDTA, with 5 % (v/v) methanol adjusted to pH 4.5 with sodium hydroxide and 0–3.0 mM octylsulfonic acid adjusted as a modifier. DA was quantified with a glassy carbon electrode and maintained at a relative potential of 0.50 V to an Ag/AgCl reference electrode (Antec Decade Elite, Zoeterwoude, The Netherlands). The limit of detection for this apparatus during these studies was 50 fg/10 μ l at a 3:1 signal to noise ratio.

2.7 Fast-scan cyclic voltammetry

A fourth cohort of rats was tested for baseline dopamine release and uptake, cocaine sensitivity, and D2 autoreceptor sensitivity using in vitro voltammetry on the day after stress and segregation. Voltammetry experiments were carried out as previously described (Brodnik and España, 2015; Brodnik et al., 2017b), and performed on the day after behavioral segregation. Rats were anesthetized for 3 min with 2.5% isoflurane before decapitation. Following decapitation, brains were quickly removed and transferred to ice cold oxygenated artificial cerebrospinal fluid (ACSF) containing, (in mM; NaCl (126), KCl (2.5), NaH₂PO₄(1.2), CaCl₂(2.4), MgCl₂(1.2), NaHCO₃(25), glucose (11), l-ascorbic acid (0.4), pH adjusted to 7.4). Coronal slices containing the NAc were prepared using a vibrating tissue slicer, and were then transferred into a continuously oxygenated ACSF bath at room temperature. Following a 30-min recovery period, slices were transferred into a testing chamber flushed with ACSF (32°). A bipolar stimulating electrode was placed on the surface of the slice and a carbon fiber microelectrode was placed within the lateral NAc. DA release was elicited with single electrical stimulation (400 μ A, 4ms, monophasic) every 5 min. Stable baseline DA release and uptake were recorded (<10% variation) before experimental compounds were applied cumulatively as previously described (Brodnik and España, 2015; Mateo et al., 2005). In these experiments, the concentration of drug was increased when stability was reached (3 stimulations with <10% variation), and in general this occurred following approximately 25 min of drug exposure.

Evoked DA release was calculated relative to post-hoc electrode calibrations. DA uptake was analyzed using a Michaelis-Menton based model (Yorgason et al., 2011). Baseline uptake was determined by setting K_m values to 0.18 μM and establishing baseline $V_{\max}$ for each individual subject. For cocaine experiments, alterations in uptake were attributed to changes in apparent K_m . Inhibition constants (K_i) were defined as the slope of a linear regression of K_m values across cocaine doses divided by baseline K_m (0.18 μM ; Calipari et al., 2015).

2.8 Cocaine self-administration

A fifth cohort of rats was trained to self-administer cocaine, and tested on the threshold self-administration paradigm following stress and segregation. Self-administration surgeries were performed as previously described (Brodnik et al., 2015; Levy et al., 2017). Rats were anesthetized using ketamine (100 mg/kg) and xylazine (10 mg/kg), and were implanted with an intravenous silastic catheter placed into the right jugular vein. The catheter was connected to a cannula which exited through the skin on the dorsal surface in the region of the scapulae. Rats received post-surgical antibiotic (Neo-Predef) and analgesic (5 mg/kg; Ketoprofen), and recovered for 6–10 days before stress and segregation. Intravenous catheters were manually flushed with saline every 2–3 days during recovery in order to maintain catheter patency.

Following stress and segregation, intravenous catheters were connected through a stainless steel spring attached to a counterbalance and rats were provided access to cocaine on the following morning. The behavioral economics of cocaine self-administration was assessed using the threshold self-administration paradigm. Rats were initially trained on a fixed ratio 1 (FR1) schedule for 0.75 mg/kg cocaine. Training was deemed complete when a rat obtained 20 injections of cocaine on three consecutive days. This training paradigm was used because previous studies have determined that this procedure has a minimal impact on cocaine consumption or on the motivation to self-administer cocaine (Roberts et al., 2007). Once rats met training criteria, they were switched to the threshold schedule of reinforcement. Under the threshold schedule, rats have access to a descending series of 11 unit doses of cocaine (421, 237, 133, 75, 41, 24, 13, 7.5, 4.1, 2.4, and 1.3 $\mu\text{g}/\text{injection}$) available on an FR1 schedule of reinforcement. Each dose is available for 10 min, and doses are available consecutively across a total 110 min session (Calipari et al., 2015; España et al., 2010). At the beginning of the session, when doses are relatively high, rats can reach preferred blood levels of cocaine with minimal responding. Later in the session, when doses are reduced, rats must respond with a greater degree of motivation to maintain preferred blood levels. These two phases of responding can be analyzed using behavioral economic principles to determine consumption at null cost (Q_0) as a proxy for defining preferred blood levels of cocaine, and maximal price paid ($P_{\max}$) for defining motivation to obtain cocaine.

We modeled responding under the threshold schedule in order to mathematically determine Q_0 and $P_{\max}$ according to previous reports (Calipari et al., 2015). Briefly, $P_{\max}$ and Q_0 values were calculated using a demand curve that was generated by curve-fitting individual animals' intake across each single session using an equation: $\log(Q) = \log(Q_0) + k \times (e^{-a \times Q_0} \times C - 1)$. For each animal, Q_0 and $P_{\max}$ were derived from the first 3 days of stable responding under the threshold schedule.

2.9 Statistics

All data were analyzed using IBM SPSS Statistics 24. Specific analyses are reported in respective figure captions.

3.0 Results

3.1 Predator odor stress results in susceptible and resilient populations

Clinical studies of PTSD distinguish affected and unaffected individuals using cutoff criteria based on symptom severity, and most iterations of the predator odor exposure model of PTSD mirror this by using behavioral cutoff criteria to define susceptible and resilient populations. However, different approaches for segregating populations have been employed across studies using the predator odor exposure model (Cohen et al., 2012; Edwards et al., 2013; Hadad et al., 2016). We first sought to characterize this model in our laboratory, and then to define a segregation procedure that is appropriate based on this characterization. Rats were tested for markers of a PTSD-like phenotype seven and eight days after odor exposure (Fig. 1A). For these experiments, we tested anxiety-like behavior in the elevated plus maze to model the ‘negative alterations in cognition and mood’ PTSD diagnostic symptom cluster (Cohen et al., 2012), context aversion to model the ‘avoidance’ symptom cluster (Edwards et al., 2013), acoustic startle response to model the ‘hypervigilance’ symptom cluster (Cohen et al., 2012), and tested for elevated corticosterone levels during the late waking phase (ZT 22:00) as a marker for HPA-axis dysregulation that is characteristic of PTSD (Dayan et al., 2017). To define behavioral cutoff criteria, we employed an iterative four-dimensional (open arm time, paired, acoustic startle response, and late waking phase corticosterone) k-means cluster analysis to define two subgroups (Supplemental Fig. 1). Clusters were defined “Cluster A” and “Cluster B” and we found that subjects in Cluster B expressed lower open arm time in the elevated plus maze, greater conditioned place avoidance, higher acoustic startle response, and higher late waking phase circulating corticosterone levels relative to Cluster A (Figure 1B–E). This analysis indicates that subjects in Cluster B express elevations in PTSD-like phenotype indicants, relative to Cluster A.

To identify which PTSD phenotype indicants can be used to define behavioral cutoff criteria that effectively represent affected and unaffected populations, we examined relationships between each PTSD-like phenotype measure. We found significant correlations between time spent in the open arms of the elevated plus maze and context avoidance with each of the other PTSD-like phenotype measures, but found that acoustic startle response and late waking phase corticosterone levels did not significantly correlate with one another (Table 1). This suggests that anxiety-like behavior in the elevated plus maze and context avoidance are particularly suited to classify the phenotypic attributes of susceptible and resilient populations in this model. Thus, a cutoff criteria algorithm was established based on time spent in the open arms of the elevated plus maze and change in time spent in the odor-paired chamber. Specifically, cutoff criteria were defined as the mean plus one standard deviation of Cluster B (Time in open arms less than 50s and Paired less than –20s), and all rats that did not meet susceptible criteria were deemed resilient. This algorithm was used for all subsequent experiments to define populations that were susceptible or resilient to predator odor stress (Fig. 1F).

3.2 Susceptible and resilient subjects express distinct syndromes

To further validate our segregation procedures, and to ensure that animals classified as susceptible display aberrant behavior relative to controls, we compared resilient, susceptible and control subjects across a panel of assays. Control subjects were exposed to the unpleasant, but not fear-inducing odor butyric acid. When viewed as a homogenous population, predator odor exposed subjects showed reduced time spent in the open arms of the elevated plus maze and significant avoidance of the odor-paired chamber, but did not show significant differences in time spent in the unpaired chamber when compared to controls (Fig. 2A–C). Individual predator odor exposed subjects were classified as susceptible when both behavioral cutoff criteria were met, and all butyric acid exposed subjects were classified as controls (Fig. 1G). Using this behavioral segregation method, we found that significantly more predator odor-exposed rats met susceptible criteria relative to butyric acid (3/61 butyric acid vs 36/96 predator odor exposed; Fig. 2D). Furthermore, we found that the percentage of susceptible rats defined using our criteria (37.5%) falls within the range of that published in other studies that use multiple PTSD-like behaviors to classify predator odor-exposed rats (i.e. 33–40%; Koresh et al., 2016; Le Dorze and Gisquet-Verrier, 2016; Levkovitz et al., 2015; Toledano and Gisquet-Verrier, 2014).

To characterize susceptible and resilient subjects defined by our behavioral cutoff criteria, we performed an extensive comparison of susceptible and resilient rats to control rats across multiple PTSD phenotypic markers (summarized in Table 2). These analyses revealed that susceptible subjects express increased anxiety like-behavior as measured by reduced open arm time in the elevated plus maze (Fig. 3A) and heightened avoidance of the predator odor-paired chamber (Fig. 3B), while resilient subjects did not differ from controls across these measures. We also found that neither susceptible nor resilient subjects differed from controls change in time spent in the unpaired chamber (Fig. 3C). Furthermore, we found that susceptible subjects express heightened acoustic startle response (Fig. 4A), reduced startle response habituation (Fig. 4B), and elevated circulating corticosterone levels during the late waking phase (Fig. 4C), while resilient subjects did not differ from controls. These studies indicate that, using the segregation procedure outlined herein, susceptible subjects express a PTSD-like phenotype, while resilient subjects do not.

In addition, we tested sucrose preference and locomotor activity following stress and segregation (Fig 5A). We found that neither susceptible nor resilient subjects expressed any differences in sucrose preference at 0.25 nor 1.0% sucrose (Fig. 5B). However, we did find that while control, resilient, and susceptible subjects did not differ in novel environment induced (Fig. 5C, D) nor saline-injection induced locomotor activity (Fig. 5C, E), susceptible subjects showed an elevated locomotor response to cocaine relative to control and resilient subjects (Fig. 5C, F).

Together, these observations indicate that predator odor stress produces two distinct sub-populations, a susceptible population that expresses a PTSD-like phenotype, and a resilient population that resembles controls. Further, we found no evidence of anhedonia-like behavior in either susceptible or resilient rats, thus it is likely that our model more accurately represents the 45–60% of PTSD patients that do not express anhedonia symptoms (Carmassi et al., 2014; Zelazny and Simms, 2015). Finally, the finding that only the susceptible

population showed an increase in locomotor sensitivity to cocaine suggests that the pharmacological response to cocaine may be fundamentally altered in this population.

3.3 Nucleus accumbens basal dopamine tone and cocaine-induced increases in dopamine are enhanced in susceptible rats

Stress-induced sensitization to the locomotor effects of cocaine has been tied to elevated dopaminergic responses to cocaine in the NAc (Garcia-Keller et al., 2013). Based on this observation, we examined if the NAc DA response to cocaine differed with stress susceptibility. For these experiments we performed in vivo microdialysis on the day after stress and segregation (Fig. 6A). Microdialysis probes were implanted into the lateral NAc (Fig. 6B), and microdialysis was performed on the following morning.

We found that, prior to cocaine treatment, basal DA levels were elevated in susceptible, but not in resilient rats (Fig. 6C). Following baseline collections, we administered a single i.p. injection of saline, and these injections did not significantly affect DA levels in any group. One hour after injections of saline, rats received a single 10 mg/kg i.p. injection of cocaine. Cocaine increased DA levels in all experimental groups; however, this effect was significantly higher in susceptible rats relative to control and resilient rats (Fig. 6D). These data demonstrate that the neurochemical effects of cocaine are differentially altered in susceptibility versus resilience to stress, with susceptible rats showing a significant sensitization to the dopaminergic effects of cocaine.

3.4 Baseline stimulated dopamine release and uptake are reduced in susceptible rats

We used ex vivo FSCV to investigate the mechanisms by which NAc DA signaling is disrupted in susceptible rats compared to resilient and control rats (Fig. 7A). Baseline evoked DA release was reduced in susceptible but not resilient rats (Fig. 7B, C). We also found a reduction in maximal uptake rate ($V_{\max}$) in susceptible rats, indicating less efficient DA uptake in this group (Fig. 7B, D).

3.5 The terminal effects of cocaine are differentially altered in susceptible and resilient rats

We next tested if cocaine's effects on DA terminals varied across groups. For these experiments, we measured changes in ex vivo DA release and uptake during bath application of increasing concentrations of cocaine (0.1–30 μ M). We found that cocaine-induced changes in DA uptake were elevated in susceptible, but not resilient rats (Fig. 8A, B).

Previous observations suggest that cocaine sensitivity may partially depend on DA transporter (DAT) availability (Chen and Reith, 2007; Nelson et al., 2009), and that $V_{\max}$ represents functional DAT availability (Ferris et al., 2013b). Given that we found decreased $V_{\max}$ and increased cocaine effects in susceptible rats, we sought to determine if differences in DA uptake rate may influence the effects of cocaine on DA uptake inhibition (K_i) across rats in this study. We found a significant positive correlation between $V_{\max}$ and K_i (Fig. 8C), suggesting that reductions in functional DAT availability may account for a portion of the increased cocaine sensitivity observed in susceptible rats.

We also measured the effects of cocaine on DA release. To our surprise, we found that the DA release-promoting effects of cocaine were enhanced in both susceptible and resilient rats (Fig. 8D). Previous research has demonstrated that the effects of cocaine on DA uptake and release are mediated through independent mechanisms (Federici et al., 2014; Venton et al., 2006). Consequently, we verified that changes in cocaine's effect on DA uptake and release were altered independently by analyzing the relationship between cocaine-induced DA uptake inhibition (K_i) and DA release (Calipari et al., 2015). As expected, we found no correlation between these two measures (Fig. 8E). Together, these observations suggest that: (i) the mechanisms mediating cocaine's DA release-promoting effects are increased in response to traumatic stress, (ii) this increase is independent of aberrant behaviors that may precipitate following traumatic stress, and (iii) this disruption is independent of changes in cocaine-induced DA uptake inhibition.

3.6 Dopamine terminal autoreceptor sensitivity is increased in resilient rats

Increases in the DA release-promoting effects of cocaine observed in resilient rats suggests that this group should express an elevated NAc DA response to cocaine in vivo. Nevertheless, the effect of cocaine did not differ between resilient and control rats in the above microdialysis studies (Fig. 6). Others have reported the occurrence of high striatal D2 receptor density in a population with a resilient-like phenotype (Jupp et al., 2016), and D2 autoreceptors function to suppress the effects of cocaine in vivo (Rouge-Pont et al., 2002). To test if differences in DA autoreceptor sensitivity play a role in normalizing the in vivo effects of cocaine in resilient rats, we determined pre-synaptic DA autoreceptor sensitivity in control, resilient, and susceptible rats. To achieve this, we measured DA release following bath application of increasing concentrations of the D2/D3 agonist quinpirole. We found increased D2/D3 autoreceptor sensitivity in resilient, but not control or susceptible rats (Fig. 9). These data suggest that increased DA terminal autoreceptor activity observed in resilient rats may counterbalance increases in the DA release-promoting effects of cocaine.

3.7 Behavioral economics of cocaine self-administration are altered in susceptible rats

Several studies indicate that terminal cocaine sensitivity corresponds with the motivation to self-administer cocaine (Calipari et al., 2015; Siciliano and Jones, 2017). We next used the threshold cocaine self-administration procedure to test if differences in DA system function correspond to alterations in the behavioral economics of cocaine self-administration (Fig. 10A; España et al., 2010; Oleson et al., 2011)). Once stable responding under the threshold schedule was achieved, data were fitted to an exponential demand curve to determine Q_0 , a measure of consumption at null cost, and P_{max} , the behavioral economic index of price (Bentzley et al., 2014; Oleson et al., 2011). Under this schedule, Q_0 represents the animal's preferred level of cocaine consumption while P_{max} represents motivation to maintain preferred blood levels of cocaine.

We found that susceptible rats showed reduced Q_0 (Fig. 10B, C) and elevated P_{max} (Fig. 10B, D). Reduced cocaine consumption at low unit prices corresponds with reduced Q_0 and indicates a reduction in preferred blood levels of cocaine, while increased P_{max} indicates an increase in motivation to maintain preferred blood levels of cocaine. Overall these data suggest that the reinforcing efficacy of cocaine is elevated in susceptible rats.

4.0 Discussion

The present studies demonstrate that susceptibility and resilience to traumatic stress drive distinct neurochemical adaptations that differentially affect DA system function and influence the reinforcing efficacy of cocaine. After defining and validating a segregation procedure for characterizing rats as susceptible and resilient following predator odor stress, we found that only susceptible rats display a sensitization to the locomotor and dopaminergic effects of cocaine. We also found that while resilient rats showed no differences in the locomotor or dopaminergic effects of cocaine *in vivo*, they nevertheless display DA terminal changes. Finally, we found that susceptible, but not resilient rats, display alterations in the behavioral economics of cocaine self-administration, with a decrease in the consumption of cocaine at null cost, and an increase in the motivation to obtain cocaine. Overall, our results suggest that changes in mesolimbic DA signaling enhance cocaine sensitivity and the motivation to obtain cocaine in susceptible rats, but resilient rats express both passive and active forms of resilience that prevent gross changes in cocaine self-administration. These findings provide the first evidence that increases in the reinforcing efficacy of cocaine are tied to the expression of aberrant behaviors observed in susceptibility to traumatic stress.

In these studies, we used a single intense and inescapable stressor because this procedure has been used previously to produce a spectrum of PTSD-like behaviors (Cohen et al., 2012; Edwards et al., 2013; Endres and Fendt, 2007; Hacquemand et al., 2013; Hadad et al., 2016; Koresh et al., 2016). We established and validated a segregation model based on data obtained in our own laboratory that relies on the expression of both context avoidance and anxiety like behavior. Avoidance behavior has repeatedly been used to discern multiple aspects of stress susceptibility (Edwards et al., 2013; Friedman et al., 2014; Krishnan et al., 2007). In our studies, the use of context avoidance behavior may be especially useful for segregation following stress because the expression of this behavior is directly tied to the stress experience and thus circumvents the potential for segregation of subjects based on inherent traits unrelated to the response to traumatic stress. Nonetheless, the utility of avoidance behavior to measure a pathological response to stress has recently been called into question as it can instead be conceptualized as an etiologically adaptive response (Desmedt et al., 2015). To avoid this, we used a more conservative approach by also considering anxiety-like behavior in the elevated plus maze. The use of this second behavior ensures that susceptible rats express a maladaptive anxiety-like behavior as well as a stress-specific behavioral response. Moreover, we found that the expression of these two behaviors significantly correlated with other markers of a PTSD-like phenotype in acoustic startle response and elevated corticosterone during the late waking phase, thus suggesting that expression of these two behaviors corresponds with a broader PTSD-like phenotype.

This segregation procedure was then validated by comparing susceptible and resilient subjects to unstressed controls across a spectrum of phenotypic markers. In these studies, we found that susceptible subjects express a spectrum of PTSD-like phenotypic markers, while resilient subjects did not. In contrast to another model of PTSD, the single prolonged stress model, we did not find any differences in sucrose preference across groups (Enman et al., 2015). This suggests that the single prolonged stress model may more closely represent

forms of PTSD with an anhedonic component, while our model more likely represents the 45–60% of patients with PTSD that do not display corresponding anhedonia (Carmassi et al., 2014; Zelazny and Simms, 2015). Importantly, patients with PTSD that do not display depressive symptoms, such as anhedonia, nevertheless show similar rates of addiction comorbidity (Kilpatrick et al., 2003). Additional behavioral experiments revealed a sensitization to the locomotor effects of cocaine that was expressed exclusively in susceptible rats. Combined, these studies suggest that changes in drug-taking behavior observed using this model may be attributed to a sensitization to the effects of cocaine rather than gross alterations in hedonic state.

We investigated the source of locomotor sensitization to cocaine by examining differences in NAc DA signaling. Despite finding no difference across groups in novel environment-induced locomotion, we did observe an increase in basal DA tone using in vivo microdialysis. While there are reports of a relationship between basal DA tone in the NAc and novel environment-induced locomotor activity (Hooks et al., 1992), others have failed to observe this relationship (Antoniou et al., 2008; Chefer et al., 2003; Ferris et al., 2013a), and thus our current finding is not without precedent. In subsequent in vitro FSCV experiments we found a reduction in evoked DA release and slower DA uptake. This observation is consistent with reports that the magnitude of evoked DA release ex vivo is inversely proportional to extracellular DA levels in vivo (Ferris et al., 2014; Zhuang et al., 2001). Importantly, both experimental (Ferris et al., 2014; Zhuang et al., 2001) and computational studies (Best et al., 2009) have demonstrated that this relationship is governed by DA uptake. Specifically, these studies suggest that slower uptake results in a shift in the finite pool of releasable DA to the extracellular space, thus decreasing the intra-terminal pool of releasable DA and producing a reduction in the magnitude of evoked DA release ex vivo (Best et al., 2009; Ferris et al., 2014; Zhuang et al., 2001). This reduction in DA uptake may be functionally derived from changes in DAT membrane expression (Calipari et al., 2014). Combined, these concepts lead to the suggestion that reductions in DA uptake rate play a role in the observed increased extracellular DA levels in susceptible rats. Nevertheless, increased DA neuron firing rate and bursting have been reported in susceptible rodents (Cao et al., 2010), and similar increases in DA neuron firing rate or bursting can result in elevated extracellular DA levels (Floresco et al., 2003). Accordingly, we cannot rule out a role for changes in DA neuron properties or the potential influence of more general circuit changes in the elevated extracellular DA levels observed in susceptible rats.

We also found that both in vivo cocaine-induced elevations of DA and ex vivo terminal cocaine sensitivity were higher in susceptible rats relative to controls. Heightened cocaine sensitivity correlated with decreased maximal DA uptake ($V_{\max}$) at baseline. This relationship is consistent with the observation that lower DAT activity corresponds with elevated cocaine sensitivity (Chen and Reith, 2007); however, it should be noted that maximal uptake rate and cocaine sensitivity may be dissociable (Calipari et al., 2015; Ferris et al., 2011; Siciliano et al., 2015). Several candidate mechanisms have been suggested to mediate this effect, including differential DAT phosphorylation state (Moritz et al., 2013), shifts in inward/outward facing DAT (Liang et al., 2009), and changes in oligomer/monomer ratios (Chen and Reith, 2007). It is possible that alterations in these processes may also contribute to the cocaine sensitization observed herein.

While increases in cocaine's effect on DA uptake rate were observed in susceptible subjects alone, we found that the DA release-promoting properties of cocaine are elevated in both susceptible and resilient subjects. The precise mechanism by which cocaine drives DA release remains an open question, nonetheless, it is known that the DA release-promoting effects of cocaine are synapsin-dependent (Venton et al., 2006), and are possibly mediated by cocaine-induced alterations to presynaptic Ca^{2+} influx (Pierce et al., 1998; Venton et al., 2006). Thus, increases in the DA release-promoting effects of cocaine observed herein may represent stress-induced changes in DA terminal synapsin function, Ca^{2+} homeostasis, or some yet unknown process by which cocaine drives DA release.

Interestingly, elevations in cocaine's release-promoting effects found in resilient subjects do not translate to increased effects of cocaine on extracellular DA levels in vivo. This apparent inconsistency can be explained by elevations in D2 autoreceptor sensitivity observed in only resilient subjects. Terminal autoreceptors are known to suppress cocaine-induced elevations in extracellular DA in vivo (Rouge-Pont et al., 2002). Thus, increases in D2 receptor sensitivity would function to counteract elevations in DA release-promoting effects of cocaine thereby normalizing the in vivo cocaine response in resilient subjects.

Increases in D2 receptor sensitivity in resilient subjects could be predicted to produce increases in DA uptake as previous reports suggest that pharmacological or genetic manipulation of both pre- and post-synaptic D2 receptors impacts DA uptake (Cass and Gerhardt, 1994; Dickinson et al., 1999). Nevertheless, studies that examined DA uptake following targeted genetic manipulation of D2 autoreceptors in DA neurons have produced conflicting results (Anzalone et al., 2012; Bello et al., 2011; Budygin et al., 2016). Consequently, it is not surprising that in the current studies heightened D2 autoreceptor sensitivity observed in resilient subjects did not correspond with alterations in DA uptake.

Finally, we tested if these differences in DA system function and cocaine sensitivity correspond with changes in cocaine self-administration using the threshold schedule. In these studies we showed that susceptible subjects display increased motivation ($P_{\max}$) to obtain cocaine, with a corresponding decrease in consumption at null cost (Q_0). This is a particularly interesting observation as motivation to obtain cocaine under the threshold schedule has recently been shown to positively correlate with several other features of an addiction-like phenotype including: (i) resistance to instrumental extinction, (ii) cue- and cocaine-induced reinstatement, and (iii) persistence of drug taking in the face of punishments (Bentzley et al., 2014). Furthermore, low initial levels of cocaine consumption were found to predict a heightened propensity to escalate cocaine taking (Bentzley et al., 2014). In consideration of the predictive nature of $P_{\max}$ and Q_0 , it may be surmised that susceptible rats express a more expansive addiction-like phenotype. Further investigation into self-administration profiles of susceptible rats will be required to confirm this. Nevertheless, the current self-administration studies clearly demonstrate that the magnitude of behavioral aberrations following traumatic stress is tied to a sensitization of the reinforcing effects of cocaine.

5.0 Conclusion

Our findings show that cocaine self-administration vulnerability is expressed exclusively in rodents that demonstrate maladaptive behaviors following predator odor traumatic stress. This feature mirrors the human condition where only individuals that develop PTSD following traumatic stress show an increased vulnerability to developing substance abuse disorders (Chilcoat and Breslau, 1998). Further, our data suggest that differential DA terminal function underlies these individual differences in the behavioral economics of cocaine self-administration following traumatic stress. Specifically, changes in terminal cocaine sensitivity may drive increases in the motivation for cocaine in rats that are susceptible to traumatic stress, while, in resilient rats, an absence of functional DAT changes and increases in DA terminal autoreceptor function may respectively act as passive and active forms of resilience that normalize cocaine self-administration behavior.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Predator odor stress results in distinct susceptible and resilient populations
- Susceptible subjects show a sensitization of the dopaminergic response to cocaine
- Susceptible subjects express increases in the motivation to self-administer cocaine
- Active and passive resilience mechanisms may normalize cocaine effects and behavior

Figure 1.

Exposure to predator odor stress generates distinct populations. **(A)** Schematic representation of the experimental timeline used for all studies described herein. **(B)** Time spent in the open arms of the elevated plus maze. Student's t-test showed a significant difference between cluster groups for change in time spent in the odor paired chamber ($t_{18} = 3.596$, $p < 0.01$) **(C)** Change in time spent in the odor-paired chamber. Student's t-test showed a significant difference between cluster groups for change in time spent in the odor paired chamber ($t_{18} = 2.749$, $p < 0.05$). **(D)** Acoustic startle response amplitude. Student's t-test showed a significant difference between cluster groups for acoustic startle response amplitude ($t_{18} = 4.051$, $p < 0.001$). **(E)** Late waking phase circulating corticosterone levels. Student's t-test showed a significant difference between cluster groups for late waking phase circulating corticosterone levels ($t_{18} = 4.404$, $p < 0.001$). **(F)** Schematic representation of the algorithm used to segregate susceptible and resilient populations. Cluster A = 12, Cluster B = 8. Student's t-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 2.

Comparison of control and predator odor-exposed subjects. **(A)** Time spent in the open arms of the elevated plus maze. Mann-Whitney U test showed a significant effect of odor exposure on time spent in the open arms of the elevated plus maze ($U = 2376$, $p < 0.05$). **(B, C)** Change in time spent in the odor-paired and unpaired chambers. Student's t-test showed a significant effect of exposure odor for change in time spent in the odor-paired chamber ($t_{161} = 3.307$, $p < 0.01$), but not the unpaired chamber ($t_{161} = 0.4566$, $p = 0.6486$). **(D)** Distribution of butyric acid and TMT exposed rats that met susceptible criteria. Chi-Square analyses showed a significant effect of TMT exposure on group distribution (Chi = 19.835, Phi = 0.343) (note that butyric acid exposed rats meeting susceptible criteria were still classified as controls). BA=54, TMT=96. Data are shown as mean ($\pm$ s.e.m.). Mann-Whitney U test: * $p < 0.05$; Student's t-test: ## $p < 0.01$; Chi-Square: ††† $p < 0.001$. TMT = 2,4,5-trimethyl-3-thiazoline, BA = Butyric acid.

Figure 3.

Susceptible rats show increased anxiety-like behavior and greater context avoidance. **(A)** Post-segregation time spent in the open arms of the elevated plus maze. One-way ANOVA revealed a significant effect of group for time spent in the open arms of the elevated plus maze ($F_{(2, 122)} = 26.97, p < 0.001$). **(B, C)** Post-segregation change in time spent in the odor-paired and unpaired chambers. One-way ANOVA revealed a significant effect of group for Paired ($F_{(2, 120)} = 9.662, p < 0.001$), but not for Unpaired. Data are shown as mean ($\pm$ s.e.m.). Control=45, Resilient=48, Susceptible=35. Bonferroni post-hoc: *** $p < 0.001$, ** $p < 0.01$ susceptible vs control and resilient. EPM = elevated plus maze, TMT = 2,4,5-trimethyl-3-thiazoline, BA = Butyric acid.

Figure 4.

Susceptible rats show increased acoustic startle response, decreased startle response habituation, and increased late waking phase serum corticosterone. **(A)** Average acoustic startle response amplitude. One-way ANOVA revealed a significant effect of group for startle response amplitude ($F_{(2, 33)} = 4.725, p < 0.05$). **(B)** Average acoustic startle response habituation. One-way ANOVA revealed a significant effect of group for startle response habituation ($F_{(2, 33)} = 4.620, p < 0.05$). **(C)** Average late waking phase serum corticosterone levels. One-way ANOVA revealed a significant effect of group for serum corticosterone levels ($F_{(2, 33)} = 6.370, p < 0.01$). Control=17, Resilient=12, Susceptible=8. Bonferroni post-hoc: * $p < 0.05$, *** $p < 0.001$ susceptible vs. control; †† $p < 0.01$, ††† $p < 0.001$ susceptible vs resilient. ASR = acoustic startle response testing.

Figure 5.

Susceptible rats display increases in the locomotor response to cocaine. **(A)** Experimental timeline used for sucrose preference and locomotor testing. **(B)** Average preference for 0.25% and 1.0% sucrose. Repeated measures two-way ANOVA with sucrose concentration as a repeated measure and group as a between subjects measure revealed no difference between groups ($F_{(39, 2)} = 1.260$, $p = 0.3020$). **(C)** Average cumulative locomotor activity during novel environment exposure, following a single vehicle injection, and following a single injection of 10 mg/kg cocaine. Repeated measures two-way ANOVA with treatment as a repeated measure and group as a between subjects measure revealed a significant effect of treatment ($F_{(39, 2)} = 88.42$, $p < 0.001$) and an interaction ($F_{(39, 4)} = 3.692$, $p < 0.01$). Locomotor activity analyzed in 5 min time bins across **(D)** novel environment exposure, and after **(E)** vehicle or **(F)** 10 mg/kg cocaine injection. Repeated measures two-way ANOVA with time as a repeated measure and group as a between subjects measure showed no difference between groups for novel environment induced-, or vehicle injection-induced locomotion, but did show a significant effect of group for cocaine injections ($F_{(39, 429)} = 3.70$, $p < 0.05$). Data are shown as mean $\pm$ s.e.m. Control $n=12$, Resilient $n=17$, Susceptible $n=12$. Bonferroni post-hoc: * $p < 0.05$, *** $p < 0.001$ susceptible vs. control; $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ susceptible vs resilient. VEH = Saline, COC = 10 mg/kg cocaine.

Figure 6.

In vivo basal DA tone and the effects of cocaine on DA are elevated in susceptible rats. **(A)** Experimental timeline for microdialysis experiments. **(B)** Schematic depiction of microdialysis probe placements. **(C)** Average basal DA levels. One-way ANOVA revealed a significant effect of group ($F_{(2,21)} = 10.02$, $p < 0.01$). **(D)** The effects of cocaine expressed as a percent of baseline. Two-way ANOVA with group as the between-subjects measure and time as the repeated measure revealed a significant effect of group ($F_{(2,21)} = 11.70$, $p < 0.001$), time ($F_{(11, 231)} = 7.612$, $p < 0.001$), and group x time ($F_{(22,231)} = 2.332$, $p < 0.001$). Data are shown as mean ($\pm$ s.e.m.) Control $n=8$, Resilient $n=10$, Susceptible $n=6$. Bonferroni post-hoc: * $p < 0.05$, *** $p < 0.001$ susceptible vs. control, $^{\dagger}p < 0.05$, $^{\dagger\dagger\dagger}p < 0.001$ susceptible vs resilient. VEH = Saline, COC = 10 mg/kg cocaine.

Figure 7.

Susceptible rats display altered DA terminal kinetics at baseline. **(A)** Experimental timeline for all ex vivo voltammetry experiments. **(B)** Example traces of evoked DA release and uptake. Insets depict traces normalized for signal amplitude with the respective resilient or susceptible trace overlaid on the dashed control trace for comparison. **(C)** Average evoked DA release. One-way ANOVA revealed a significant effect of group ($F_{(2,42)} = 5.419$, $p < 0.01$). **(D)** Average uptake rate ($V_{\max}$). One-way ANOVA revealed a significant effect of group ($F_{(2,42)} = 19.46$, $p < 0.001$). Control $n=15$, Resilient $n=20$, Susceptible $n=10$. Bonferroni post-hoc: $*p < 0.05$, $***p < 0.001$ susceptible vs. control; $^{\dagger}p < 0.01$ $^{\dagger\dagger}p < 0.001$ susceptible vs. resilient.

Figure 8.

Traumatic stress differentially alters DA terminal cocaine sensitivity in susceptible and resilient rats. **(A)** Example color plots (above) and current vs time plots (below) of evoked DA release in the presence of 30 μ M cocaine. **(B)** The effect of cocaine on DA uptake (K_m). Two-way ANOVA with group as the between-subjects measure and cocaine concentration as the repeated measure revealed a significant effect of group ($F_{(2, 20)} = 3.586$, $p < 0.05$), concentration ($F_{(4, 83)} = 3.586$, $p < 0.001$), and concentration $\times$ group ($F_{(8, 83)} = 3.186$, $p < 0.01$). **(C)** The correlation between baseline DA uptake rate (V_{max}) and cocaine inhibition constant (K_i ; Pearson's correlation: $r^2 = 0.3393$, $p < 0.01$). **(D)** The effects of cocaine on DA

release as a percent of baseline. Two-way ANOVA with group as the between-subjects measure and cocaine concentration as the repeated measure revealed a significant effect of group ($F_{(2,20)} = 8.086$, $p < 0.01$), concentration ($F_{(4,83)} = 38.75$, $p < 0.001$), and concentration x group ($F_{(8,83)} = 2.201$, $p < 0.05$). (E) Correlation of DA release as a percent of baseline in the presence of 10 μ M cocaine and cocaine inhibition constant (K_i ; Pearson's correlation: $r^2 = -0.1767$, $p = 0.4201$). Control n=7, Resilient n=11, Susceptible n=5. Bonferroni post-hoc: * $p < 0.05$, *** $p < 0.001$ susceptible vs. control; $^{\dagger\dagger\dagger}p < 0.001$ susceptible vs resilient; # $p < 0.05$, ## $p < 0.01$ resilient vs control.

Figure 9.

DA terminal autoreceptors are sensitized in resilient rats. **(A)** Example traces of evoked DA release for control, resilient, and susceptible rats across applied concentrations of quinpirole. **(B)** The effects of the D2/D3 agonist, quinpirole, on DA release as a percent of baseline. Two-way ANOVA with group as the between-subjects measure and quinpirole concentration as the repeated measure revealed a significant effect of group ($F_{(2,18)} = 7.015$, $p < 0.01$), concentration ($F_{(4,72)} = 519.8$, $p < 0.001$), and concentration $\times$ group ($F_{(8,72)} = 3.091$, $p < 0.01$). Data are shown as mean ($\pm$ s.e.m.). Control $n=6$, Resilient $n=10$, Susceptible $n=5$. Bonferroni post-hoc: $^{\dagger\dagger} p < 0.01$ susceptible vs resilient; $^{##} p < 0.01$ resilient vs control.

Figure 10.

The behavioral economics of cocaine self-administration are altered in susceptible rats. **(A)** Experimental timeline used for cocaine self-administration behavioral economics experiments. **(B)** Average consumption and lever presses during threshold self-administration in control, resilient and susceptible rats. **(C)** Average Q_0 . One-way ANOVA revealed a significant effect of group ($F_{(2, 19)} = 5.528, p < 0.05$). **(D)** Average P_{max} . One-way ANOVA revealed a significant effect of group ($F_{(2, 19)} = 14.56, p < 0.001$). Control $n=7$, Resilient $n=10$, Susceptible $n=5$. Bonferroni post-hoc: $*p < 0.05$, $***p < 0.001$ for susceptible vs control and resilient.

Table 1

Correlation coefficients across markers of a PTSD-like phenotype following predator odor exposure.

	Context Avoidance	Acoustic Startle	Corticosterone
Open Arm Time	0.530 *	-0.472 *	-0.604 **
Context Avoidance		-0.591 **	-0.492 *
Acoustic Startle			0.350

For all analyses the number of TMT exposed subjects was 20. Pearson's correlation:

* $p < 0.05$,

** $p < 0.01$.

Table 2

Susceptible and resilient rats express distinct syndromes.

	Control		Resilient		Susceptible	
	n	n	effect	n	effect	
Anxiety-like behavior (open arm time in the elevated plus maze)	45	60	↔	36	↑***	
Context avoidance (paired)	45	60	↔	36	↑**	
Acoustic startle response	17	12	↔	8	↑***	
Startle response habituation	17	12	↔	8	↓*	
Late waking phase corticosterone	17	12	↔	8	↑*	
0.25% Sucrose preference	12	17	↔	12	↔	
1.0% Sucrose preference	12	17	↔	12	↔	
Novel environment-induced locomotor activity	12	17	↔	12	↔	
Saline injection-induced locomotor activity	12	17	↔	12	↔	
Cocaine-induced locomotor activity	12	17	↔	12	↑***	

Arrows indicate significance relative to controls. Bonferroni post hoc: ↔ = no difference relative to controls; ↓ = decrease relative to controls; ↑ = increase relative to controls.

* $p < 0.05$,** $p < 0.01$,*** $p < 0.001$.