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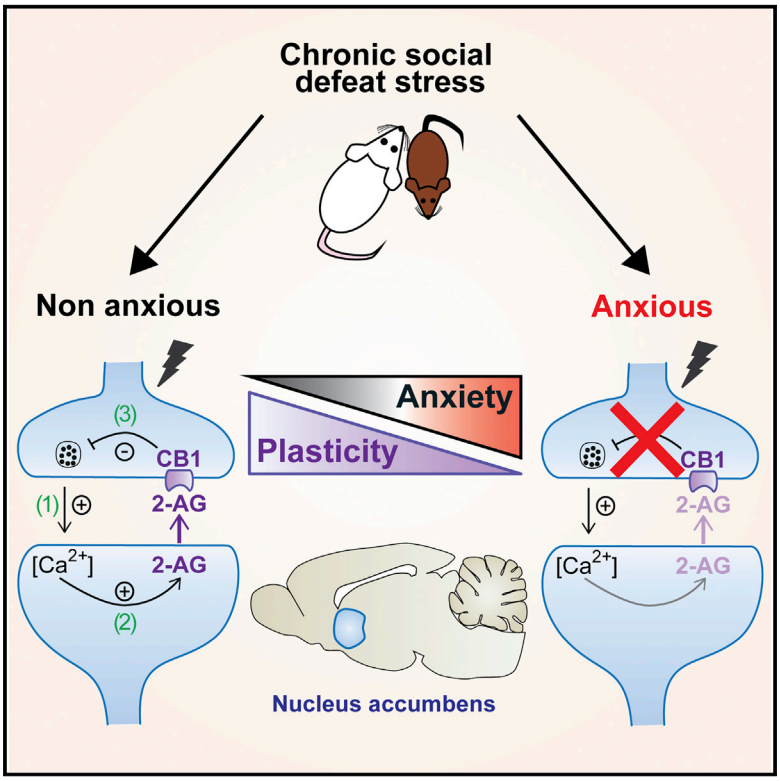
Endocannabinoid-Mediated Plasticity in Nucleus Accumbens Controls Vulnerability to Anxiety after Social Defeat Stress

Graphical Abstract



Highlights

- Socially defeated mice were clustered into anxious and non-anxious groups
- Spike-timing endocannabinoid plasticity was abolished in anxious mice
- Elevation of 2-AG levels in the nucleus accumbens restores behavior and plasticity
- Endocannabinoid plasticity is a synaptic marker of anxiety following social defeat

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In Brief

Bosch-Bouju et al. used cluster analysis to segregate mice into anxious and non-anxious populations following social defeat. Endocannabinoid spike-timing-dependent plasticity is abolished in anxious mice only. Enhancement of endocannabinoid signaling in the nucleus accumbens restores anxiety-like behaviors and synaptic plasticity. Endocannabinoid plasticity is thus a synaptic marker of anxiety following social defeat.

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Cell Reports
Report

Endocannabinoid-Mediated Plasticity in Nucleus Accumbens Controls Vulnerability to Anxiety after Social Defeat Stress

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SUMMARY

Chronic social defeat stress (CSDS) is a clinically relevant model of mood disorders. The relationship between the CSDS model and a physiologically pertinent paradigm of synaptic plasticity is not known. Here, we found that cluster analysis of the emotional behavior states of mice exposed to CSDS allowed their segregation into anxious and non-anxious groups. Endocannabinoid-mediated spike-timing dependent plasticity (STDP) in the nucleus accumbens was attenuated in non-anxious mice and abolished in anxious mice. Anxiety-like behavior in stressed animals was specifically correlated with their ability to produce STDP. Pharmacological enhancement of 2-arachidonoyl glycerol (2-AG) signaling in the nucleus accumbens normalized the anxious phenotype and STDP in anxious mice. These data reveal that endocannabinoid modulation of synaptic efficacy in response to a naturalistic activity pattern is both a molecular correlate of behavioral adaptability and a crucial factor in the adaptive response to chronic stress.

INTRODUCTION

The neural and molecular mechanisms responsible for individual vulnerability and resilience to neuropsychiatric illnesses such as depression and anxiety disorders are poorly understood. Endocannabinoids have been linked to psychiatric illness, in particular the pathophysiology of depressive- and anxiety-like behaviors (Lafourcade et al., 2011; Hill and Gorzalka, 2009; Hillard et al., 2012; Mangieri and Piomelli, 2007; Mechoulam and Parker, 2013; Vinod and Hungund, 2006). In depressed patients, blood levels of endocannabinoids (eCBs) are decreased (Hill et al., 2009), while in animal models of depression, altered brain levels of eCBs and functionality of the cannabinoid type 1 receptor (CB1R) are reported (Bluett et al., 2014; Hill et al., 2005; Qin et al., 2015). In addition, pharmacological and genetic disruption of CB1R or eCB production results in enhanced anxiety, stress, and fear response (Hill and Patel, 2013; Jenniches et al., 2016; Marsicano et al., 2002; Qin et al., 2015; Shonesy et al., 2014; Steiner et al., 2008), reinforcing the idea that this system may play a significant role in the pathogenesis of neuropsychiatric diseases.

Endocannabinoids are lipid mediators with essential modulatory functions in the brain (Katona and Freund, 2012). Produced in the postsynapse, the two major eCBs, anandamide and 2-arachidonoyl glycerol (2-AG), signal in a retrograde direction to modulate synaptic strength via presynaptic CB1R (Castillo et al., 2012). By integrating and translating environmental changes into synaptic changes, eCBs regulate a range of brain functions (for review, see Morena et al., 2016). Activation of CB1R leads to acute depression of synaptic transmission, which with extended eCB signaling engages an endocannabinoid-mediated long-term depression (LTD) originally discovered in the nucleus accumbens (Robbe et al., 2002), a key structure to stress resiliency (Duval et al., 2015; Francis et al., 2015; Levita et al., 2012; McLaughlin et al., 2014; Vialou et al., 2010). However, it is not known whether eCBs produced in response to a naturalistic pattern of synaptic activity participate in stress resiliency.

Here, we focused on eCB spike-timing dependent plasticity (STDP) at excitatory synapses in the accumbens in a clinically relevant model of anxiety- and depressive-like behaviors: chronic social defeat stress (CSDS) (Berton et al., 2006; Krishnan et al., 2007; Larrieu et al., 2014). CSDS induces individual differences across behavioral endpoints (Krishnan et al., 2007). We automated classification of behavioral endpoints to segregate defeated mice based on their anxiety-like behaviors. Our findings demonstrate that impairment of eCB STDP in the accumbens is a synaptic signature of anxiety-like behavior after social defeat stress. The restoration of eCB signaling in the accumbens through the enhancement of 2-AG signaling protects against CSDS-induced anxiety-like behavior. Altogether, these data establish eCB STDP in the accumbens as a central regulator of adaptive capacity in animals exposed to CSDS, offering a

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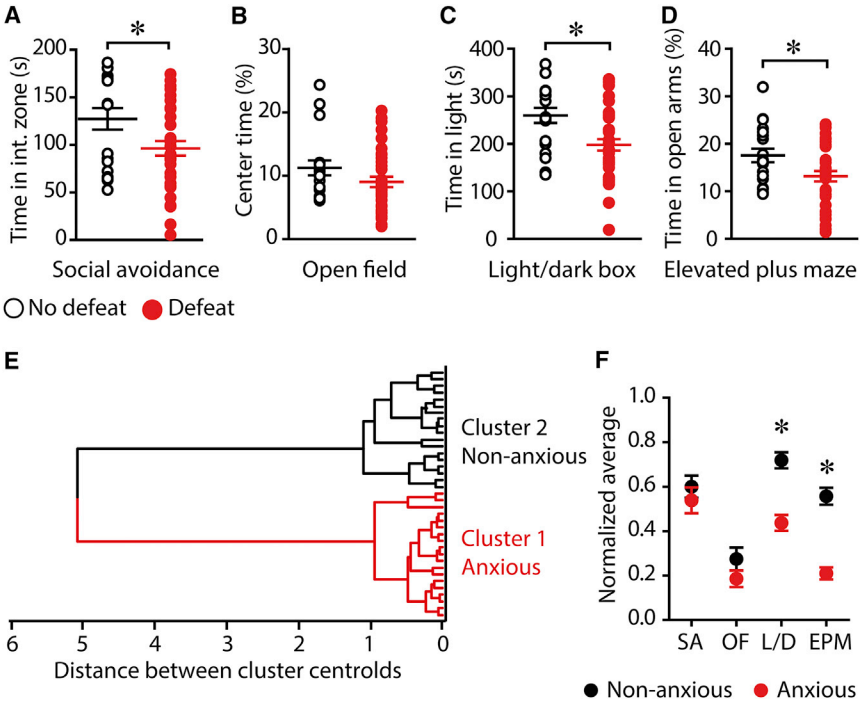

Figure 1. Behavioral Clustering in Socially Defeated Mice

(A–D) Behavioral portraits of undefeated mice (white) and defeated mice (red) show increased anxiety in defeated mice. *p < 0.05, unpaired t test. Data are presented as mean ± SEM. (A) Time in the interaction zone. Undefeated: 127 ± 11 s, n = 19; defeated: 96 ± 8 s, n = 34. t_{51} = 2.336, *p = 0.0235. (B) Time in the center of the open field. Undefeated: 11.3% ± 1.2%, n = 19; defeated: 9.0% ± 0.8%, n = 36. t_{53} = 1.570, p = 0.1223. (C) Time in the light compartment. Undefeated: 260 ± 16 s, n = 19; defeated: 198 ± 12 s, n = 37. t_{54} = 3.048, *p = 0.036. (D) Time in open arms of the elevated plus maze. Undefeated: 17.5% ± 1.4%, n = 19; defeated: 13.2% ± 1.1%, n = 37. t_{54} = 2.385, *p = 0.0208. (E) Clustering analysis of socially defeated mice's behavior reveals a dendrogram with two clusters corresponding to anxious animals (red) and non-anxious animals (black). (F) Normalized average values for all behavioral parameters of the cluster analysis in anxious and non-anxious groups. Data are presented as mean ± SEM. From left to right, non-anxious (n = 18) versus anxious (n = 18), respectively: SA, social avoidance test, 0.60 ± 0.05 versus 0.54 ± 0.06; OF, open field test, 0.28 ± 0.05 versus 0.19 ± 0.04; L/D, light/dark box test, 0.72 ± 0.04 versus 0.44 ± 0.04; EPM, elevated plus maze test, 0.56 ± 0.04 versus 0.21 ± 0.03. *p < 0.05, two-way ANOVA with Bonferroni post-test, with cluster ($F_{(1,105)}$ = 42.96, p < 0.0001) and behavioral parameter ($F_{(3,105)}$ = 29.99, p < 0.0001) as factors. Interaction: $F_{(3,105)}$ = 5.39, p = 0.0017.

pharmacologically amenable mechanism to promote resiliency to stressful events.

RESULTS

Segregation of Defeated Animals into Anxious and Non-anxious Populations Using Cluster Analysis

Naive C57BL/6J mice were subjected to ten daily bouts of social defeat by an aggressive CD1 male mouse. CSDS is known to induce individual differences to stress responses, and defeated animals can be separated into susceptible and resilient based on the measure of their social interaction (Figure S1) (Golden et al., 2011; Krishnan et al., 2007). In the present study, we used an alternative method to segregate defeated mice based on their emotional behaviors in open field, social avoidance, light/dark box, and elevated plus maze tests (Figures 1A–1D; Figure S2). This unbiased cluster analysis approach revealed that defeated mice can be segregated in two populations based on their emotional behaviors (Figure 1E): 52% of defeated mice showed severe anxiety-like behaviors and were hereafter labeled anxious, while the remaining 48% that display anxiety-like behaviors similar to those of undefeated mice were labeled non-anxious. By comparison with the classical segregation of susceptible and resilient mice, we found that 50% of resilient and 55% of susceptible mice were anxious (Figure S1). Consequently, both anxious and non-anxious mice displayed an increase in social avoidance following CSDS, but only anxious mice exhibited elevated anxiety-like behaviors, as revealed by an aversion to the open arms of an elevated plus maze and the light compartment of a light/dark box (Figure 1F; Figure S2). Anxious and non-anxious mice displayed a similar increase in plasma corticosterone levels, adrenal weight, and body weight (Figure S2), suggesting that the two populations of defeated mice do not differ in their metabolic stress response.

Endocannabinoid Spike-Timing Dependent Depression Covariates with Anxiety-like Behavior

We next analyzed the consequences of CSDS on synaptic Hebbian learning in the accumbens, a key structure that contributes to the etiology of mood and anxiety disorders (Bagot et al., 2015; Calhoon and Tye, 2015; Shin et al., 2015; Vialou et al., 2010). We first established that CB1R mediates Hebbian STDP (Abbott and Nelson, 2000; Fino et al., 2010) in the accumbens. In undefeated mice, presynaptic stimulation of 100 pairings at 1 Hz coupled to a single postsynaptic spike delayed by ~15 ms induced significant LTD (51% of baseline) of excitatory postsynaptic currents (EPSCs) (Figures 2A–2C). As expected for eCB-mediated plasticity, the CB1R antagonists AM251 and SR141716A both blocked STDP-LTD (Figure S3). In anxious mice, STDP-LTD was abolished (111% of baseline) (Figure 2A). Non-anxious mice displayed an intermediary phenotype: STDP-LTD was attenuated but still present (83% of baseline) (Figures 2A–2C). In the bed nucleus of the stria terminalis, stress transforms CB1R-dependent LTD to long-term potentiation

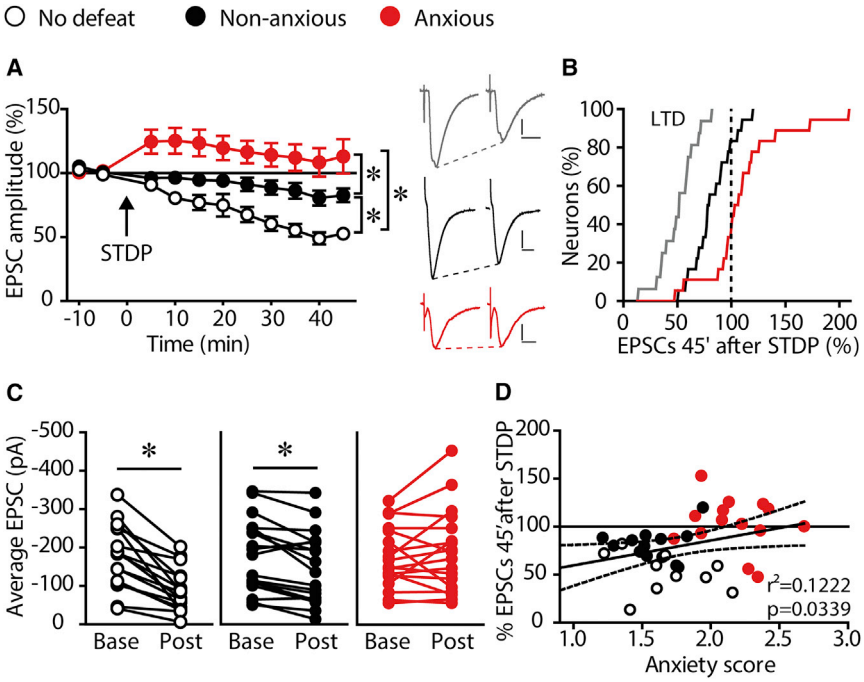

Figure 2. Spike-Timing-Dependent LTD Is a Synaptic Marker in Anxious Mice

(A) Time course of STDP-LTD in undefeated (white), non-anxious (black), and anxious (red) mice. EPSC normalized amplitude 45 min after STDP. Undefeated: 51.28% ± 4.36%, n = 16; non-anxious: 82.13% ± 4.32%, n = 18; anxious: 110.80% ± 8.71%, n = 18. *p < 0.05, two-way ANOVA with Bonferroni post-test, with group ($F_{(2,512)}$ = 104.99, p < 0.0001) and time ($F_{(10,512)}$ = 5.11, p < 0.0001) as factors. Interaction: $F_{(20,512)}$ = 2.90, p < 0.0001. Data are presented as mean ± SEM. Illustration traces: example of ten averaged EPSCs during baseline (left) and 45 min after STDP (right). Upper (gray), undefeated; middle (black), non-anxious; bottom (red), anxious. Scale bar, 50 pA, 10 ms.

(B) Cumulative probability distribution of normalized EPSCs after STDP protocol. Same color code as (A).

(C) EPSCs before and after STDP-LTD induction for undefeated (white), non-anxious (black), and anxious (red) mice. No defeat: before, −177 ± 21 pA, versus after, −88 ± 14 pA; n = 16, p < 0.0001. Non-anxious: before, −181 ± 22 pA, versus after, −150 ± 22 pA; n = 18, p = 0.0025. Anxious: before, −175 ± 19 pA, versus after, −190 ± 26 pA; n = 18, p = 0.3365. Each line represents one neuron, *p < 0.05, paired t test.

(D) The anxiety score positively correlates with the expression of LTD (r^2 = 0.1222, p = 0.0339, Pearson test). Each dot represents one mouse.

(LTP) (Glangetas et al., 2013). Our data support this idea that the STDP protocol triggered LTD in all and 55% of the neurons from undefeated and non-anxious mice, respectively, in contrast to anxious mice, among which only 11% of the neurons expressed LTD and up to 33% exhibited LTP (LTD threshold, 85% of baseline EPSC; LTP threshold, 115%) (Figure 2B). To strengthen the association between eCB-mediated plasticity and anxiety, we computed an anxiety score and found that this behavioral index positively correlated with eCB-LTD in the accumbens (Figure 2D). Specifically, eCB-LTD was significantly correlated with anxiety measured in open field, light/dark box, and elevated plus maze tests (Table S1). In contrast, we did not observed a correlation between eCB-LTD and corticosterone levels in defeated mice (Figure S3). These data suggest that both groups experienced similar levels of neuroendocrine stress response and dissociate general hypothalamic-pituitary-adrenal axis reactivity to stress from social defeat stress-induced eCB-plasticity deficits. Basic intrinsic and synaptic properties of accumbens output neurons were similar in anxious and non-anxious mice (Figure S3), suggesting that modification of neuronal excitability or network activity is a minor contributor to the lack of eCB-mediated plasticity.

Pharmacological Enhancement of eCB Signaling Normalizes Both Anxiety-related Behavior and Synaptic Plasticity within the Accumbens

We next investigated whether upregulation of 2-AG signaling could normalize anxiety-like behavior and eCB-LTD in defeated mice. We used JZL184, a monoacylglycerol lipase (MAGL) inhibitor, to prevent 2-AG degradation and increase its accumulation at the synapse (Jung et al., 2012). Mice treated with JZL184 (16 mg/kg, intraperitoneal [i.p.]) 1 day after the last session of CSDS showed anxiety-like behavior that was undistinguishable from that of undefeated mice (Figure 3A). To discern the contribution of 2-AG elevation within the accumbens in the systemic effects of JZL184, we repeated experiments directly infusing the MAGL inhibitor into the accumbens (1 μg, 0.5 μl/side). Similar to the systemic protocol, local infusion of JZL184 prevented anxiety-like behaviors in defeated mice (Figure 3C). We also found that JZL184 (1 μM) restored eCB-LTD in defeated mice (Figures 3B–3D; Figure S3), reinforcing the link between eCB-mediated STDP-LTD in the accumbens and behavioral anxiety following CSDS. As a control, the effect of JZL184 on STDP-LTD was blocked by the CB1R antagonist SR141716A (Figure S3). Altogether, these data favor the idea that elevation of 2-AG in the accumbens can normalize anxiety behavior in defeated mice.

DISCUSSION

There is a considerable interest in understanding neurobiological correlates of adaptive response and resiliency to chronic social stress. In the current study, we provide clear evidence that eCB-mediated Hebbian learning at medium spiny neuron excitatory synapses in the nucleus accumbens contributes to behavioral adaptations to social stress. Furthermore, our results indicate that this synaptic plasticity is a pharmacologically targetable neurobiological mechanism that may promote resistance to anxiety following chronic stress.

A unique feature of CSDS, distinguishing it from other environmental stressors, is that CSDS induces a range of individual

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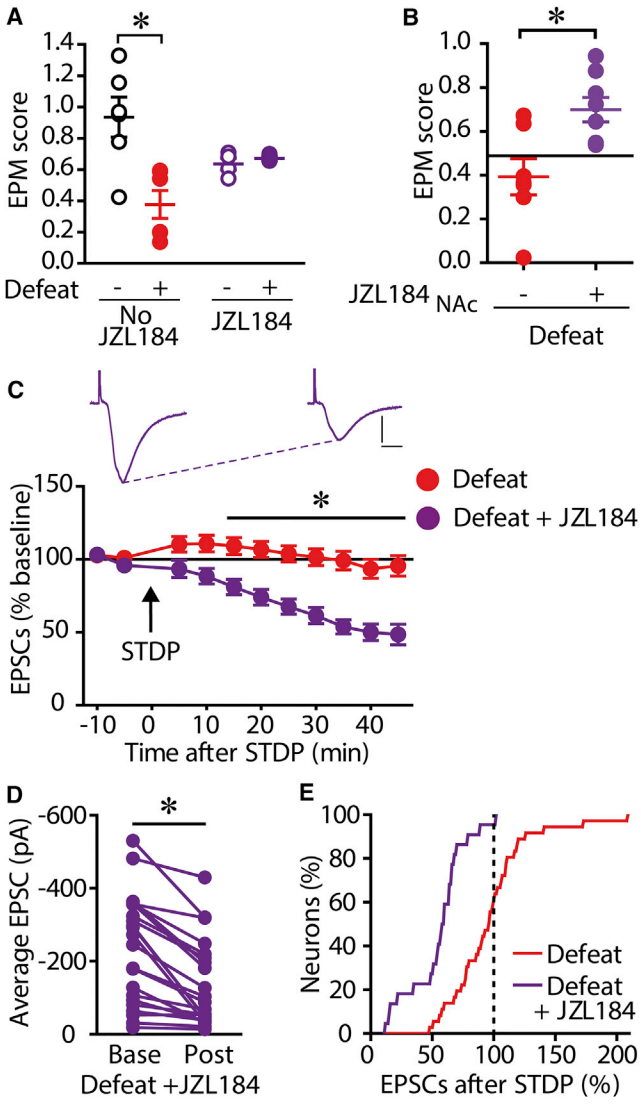

Figure 3. Enhancement of Circulating 2-AG Normalizes Anxious Behavior and Synaptic Depression in Defeated Mice

(A) A single injection of JZL184 (16 mg/kg) is sufficient to restore a normal anxiety-like behavior. Elevated plus maze (EPM) score is the average of normalized measures for number of head dippings, percentage of time in the open arms, and number of entries in the open arms. Undefeated: 0.94 ± 0.13, n = 6; defeated: 0.38 ± 0.09, n = 6; undefeated + JZL184: 0.64 ± 0.04, n = 4; defeated + JZL184: 0.67 ± 0.01, n = 3. Two-way ANOVA with Bonferroni post-test, with JZL184 ($F_{(1,15)}$ = 0.0005, p = 0.9821) and CSDS ($F_{(1,15)}$ = 5.983), p = 0.0273) as factors. Interaction: $F_{(1,15)}$ = 7.713, p = 0.0141. *p < 0.05.

(B) Infusion of JZL184 bilaterally in the nucleus accumbens also restores anxiety behavior of defeated mice. EPM score. Defeated: 0.39 ± 0.08, n = 7; defeated + $JZL184_{NAc}$: 0.70 ± 0.06, n = 7. t_{13} = 3.158, *p = 0.0076, unpaired t test.

(C) Time course of STDP-LTD in defeated mice without JZL184 (red) and with JZL184 (purple). EPSC normalized amplitude 45 min after STDP. Defeated: 96.88% ± 5.36%, n = 36; defeated + JZL184: 55.13% ± 4.96%, n = 22. *p < 0.05, two-way ANOVA with Bonferroni post-test, with JZL184 ($F_{(1,572)}$ = 140.07, p < 0.0001) and time ($F_{(10,572)}$ = 8.08, p < 0.0001) as factors. Interaction: $F_{(10,572)}$ = 4.02, p < 0.0001. Illustration traces: example of ten averaged EPSCs during baseline (left) and 45 min after STDP (right). Scale bar, 100 pA, 10 ms. (A–C) Data are presented as mean ± SEM.

(D) EPSC amplitude before and after STDP protocol for defeated mice with JZL184 bath application. Before, −233 ± 32 pA, versus after, −131 ± 25 pA; n = 22, *p < 0.0001, paired t test. Each line represents one neuron.

(E) Cumulative probability distribution of normalized EPSCs after STDP protocol. Same color code as (C).

responses (Golden et al., 2011; Krishnan et al., 2007) similar to those observed after traumatic stress in humans. This particularity makes CSDS in rodents a useful model for studying the mechanisms that underlie anxiety and depression onset. Here, we used several measures of emotional behavior to classify a population of CSDS-exposed mice into anxious and non-anxious groups using cluster analysis. Using this approach, we identified a non-anxious set of CSDS animals corresponding to approximately half of the entire population that failed to develop anxiety-related behavior and exhibited a behavioral phenotype comparable to that of undefeated mice. Both anxious and non-anxious mice showed strong generalized social avoidance. This study therefore reports individual differences in anxiety-like behaviors in mice exposed to CSDS. In the nucleus accumbens, CB1R is expressed at both excitatory and inhibitory synapses (Pickel et al., 2004). However, STDP protocol requires activation of pre- and post-synaptic elements of the stimulated synapses, which rules out the contribution of interneurons' plasticity in the observed STDP-LTD.

It has been previously reported that anxiety-like behavior may be correlated to levels of eCBs in the brain (Hill and Patel, 2013; Qin et al., 2015), but the effects on the eCB synaptic plasticity remained unexplored. In our study, we demonstrated that anxiety behavior induced by CSDS covariates with eCB-dependent plasticity. Here, we used a STDP protocol to reveal the functionality of the eCB system. Hebbian synaptic plasticity induced by STDP has been described in intact brains in the human cortex and in sensory systems and is thought to be a neurobiological basis for associative learning (Letzkus et al., 2007). However, the significance of STDP in non-sensory or motor systems remains to be clarified. We demonstrated that non-anxious mice display an attenuation in eCB STDP but that this form of plasticity is abolished in anxious mice. This suggests that eCB Hebbian plasticity constitutes a system for adaptive synaptic plasticity in the accumbens that allows behavioral adaptability and thus avoids the development of strong anxiety-like behavior following CSDS.

The role of the eCB system in stress and anxiety disorders may rely on its reciprocal interactions with the hypothalamic-pituitary-adrenal axis, which is responsible for stress response in the body (Gorzalka and Hill, 2009; Hill and Tasker, 2012; McEwen et al., 2015). It has been previously shown that stress-induced modulation of corticosterone affects the eCB system in the amygdala and the hypothalamus (Gray et al., 2015; Qin et al., 2015; Wamsteeker et al., 2010). In the current study, basal corticosterone levels were increased in both anxious and non-anxious defeated groups, without apparent correlation to the level of eCB-LTD. Whether hypothalamic-pituitary-adrenal axis reactivity due to acute stress might be different between groups has yet to be determined, but this is a focus of future research for our group. Recent findings report preexisting individual differences in the peripheral immune system that

predict and promote stress susceptibility (Hodes et al., 2014). In our study, innate differences in the functionality of the eCB system could be responsible for vulnerability to social defeat-induced emotional alteration.

The lack of eCB plasticity in anxious mice may arise from reduced eCB levels. Reduction in circulating levels of the eCB 2-AG has been found in individuals exposed to traumatic stress, as well as in rodents exposed to chronic stress (Hill et al., 2009), while enhancement of 2-AG levels increases behavioral resiliency to chronic stress in mice (Zhang et al., 2015; Zhong et al., 2014). Thus, we tested whether increased anxiety-related behavior could be overcome by enhancing 2-AG signaling, the main endocannabinoid involved in LTD in the accumbens (Castillo et al., 2012). We found that pharmacological blockade of intracellular 2-AG hydrolysis in mice subjected to CSDS alleviated the anxiety-related behavior and restored eCB-dependent plasticity in a CB1R-dependent manner. This suggests that CSDS-induced anxiety alters the bioavailability of eCB.

In conclusion, we found that impairment of eCB plasticity in the accumbens is a synaptic signature for behavioral adaptability following social stress. The restoration of eCB signaling through the improvement of 2-AG signaling protects against CSDS-induced anxiety-like behavior. Finally, exploring emotional processes at the synaptic level would lead to a better understanding of how chronic stress affects our brain and offer a pharmacologically amenable mechanism to promote resiliency to stressful events.

EXPERIMENTAL PROCEDURES

CSDS and Behavioral Testing

All experiments were performed according to criteria of the European Communities Council Directive (50120103-A) on C57BL/6J adult male mice. The social defeat protocol was performed as previously described (Larrieu et al., 2014). Behavioral tests were performed 24–48 hr after the last session of social defeat using a social avoidance test, open field test, light/dark box test, and elevated plus maze test (see supplemental Information for details).

The anxiety score was calculated as the algebraic sum of standardized scores ((x − min value) / (max value − min value)) of each of the six analyzed parameters of the three anxiety-related behavior tests. When more than one parameter was used for one test, normalized values of parameters were averaged so that the power of each of the three anxiety tests was equal to 1. This procedure yields scores that are distributed along a scale from 0 to 3, with 3 reflecting high anxiety.

Electrophysiology

Brain slices were prepared 24 hr after the last behavioral test for each animal. Signals were amplified and recorded with Multiclamp 700B, controlled with pClamp 10.3 software via a Digidata 1440A interface (Molecular Devices). STDP protocol consists of pairing pre- and postsynaptic stimulations 100 times at 1 Hz, with a delay of ~15 ms and the neuron held at −70 mV in the current clamp configuration. Medium spiny neurons were recorded for 10 min of stable baseline (0.1 Hz) and for at least 35 min after the STDP protocol. Chemicals used were AM251 (4 μM), SR141716A (1 μM), and JZL184 (1 μM).

In Vivo Pharmacological Experiments

For JZL184 i.p. injections, mice were given i.p. injections of vehicle (22.5:100 HBC:H_2O) or JZL184 (16 mg/kg) 6 hr before the elevated plus maze test. For JZL injections in the nucleus accumbens, animals were implanted 1 week before CSDS started with bilateral guide cannulas (PlasticsOne) 1 mm above the nucleus accumbens. JZL184 (1 μg/0.5 μl DMSO 10% in saline/hemisphere) or vehicle was injected into the nucleus accumbens 1 hr before the elevated plus maze test.

Statistical Analyses

Statistical tests were performed with Prism (GraphPad) using a critical probability of $p < 0.05$. All values are given as mean ± SEM. The dendrogram was obtained with XLStat (Addinsoft) using centroid hierarchical cluster analysis (Euclidienne distance and Ward method) to separate the defeated mice into anxious and non-anxious phenotypes. See Supplemental Information for details.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, three figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2016.06.082.

AUTHOR CONTRIBUTIONS

Conceptualization, C.B.-B., T.L., O.J.M., and S.L.; Methodology, C.B.-B., T.L., and S.L.; Investigation, C.B.-B., T.L., and L.L.; Writing C.B.-B., T.L., O.J.M. and S.L., Funding Acquisition, S.L. and O.J.M.; Supervision, S.L. and O.J.M.

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Supplemental Information

Endocannabinoid-Mediated Plasticity in Nucleus Accumbens Controls Vulnerability to Anxiety after Social Defeat Stress

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SUPPLEMENTAL INFORMATION

EXPERIMENTAL PROCEDURES

Animals

All experiments were performed according to criteria of the European Communities Council Directive (50120103-A). Behavioral and biochemical experiments were performed on C57Bl6/J mice obtained from Janvier Labs (France). Mice were maintained under standard housing conditions on corn cob litter in a temperature- (23 ± 1 °C) and humidity (40 %) -controlled animal room with a 12-h light/dark cycle (0700–1900 hours), with *ad libitum* access to food and water. Retired CD1 breeders used as the aggressors in the social defeat experiments were obtained from Janvier Labs. All tests were conducted during the light period. C57BL6/J male mice were housed two per cage and maintained in a temperature- and humidity-controlled facility on a 12-h light dark cycle with food and water *ad libitum*. Mice were 3-month-old when the behavioral, biochemical, and electrophysiological analysis were conducted.

Chronic social defeat stress

Social defeat protocol was performed as previously described (Larrieu et al., 2014). Briefly, intruder mice were exposed individually to an aggressive CD1 mouse for 5 min per day, during which they were attacked and displayed subordinate posturing. Each episode of stress was followed by 3 h of protected sensory contact with their aggressor, where the intruder and CD1 mice were separated by a perforated Plexiglas divider in the home cage of the CD1 mouse. C57Bl6/J mice were exposed to a different aggressor each day for 10 days in order to prevent any habituation to the resident aggressor. Undefeated mice were placed in pairs within a home cage set-up identical to that of the defeated mice, with one undefeated mice per side

separated by a perforated Plexiglas divider for the duration of each sensory contact session. Each mouse was weighted after the 3h-sensory- contact period for 10 days. Twenty-four hours after the last session of stress, social avoidance test was performed to verify that CSDS can cause social avoidance and open-field test to assess anxiety-like behavior. Two days after the last episode of social defeat, anxiety-like behavior was further analyzed in a light/dark box test and elevated plus maze test. The same animals were used for plasmatic corticosterone levels as well as electrophysiological analyses. Basal condition group is indicated as 'undefeated' and social defeat condition group as 'defeated'. To avoid maximum suffering, CSDS was carried out with the minimum number of animals.

Behavioral testing

Social avoidance. Mice were transferred to a new cage (40 × 40 cm). A social exploration session comprised 5 min without target where the open-field contained an empty wire mesh in the corner, followed by 5-min exposure of an unfamiliar adult CD1 male enclosed in a wire mesh placed in the corner of the open-field. The number of active investigatory behavior (mainly sniffing the anogenital region, mouth, ears, trunk and tail) was manually counted by an experimenter blind to the conditions. For supplemental analysis, the social interaction ratio was calculated as previously described (Golden et al., 2011). The social interaction ratio (SI ratio) is obtained by dividing the time spent in the interaction zone when the target is present by the time spent in the interaction zone when the target is absent. Historically, a SI ratio equal to 1 has been used as the threshold for dividing defeated mice into the susceptible and resilient categories (Golden et al., 2011).

Open-field. The apparatus consisted of a Plexiglas open-field (40 x 40 cm) with 16-cm-high walls. Lighting consisted of four fluorescent bulbs at a height of 2 m above

the floor of the open-field apparatus placed on each corner of the experiment room (light intensity of 30 Lux). The floor was cleaned between each trial. Each mouse was transferred to an open-field facing a corner and was allowed to freely explore for 10 minutes the open-field. A video tracking system (Smart, Panlab, Barcelona, Spain) recorded the exact track of each mouse as well as total distance travelled (cm) and the time spent exploring the center of the arena.

Light/Dark box (L/D). The L/D box test uses a 44 × 21 × 21 cm high Plexiglas box divided into a dark (14 x 21 x 21 cm) and a light (30 x 21 x 21 cm; 200 lux illuminated) compartments separated by an open door (5 x 5 cm) located in the center of the partition at floor level. Each mouse was placed into the dark chamber and the door was opened 5 seconds later. The door is used in order to avoid that mice escape from experimenter in the light side. Mice were allowed to freely explore the apparatus for 10 min. Time spent in the light compartment and number of crosses between the dark and light compartments was measured as an indicator of anxiety-like behavior.

Elevated plus maze. The elevated plus maze consisted of two open arms (30 x 8 cm) and two closed arms (30 x 8 x 15 cm). A neutral area of (8 x 8 cm) interconnected all four arms. Arms that are arranged to form a plus shape. The open arm had a rim with a height of 0.5 cm to facilitate the grip on the open arms. The elevated plus maze was located 120 cm above the floor. The apparatus was novel to the mice at the time of testing and each mouse was tested only once. Each mouse was placed onto the central platform facing an open arm and allowed to freely explore the apparatus for 5 min. Session was performed in a room weakly illuminated with a light intensity of 15 Lux. Mice were tracked with Smart software to measure the percent time spent on

the open-arms (expressed as time spent in open-arms / time spent in open and closed arms x 100) and the number of transitions to the open arms. The number of head dipping was manually counted by an experimenter blind to the conditions.

Anxiety score. It was calculated as algebraic sum of standardized scores of each of the 6 analyzed parameters of the three anxiety-related behavior tests (open-field (time spent in the center (%)), light/dark box (Time spent in the light compartment (%) and number of transitions to the light compartment (%)) and elevated plus maze (Time spent in the open arms (%), number of head dipping and number of transitions to the open arms). When more than one parameter was used for one test, normalized values of parameters were averaged so that the power of each of the three anxiety tests was equal to 1. Standardization consisted in subtracting the value of each animal to the minimum value of the whole population and then dividing this number by the maximum value of the whole population minus minimum value of the whole population: (x – min value) / (max value – min value). This procedure yields scores which are distributed along a scale from 0 to 3, 3 reflecting high anxiety.

Hypothalamic-pituitary-adrenal axis analysis

Trunk blood collection in ethylenediaminetetraacetic acid-lined tubes (EDTA) was performed early in the morning (08:00) before brain slicing. Corticosterone was measured with an in-house radioimmunosorbent assay in the plasma as previously described (Larrieu et al., 2014). Briefly, after steroid extraction with absolute ethanol, total corticosterone was measured by competition between cold corticosterone (B) and ^{3}H-B (B*) by a specific anti-corticosterone antibody provided by Dr H Vaudry (University of Rouen, France). We conducted corticosterone analysis in basal condition, one day after the last behavioral test in order to overcome the stress

induced by the test. Adrenal glands were removed, dissected free of adhering fat, and weighted. Organ weights are expressed in milligram.

Electrophysiology

Twenty-four hours after the last behavioral test, mice were briefly anaesthetized with isoflurane and decapitated. The brain was removed from the skull and 350 µm-thick slices were cut in the parasagittal plan with a ~10°-angle using a vibratome (VT1000S, Leica Microsystems). The composition of the extracellular solution was (mM): 125 NaCl, 2.5 KCl, 25 glucose, 25 NaHCO3, 1.25 NaH2PO4, 2CaCl2, 1MgCl2, bubbled with 95% O2 and 5% CO2. For the slicing procedure and rest of slices, 10 µM pyruvic acid was added to the extracellular solution. The brain was sliced at 4°C and slices had a rest period of 1h at 34°C before recording started.

Whole-cell recordings were performed at 32° using a temperature controller (5TC-344B, Warner Instrument Corporation) with borosilicate glass pipettes of 4-8 MΩ resistance containing (mM): 128 K-gluconate, 20 NaCl, 10 Hepes, 1 $MgCl_2$, 0.3 $CaCl_2$, 2 Na_2-ATP, 0.3 NA-GTP, 0.2 cAMP, 1 EGTA, adjusted to pH 7.35 with KOH. Slices were continuously superfused at 2-3 ml/min with the extracellular solution. Individual medium-size spiny neurons in the accumbens were identified using infrared-differential interference contrast microscopy with CCD camera (Roper Scientifc). Signals were amplified and recorded with Multiclamp 700B (Molecular Devices) controlled with pClamp 10.3 software via a Digidata 1440A interface (Molecular Devices). Data were sampled at 20 kHz and filtered at 5 kHz. A bipolar concentric electrode (Phymep) was place in the deep layers of prefrontal cortex, and EPSCs were evoked with 0.1Hz monophasic stimulation at constant current (0.5 to 2

mA, 150 µs pulse width). EPSCs were recorded in MSNs in voltage-clamp configuration with a membrane potential maintained between -60 and -80 mV. STDP protocol consists in pairing of pre- and postsynaptic stimulations 100 times at 1 Hz with a decay of ~15ms with neuron held at -70 mV in current clamp configuration. Postsynaptic stimulation correspond to a spike evoked by a depolarizing step evoked (200-350 pA for 10-20 ms) and delay between pre- and post-synaptic stimulations is calculated between the onset of the synaptic response and the peak of the spike. Neurons were recorded for 10 min of stable baseline and for at least 35 min after STDP protocol, and LTD was measured between 35 and 45 min after STDP protocol. Series resistances were measured with a -5 mV (50 ms) step throughout the experiment and a variation above 20% led to the rejection of the experiment. AMPA/NMDA ratio and mEPSCs were recorded with the following intracellular solution (in mM): 125 Cesium-Methisulfonate, 20 NaCl, 10 Hepes, 1 $MgCl_2$, 0.3 $CaCl_2$, 2 Na_2-ATP, 0.3 Na-GTP, 0.2 cAMP, 1 EGTA, adjusted to pH 7.35 with CsOH. For AMPA/NMDA ratio, 20 stimulations at 0.1 Hz at -60 mV (AMPA component) and 20 stimulations at +40 mV (NMDA component) were averaged for each tested neuron. For mEPSCs, 10s of recording in voltage clamp configuration at -60 mV were analyzed for each neuron.

AM251 (4µM), SR141716A (1µM) and JZL184 (1µM) were provided by Tocris (USA) and were dissolved in DMSO at a final concentration of DMSO of 0.1% in the extracellular solution. Experiments were performed deliberately without GABA-A blockers because they greatly change the excitability of MSNs and can consequently affect the response of MSN to STDP protocol (Paille et al., 2013).

In vivo pharmacological experiments

To examine the role of endogenous 2-AG in the behavioral changes measured in defeated mice, 2-AG supplementation was pharmacologically created. Twenty-four hours after the last session of social defeat, undefeated and defeated mice were given intraperitoneal (i.p.) injections of vehicle (22.5:100 2-Hydroxylpropyl-β-cyclodextrin (HBC):H_2O) or JZL184 (16mg per kg; 0.1ml per 10g of mouse) 6 hours before elevated plus maze test. JZL184 was freshly dissolved in HBC vehicle and mix thoroughly during 30 minutes.

To reinforce the role of 2-AG increase in the NAc on anxiety behavior, we injected JZL184 directly into the NAc. For this experiment, animals were implanted 1 week before CSDS starts. For surgery, mice were anaesthetized with isoflurane (1-2 %) inhalation and positioned in a standard stereotaxic frame (Kopf Instruments). Guide cannulas (26G, PlasticsOne) were implanted bilaterally 1 mm above the NAC with an angle of 6° with the following coordinates from bregma: anteroposterior: + 1.8 mm; mediolateral: +/- 1.58 mm; dorsoventral: - 2.77 mm. Guide cannulas were fixed to the skull with dental acrylic cement and anchored with three surgical screws placed in the skull. Stylets (33G, PlasticsOne) were inserted into guide cannulas to prevent clogging. Mice were given tolfedine 0.4 mg/kg s.c. for recovery. Mice were handled and trained for head restraint daily for one week. After CSDS was completed, JZL 184 (1 µg / 0.5 µl DMSO 10 % in saline / hemisphere, 0.1µl/min) (Morena et al., 2015) was injected into the NAc 1 h before the EPM with an injection cannula (33G, PlasticsOne) that protruded the guide cannula from 1 mm (final depth into the brain : -3.77 mm). Sham animals were injected with the same volume of DMSO 10 %. For histological control, mice were injected with 0.5 µl / side with evans blue, brains were frozen and 40µm-slices were performed with a cryostat (Leica). Animals with cannulas that were not in the NAc were discarded from the final analysis.

Statistical analyses

Statistical tests were performed with GraphPad Prism (GraphPad software, San Diego, CA, USA) using a critical probability of $P<0.05$. All values are given as mean ± s.e.m. Statistical analyzes performed for each experiment are summarized in each figure legend with the chosen statistical test, n and P-values, as well as degree of freedom and F/t values. Dendrogram was obtained with XLStat (Addinsoft) using centroid hierarchical cluster analysis (Euclidienne distance and Ward method) to separate the defeated mice into two phenotypes: non-anxious and anxious. As appropriate, we used paired t-test, unpaired t-test, 1-way ANOVA with Dunn's multiple comparison test and 2-way ANOVA followed by the Bonferroni post hoc test. Correlation analyses were performed with Pearson correlation coefficients (r).

SUPPLEMENTARY FIGURES

Supplementary Table 1: Table of correlation values between LTD and behavioral measures. Linear regression with Pearson tests.

Supplementary Figure 1: Classification of defeated mice into resilient and susceptible groups, related to Figure 1. CSDS induces social avoidance in susceptible mice. (A) The social interaction ratio was used to segregate defeated mice between susceptible (orange) and resilient (gray). Social interaction ratio: resilient: 1.61 ± 0.08, n=25; control: 1.27 ± 0.08, n=18; susceptible: 0.71 ± 0.08, n=11. (B, C) Time spent in interaction (B) and corner (C) zones, with and without target for control, resilient and susceptible mice. 2-way ANOVAS

(D) Number of resilient and susceptible mice that were classified anxious and non-anxious, based on the cluster analysis of emotional behaviors. Resilient: 13 anxious and 13 non-anxious mice; susceptible: 6 anxious and 5 non-anxious mice.

(E-I) Behavioral portraits of control (white, n=20), resilient (gray, n=26) and susceptible (orange, n=11) mice, Kruskal-Wallis 1-way ANOVA test. (E) Time spent in center of open field: control: 10.9 ± 1.2 %; resilient: 10.4 ± 1.4 %; susceptible: 8.2 ± 1.4 %, p=0.2844. (F) Time in the light compartment: control: 253 ± 17 s; resilient: 198 ± 15 s; susceptible: 197 ± 21 s, p=0.0531. (G) Time in the open arms: control: 16.8 ± 1.5 %; resilient: 13.9 ± 1.4 %; susceptible: 14.6 ± 2.5 %, p=0.3906. (H) Anxiety score: control: 1.63 ± 0.09; resilient: 1.92 ± 0.08; susceptible: 1.95 ± 0.12, p=0.0566. (I): corticosterone levels: control: 28.2 ± 3.5 nM; resilient: 52.0 ± 5.0 nM; susceptible: 78.8 ± 24.8, p=0.0010 with Dunn's multiple comparison: control vs. resilient: $p<0.01$, control vs. susceptible $p<0.05$.

Supplementary Figure 2: Behavioral assessment of undefeated, non-anxious and anxious, related to Figure 1.

(A-C) Behavioral portraits of undefeated (white) and defeated (red) mice. *$p<0.05$, unpaired t-test. (A) Left, illustrative video track of undefeated (left) and defeated (right) mice in the social avoidance test. Right, defeated mice displayed social avoidance, with significantly less contacts with the target mouse, compared to undefeated mice, $t_{54}=5.456$, *$P<0.0001$, unpaired t-test, 56.8 ± 2.9 contacts, n=19 (undefeated) vs. 37.3 ± 2.1 contacts, n=37 (defeated) and increased time in the corner zone, $t_{50}=2.656$, *$P<0.0106$, unpaired t-test, 42 ± 4 s, n=19 (undefeated) vs. 60 ± 5 s, n=34 (defeated) (B) Left, illustrative video track of undefeated (left) and defeated (right) mice in the light/dark box. Light blue, light compartment, dark blue,

dark compartment (no tracking). Right, defeated mice made less transitions to the light compartment t_{54}=3.206, *p=0.023, undefeated mice: 37.3 ± 1.5 transitions, n=19; defeated mice: 20.9 ± 1.2 transitions, n=37. (C) Left, illustrative video track of undefeated (left) and defeated (right) mice in the elevated plus maze. Red, open arms, orange, risk zone, green, closed arms, blue, safe zone. Right, defeated mice decreased the number of head dipping t_{54}=3.943, *p=0.0002, undefeated mice: 8.68 ± 1.25 head dipping, n=19; defeated mice: 4.06 ± 0.54 head dipping, n=37) and decreased the number of entries in open arms t_{52}=2.013 *p=0.0493, undefeated mice: 13.2 ± 1.0 entries, n=19; defeated mice: 10.3 ± 0.9 entries, n=35..

(D-G) Behavioral results for non-anxious (black) and anxious (red) clustered mice. Dashed line corresponds to the mean value for undefeated mice. (D) Anxious and non-anxious mice displayed similar levels of social avoidance: number of contacts: t_{35}=0.03170, P=0.7532, unpaired t-test, non-anxious: 39.0 ± 2.7 contacts, n=18; anxious: 35.6 ± 3.2 contacts, n=19; time in interaction zone: t_{35}=0.7000, P=0.4885, unpaired t-test, non-anxious: 111 ± 10 s, n=18; anxious: 99 ± 13 s, n=19; time in corner zone: t_{35}=0.3658, P=0.7167, unpaired t-test, non-anxious: 59 ± 8 s, n=18; anxious: 55 ± 7 s, n=19 (E) No difference for the time spent in center of open field t_{35}=1.611, P=0.1162, unpaired t-test, non-anxious: 11.3 ± 1.7 %, n = 18; anxious: 8.3 ± 1.2 %, n = 19. (F) Compared to non-anxious mice, anxious mice spent less time in light t_{35}=5.167, *p<0.0001, unpaired t-test, non-anxious: 244 ± 14 s, n=18; anxious: 154 ± 13 s, n=19 and made fewer transitions to the light compartment t_{35}=4.953, *P<0.0001, unpaired t-test, non-anxious: 25.7 ± 1.3 transitions, n=18; anxious: 16.3 ± 1.4 transitions, n=19. (G) Compared to non-anxious mice, anxious mice spent less time in open arms, t_{35}=5.245, *P<0.0001, unpaired t-test, non-anxious: 19.1 ± 1.4 %, n=18; anxious: 9.3 ± 1.3 %, n=19, made fewer head dipping in an elevated plus

maze, t_{35}=5.355, *P<0.0001, unpaired t-test, non-anxious: 6.50 ± 0.76 head dipping, n=18; anxious: 2.26 ± 0.39 head dipping n=19, and made fewer entries in open arms, t_{35}=5.283, *P<0.0001, unpaired t-test, non-anxious: 19.1 ± 1.4 entries, n=18; anxious: 9.3 ± 1.3 entries n=19. (H) Time course of body weight of undefeated (white), non-anxious (black) and anxious mice (red), from the first day of CSDS to the last day before electrophysiological recording. Body weight are significantly different between undefeated and non-anxious mice from day 7 and between undefeated and anxious mice from day 10, *p<0.001, 2-way ANOVA with Bonferroni post-test with "group" ($F_{(2,636)}$=7.30, p=0.0016) and "time" ($F_{(12,636)}$=100.68, p<0.0001) as factors; interaction: $F_{(24,636)}$=4.89, p<0.0001. (I) Absolute adrenal weight of undefeated (white), non-anxious (black) and anxious (red) mice F(2,53)=27.26; p<0.0001, 1-way ANOVA with Dunn's multiple comparison test *p<0.001, undefeated: 4.966 ± 0.26, n=20; non-anxious: 8.215±0.39, n=18; anxious: 8.409±0.46, n=19. (J) Total corticosterone plasma levels are similar in the non-anxious and anxious groups $F_{(2,50)}$=6.307; p=0.036 1-way ANOVA with Dunn's multiple comparison test *p<0.05, Undefeated (white): 28.15 ± 3.52 nM, n=20, non-anxious (black): 49.50 ± 6.55 nM, n=16; anxious (red): 50.65 ± 5.71 nM, n=17.

Supplementary Figure 3: CB1R-dependency of STDP-LTD and membrane / synaptic properties of Accumbens neurons, related to Figure 2 and 3.

(A) STDP-LTD is mediated by CB1R in undefeated mice (white, 51.3 ± 4.4 % of baseline, n = 16, p<0.0001). Bath application of the CB1R antagonists AM251 (light gray, 96.8 ± 10.9 % of baseline, n = 11, p=0.7769), or SR141716A (dark gray, 82.7 ± 12.7 % of baseline, n = 4, p=0.6250) completely blocked STDP induction. *p<0.0001, Wilcoxon signed rank test with the theoretical value of 100. Illustration traces:

example of EPSCs (average of 10 sweeps) during baseline (left) and 45 min after STDP (right) in presence of AM251 in the bath. (B) Total corticosterone plasma levels (nM) and endocannabinoid-LTD (% EPSC 45 min after STDP) are not correlated (r^2=0.07236, p=0.1025, Pearson test). Each dot represents one mouse.

(C) Representative raw traces of individual voltage responses of neurons to series of 600-ms current pulses with 20 pA increment, starting at -100 pA (left) and 30 pA after rheobase (right) for undefeated (gray, top), non-anxious (black, middle) and anxious (red, bottom) mice. (D) Averaged current-voltage (I-V) curves recorded in neurons of undefeated (white), non-anxious (black) and anxious (red) mice. 2-way ANOVA with "current" ($F_{(35,1899)}$=509.25, p<0.0001) and "group" ($F_{(2,1899)}$=31.56, p<0.0001 as factors; interaction: $F_{(70,1899)}$=4.17, p<0.0001. Bonferroni post-test revealed a significant difference between non-anxious and anxious groups, *p<0.01 between 80 and 110 pA and *p<0.001 between 120 and 190 pA, (E) Passive membrane properties (left, RMP: resting membrane potential; middle, rheobase; right, membrane potential threshold for the first action potential) for undefeated (white), non-anxious (black) and anxious (red) mice. (F) AMPA/NMDA ratio for undefeated (white) and defeated (red) mice. (G): average mEPSC frequency (left) and mEPSC peak amplitude (right) show no difference between undefeated (white, n=5) and defeated (red, n=5) mice. P>0.05, unpaired t-test.

(H) The effects of JZL184 are mediated by CB1R. In control conditions STDP-LTD is conserved in non-anxious mice (black, 83.0 ± 4.4 % of baseline, n = 18, p=0.0013) and abolished anxious mice (red, 108.0 ± 7.4 % of baseline, n = 18, p=0.3633). In slices treated with JZL184, STDP-LTD was indistinguishable in non-anxious (purple, 54.4 ± 6.5 % of baseline, n = 10, p<0.0001) and anxious (pink, 55.7 ± 7.6 % of baseline, n = 12, p=0.0001) mice. *p<0.01, one sample t-test with the theoretical

value of 100. (I) STDP protocol does not induce plasticity in defeated mice (red, 96.9 ± 5.4 % of baseline, n = 36, p=0.1949) but bath application of JZL184 induces a significant LTD following STDP protocol (purple, 55.1 ± 5.0 % of baseline, n = 22, p<0.0001) and this is blocked by co-application of SR141716A (blue, 111.2 ± 29.4 n = 3, p=0.7500). Each dot represents one neuron.

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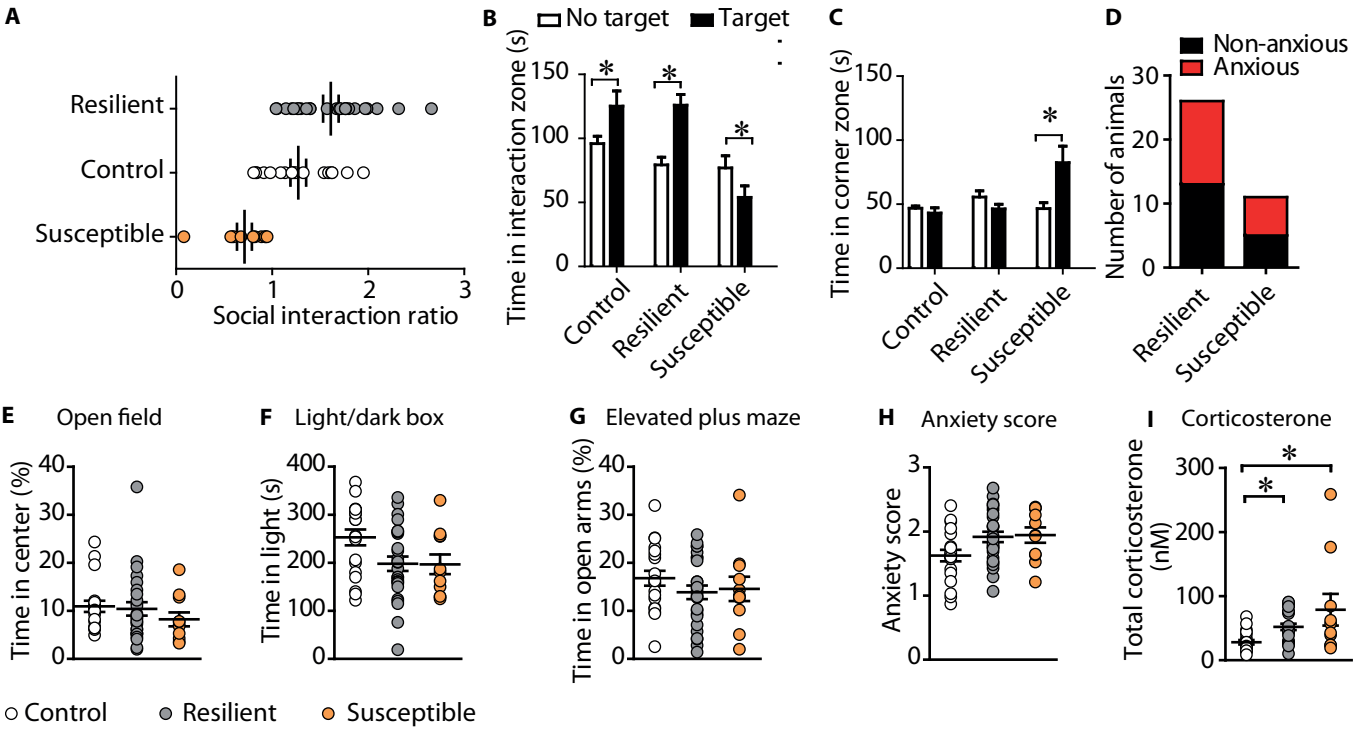
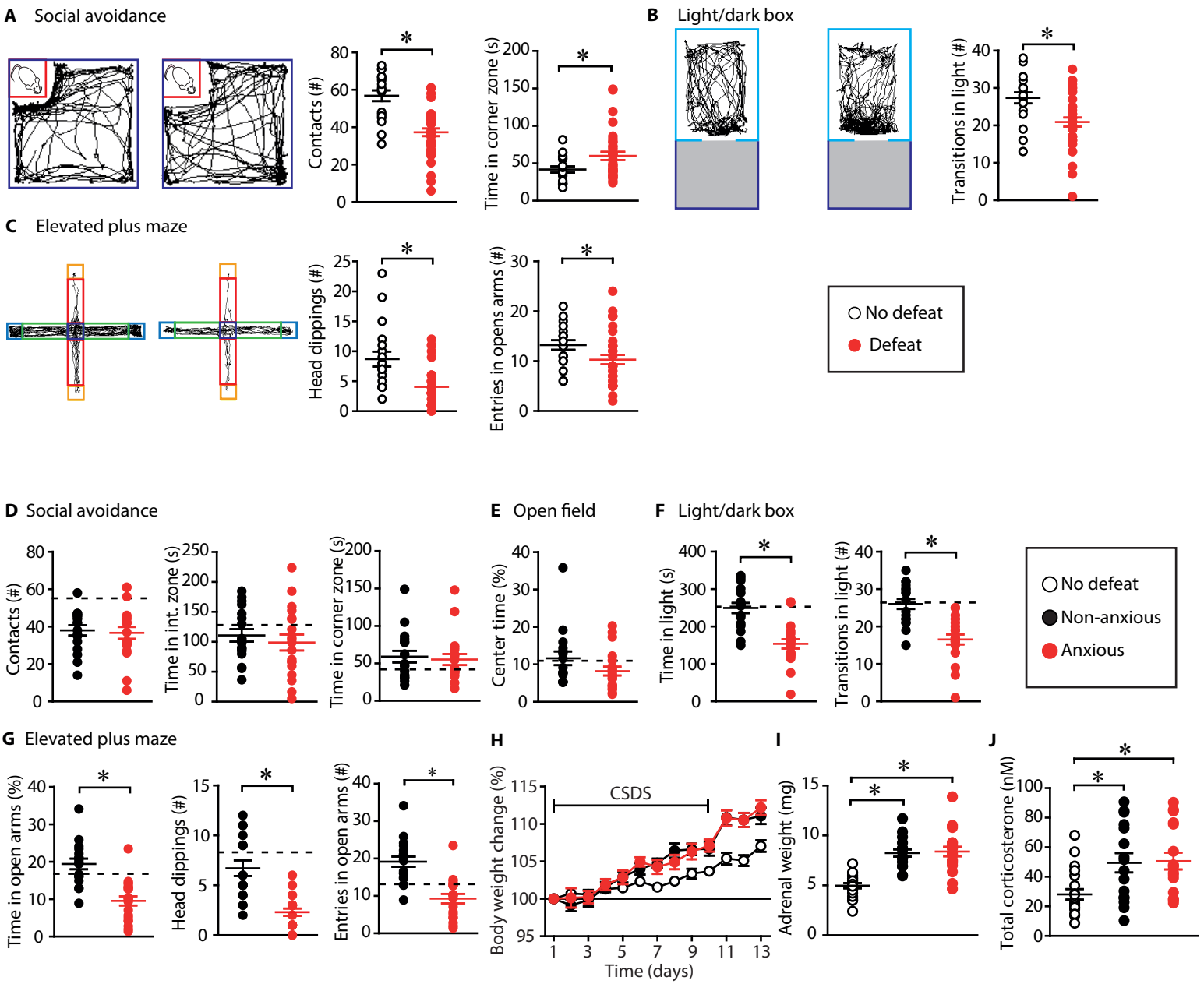
Supplemental Table 1

	Behavior correlated to LTD	Pearson r	r^2 value	p value
Social interaction	**# of contacts**	-0.4587	0.2105	**0.0038**
	Time in corner zone (s)	0.2554	0.0653	0.1217
	Time in interaction zone (s)	-0.2785	0.0775	0.0905
Open field	**Distance (cm)**	-0.3262	0.1064	**0.0456**
	Time in center (%)	0.1003	0.0101	0.5490
Light/dark	**# transitions to light**	-0.3202	0.1025	**0.0500**
	Time in light (s)	-0.4927	0.2428	**0.0017**
Elevated plus maze	**# of head dipings**	-0.3402	0.1157	**0.0366**
	# entries open arms	-0.2180	0.0476	0.1885
	Time in open arms (%)	-0.3064	0.09388	0.0614
Anxiety score (OF + L/D + EPM)		0.3459	0.1222	**0.0339**

Supplementary Figure 1



Supplementary Figure 2



Supplementary Figure 3