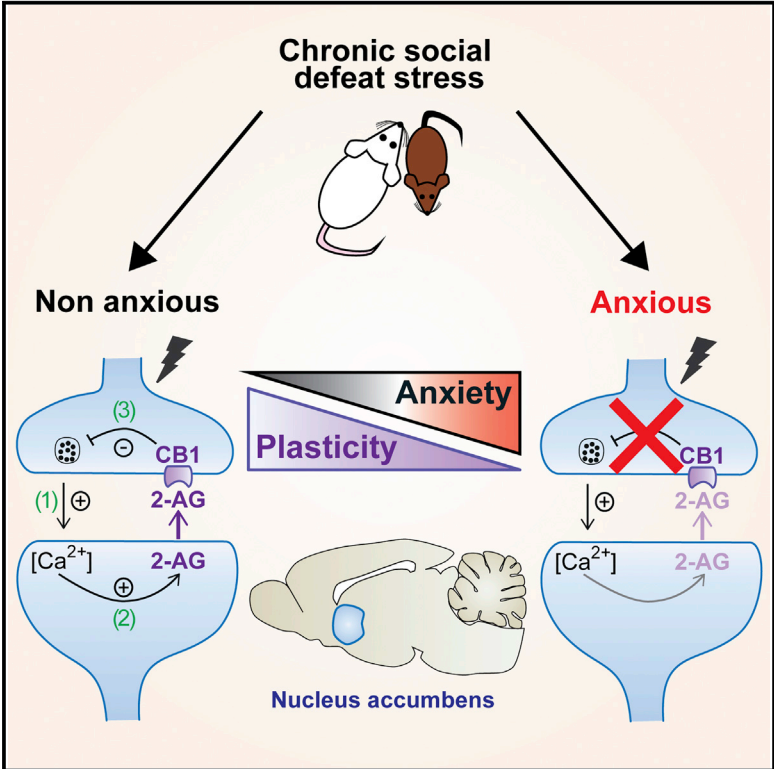# Cell Reports

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

Graphical Abstract

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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.

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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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
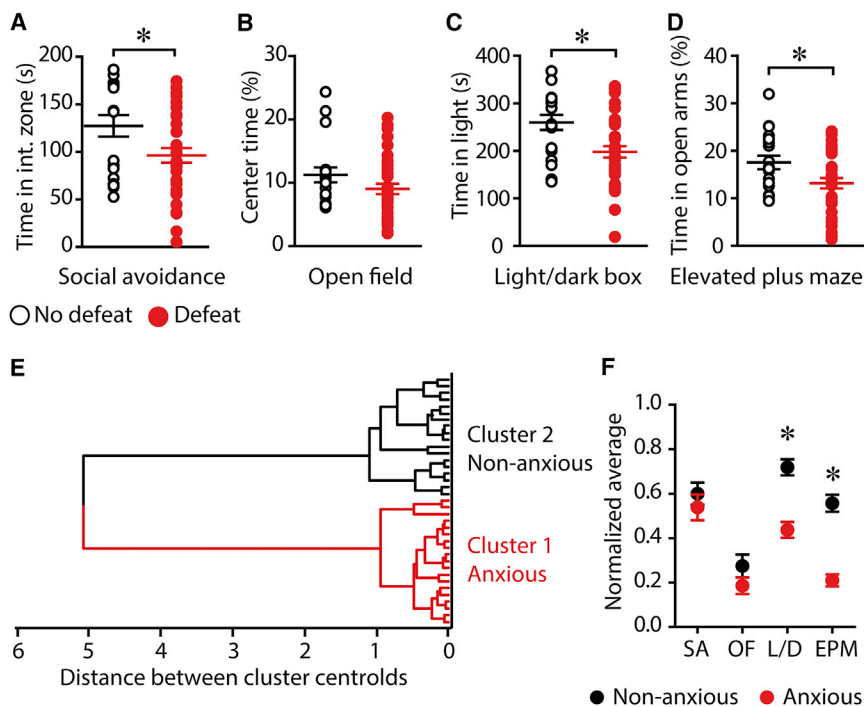
Figure 1. Behavioral Clustering in Socially Defeated Mice

(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
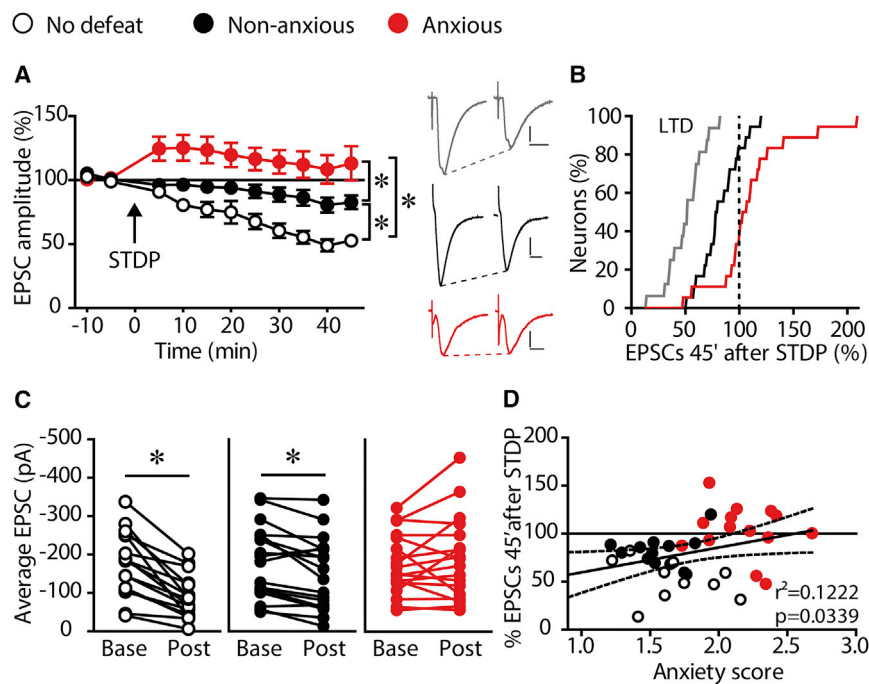
○ No defeat   ● Non-anxious   ● Anxious

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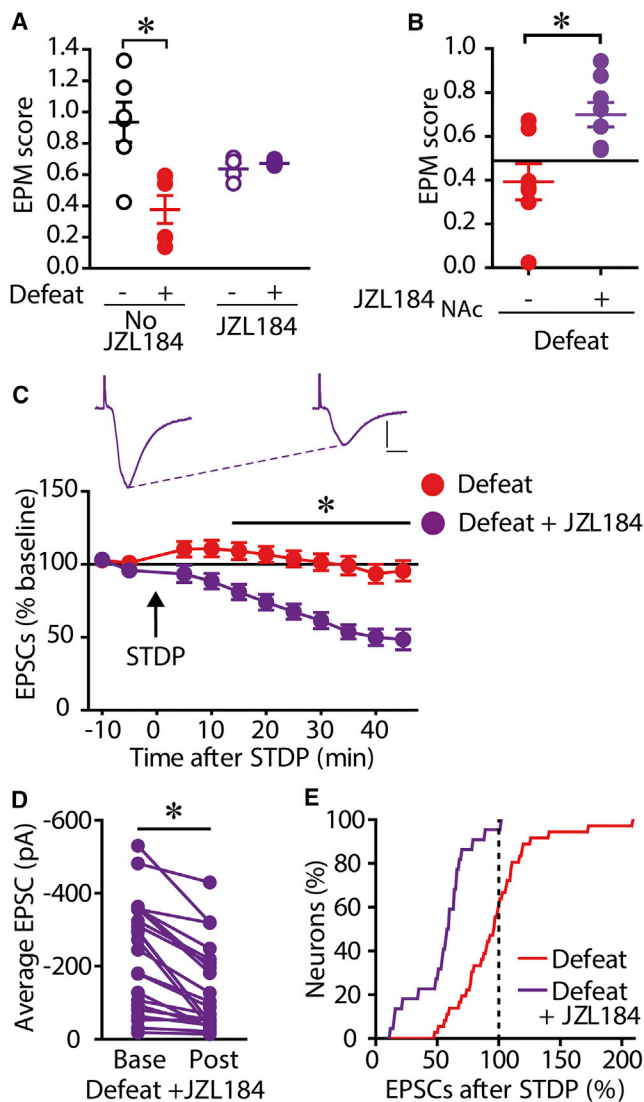
Figure 3. Enhancement of Circulating 2-AG Normalizes Anxious Behavior and Synaptic Depression in Defeated Mice

(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: $96.88\% \pm 5.36\%$, n = 36; defeated + JZL184: $55.13\% \pm 4.96\%$, 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.

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

(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).

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.

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