Behavioral/Systems/Cognitive

Chronic Psychoemotional Stress Impairs Cannabinoid-Receptor-Mediated Control of GABA Transmission in the Striatum

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Exposure to stressful events has a myriad of consequences in animals and in humans, and triggers synaptic adaptations in many brain areas. Stress might also alter cannabinoid-receptor-mediated transmission in the brain, but no physiological study has addressed this issue so far. In the present study, we found that social defeat stress, induced in mice by exposure to aggression, altered cannabinoid CB_1-receptor-mediated control of synaptic transmission in the striatum. In fact, the presynaptic inhibition of GABAergic IPSCs induced by the cannabinoid CB_1 receptor agonist HU210 [(6a*R*)-trans-3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol] was reduced after a single stressful episode and fully abolished after 3 and 7 d of stress exposure. Repeated psychoemotional stress also impaired the sensitivity of GABA synapses to endocannabinoids mobilized by group I metabotropic glutamate receptor stimulation, whereas the cannabinoid CB_1-mediated control of glutamate transmission was unaffected by repeated exposure to an aggressor. Corticosteroids released in response to the activation of the hypothalamic–pituitary–adrenal axis played a major role in the synaptic defects observed in stressed animals, because these alterations were fully prevented by pharmacological blockade of glucocorticoid receptors and were mimicked by corticosterone injections. The recovery of stress-induced synaptic defects was favored when stressed mice were given access to a running wheel or to sucrose consumption, which function as potent natural rewards. A similar rescuing effect was obtained by a single injection of cocaine, a psychostimulant with strong rewarding properties. Targeting cannabinoid CB_1 receptors or endocannabinoid metabolism might be a valuable option to treat stress-associated neuropsychiatric conditions.

Key words: **cocaine; electrophysiology; glucocorticoid; natural reward; running wheel; sucrose**

Introduction

Interference with activity-dependent plasticity is a major synaptic effect of stress in the CNS. Accordingly, a brief experience of acute inescapable stress impairs hippocampal long-term potentiation (LTP) (Shors et al., 1989; Kim et al., 1996; Yang et al., 2004) and facilitates long-term depression (LTD) (Kim et al., 1996; Xu et al., 1997; Yang et al., 2004, 2005; Chaouloff et al., 2007; Wong et al., 2007), whereas it causes opposite effects in the amygdala: promotion of LTP (Vouimba et al., 2004; Rodríguez Manzanares et al., 2005) and inhibition of LTD (Maroun, 2006). Furthermore, stress induces per se LTP in the ventral tegmental area (Saal et al., 2003). These effects have been claimed to mediate critical neuropsychological and behavioral effects of stress, such as memory loss (Kim and Diamond, 2002), avoidance behavior (Shors et al., 1992), and relapse to addictive drug assumption (Saal et al., 2003).

Exposure to stressful events has a myriad of consequences in animals and in humans, and it is unlikely that the currently described synaptic effects of stress may account for all these consequences. Recent evidence, for example, suggested that stress alters endocannabinoid transmission in the brain. Accordingly, stress elicits the rapid formation of endocannabinoids in the periaqueductal gray matter of the midbrain (Hohmann et al., 2005) and alters endocannabinoid content in limbic forebrain, amygdala, striatum, and prefrontal cortex (Patel et al., 2005a; Rademacher et al., 2008). The activation of the endocannabinid system during stress modulates complex responses, such as stress-induced analgesia (Hohmann et al., 2005), escaping behavior (Patel et al., 2005a), suppression of reproductive behavior (Coddington et al., 2007), and sensitivity to natural reward (Rademacher and Hillard, 2007). Although these studies advanced an association between stress and endocannabinoid-mediated neurotransmission, no physiological study so far has addressed the question of whether stress alters the synaptic responses to

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cannabinoid receptor activation. To fill this gap, here, we performed neurophysiological recordings from striatal neurons of mice exposed to social defeat stress. The dorsal striatum is an ideal structure to study the possible stress–cannabinoid interaction, because this brain area plays a central role in motor, cognitive, and emotional functions modulated by stress (White and Salinas, 2003; Balleine et al., 2007) and contains high levels of cannabinoid receptors controlling both excitatory and inhibitory synaptic transmission (Szabo et al., 1998; Gerdeman and Lovinger, 2001; Huang et al., 2001; Piomelli, 2003; Andersson et al., 2005; Ade and Lovinger, 2007; Centonze et al., 2007a,b).

Our results show that the behavioral effects of social stress in mice were associated with a selective alteration of the sensitivity of GABA synapses to cannabinoid receptor activation by exocannabinoids and endocannabinoids. These synaptic alterations were mimicked by corticosterone injections, were prevented by the glucocorticoid receptor antagonist 11β-(4-dimethyl-amino)-phenyl-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one (RU486), and were rescued after exposure to natural rewards and to the psychostimulant cocaine.

Materials and Methods

Male C57BL/6 mice (6–7 weeks of age) were used for all the experiments. All animals were housed, four per cage, on a 12 h light/dark cycle with lights on at 6:00 A.M. All efforts were made to minimize animal suffering and to reduce the number of mice used, in accordance with the European Communities Council Directive of November 24, 1986 (86/609/EEC).

Social defeat. Chronic psychoemotional stress induced by negative experience of social defeats in intermale confrontations is well known to lead to the development of anxious-depressive symptoms in male mice. In the present work, psychoemotional stress was induced by using a protocol already published by Avgustinovich et al. (2005) and Berton et al. (2006). Briefly, 6- to 7-week-old C57B6 mice were subjected to daily bouts for 10 min with an aggressive CD1 resident mouse, followed by 3 h protected sensory contact with their aggressor. Mice were exposed to a different aggressor each day for 1, 3, or 7 consecutive days.

Behavior. The elevated plus maze represents one of the most widely used tests for assessing anxiety in rodents (Lister, 1987). Each mouse was placed in the center of the maze with its nose in a closed arm. The time spent in the open arms and in the closed arms of the maze was recorded as measure of anxious state. The time spent in each compartment was expressed as percentage of the total 5 min test time. The entry with all four feet into one arm was defined as an arm entry. At the end of each trial, the maze was wiped clean.

Electrophysiology. Corticostriatal coronal slices (200 μm) were prepared from tissue blocks of the mouse brain with the use of a vibratome (Centonze et al., 2005, 2007a,b). A single slice was then transferred to a recording chamber and submerged in a continuously flowing artificial CSF (ACSF) (32°C; 2–3 ml/min) gassed with 95% O_2–5% CO_2. The composition of the control solution was as follows (in mM): 126 NaCl, 2.5 KCl, 1.2 $MgCl_2$, 1.2 NaH_2PO_4, 2.4 $CaCl_2$, 11 glucose, 25 $NaHCO_3$. The striatum could be readily identified under low-power magnification, whereas individual neurons were visualized *in situ* using a differential interference contrast (Nomarski) optical system. This used an Olympus BX50WI noninverted microscope with 40× water-immersion objective combined with an infrared filter, a monochrome CCD camera (COHU 4912), and a PC compatible system for analysis of images and contrast enhancement (WinVision 2000; Delta Sistemi). Whole-cell patch-clamp recordings were made with borosilicate glass pipettes (1.8 mm outer diameter; 2–4 MΩ), in voltage-clamp mode, at the holding potential of −80 mV. Recording pipettes were advanced toward individual striatal cells in the slice under positive pressure, and, on contact, tight gigaohm seals were made by applying negative pressure. The membrane patch was then ruptured by suction and membrane current and potential monitored using an Axopatch 1D patch clamp amplifier (Molecular Devices). Whole-cell access resistances measured in voltage clamp were in the range of 5–20 MΩ. To detect evoked IPSCs (eIPSCs), spontaneous IPSCs (sIPSCs), and miniature $GABA_A$-mediated IPSCs (mIPSCs), intraelectrode solution had the following composition (in mM): 110 CsCl, 30 K^+-gluconate, 1.1 EGTA, 10 HEPES, 0.1 $CaCl_2$, 4 Mg-ATP, and 0.3 Na-GTP; 30 μM (5*S*,10*R*)-(+)-5-methyl-10,11-dihydro-5*H*-dibenzo-[a,d]cyclohepten-5,10-imine maleate (MK-801) and 10 μM CNQX were added to the external solution to block NMDA and non-NMDA glutamate receptors, respectively. Conversely, to study the intrinsic properties of the neurons and evoked and spontaneous glutamate-mediated EPSCs (eEPSCs; sEPSCs), the recording pipettes were filled with internal solution of the following composition (in mM): 125 K^+-gluconate, 10 NaCl, 1.0 $CaCl_2$, 2.0 $MgCl_2$, 0.5 BAPTA, 19 HEPES, 0.3 GTP, 1.0 Mg-ATP, adjusted to pH 7.3 with KOH. Bicuculline (10 μM) was added to the perfusing solution to block $GABA_A$-mediated transmission.

Spontaneous and miniature synaptic events were stored by using P-CLAMP 9 (Molecular Devices) and analyzed off-line on a personal computer with Mini Analysis 5.1 (Synaptosoft) software. The detection threshold of spontaneous and miniature excitatory and inhibitory events was set at twice the baseline noise. The fact that no false events would be identified was confirmed by visual inspection for each experiment. Off-line analysis was performed on spontaneous and miniature synaptic events recorded during fixed time epochs (2–3 min; 5–10 samplings), sampled every 2–3 min. Only cells that exhibited stable frequencies in control (<20% changes during the control samplings) were taken into account. Events with complex peaks were eliminated. Evoked synaptic events (eEPSCs; eIPSCs) were elicited at 0.1 Hz frequency by using bipolar electrodes located either in the white matter between the cortex and the striatum (to activate corticostriatal glutamatergic fibers) or within the striatum (to stimulate intrastriatal GABAergic terminals). P-CLAMP 9 (Molecular Devices) was used to store the data. In distinct neurons, eEPSCs or eIPSCs of similar amplitude were obtained with variable intensities of stimulation, mainly depending on the distance between the stimulating and recording sites. eEPSCs normally ranged between 50 and 400 pA, whereas eIPSCs ranged between 30 and 200 pA.

Statistical analysis. For data presented as the mean ± SEM, statistical analysis between two groups was performed using paired or unpaired Student's *t* test or Wilcoxon's test. Multiple comparisons were analyzed by one-way ANOVA followed by Tukey's honestly significant difference. The analyses were performed on a per-cell basis. The significance level was established at $p < 0.05$. To determine differences between two cumulative distributions, the Kolmogorov–Smirnov test was used. The analyses were performed on a per-cell basis. Throughout the text, *n* refers to the number of cells. One to six neurons per animal were recorded. Each electrophysiological experiment in control and stressed mice was obtained by pooling data from at least six different animals. Only one animal per day was used.

Drugs. In some experiments, RU486 (Sigma/RBI; 25 mg/kg) was dissolved in 100 μl of DMSO and injected intraperitoneally 5–10 min before each session of 3 consecutive days of stress. In other experiments, RU486 was emulsioned through sonication in 200 μl of saline (0.9% NaCl). The data were not different between the two groups of experiments and were pooled together. For cocaine experiments, a single dose of cocaine (15 mg/kg; in 200 μl of saline) was injected immediately after the third of three sessions of stress. Corticosterone (Sigma/RBI) was administrated subcutaneously once a day in a volume of 10 ml/kg for 3 consecutive days (20 mg/kg; suspended in physiological saline containing 0.1% DMSO and 0.1% Tween 80). Mice receiving injections of the appropriate vehicle were used as controls.

Drugs used for the electrophysiological experiments were first dissolved in DMSO [*N*-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1*H*-pyrazole-3-carboxamide (AM251); (6a*R*)-11β-(4-dimethyl-amino)-phenyl-17β-hydroxy-17-(1-propynyl)-estra-4,9-dien-3-one 3-(1,1-dimethylheptyl)-6a,7,10,10a-tetrahydro-1-hydroxy-6,6-dimethyl-6*H*-dibenzo[b,d]pyran-9-methanol (HU210)] or water, and then in the bathing ACSF to the desired final concentration. The concentrations of the various drugs were chosen according to previous *in vitro* studies on corticostriatal brain slices (Centonze et al., 2005, 2007a,b) and were as follows: baclofen (10 μM), CNQX (10 μM), (*S*)-3,5-dihydroxyphenylglycine (3,5-DHPG) (50 μM), AM251 (10 μM), HU210 (1 μM), MK-801 (30 μM), tetrodotoxin (TTX; 1 μM; Tocris), and bicu-

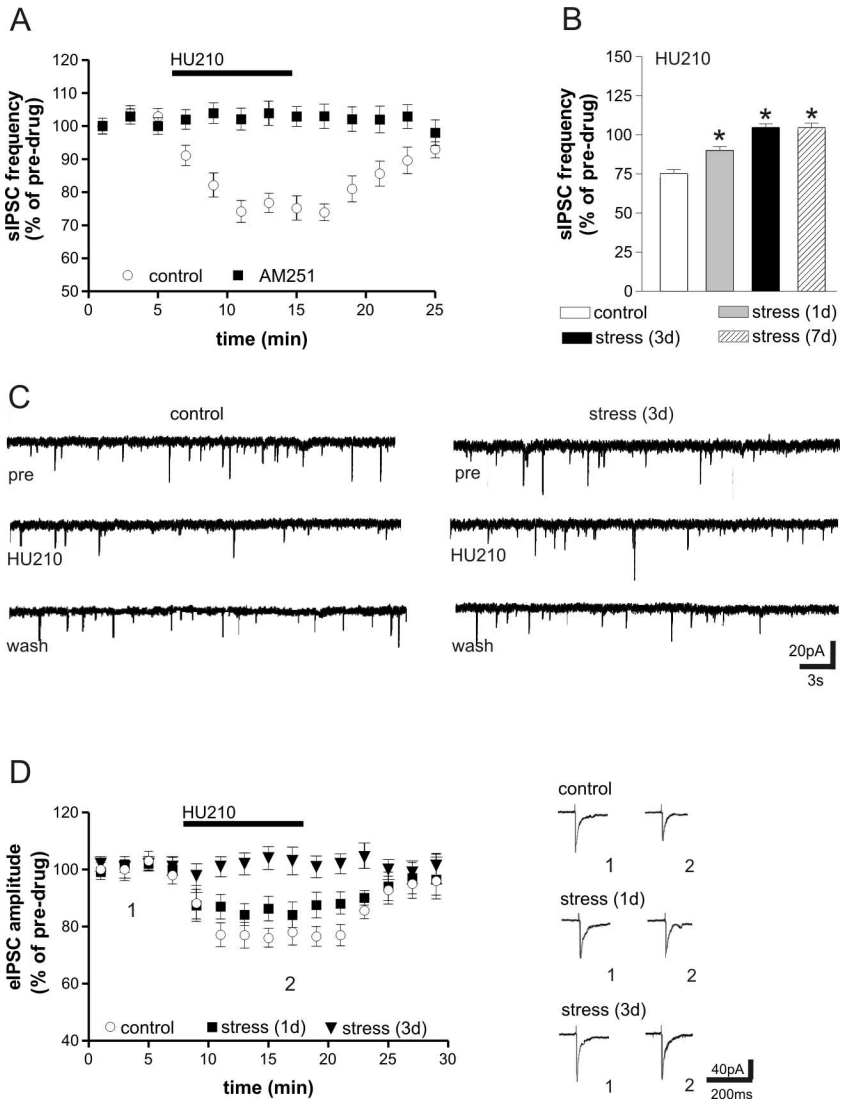

Figure 1. Stress alters the sensitivity of striatal GABA synapses to the stimulation of cannabinoid CB_1 receptors. ***A***, HU210, agonist of CB_1 receptors, reduced sIPSC frequency in control mice. This effect was fully prevented by preincubation with the CB_1 receptor antagonist AM251. ***B***, The graph shows that the depressant effect of HU210 on sIPSC was attenuated in striatal mice exposed to one session of stress, and it was completely abolished in neurons from mice exposed to stress for 3 and 7 consecutive days. *$p < 0.05$. ***C***, The electrophysiological traces are examples of voltage-clamp recordings in the presence of TTX showing that HU210 (10 min) failed to reduce mIPSC frequency in a mouse exposed to 3 d of stress. ***D***, The graph shows that HU210 failed to reduce eIPSC amplitude in animals stressed for 3 d, but not in control animals or in mice exposed to one session of stress. Error bars indicate SEM. The traces on the right are examples of eIPSCs recorded in control and stressed mice before (1) and during (2) the application of HU210.

culline (10 μM) (Sigma/RBI). In the experiments with drugs dissolved in DMSO, the control samplings were obtained during DMSO and ACSF applications.

Results

Behavioral effect of the social defeat paradigm

To evaluate the behavioral effects associated with 3 consecutive days of social defeat, we used the elevated plus maze paradigm. This test was used to verify the effectiveness of our social defeat protocol to induce an anxious state 24 h after the last aggression.

All elevated plus maze measures showed significant difference between stress group (exposed animals; $n = 8$) and control group (unexposed animals; $n = 8$). When compared with unexposed mice, exposed animals showed a significant reduction in the time spent in the open arms (6.6 ± 2.2 vs 17.5 ± 4.5%; $p < 0.05$) and an increase in the time spent in the closed arms (80.8 ± 3.0 vs 67.2 ± 4.5%; $p < 0.05$) (data not shown).

Effects of HU210 on GABA transmission in stressed mice

As described previously (Centonze et al., 2007a,b), application of the cannabinoid CB_1 receptor agonist HU210 (10 min; $n = 14$) significantly ($p < 0.01$) reduced the frequency of sIPSCs in control striatal neurons, an effect prevented by preincubating the slices with the selective antagonist of CB_1 receptors AM251 ($n = 6$; $p > 0.05$) (Fig. 1*A*). Despite the hydrophobic properties of HU210, its depressant effect was slowly reversible at the wash of the drug.

In striatal neurons of mice exposed to 1 d of social defeat ($n = 13$), HU210 effects were still present, although they were significantly attenuated ($p < 0.05$). In neurons from mice receiving 3 ($n = 28$) and 7 d ($n = 13$) of psychoemotional stress, HU210 effects were completely abolished ($p > 0.05$) (Fig. 1*B*). In these mice, basal sIPSC frequency [stress (3 d), 1.37 ± 0.06 Hz; stress (7 d), 1.32 ± 0.05 Hz; control, 1.40 ± 0.04 Hz] and amplitude [stress (3 d), 31.6 ± 1.5 pA; stress (7 d), 32.1 ± 1.7 pA; control, 30.8 ± 1.4 pA] were normal (n = at least 10 and $p > 0.05$ for each experimental and control group). HU210 was also ineffective in reducing the frequency of mIPSCs ($n = 14$; 101.7 ± 3%) and the amplitude of eIPSCs ($n = 12$) in neurons from stressed (three sessions) mice ($p > 0.05$ for both parameters), whereas it caused a significant inhibition of both mIPSC frequency ($n = 12$; 82.4 ± 4%; $p < 0.01$) and eIPSC amplitude ($n = 10$; $p < 0.01$) in control animals (Fig. 1*C*,*D*).

We also investigated the sensitivity of GABA synapses to the stimulation of cannabinoid CB_1 receptors 3 and 7 d after the last of three consecutive sessions (one per day) of social stress. In mice killed 3 or 7 d after the last session of exposure to an aggressor, the depressant effects of HU210 on eIPSC amplitude ($n = 7$ for both groups) and on sIPSC frequency ($n = 8$ for both groups) recovered ($p < 0.01$) and were similar to those recorded in control animals ($p > 0.05$) (Fig. 2).

Isolated application of AM251 failed to alter the frequency of sIPSCs in control and stressed (1, 3, and 7 d) mice (n = at least 7 cells and $p > 0.05$ for each experimental group) (data not shown), ruling out the involvement of endocannabinoid tone change in the observed stress effects.

Effects of HU210 on glutamate transmission in stressed animals

In the striatum, stimulation of cannabinoid CB_1 receptors presynaptically reduces glutamatergic transmission (Gerdeman and Lovinger, 2001; Huang et al., 2001; Centonze et al., 2005).

Thus, we wondered whether the altered response to HU210

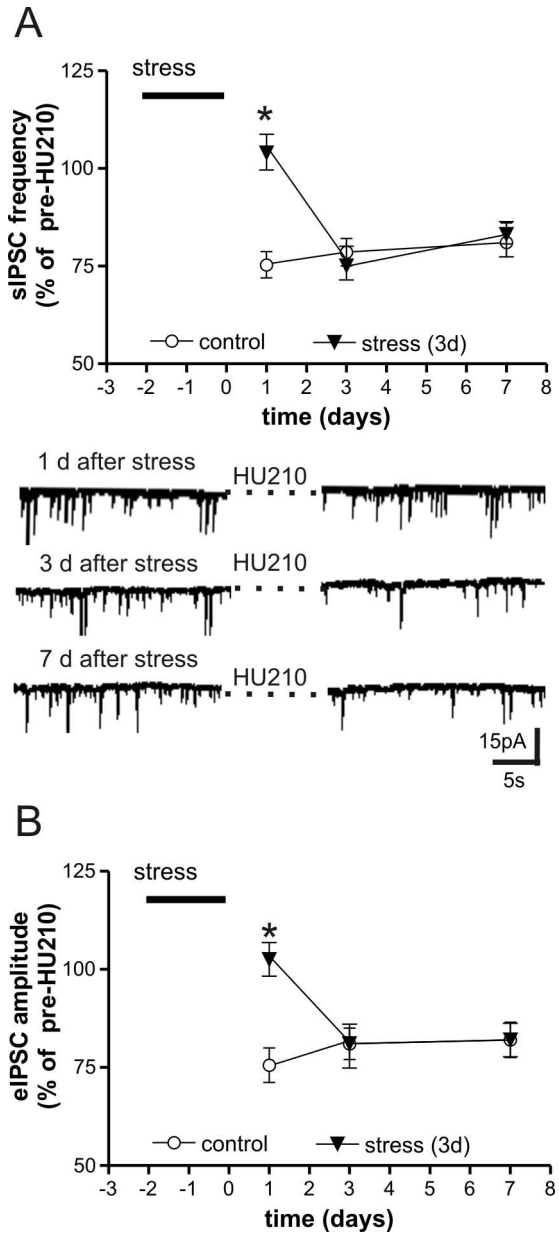

Figure 2. Effects of time on stress-induced inhibition of HU210 responses. ***A***, ***B***, The graphs show that the reduction of sIPSC frequency (***A***) and of eIPSC amplitude (***B***) induced by HU210 in mice exposed to stress for 3 consecutive days was lost 3 and 7 d after the last stress session. The electrophysiological traces are examples of voltage-clamp recordings showing that HU210 reduces normally sIPSC frequency in striatal neurons 3 and 7 d after stress. Error bars indicate SEM. $^*p < 0.05$.

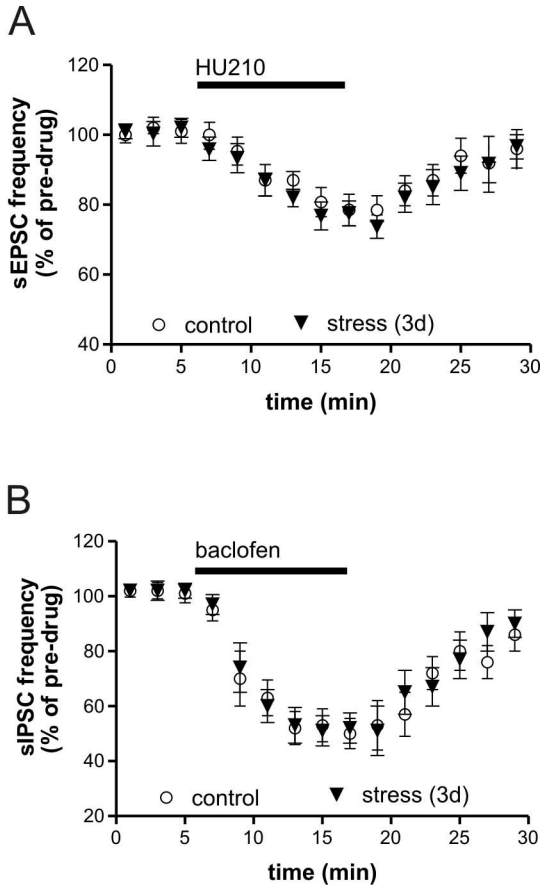

Figure 3. Stress does not alter the sensitivity of glutamatergic synapses to HU210 and the sensitivity of GABAergic synapses to baclofen. ***A***, The graph shows that the depressant action of HU210 on sEPSC frequency was similar in control and stressed (three sessions) mice. ***B***, The depressant effect of baclofen on sIPSC frequency was similar in control and stressed (three sessions) mice. Error bars indicate SEM.

found in stressed mice was restricted to GABA-mediated transmission or also involved glutamate synapses. sEPSC frequency [stress (3 d), 2.62 ± 0.17 Hz; control, 2.53 ± 0.10] and amplitude [stress (3 d), 11.6 ± 1.2 pA; control, 10.2 ± 0.9 pA] were unaffected by stress (n = at least 9 and $p > 0.05$ for both groups and parameters). HU210 (10 min) reduced the frequency of sEPSCs in both experimental groups ($p < 0.01$). The depressant action of HU210 on sEPSC frequency recorded from stressed mice (n = 16) was similar ($p > 0.05$) to that of the control animals (n = 13), indicating that stress selectively alters the sensitivity of striatal GABAergic nerve terminals to the stimulation of cannabinoid receptors (Fig. 3*A*). Accordingly, HU210 also reduced to a similar extent ($p > 0.05$) the amplitude of eEPSCs in both control (77.3 ± 6.5%; n = 14; $p < 0.01$) and defeated (3 d) (75.1 ± 7.0%; n = 13; $p < 0.01$) mice (data not shown).

Effects of baclofen on GABA transmission after stress

Many receptors participate in the presynaptic modulation of GABA transmission in the striatum, including the GABAB receptors (Calabresi et al., 1991). Thus, we investigated whether the abnormal control of GABA synapses observed in stressed mice was specific for the CB_1 receptors, or also involved other presynaptic receptors. Application of the $GABA_B$ receptor agonist baclofen (10 min; n = 9) significantly ($p < 0.01$) reduced striatal sIPSC frequency in control mice. In neurons from stressed (3 d) mice, the effects of baclofen were similar to those observed in the respective control groups (n = 11; $p > 0.05$ compared with baclofen in nonstressed mice) (Fig. 3*B*).

Effects of 3,5-DHPG on GABA transmission in stressed animals

It has been recently reported that activation of metabotropic glutamate receptors 5 by 3,5-DHPG mobilizes endocannabinoids in the striatum (Jung et al., 2005; Maccarrone et al., 2008) and that this effect results in the inhibition of GABA-mediated sIPSCs and of mIPSCs through the stimulation of cannabinoid CB_1 receptors (Centonze et al., 2007b; Maccarrone et al., 2008). Thus, to see

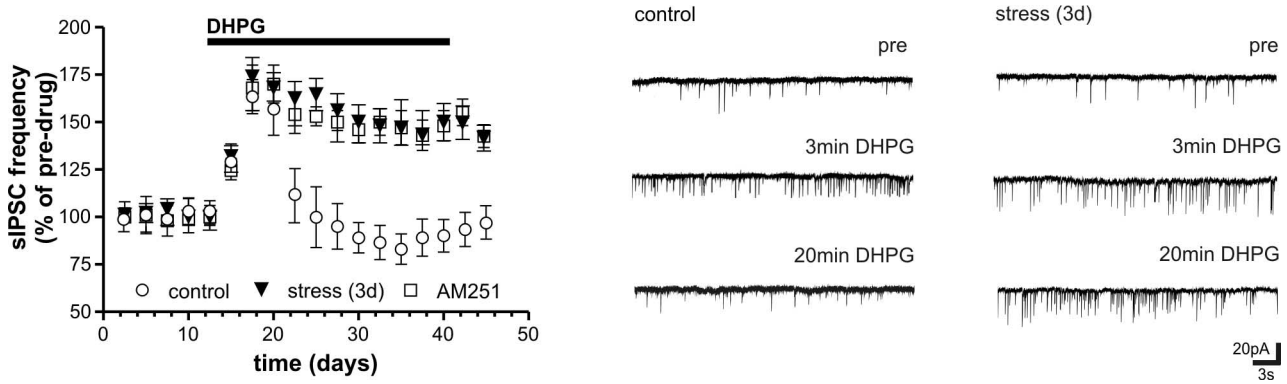

Figure 4. Effects of 3,5-DHPG on GABA transmission in stressed mice. The graph shows the early increase and the late reduction of sIPSC frequency after the application of a group I metabotropic glutamate receptor agonist 3,5-DHPG in control mice. Preincubation with AM251 or three sessions of stress prevented the late endocannabinoid-dependent inhibition of sIPSCs. Error bars indicate SEM. Examples of voltage-clamp recordings of sIPSC before and during the application of 3,5-DHPG in control and stressed mice are shown on the right.

whether stress altered the sensitivity of GABA synapses not only to the synthetic cannabinoid HU210 but also to endocannabinoids, we measured the effects of 3,5-DHPG on striatal sIPSCs recorded from mice exposed to three sessions of stress. Application of 3,5-DHPG (30 min; $n = 18$) caused a biphasic effect on sIPSCs in control mice, because it initially increased ($p < 0.01$ at 5 min), and then reduced the frequency of these events ($p < 0.05$ at 20 min). As reported previously (Centonze et al., 2007b), the initial increase of sIPSC frequency was endocannabinoid independent, whereas the subsequent decrease of sIPSC frequency was prevented by the antagonist of the cannabinoid CB_1 receptor AM251 ($n = 7$). According to the idea that social stress altered the sensitivity of striatal GABA synapses also to endocannabinoids, we observed that 3,5-DHPG produced only a sustained increase of sIPSC frequency in stressed mice ($n = 13$; $p < 0.05$ at 5, 10, and 20 min), because the late sIPSC inhibition was absent (Fig. 4).

Role of corticosteroids on stress-induced inhibition of HU210 responses

Many effects of stress in the nervous system are secondary to the activation of the hypothalamic–pituitary–adrenal axis and to the subsequent increase in plasma concentrations of corticosteroids (Piazza and Le Moal, 1998; Chrousos and Kino, 2007). To see whether the altered response to cannabinoid receptor stimulation seen in defeated mice was caused by corticosteroids, we pretreated mice with intraperitoneal injections of RU486 ($n = 8$), antagonist of glucocorticoid receptors, or with vehicle ($n = 8$), 5–10 min before exposure to each session of the stress protocol (3 consecutive days). RU486 did not alter basal sIPSC frequency (1.29 ± 0.05 vs 1.33 ± 0.05 Hz; $p > 0.05$) and amplitude (30.6 ± 1.9 vs 31.5 ± 1.3 pA; $p > 0.05$), but it was able to prevent the effects of stress on HU210 responses ($n = 16$; $p < 0.01$), whereas intraperitoneal vehicle did not ($n = 16$; $p > 0.05$).

In another set of experiments, unstressed mice ($n = 6$) were administrated subcutaneously with corticosterone once a day for 3 consecutive days. A similar corticosterone injection paradigm has been reported to cause a persistent elevation of plasma corticosterone lasting for ~24 h (Sousa et al., 1998). In these mice, sIPSC frequency (1.30 ± 0.04 vs 1.32 ± 0.05 Hz; $p > 0.05$) and amplitude (34.3 ± 1.5 vs 31.9 ± 1.2 pA; $p > 0.05$) were normal, but HU210 was ineffective in reducing GABA transmission ($p > 0.05$; $n = 14$), as described for stressed mice (Fig. 5*A*).

Effects of natural rewards on stress-induced inhibition of HU210 responses

We also investigated whether enriched environment with rewarding properties might be able to reverse the effects of stressful events on cannabinoid transmission. Access to running wheel or to sucrose consumption have been shown to act as potent natural rewards in rodents (Werme et al., 2000, 2002; Mahler et al., 2007; Rademacher and Hillard, 2007). In an additional set of experiments, stressed mice (three sessions; $n = 8$) were introduced for 24 h in a novel cage containing a running wheel immediately after the completion of the social defeat protocol. The sensitivity of striatal sIPSCs to HU210 was normal in these mice ($n = 18$; $p < 0.01$), indicating a rescue of the effects of stress (Fig. 5*B*). Similar results were obtained in stressed mice (3 d; $n = 6$) that were allowed, immediately after the third stress session, to consume *ad libitum* a drinking fluid containing sucrose (3% in tap water) for 24 h. Also, in these animals, in fact, the effect of HU210 on GABAergic sIPSC frequency was normal ($n = 14$; $p < 0.01$) (Fig. 5*B*). Notably, neither running wheel (six mice) nor sucrose (six mice) affected basal sIPSC frequency (running wheel, 1.24 ± 0.06 Hz; sucrose, 1.30 ± 1.0 Hz; $p > 0.05$) and amplitude (running wheel, 31.0 ± 1.6 pA; sucrose, 32.2 ± 1.6 pA; $p > 0.05$), nor produced significant effects on HU210-induced sIPSC inhibition ($n = 15$ and $p < 0.01$ for both experimental groups) when administered in nonstressed mice (Fig. 5*D*).

Effects of cocaine on stress-induced inhibition of HU210 responses

The psychostimulant cocaine has strong rewarding properties and might mimic the effects of running wheel and of sucrose in defeated animals. Striatal sIPSCs recorded from mice ($n = 6$) receiving a single intraperitoneal injection of cocaine, but not of saline ($n = 6$; $p > 0.05$), immediately after the third session of stress had a normal sensitivity to HU210 ($n = 14$; $p < 0.01$). Not surprisingly, HU210-mediated response was still fully abolished in mice exposed to 3 d of social stress and receiving a single intraperitoneal injection of saline ($n = 6$; $p > 0.05$). According to a previous study by Centonze et al. (2007a), a single intraperitoneal cocaine injection in control animals failed to alter per se the sensitivity of GABA synapses to HU210 (Fig. 5*C*,*D*).

Discussion

To our knowledge, this is the first physiological study demonstrating that stressful events alter cannabinoid transmission in

the nervous system. We have shown, in fact, that a social defeat stress paradigm able to induce anxiety-like behavior, caused a dramatic rearrangement of cannabinoid CB_1-receptor-mediated control of GABA transmission in the striatum. The synaptic responses to a selective cannabinoid CB_1 receptor agonist, in fact, were reduced after a single stressful episode, and fully abolished after 3 and 7 d of stress exposure. Notably, the striatum is a brain area involved in the control of complex motor, cognitive, and emotional functions, which are all modulated by stress (White and Salinas, 2003; Balleine et al., 2007).

Stress exposure did not result in a widespread dysregulation of GABA synaptic transmission, because it did not alter the basal properties of sIPSCs nor the sensitivity of these synaptic currents to the stimulation of GABAB receptors. These data support the hypothesis that stress selectively alters the sensitivity of cannabinoid receptors, rather than downstream effectors.

Furthermore, the stress effect was specific for cannabinoid receptors controlling GABA transmission, whereas the sensitivity of glutamate synapses to CB_1 receptor stimulation was unaltered, indicating a possible differential regulation of distinct cannabinoid receptors. In line with this observation, we recently reported that the sensitivity of GABA synapses to the cannabinoid receptor stimulation was selectively reduced in Huntington's disease mice (Centonze et al., 2005), and selectively enhanced in rats chronically treated with cocaine (Centonze et al., 2007a). Importantly, disturbances of GABA–glutamate balance have long been implicated in anxiety disorders (Millan, 2003), and it is therefore conceivable that the differential alteration of cannabinoid receptors controlling excitatory and inhibitory transmission might contribute to this effect.

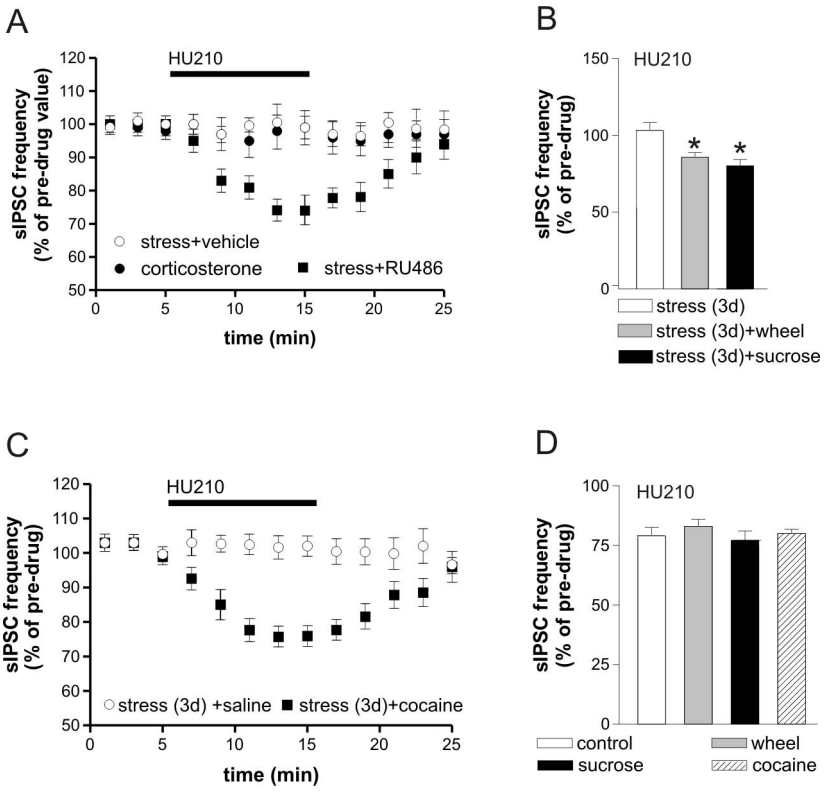

Figure 5. Blockade of glucocorticoid receptors, natural rewards and cocaine reverse the inhibition of HU210 responses induced by stress. ***A***, The graph shows that pretreatment with RU486, antagonist of glucocorticoid receptors, prevented the effects of three sessions of stress on HU210-induced reduction of sIPSC frequency and that corticosterone treatment mimicked the stress-induced effects. ***B***, The graph shows that natural rewards like wheel running or sucrose drinking were able to rescue the effect of HU210 on sIPSC frequency in mice exposed to 3 d of stress. $*p < 0.05$. ***C***, The sensitivity of striatal sIPSCs to HU210 was normal in mice receiving a single injection of cocaine after the third session of stress. ***D***, The graph shows that neither 24 h of running wheel or sucrose drinking, nor a single injection of cocaine altered per se the sensitivity of GABA synapses to HU210. Error bars indicate SEM.

The source of GABA inputs to striatal neurons is dual. Accordingly, striatal GABAergic principal neurons, in addition to inhibiting basal ganglia output nuclei, form functional synapses through their recurrent axon collaterals, establishing a feedback control over striatal neuron activity (Tunstall et al., 2002; Guzmán et al., 2003; Koos et al., 2004; Gustafson et al., 2006). Inputs from GABAergic interneurons are another important source of synaptic inhibition of projection neurons, giving rise to a feedforward inhibitory pathway that is independent of striatal output (Plenz, 2003; Tepper et al., 2004; Gustafson et al., 2006). Both the feedback and the feedforward intrastriatal GABAergic pathways are likely modulated by cannabinoid CB_1 receptors in control condition (Centonze et al., 2007b), because these receptors are expressed at very high concentrations on both axon collaterals of striatal projection neurons and on GABA interneurons (Herkenham et al., 1991; Hohmann and Herkenham, 2000; Piomelli, 2003).

We also showed that social stress altered the synaptic effects not only of exogenous cannabinoids but also of endocannabinoids mobilized in the striatum in response to mGlu5 receptor stimulation. This finding lends support to the notion that stress-induced alteration of cannabinoid transmission may have relevant synaptic consequences during the physiological activity of the striatum, mainly driven by glutamate inputs originating from the cortex and the thalamus (Wilson and Kawaguchi, 1996; Stern et al., 1998).

Furthermore, we have shown that corticosteroids released in response to the activation of the hypothalamic–pituitary–adrenal axis play a major role in the synaptic defects of stressed animals, because these alterations were mimicked by corticosterone injections and were fully prevented by pharmacological blockade of glucocorticoid receptors. In this respect, a recent study showed that CB_1 receptors are under a negative regulation by glucocorticoids in the hippocampus, and suggest that hippocampal cannabinoid CB_1 receptor signaling could be reduced under conditions associated with hypersecretion of glucocorticoids, such as chronic stress (Hill et al., 2008).

The relationship between stress and endocannabinoid system is complex, because it has been reported that stressful events increase endocannabinoid levels in several brain areas, likely in response to the stimulation of glucocorticoid receptors (Di et al., 2005; Malcher-Lopes et al., 2006). The resulting activation of cannabinoid receptors mediates specific behav-

ioral responses to stress, such as stress-induced analgesia (Hohmann et al., 2005), stress-induced inhibition of reproductive behaviors (Coddington et al., 2007), and stress-induced increased emotionality (Hill and Gorzalka, 2006). Stimulation of cannabinoid receptors is also critical for the activation of the amygdala during stress (Patel et al., 2005b). However, it has been shown that stress impairs cannabinoid CB_1-receptor-mediated transmission (Hill et al., 2005; present study), and evidence exists that this effect may mediate some aspects of the stress response. It has also been shown, in fact, that stimulation of CB_1 receptors reduces, rather than enhances, the expression of active escape behavior during an acute stress episode (Patel et al., 2005a), attenuates stress-induced anhedonia (Rademacher and Hillard, 2007) and other stress-induced depressive manifestations (Gobbi et al., 2005), and reduces hippocampus-dependent cognitive impairment induced by chronic stress (Hill et al., 2005). Furthermore, genetic and pharmacological inactivation of CB_1 receptors promotes passive stress-coping behavior (Steiner et al., 2008) and stress-induced motor inhibition (Fride et al., 2005), whereas pharmacological stimulation of the endocannabinoid system reduces the suppression of hippocampal cell proliferation and the increase in defensive behaviors seen in rats exposed to predator odor (Hill et al., 2006). To reconcile these discrepant results, it can be proposed that duration, type, and context of the stress paradigm are all important to determine whether cannabinoids have a prevailing facilitatory or inhibitory role in the resulting stress-induced behavioral changes, possibly because of a differential activation of the hypothalamus–pituitary–adrenal axis during different stress conditions.

Some evidence supports the concept of a bidirectional functional relationship between glucocorticoids and the endocannabinoid system. The hypothalamus–pituitary–adrenal axis, in fact, regulates endocannabinoid production (Di et al., 2005; Malcher-Lopes et al., 2006) and CB_1 receptor expression (Hill et al., 2008) in the brain, and it is, in turn, regulated by endocannabinoids. Furthermore, inactivation of cannabinoid CB_1 receptors increases adrenocorticotropin and corticosterone plasma concentrations (Manzanares et al., 1999; Haller et al., 2004), whereas stimulation of these receptors has an opposite effect (Patel et al., 2004). In this respect, a novel finding of our study consists in the demonstration that stress-induced loss of cannabinoid CB_1 receptor sensitivity in the striatum can be fully prevented by inhibiting glucocorticoid receptors and mimicked by enhancing glucocorticoid levels.

The effects on cannabinoid responses observed in this study disappeared within a few days after the cessation of the stress sessions. These data are consistent with previous electrophysiological work showing that stress-induced defects of hippocampal synaptic plasticity are labile and undergo a passive rundown when the animals are no longer exposed to stress (Yang et al., 2004).

Of note, the recovery of stress-induced synaptic defects were accelerated when the mice were given access to a running wheel or to sucrose consumption, which function as potent natural rewarding stimuli (Werme et al., 2000, 2002; Mahler et al., 2007; Rademacher and Hillard, 2007). A similar rescuing effect was obtained by a single injection of cocaine, a psychostimulant substance with strong rewarding properties (Ungless et al., 2001; Saal et al., 2003). Enriched environment and novelty exploration has been reported to accelerate the reversal of stress-induced synaptic defects in the hippocampus (Yang et al., 2006, 2007), a result consistent with our findings.

In conclusion, here we have identified a novel synaptic alteration induced by stress in the nervous system and found that this alteration was sensitive to the inhibition of glucocorticoid receptors and to the activation of the central reward system. Targeting cannabinoid CB_1 receptors or endocannabinoid metabolism might be a valuable option to treat stress-associated neuropsychiatric conditions and anxiety disorders.

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