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Stress Impairs GABAergic Network Function in the Hippocampus by Activating Nongenomic Glucocorticoid Receptors and Affecting the Integrity of the Parvalbumin-Expressing Neuronal Network

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Stress facilitates the development of psychiatric disorders in vulnerable individuals. It affects physiological functions of hippocampal excitatory neurons, but little is known about the impact of stress on the GABAergic network. Here, we studied the effects of stress and a synthetic glucocorticoid on hippocampal GABAergic neurotransmission and network function focusing on two perisomatic interneurons, the parvalbumin (PV)- and the cholecystokinin (CCK)-positive neurons. In acute hippocampal slices of rat, application of the potent glucocorticoid receptor (GR) agonist dexamethasone (DEX) caused a rapid increase in spontaneous inhibitory postsynaptic currents (sIPSCs) in CA1 pyramidal neurons. This effect was mediated by a nongenomic GR that evoked nitric oxide (NO) release from pyramidal neurons. Retrograde NO signaling caused the augmentation of GABA release from the interneurons and increased CCK release, which in turn further enhanced the activity of the PV-positive cells. Interestingly, chronic restraint stress also resulted in increased sIPSCs in CA1 pyramidal neurons that were Ca^{2+}-dependent and an additional DEX application elicited no further effect. Concomitantly, chronic stress reduced the number of PV-immunoreactive cells and impaired rhythmic sIPSCs originating from the PV-positive neurons. In contrast, the CCK-positive neurons remained unaffected. We therefore propose that, in addition to the immediate effect, the sustained activation of nongenomic GRs during chronic stress injures the PV neuron network and results in an imbalance in perisomatic inhibition mediated by the PV and CCK interneurons. This stress-induced dysfunctional inhibitory network may in turn impair rhythmic oscillations and thus lead to cognitive deficits that are common in stress-related psychiatric disorders.
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INTRODUCTION

Severe or chronic stress can facilitate the development of psychiatric illnesses in genetically predisposed individuals. Preclinical studies document that chronic stress has a significant impact on neuroplasticity in limbic brain areas and that these changes have been suggested to contribute to the pathophysiology of mood disorders (Drevets *et al*, 2008; McEwen, 2007; Pittenger and Duman, 2008). Stress increases the levels of circulating corticosteroids whose actions are mediated by mineralocorticoid and glucocorticoid receptors (MRs and GRs), which are abundantly expressed in the brain and influence emotional and cognitive functions (Joëls *et al*, 2007; McEwen *et al*, 1986). As MRs and GRs are expressed predominantly in principal cells, previous studies concerning corticosteroid effects on neuronal activity in the hippocampus focused mainly on these neurons (Joëls *et al*, 2007; Joëls, 2008; Karst *et al*, 2005), whereas the GABAergic network remained relatively unexplored. However, microdialysis studies report on increased extracellular GABA levels in the hippocampus of rats exposed to a psychological stressor (de Groote and Linthorst, 2007). Stress or artificially elevated glucocorticoid levels, which have been shown to regulate the expression of

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glutamic acid decarboxylase (GAD) (Bowers *et al*, 1998; Stone *et al*, 2001), caused an increase in the magnitude of inhibitory postsynaptic currents (IPSCs) in the hippocampus (Maggio and Segal, 2009). Parallel to these findings, accumulating evidences from clinical studies suggest a dysregulation of the GABAergic system in depressed patients (Brambilla *et al*, 2003; Hasler *et al*, 2007; Krystal *et al*, 2002; Sanacora *et al*, 1999).

Diverse subtypes of GABAergic interneurons provide networks of inhibition that sculpt the firing pattern of pyramidal cells and orchestrate network oscillations that represent distinct brain states (Buzsáki and Draguhn, 2004; Somogyi and Klausberger, 2005). A specific subtype of GABAergic neurons that innervate the perisomatic domain of principal neurons has a pivotal role in generating synchronized network oscillations (Somogyi and Klausberger, 2005; Klausberger *et al*, 2005). Driven by external and internal stimuli, these neurons evoke rhythmic perisomatic hyperpolarizations in principal neurons to accurately control the timing of firing and to synchronize the activity of a large number of pyramidal cells (Somogyi and Klausberger, 2005; Klausberger *et al*, 2005; Sohal *et al*, 2009). Precise synchronous activity of oscillating networks is essential for mediating complex cognitive processes such as perception and memory (Buzsáki and Draguhn, 2004; Somogyi and Klausberger, 2005).

In this study, we focus on the two major perisomatic interneurons: parvalbumin (PV) and cholecystokinin (CCK)-positive neurons. These two interneuron subtypes provide functional dichotomy in the inhibitory network because of their distinct membrane properties, expression patterns of receptors, and their presynaptic modulations (Klausberger *et al*, 2005; Freund and Katona, 2003). The PV-positive (PV+) neuron syncytium is regarded as a non-plastic precision clockwork for network oscillations, whereas the CCK-positive (CCK+) neurons are considered to function as a plastic fine-tuning device that modulates synchronous activities as a function of subcortical inputs (Freund and Katona, 2003). Malfunctioning of the CCK+ inhibitory network has been suggested to contribute to emotional disorders including anxiety (Freund and Katona, 2003), although to the best of our knowledge this concept has not been tested experimentally.

We examined how hippocampal GABAergic transmission was altered by the potent synthetic GR agonist dexamethasone (DEX) as well as by acute and chronic restraint stress using whole cell patch-clamp recordings. In addition, we studied the functional dichotomy of PV+ and CCK+ neurons in the context of chronic stress, specifically the differential effects of chronic stress on PV and CCK interneurons with regard to their ability to generate rhythmic spontaneous IPSCs.

MATERIALS AND METHODS

Animals and Chronic Restraint Stress

Adult male Sprague Dawley rats ($n = 48$, Harlan Winkelmann, Borchen, Germany) weighing 170–200 g at the beginning of the experiment were group housed (3–4 animals/cage). The experiments were performed in accordance with the European Communities Council Directive of 24 November 1986 (86/EEC) and the US National Institutes of Health Guide for the Care and Use of Laboratory Animals, and were approved by the Lower Saxony Federal State Office for Consumer Protection and Food Safety, Germany. Animals were kept under an inverse light cycle (light off from 0700 to 1900 hours), and all experimentation including daily weighing and handling of controls was performed under dimmed red light. Animals of the acute stress group were restrained for 30 min in well-ventilated polypropylene tubes and decapitated immediately thereafter. During restraint, animals were not physically compressed and did not experience pain. Animals of the chronic stress group were restrained daily for 6 h without access to food and water (from 0800 to 1400 hours, which is during their active period) for a total of 21 days (McLaughlin *et al*, 2007). Food was also withheld from the controls during the restraint period to ensure that effects on body weight gain were not simply a result of limited food availability. Throughout the entire experiment, body weight was recorded daily before the onset of restraint. Increased adrenal weight is an indicator of sustained stress. Therefore, in the experiments where brains were perfused for immunocytochemistry, adrenal glands were removed immediately after perfusion and weighed. Adrenal weight was expressed as milligrams per gram of body weight on the last experimental day.

Whole-Cell Recordings

Whole-cell voltage-clamp recordings were made in acute coronal hippocampal slices (350 μm thickness) from CA1 pyramidal neurons of the dorsal hippocampus. The bath solution in all experiments consisted of 125 NaCl, 2.5 KCl, 1.25 Na_2HPO_4, 2 $MgSO_4$, 26 $NaHCO_3$, 1.5 $CaCl_2$, 1 ascorbic acid, and 14 glucose (in mM) (pH 7.4, aerated with 95% O_2—5% CO_2) and were kept at 30°C. The pipette solution for all experiments contained 140 KCl, 1 $CaCl_2$, 10 EGTA, 2 $MgCl_2$, 0.5 Na_2-GTP, 4 Na_2-ATP, and 10 HEPES (in mM); pH was adjusted to 7.2 with KOH. Spontaneous GABAergic inhibitory postsynaptic currents (sIPSCs) were recorded at a holding potential of −70 mV in the presence of the 10 μM AMPA antagonist 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), 40 μM NMDA antagonist 2-amino-5-phosphonovaleric acid (APV), and 1 μM glycine receptor antagonist strychnine. Miniature IPSCs (mIPSCs) were recorded in the presence of 0.5 μM tetrodotoxin. Signals with amplitudes at least twofold above the background noise were analyzed. There were no significant differences in noise levels between control and stress animals. Patches with a serial resistance of >20 MΩ, a membrane resistance of <0.8 GΩ, or leak currrents of >150 pA were excluded. The membrane currents were filtered by a four-pole Bessel filter at a corner frequency of 2 kHz, and digitized at a sampling rate of 5 kHz using the DigiData 1322A interface (Axon Instruments/Molecular Devices, Sunnyvale, CA). Data acquisition was performed using commercially available software (pClamp 10.1; Axon Instruments/Molecular Devices). MiniAnalysis 6.0.9 (Synaptosoft, Decatur, GA) was used to perform amplitude and frequency analysis of sIPSCs and mIPSCs. To observe the influence of intracellular $[Ca^{2+}]$ on the effect of chronic stress, the slices were incubated in the cell-permeable Ca^{2+} chelator EGTA-AM (100 μM in the

recording solution) at room temperature. EGTA-AM was washed off after 30 min in incubation. For analysis of theta rhythms, power spectrum and autocorrelations were performed in Clampfit 10.1. We calculated a value of 'relative theta power' for each cell by summing the spectral power between 4 and 14 Hz, and dividing this by the total spectral power between 1 and 50 Hz during 10 s of sIPSC activity (Karson *et al*, 2008).

Drugs

CCK-8, LY225910 were obtained from Tocris. The DEX-BSA conjugate was obtained from Steraloids (Newport, RI). EGTA-AM was obtained from Molecular Probe (Invitrogen, Carlsbad, CA). All other chemicals were obtained from Sigma-Aldrich (St Louis, MO).

Perfusion and Immunocytochemistry

Control and chronically stressed rats ($n = 6$/group) were terminally anesthetized with an overdose of ketamine (50 mg/ml), xylazine (10 mg/ml), and atropine (0.1 mg/ml), and perfused transcardially with ice-cold 0.9% saline, followed by 200 ml of ice-cold fixative containing 4% paraformaldehyde in 0.1 M sodium-phosphate buffer (PBS, pH 7.2) for 15 min. The descending aorta was clamped so that adrenal glands were not perfused. To prevent post-perfusion artifacts in the brains, the heads were post-fixed overnight in fresh fixative at 4°C. On the following day, the brains were gently removed, washed thoroughly in PBS, and immersed in 30% sucrose in PBS at 4°C for 24 h for cryoprotection. After calibration, brains were frozen and coronal sections (50 μm) were cut in a cryostat. Samples from the two groups were processed in parallel to avoid any nonspecific effect of the staining procedure. First, sections were washed in PBS and then treated with 1% H_2O_2 in PBS for 30 min, rinsed three times in PBS, and preincubated in PBS containing 3% normal goat-serum and 0.5% Triton X-100. After preincubation, sections were incubated either with a polyclonal rabbit anti-cholecystokinin-8 antibody (AbCam, Cambridge, MA, AB43842, dilution 1 : 10000) or with a monoclonal mouse anti-parvalbumin antibody (Chemicon/Millipore, Temecula, CA; MAB1572, dilution 1 : 3000) overnight at 4°C. The next day, sections were rinsed three times in PBS and then incubated for 2 h at room temperature either with biotinylated goat anti-rabbit (diluted 1 : 200; Vector Laboratories, Burlingame, CA) or with biotinylated goat anti-mouse (diluted 1 : 200, Vector Laboratories) secondary antibody. After washing, the sections were incubated with the avidin-biotin-horseradish peroxidase complex (Vectastain *Elite* ABC Kit, Vector Laboratories) for 1 h, rinsed again in PBS, developed for 5 min in diaminobenzidine (DAB Peroxidase Substrate Kit, Vector Laboratories), and then thoroughly rinsed in PBS. The sections were mounted on glass slides in a 0.1% gelatin solution and dried overnight, after which they were dehydrated through a series of alcohols, cleared in xylene for 30 min and coverslipped with Eukitt (Kindler, Freiburg, Germany).

Quantification of CCK and PV-Immunoreactive Neurons

Every sixth section throughout the dorsal hippocampus was examined and found to yield a mean of six sections per animal. Cell numbers are expressed as densities, ie, the number of counted cells was divided by the volume of the analyzed hippocampal area.

Statistical Analysis

Data are presented as mean ± SEM. Unless noted, data on drug effects were analyzed by paired-sample Student's *t*-test. Data on the time course of the DEX effect were analyzed by one-way ANOVA followed by Dunnett's Multiple Comparison. Data on stress effect on sIPSCs were analyzed by unpaired *t*-test or ANOVA as indicated in Results section. Data on PV + and CCK + cell numbers within the different hippocampal subareas of control and chronically stressed rats were analyzed using one-way ANOVA followed by Tukey's *post hoc* test.

RESULTS

The Glucocorticoid DEX Facilitates GABAergic Transmission through a Rapid Non-Classical GR Mechanism

It is well known that stress affects the function of hippocampal excitatory transmission through glucocorticoid action. Here, we hypothesize that GR activation may also affect the inhibitory network. To directly test this, we applied the potent and selective GR agonist DEX on hippocampal slices of rats. After the application of DEX (25 nM), we detected a rapid increase in sIPSC frequency (6.0 ± 0.6 Hz before and 9.2 ± 0.7 Hz after DEX, $t_8 = 8.386$, $P < 0.0001$, paired *t*-test; Figure 1a and c1) and amplitude (98.9 ± 14.4 pA before and 140.8 ± 25.5 pA after DEX, $t_8 = 3.084$, $P = 0.015$, Figure 1a and c2). In all experiments, the initial significant facilitating effect of DEX occurred 5 min after exposure to DEX. This effect accelerated for another few minutes and gradually subsided afterward (Figure 1b1 and b2, one-way ANOVA followed by *post hoc* Dunnett's Multiple Comparison). In addition, in six of the nine cells recorded, DEX caused burst-like activities that were not detected before DEX application (Figure 1a, arrow in the lower trace). Such a fast DEX effect has not been described before (Maggio and Segal, 2009). By contrast, miniature IPSCs (mIPSCs) showed no significant changes in response to DEX application (frequency: 2.3 ± 0.3 Hz before and 2.2 ± 0.3 Hz after DEX, $t_6 = 0.6281$, $P = 0.5575$; amplitude: 52.3 ± 2.8 pA before and 54.0 ± 2.3 pA after DEX, $t_6 = 0.3932$, $P = 0.7104$, paired *t*-test; Figure 1d1 and d2), indicating that in contrast to the previously observed slow effect of DEX (Maggio and Segal, 2009), the rapid effect of DEX that was observed here does not act on the terminals.

This rapid onset of the stimulatory effect of DEX on GABA transmission was quite unexpected. Although it has been reported that the activity of the hippocampal pyramidal neurons can be promoted through a nongenomic corticosterone action (Karst *et al*, 2005), the observed stimulation of sIPSCs could not be due to an increase in the excitatory drive because all the glutamatergic transmission

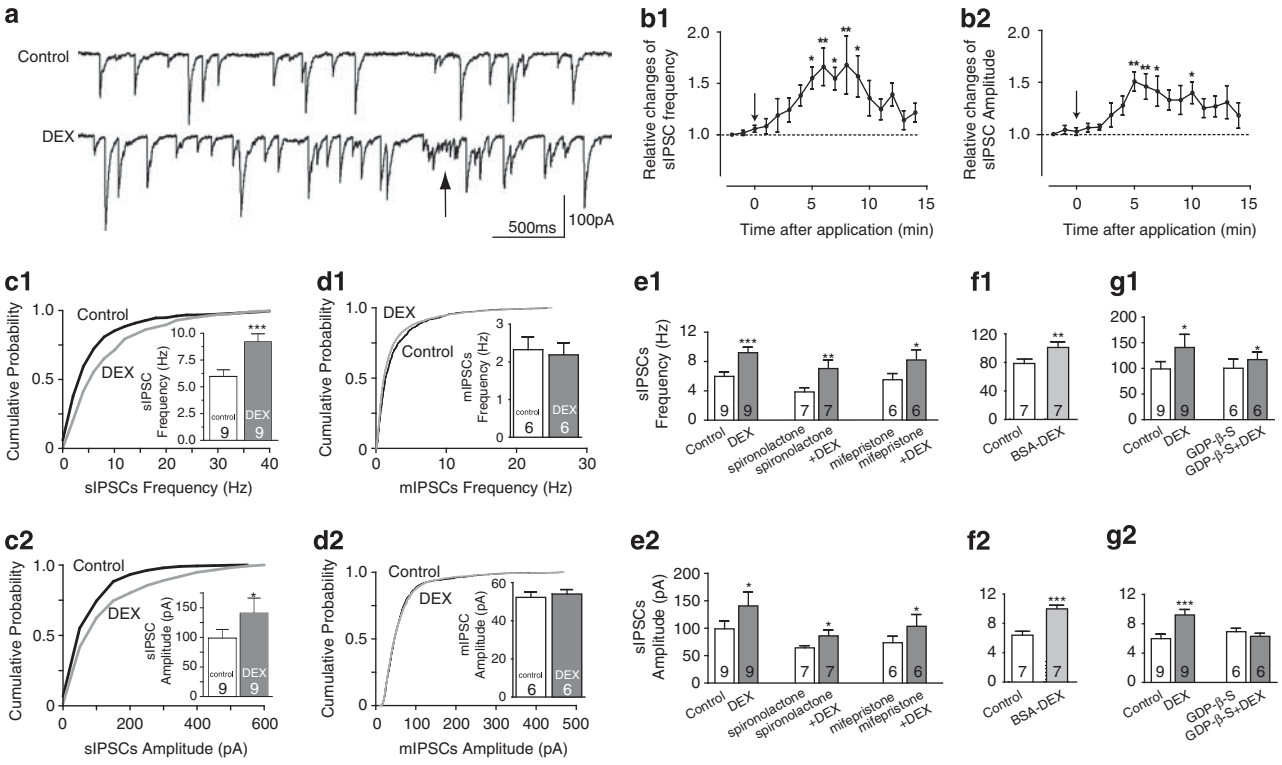


Figure 1 Glucocorticoid receptor stimulation facilitates GABAergic transmission through a nongenomic mechanism. (a) Representative recordings of sIPSCs in a CA1 pyramidal cell before and ~7 min after the application of the GR agonist DEX, which rapidly increases both the frequency and amplitude of sIPSCs. (b) Time course of DEX effect on sIPSC frequency (b1) and amplitude (b2) (1-min bins; graphs show relative changes). (c) Numerical data of the means and cumulative plot of frequency (c1) and amplitude (c2) of sIPSCs before (white bars) and 5–9 min after DEX (gray bars). (d) Numerical data of the means and cumulative plot of frequency (d1) and amplitude (d2) of mIPSCs before (white bars) and 5–9 min after DEX (gray bars). (e–g) The stimulatory effect of DEX on sIPSCs is mediated by a nongenomic GR. (e1, e2) The DEX effect was resistant to the wash-in of spironolactone and mifepristone, which are antagonists at nuclear MRs/GRs. (f1, f2) Membrane-impermeable BSA-DEX could retain the DEX effect. (g1, g2) Intracellular application of GDP-β-S, which irreversibly inactivates G proteins, blocked the DEX effect. Data are means ± SEM. Numbers indicate the number of neurons tested for each experimental condition, the same cells in all conditions. Six normal adult male rats were used; $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

was blocked in the presence of CNQX and APV (antagonist for AMPA and NMDA receptor, respectively) to isolate sIPSCs. Therefore, we performed a series of experiments to examine the cellular target of DEX. First, the DEX effect described above remained in the presence of the nuclear MR and GR antagonists spironolactone (10 μM) (frequency: 3.9 ± 0.6 Hz before and 7.0 ± 1.1 Hz after DEX, $t_6 = 4.854$, $P = 0.0028$; amplitude: 64.3 ± 4.6 pA before and 89.1 ± 12.1 pA after DEX, $t_6 = 2.560$, $P = 0.043$, paired *t*-test; Figure 1e) and mifepristone (10 μM; frequency: 5.5 ± 0.8 Hz before and 8.2 ± 1.4 Hz after DEX, $t_5 = 3.758$, $P = 0.013$; amplitude: 73.6 ± 11.8 pA before and 103.5 ± 21.6 pA after DEX, $t_5 = 2.770$, $P = 0.039$; paired *t*-test; Figure 1e). The antagonists by themselves showed no significant effects on the sIPSCs. Second, bath application of the membrane-impermeable BSA-DEX conjugate (250 nM) retained the stimulatory effect of DEX on GABA release (frequency: 6.4 ± 0.5 Hz before and 10.0 ± 0.5 Hz after BSA-DEX, $t_6 = 16.59$, $P < 0.0001$; amplitude: 78.5 ± 6.2 pA before and 101.0 ± 8.0 pA after BSA-DEX, $t_6 = 4.654$, $P = 0.0035$; paired *t*-test; Figure 1f). Finally, intracellular application of the G-protein inhibitor GDP-β-S (0.5 mM) through the pipette solution blocked the DEX-induced increase in sIPSC frequency (6.9 ± 0.5 Hz before and 6.3 ± 0.4 Hz after DEX, $t_5 = 1.728$, $P = 0.145$; Figure 1g). Taken together, our data strongly suggest that the rapid effect of DEX (5 min after the application) was mediated by a nongenomic membrane-bound GR that activated a G protein-dependent signaling pathway. This fast mechanism differs from genomic GR-mediated processes (>25 min, Maggio and Segal, 2009). Furthermore, as the GDP-β-S application was restricted to the postsynaptic pyramidal neuron, this result suggests that DEX, at least partially, acts on the postsynaptic cell and that retrograde messenger(s) mediate the stimulatory effect on GABA release.

DEX-Induced Facilitation of GABAergic Transmission is Mediated by Retrograde Nitric Oxide Signaling

We then examined which retrograde messenger system may be involved in the stimulatory effect of DEX on GABA release. Earlier studies have shown that in the hippocampus, endocannabinoids mediate the activity-dependent suppression of GABAergic inhibition through activation of CB1 receptors that are expressed specifically by CCK+ cells (Freund and Katona, 2003). It is very unlikely that the same retrograde CB1-mediated messenger pathway is responsible for both facilitating and suppressing GABAergic transmission. On the other hand, NO-sensitive guanylyl cyclase has been reported to be present in the axon terminals of both PV+ and CCK+ cells (Szabadits *et al*, 2007). We therefore focused on the retrograde nitric oxide (NO) pathway.

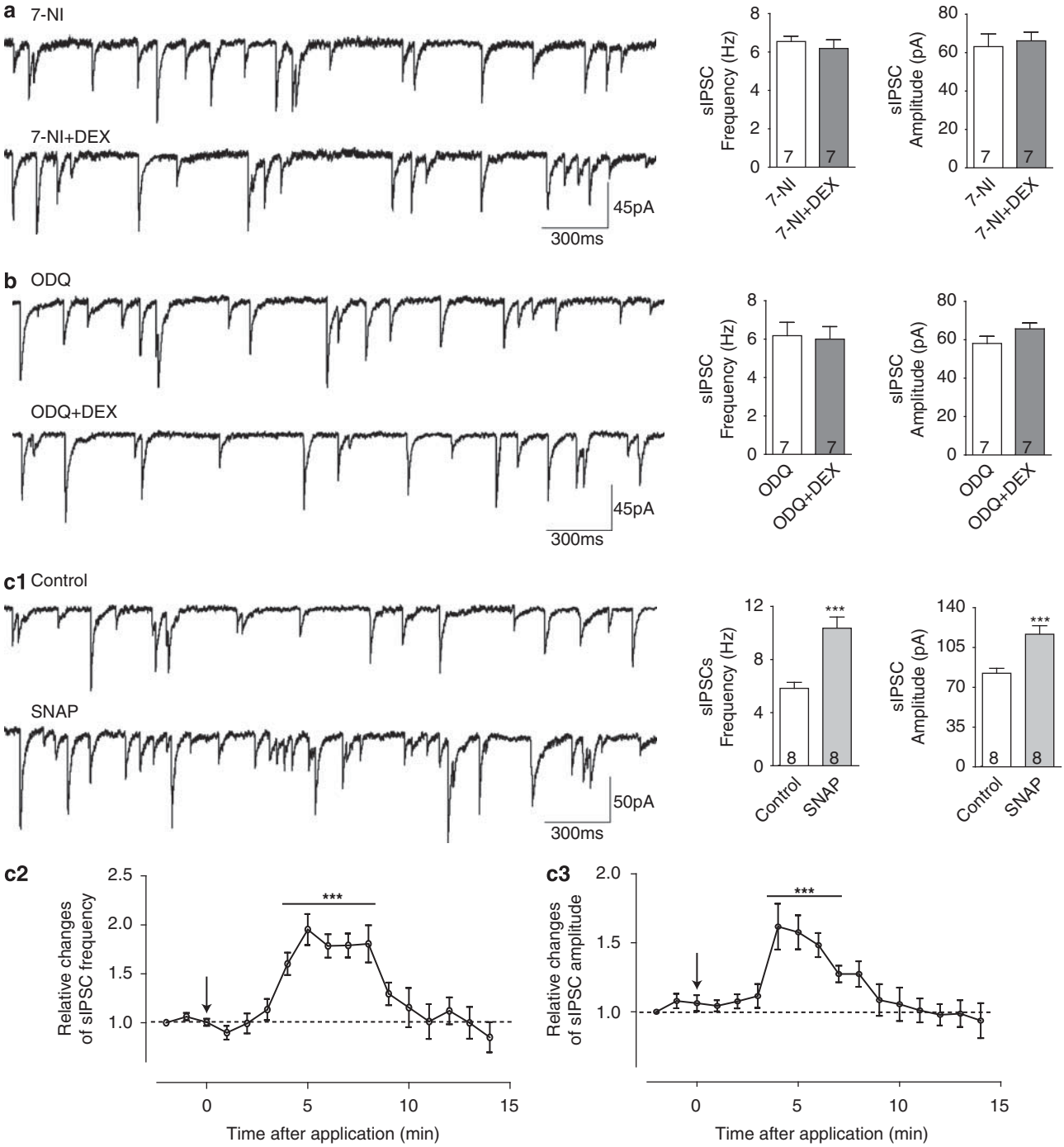


Figure 2 The DEX-induced facilitation of GABA transmission is mediated by the nitric oxide retrograde pathway. (a) The DEX effect is abolished in the presence of an NO synthase inhibitor 7-NI. (Left) Representative recordings of sIPSCs from a CA1 pyramidal cell before and ~7 min after DEX application; 7-NI was applied intracellularly through the pipette solution. (Right) Numerical data of mean sIPSCs before and after DEX. (b) The DEX effect is abolished in the presence of an inhibitor of NO-sensitive guanylyl cyclase, ODQ. (Left) Representative recordings of sIPSCs from a CA1 pyramidal cell before and after DEX application; slices were incubated in ODQ for ~30 min. (Right) Numerical data of mean sIPSCs before and after DEX. (c) Bath application of the NO donor SNAP mimicked the DEX effect. (c1) left: Representative sIPSC recordings from a CA1 pyramidal cell before and ~6 min after SNAP. (c1) right: Numerical data of mean sIPSCs before and 5–9 min after SNAP. (c2, c3) Time course of the SNAP effect on sIPSCs (1-min bins; graphs show relative changes). Data are mean ± SEM. Numbers within the bar graphs indicate the number of neurons tested for each experimental condition, the same cells in all conditions; six normal adult male rats were used; ***$P < 0.001$.

Intracellular application of 7-nitroindazole (7-NI, 100 μM), a selective inhibitor of neuronal NO synthase, which did not show any significant effect on hippocampal sIPSCs by itself, completely blocked the DEX-induced increase in sIPSC amplitude and frequency (amplitude in 7-NI: 63.1 ± 6.6 pA; amplitude in 7-NI plus DEX: 66.2 ± 4.6 pA; NS; frequency in 7-NI: 6.5 ± 0.3 Hz; frequency in 7-NI plus DEX: 6.2 ± 0.5 Hz; NS; Figure 2a). Similarly,

a 30 min incubation of the slices with ODQ (50 μM in aCSF), a selective inhibitor of NO-sensitive guanylyl cyclase (NOsGC), completely blocked the effect of DEX on GABA release (sIPSC amplitude in ODQ: 58.1 ± 3.7 pA, amplitude in ODQ plus DEX: 65.7 ± 3.1 pA, NS; frequency in ODQ: 6.2 ± 0.7 Hz, frequency in ODQ plus DEX: 6.0 ± 0.6 Hz, NS; Figure 2b). However, incubation with ODQ caused a small reduction in the sIPSC amplitude ($t_{14} = 2.418$, $P = 0.0298$; unpaired *t*-test) but not in the frequency, which is most likely due to weak activation of NOsGC at baseline level. These results show that inhibiting NO synthesis or silencing NO downstream signaling can completely block the fast DEX effect mentioned above. In addition, the NO donor SNAP (*S*-nitroso-*N*-acetylpenicillamine) mimicked the facilitating effect of DEX on sIPCSs. Similar to the effect elicited by DEX, bath application of SNAP (100 μM) increased sIPSC amplitude and frequency (baseline amplitude: 82.3 ± 4.4 pA; amplitude after SNAP 116.7 ± 7.4 pA; $P < 0.005$; baseline frequency: 5.8 ± 0.4 Hz; frequency after SNAP 10.4 ± 0.8 Hz; $P < 0.005$; paired *t*-test; Figure 2c). Furthermore, SNAP (100 μM) also mimicked the time course of the fast DEX effect (c.f. Figure 2c2/c3 to Figure 1b1/b2). Taken together, these results indicate that the fast DEX-induced facilitating effect on GABA transmission is mediated by NO retrograde signaling.

DEX-Induced Facilitation of GABAergic Transmission is Partially Mediated by NO-Induced Release of CCK

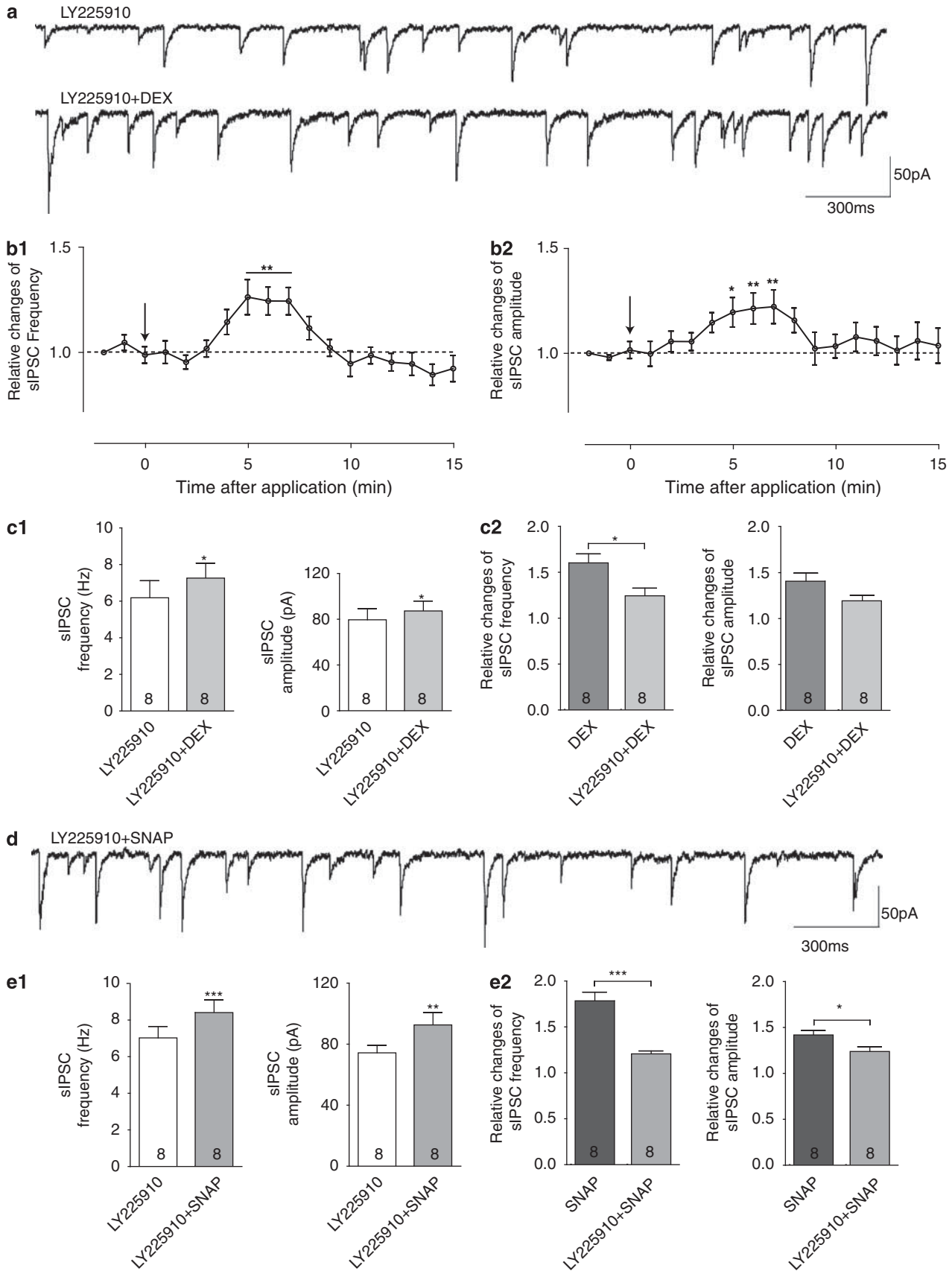
There is substantial evidence that the neuropeptide CCK is involved in stress response (Becker *et al*, 2008; Hebb *et al*, 2005). Therefore, we tested whether the enhanced inhibitory activities in the presence of DEX would induce CCK release from CCK interneurons, which would further modulate perisomatic GABA release (Földy *et al*, 2007). We incubated the slices with the selective CCK_2 receptor antagonist LY225910 (20 μM in aCSF) for 30 min before recording. The antagonist by itself had no effect on sIPSCs, but it blocked the response to further CCK application (data not shown). Then, we applied 25 nM DEX and observed an increase in sIPSC amplitude and frequency (amplitude in LY225910: 79.6 ± 9.8 pA, amplitude in LY225910 plus DEX: 89.8 ± 7.0 pA, $t_7 = 2.504$, $P = 0.04$; frequency in LY225910: 6.2 ± 0.9 pA, frequency in LY225910 plus DEX: 7.3 ± 0.8 pA, $t_7 = 4.788$, $P = 0.002$; paired *t*-test; Figure 3a and c1). However, compared with the condition without LY225910 pre-incubation, the extent of the increase was much lower (increase of amplitude: 19.5 ± 6.0 *vs* 40.8 ± 8.8%, unpaired *t*-test $t_{15} = 1.946$, $P = 0.07$; increase of frequency: 24.4 ± 8.3 *vs* 60.2 ± 9.9%, unpaired *t*-test $t_{15} = 2.733$, $P = 0.015$; Figure 3c2). It is interesting to note that the duration of fast DEX effect was shortened in the presence of LY225910 (3 *vs* 5 min; Figure 3b), whereas the onset time was unaffected (one-way ANOVA with Dunnett's multiple comparison test, Figure 3b). Furthermore, after the incubation with LY225910, the stimulatory effect of the NO donor SNAP on GABAergic transmission was also significantly reduced (increase of amplitude: 23.9 ± 5.1 *vs* 42.0 ± 4.9%, unpaired *t*-test, $t_{14} = 2.550$, $P = 0.02$; increase of frequency: 20.8 ± 3.1 *vs* 78.3 ± 9.6%, unpaired *t*-test, $t_{14} = 5.718$, $P < 0.0001$; Figure 3d and e). The fact that the CCK_2 receptor antagonist partially blocked the fast effects of DEX and SNAP suggests that CCK additionally contributes to DEX action and that NO signaling induces endogenous CCK release, which in turn further facilitates GABAergic transmission.

Acute Stress Enhances Hippocampal GABAergic Transmission

The above data show that the acute application of DEX caused a rapid facilitation of GABAergic transmission in the hippocampal CA1 area through a nongenomic GR mechanism. As glucocorticoids mediate not only the stress response, it is important to ask whether exposure to real life stress also elicits the enhancement of inhibitory transmission. To answer this, we performed a series of experiments with rats exposed to short-term (acute) restraint stress.

Immediately after exposing animals to 30 min restraint stress, we found an increase in sIPSC frequency in the hippocampal slices of stressed rats compared with controls (control: 7.3 ± 0.3 Hz; acute stress: 11.8 ± 0.7 Hz; $t_{15} = 5.424$ $P < 0.0001$; unpaired *t*-test; Figure 4a and b1), whereas the amplitude of the sIPSCs remained unaffected (control: 90.01 ± 9.3 pA; acute stress: 98.41 ± 11.2 pA; $t_{15} = 0.5772$ $P = 0.5723$; Figure 4a and b2). In addition, in 7 out of the 10 cells recorded from the acutely stressed animals, burst-like activities were frequently observed, whereas in the control group, this phenomenon was sparsely detected (2 of the 7 cells, data not shown). These data show that acute stress exposure results in an enhancement in hippocampal GABAergic transmission that is similar though not identical to the effect elicited by DEX (see Figure 1 and Maggio and Segal, 2009).

Figure 3 The DEX-induced increase in GABAergic transmission is partially mediated by endogenous CCK release downstream from the nitric oxide signaling. (a) Representative sIPSCs from a CA1 pyramidal cell after incubation with the CCK_2 receptor antagonist LY225910 for 30 min and then ~7 min after DEX application. (b) Time course of the DEX effect following 30 min-incubation with LY225910 (1-min bins; graphs show relative changes), showing that the duration of significant DEX effect was shortened by blocking CCK_2 receptor. (c) Numerical data of mean sIPSCs before and 5–7 min after DEX exposure in the presence of LY225910 (c1) and mean relative changes of sIPSCs (c2) in response to DEX without and with LY225910, respectively. Bath application of DEX still induced a significant increase in sIPSCs. However, the increase was smaller compared with the condition without LY225910 preincubation, revealing that CCK-mediated signaling is also involved in the DEX-induced enhancement of sIPSCs. (d) Representative sIPSCs after bath application of the NO donor SNAP in the presence of LY225910. (e) Numerical data of mean sIPSCs (e1) and mean relative changes of sIPSCs (e2) in response to SNAP without and with LY225910, respectively, showing that LY225910 partially blocked the stimulatory effect of SNAP. This indicates that NO signaling induces endogenous CCK release, which in turn facilitates GABAergic transmission. Data are mean ± SEM. Numbers within the bar graphs indicate the number of neurons tested for each experimental condition, same cells in all conditions; seven normal adult male rats were used; $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.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e1
sIPSC frequency (Hz)
sIPSC amplitude (pA)
LY225910
LY225910+SNAP
e2
Relative changes of sIPSC frequency
Relative changes of sIPSC amplitude
SNAP
LY225910+SNAP

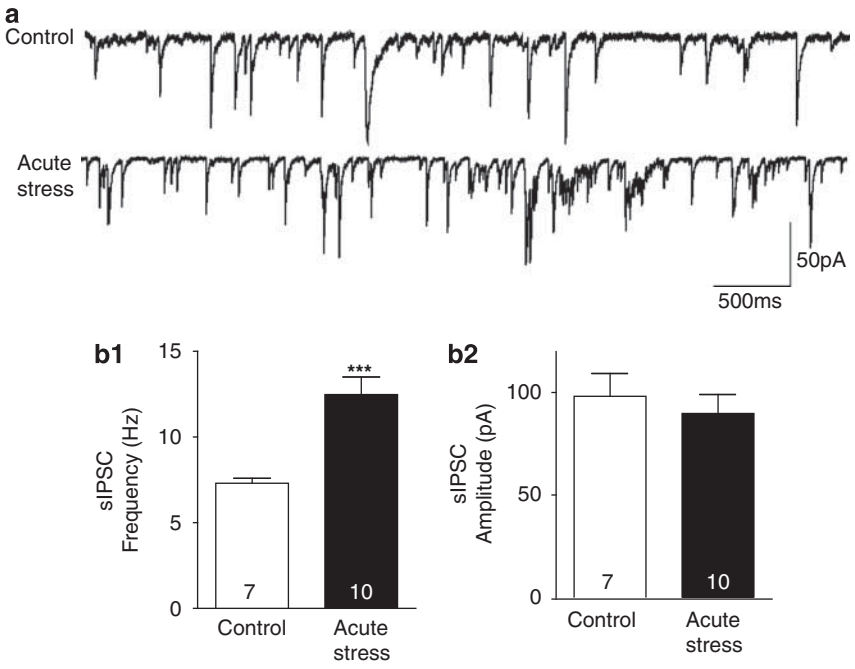

Figure 4 Acute stress increases hippocampal GABAergic transmission. (a) Representative voltage-clamp recordings of sIPSCs in CA1 pyramidal cells of control and acutely stressed rats. Acute stress significantly increased sIPSC frequency (b1), whereas the amplitude (b2) was not affected. Data are mean ± SEM; numbers within the bars indicate the number of neurons recorded; ***$P<0.001$.

Chronic Stress Results in a Ca^{2+}-Dependent Elevation of GABAergic Transmission

We then asked whether the elevation in hippocampal sIPSCs sustains after long-term stress during which the system is exposed to a prolonged high level of stress hormones including corticosterone. To examine this, we used rats exposed to 3 weeks of restraint stress. To assess the physiological effects of chronic restraint stress, body weights were recorded daily throughout the experiment and adrenal weights were determined at the end of the experiment. Stress significantly reduced body weight gain, and two-way ANOVA (stress × time) revealed a significant main effect of stress ($F_{1,27}=606.6$, $P<0.001$; Figure 5a). Furthermore, chronic stress increased the relative adrenal weights (two-tailed unpaired *t*-test: $t_{10}=2.579$, $P<0.05$; Figure 5b), which is in line with earlier findings that increased adrenal weights are reliable indicators of sustained HPA (hypothalamus-pituitary-adrenal) axis hyperactivity (Magariños and McEwen, 1995; McLaughlin *et al*, 2007).

We next investigated whether long-term stress affected the occurrence of sIPSCs in CA1 pyramidal neurons. Exposure to chronic restraint stress resulted in a significant increase in the frequency of hippocampal sIPSCs (control: 5.9 ± 0.3 Hz; stress: 10.4 ± 0.5 Hz; $t_{48}=9.279$; $P<0.0001$; Figure 6a and b), without significant changes in the amplitude of the sIPSCs (control: 96.1 ± 6.6 pA; stress: 97.2 ± 8.1 pA; NS; Figure 6a and c). Burst-like activities were also observed in 10 of 16 cells recorded from this group.

Furthermore, stress-induced enhancement of the sIPSC frequency could be normalized by the membrane-permeable Ca^{2+} chelator EGTA-AM, which reduces the concentration of intracellular free Ca^{2+}. One-way ANOVA revealed a significant difference in the sIPSC frequency between the groups ($F_{(3,60)}=35.19$; $P<0.0001$). Tukey's *post hoc* test showed a significant difference between the control and the acute stress groups ($q=13.05$; $P<0.001$). Bath application of EGTA-AM to the hippocampal slices of chronically stressed animals normalized the sIPSC frequency to control level (100 μM; stress + EGTA-AM: 6.4 ± 0.4 Hz; $q=7.884$, $P<0.001$ *vs* stress; $q=1.208$, $P>0.05$ *vs* control; Figure 6a, b, and c), whereas in the control slices, EGTA-AM caused no significant changes in sIPSCs (Figure 6a, b, and c). This indicates that chronic stress induced an enhancement in GABAergic transmission, which is concomitant with an increased intracellular-free Ca^{2+} in hippocampal interneurons.

Importantly, bath application of DEX (25 nM) to the slices from the chronically stressed rats failed to elicit any significant change in sIPSCs of the CA1 pyramidal neurons (frequency: 10.2 ± 0.9 Hz before and 10.3 ± 1.0 Hz after DEX, paired *t*-test, NS; amplitude: 87.2 ± 6.4 pA before and 85.3 ± 5.1 pA after DEX, paired *t*-test, NS, Figure 7). The origin of the loss of the DEX effect after chronic stress is yet to be determined (see Discussion section).

Chronic Stress Reduces the Number of the PV-Immunoreactive Neurons, whereas the Number of CCK-Immunoreactive Neurons Remains Unchanged

Considering (i) the theory of functional dichotomy of PV + and CCK + neurons proposed by Freund (Freund and Katona, 2003) and (ii) our present results that indicate that DEX facilitates the release of endogenous CCK (Figure 3), which specifically stimulates PV + neurons, we asked

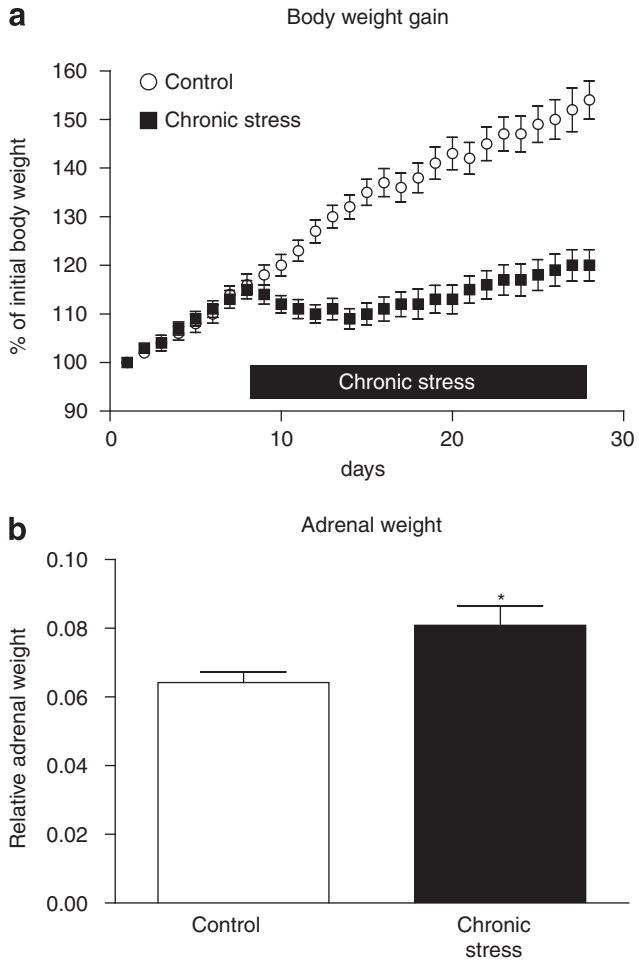

Figure 5 Physiological parameters showing the responses to chronic stress. (a) Body weight gain (control: $n=23$; Stress: $n=22$), (b) Relative weight of the adrenal glands (mg per 100 g body weight, six animals per group). Data are mean ± SEM. $^{*}P<0.05$.

whether these two subtypes of perisomatic interneurons are differentially affected by chronic stress. We first examined the effects of chronic restraint stress on the structural integrity of PV + and CCK + neurons. A number of these neurons in the dorsal hippocampus of control and chronically stressed rats were quantified. We found that chronic stress decreased the number of PV-immunoreactive neurons in all hippocampal subregions: 31% reduction in dentate gyrus ($P<0.01$, Figure 8c), 23% reduction in CA2-3 ($P<0.01$, Figure 8c), and 36% reduction in CA1 ($P<0.01$, Figure 8a1, a2, and c). In contrast, the number of CCK-immunoreactive neurons remained unaltered (Figure 8b1, b2, and d).

Chronic Stress Impairs CCK-Induced Rhythmic Burst Activity Originating from the PV Interneurons

We then ask whether the above differential effects of chronic stress on the number of PV- and CCK-immunoreactive neurons also affect the function of PV + and CCK + neurons. It is reported that an elaborately regulated Ca^{2+} signaling is essential for the temporal precision of the output from interneurons (Hefft and Jonas, 2005). As our data indicated that chronic stress induced an excessive intracellular free Ca^{2+} in the hippocampal interneurons, we decided to test whether chronic stress affects the ability of the perisomatic interneurons to generate rhythmic firing, which is of great importance in controlling the timing of pyramidal neuron action potentials. It is known that in the hippocampus, both CCK analogs and the muscarinic acetylcholine receptor agonist carbachol can provoke rhythmic sIPSCs *in vitro*. Carbachol-induced rhythmic IPSCs are inhibited by endocannabinoids, by N-type calcium channel blockers, and by activation of $GABA_B$ receptors (Karson *et al*, 2008). In contrast, CCK-triggered IPSCs are inhibited by P/Q-type calcium channel blockers, but are insensitive to endocannabinoids (Karson *et al*,

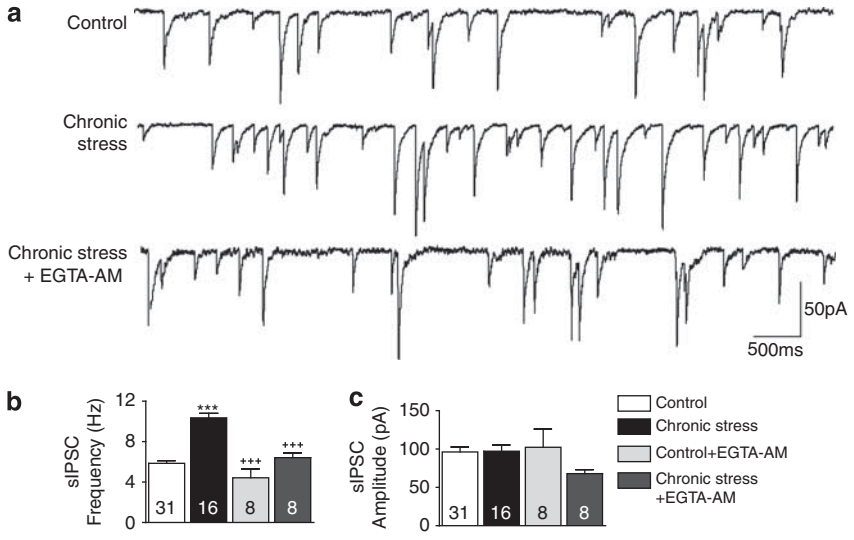

Figure 6 Chronic stress increases hippocampal GABAergic transmission. (a) Representative voltage-clamp recordings of sIPSCs in CA1 pyramidal cells of a control and a chronically stressed rat. Stress significantly increased sIPSC frequency (b), whereas the amplitude (c) was not affected (control: $n=14$; stress: $n=12$). The sIPSC frequency ,which was elevated by stress, was normalized by EGTA-AM (seven animals per group). Data are mean ± SEM; numbers within the bars indicate the number of neurons recorded; $^{***}P<0.001$, *vs* control, $^{+++}P<0.001$, *vs* stress.

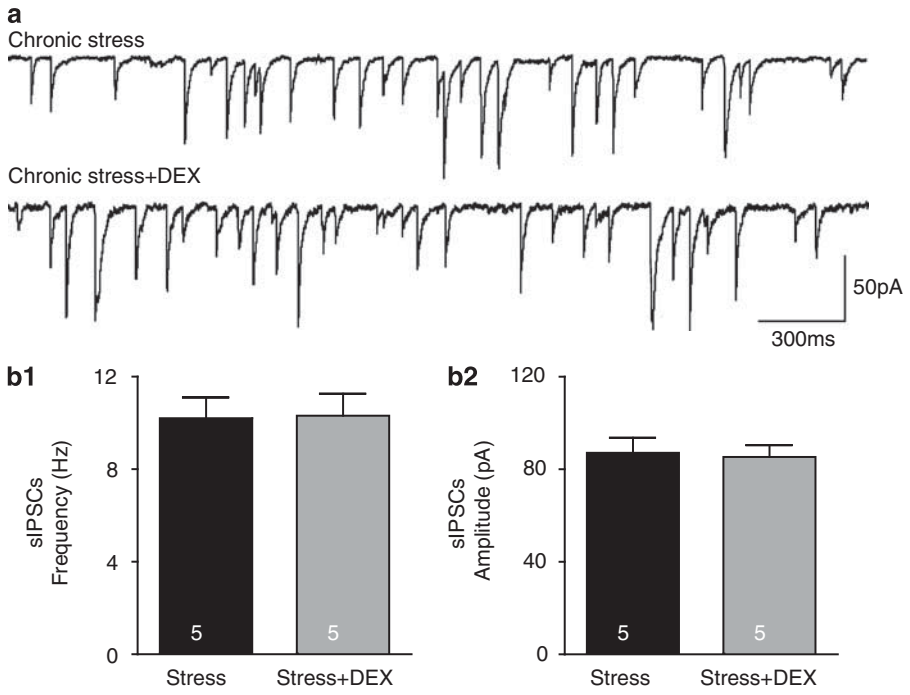

Figure 7 Bath application of DEX (25 nM) failed to elicit any significant change in sIPSCs in the hippocampus of chronically stressed rats. (a) Representative recordings of sIPSCs in a CA1 pyramidal cell from a stressed rat before and after the application of the GR agonist DEX. (b) Numerical data of the means of frequency (b1) and amplitude (b2) of sIPSCs before (black bar) and after DEX (gray bar) (five neurons recorded from five stressed animals). Data are mean ± SEM.

2008). Given the distinct cellular properties of CCK+ and PV+ neurons, carbachol presumably triggers rhythmic sIPSCs originating from CCK+ cells, whereas CCK stimulates sIPSCs from PV+ cells (Karson *et al*, 2008). In this study, the bath application of carbachol (5 μM) induced a rhythmic pattern of sIPSCs in hippocampal slices of control rats (Figure 9a1 and a2). Autocorrelation analysis of 10-s stretches of sIPSCs (selected from shortly after the onset of the enhancement) in all 8 cells showed regular-repeating peaks. After carbachol application, power spectral analysis of the same stretches from each cell revealed a significant enhancement in the total power and, more importantly, a sharp peak power within the theta frequency range, 4–14 Hz (relative theta power was 46.7 ± 1.1% before carbachol and 57.7 ± 1.1% after carbachol; $t_7 = 8.326$, $P < 0.0001$; paired *t*-test; Figure 9a2 and e). Similarly, the bath application of the CCK analog CCK8-S (1 μM) induced rhythmic sIPSCs that were manifested by regular-repeating peaks in the autocorrelation analysis, and a significant increase of power specifically in the theta frequency range in all eight tested cells (relative theta power was 47.1 ± 1.0% before and 60.2 ± 1.9% after CCK application; $t_7 = 6.374$, $P < 0.0001$; paired *t*-test; Figure 9c1, c2, and e). Consistent with previous studies, the rhythmicity triggered by the CCK analog was less variable and more persistent compared with that induced by carbachol.

In hippocampal slices from chronically stressed rats, the response to carbachol was similar to that in controls (relative theta power before carbachol: 46.2 ± 1.5%, after carbachol: 59.4 ± 1.9%; $t_7 = 12.28$, $P < 0.0001$; Figure 9b1, b2, and e). Two-way ANOVA of repeated measures on data from the control and the chronic stress groups also revealed no significant effect of stress ($F = 0.1567$; $P = 0.6991$). However, CCK8-S failed to induce rhythmicity in all 10 tested cells. In contrast to control, the CCK agonist induced an arrhythmic pattern in the slices from stressed rats as indicated by flat autocorrelation curves. Similarly, CCK8-S did not change relative theta power in stressed rats (before CCK 49.4 ± 1.2%; after CCK 48.7 ± 1.6%; $t_9 = 0.730$, $P = 0.48$; Figure 9d1, d2, and e) despite a massive increase in the total power. This shows that in addition to the enhancement of GABAergic transmission, the temporal precision in firing of the PV+ cells is impaired after chronic stress, whereas the same parameter remains unaffected in CCK+ cells.

DISCUSSION

This study provides four novel findings: (1) acute application of a GR agonist rapidly elicits an increase in sIPSCs in the hippocampus, notably through a membrane-bound GR and by retrograde NO signaling; (2) exposing animals to a brief acute stress causes a similar enhancement of hippocampal sIPSCs; (3) chronic stress also results in elevated GABAergic transmission in the hippocampus, which is Ca^{2+}-dependent, and DEX application elicits no further increase in sIPSCs; (4) chronic stress impairs the generation of rhythmic spontaneous IPSCs originating specifically from PV+ neurons. Given the pivotal role of perisomatic inhibition in synchronizing activities of pyramidal neurons, the chronic stress-induced deficit of the PV interneurons to generate rhythmic firing may result in abnormal network oscillations and thus contribute to cognitive impairments that are often observed in patients

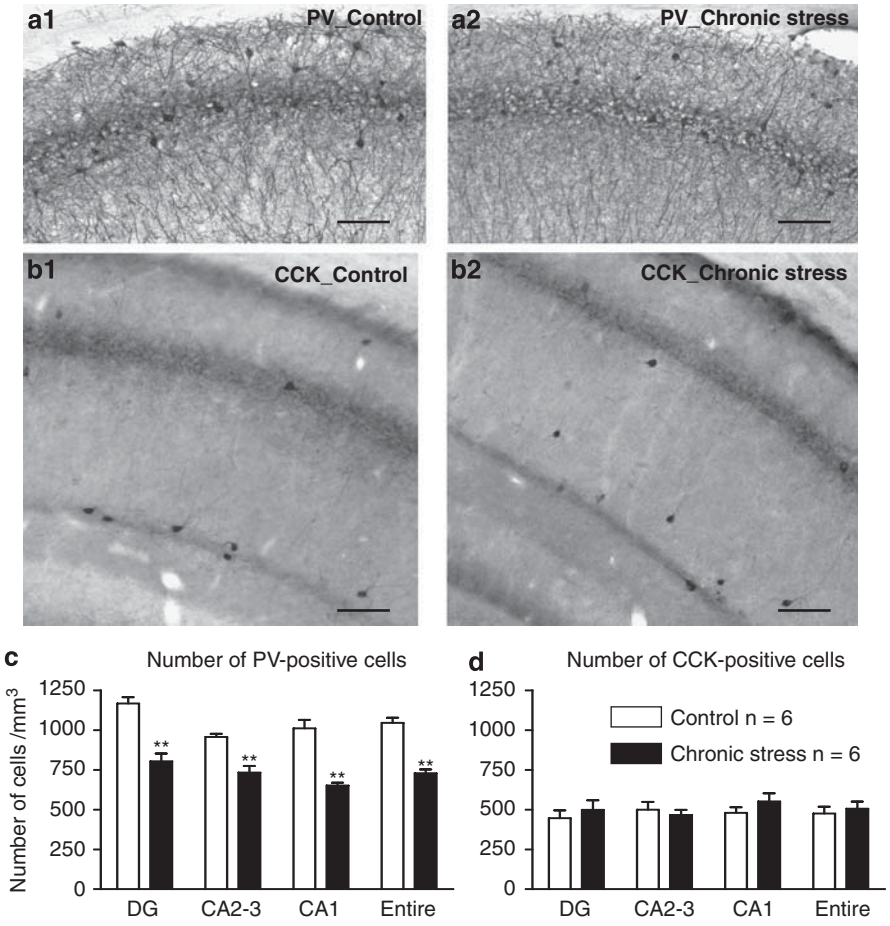

Figure 8 Chronic stress reduces the number of PV-immunoreactive cells, whereas the number of CCK-immunoreactive cells remains unaltered. (a) Representative examples of parvalbumin-stained CA1 sections from a control (a1) and a stressed rat (a2). (b) Representative examples of cholecystokinin-stained CA1 sections from a control (b1) and a stressed (b2) rat. Stress significantly decreased the number of PV+ cells in all hippocampal subareas (c), whereas the number of CCK+ cells was not affected (d). This indicates that chronic stress affects the structural integrity of PV+ but not of CCK+ cells. Data are mean ± SEM; six animals per group were analyzed; $^{**}P<0.01$. DG, dentate gyrus. Scale bars: 100 μm.

with stress-related psychiatric disorders. An association between the functional deficit of PV interneurons and the altered γ-oscillation has been suggested as an underlying mechanism in working memory deficits in schizophrenic patients (Lewis *et al*, 2005; Lodge *et al*, 2009).

Glucocorticoids Facilitate GABAergic Transmission by Activating Nongenomic GRs

Stress activates the HPA axis to increase release of corticosteroids from the adrenal glands. The cellular effects of corticosteroids are mediated chiefly through slow genomic mechanisms (De Kloet *et al*, 1998; Maggio and Segal, 2009). However, the existence of a rapid nongenomic pathway recently gained substantial credit (Di *et al*, 2005, 2009; Haller *et al*, 2008; Karst *et al*, 2005; Campolongo *et al*, 2009). In the hypothalamus, corticosterone elicits rapid opposing effects on glutamate and GABA release of magnocellular neurons through retrograde endocannabinoid and NO signaling through divergent nongenomic pathways (Di *et al*, 2005, 2009). In the basolateral nucleus of the amygdala, corticosterone was suggested to activate a G_s-associated membrane receptor and to induce endocannabinoid release, which eventually facilitates the consolidation of aversive memories (Campolongo *et al*, 2009). In the hippocampus, corticosterone can boost glutamate release and promote neuronal excitability through a nongenomic MR pathway (Karst *et al*, 2005). To the best of our knowledge, we are the first to provide functional evidence for the presence of membrane-bound GRs in the hippocampus. We show here that the acute application of DEX elicits a surprisingly rapid facilitation of GABAergic transmission in the hippocampus of control animals. The fast onset of the DEX effect shown in this study is substantially different from the time course of DEX effect described in a recent paper (Maggio and Segal, 2009). There, the authors reported that DEX caused an increase in the amplitude but not in the frequency of sIPSCs in the dorsal hippocampus of the rat. More importantly, Maggio and Segal (2009) described a slow onset and long-lasting response, detected 30–45 min after DEX application. They suggested that this slow effect was caused by the activation of genomic GR. In this paper, the fast DEX effect on sIPSCs, which act through the nongenomic mechanism, occurs within a narrow time window (about 5–10 min after DEX application, see Figure 1). There are at least three

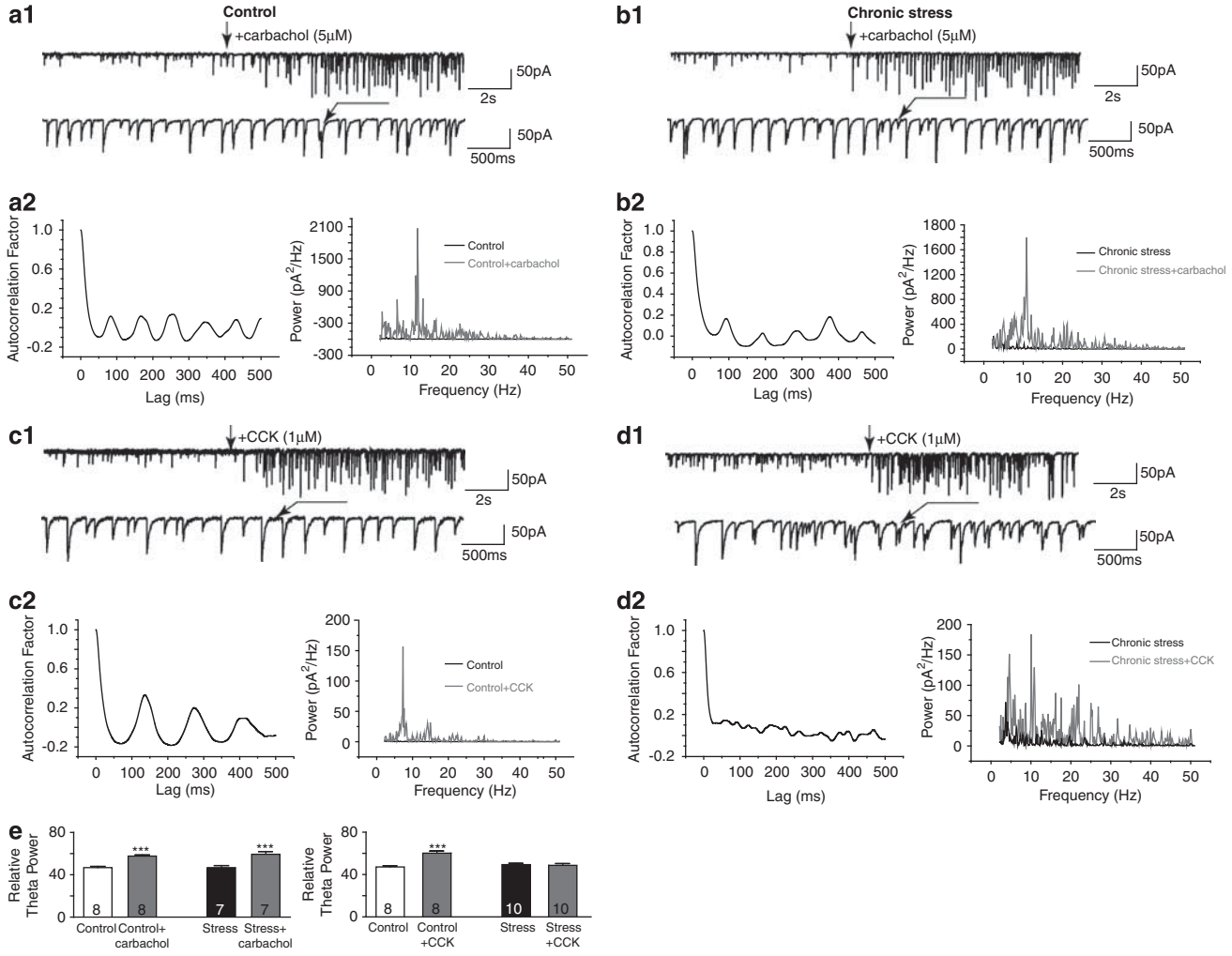

Figure 9 Chronic stress results in a functional deficit of PV+ neurons, whereas CCK+ neurons remain functionally intact. (a, c) Representative data from a control animal. Bath application of carbachol (a) or CCK-8S (c) induced rhythmic sIPSCs. (a1, c1) Representative recordings of sIPSCs in a CA1 pyramidal cell before and during washing in of carbachol (a1) or CCK-8S (c1). Lower traces are time expanded from the indicated segments in the upper traces. (a2, c2) Autocorrelation (left) and spectral power analysis (right) of 10-s stretches from the representative recordings after the experimental treatment. Graphs are representative of autocorrelation and spectra from all recorded cells. (b, d): Representative data from a stressed animal. In slices from chronically stressed rats, carbachol had the same effect as in controls (b). In contrast to control, CCK-8S failed to induce a rhythmic sIPSC pattern in stressed animals (d). This indicates that the temporal precision in firing of the PV+ cells is impaired after chronic stress, whereas the same property of CCK+ cells remains unaffected. (e) Numerical data of relative theta powers before and after the treatments. Data are mean ± SEM. Numbers within the bars indicate the number of neurons tested for each animal group (seven animals per group) and experimental condition, with the same cells in all conditions; ***$P<0.001$.

possibilities that can explain the discrepancy between the two studies. (i) In the study by Maggio and Segal (2009), the perfusion rate during the recordings was quite slow, which meant that the equilibrium could be reached only after 6 min, whereas in our case the perfusion rate was much faster; thus the equilibrium could be reached already within 1 min. The slow perfusion rate may confound the fast onset of DEX effect. (ii) The numerical values of sIPSC frequency and amplitude were calculated as the total number of events in 7 min of recording epochs (Maggio and Segal, 2009), which exceeds the narrow time window of the fast DEX effect shown in Figure 1. (iii) In the study by Maggio and Segal (2009), the sIPSCs were recorded using a CsCl-containing pipette solution. This may explain why the recorded sIPSCs were small in amplitude and sparse in frequency. Therefore, the fast-onset enhancement of sIPSCs in response to DEX may have been concealed.

We show here that the DEX effect is mediated by a membrane-bound receptor as the membrane-impermeable BSA-DEX retained the effect. In addition, activation of the nongenomic GRs induces downstream retrograde NO signaling, which eventually augments GABAergic transmission from presynaptic interneurons. The involvement of NO in modulating GABAergic transmission is further supported by studies showing that hippocampal GABAergic synapses possess the molecular machinery for retrograde NO signaling (Szabadits *et al*, 2007). Furthermore, NO signaling has been reported to facilitate action potentials in the peptidergic neurons of the posterior pituitary (Klyachko *et al*, 2001). It is tempting to speculate that NO signaling induced by nongenomic GR action in the hippocampus also modulates the opening of ion channels involved in certain phase(s) of an action potential; therefore, mIPSCs were not affected by DEX in our experiment. Taken together, our

results suggest that the activation of nongenomic GRs, by evoking retrograde NO signaling, facilitates the firing activity of the hippocampal interneurons. Given the association between GABAergic inhibition and network oscillation, the cross talk between glucocorticoids and NO signaling may have a role in boosting oscillations to enhance cortical circuit performance in stressful situations.

Furthermore, we observed that the DEX-induced facilitation of GABA release was partially blocked by the CCK_2 receptor antagonist LY225910. Moreover, the facilitating effect of the NO donor SNAP on sIPSCs was also significantly reduced in the presence of LY225910. The most likely interpretation for this is that DEX induces retrograde NO signaling, which activates the NO-sensitive guanylyl cyclase and thus leads to cGMP production. On the one hand, cGMP increases GABA transmission from both PV+ and CCK+ cells, and on the other, it induces CCK release from CCK+ cells. However, it remains to be elucidated how cGMP is associated with CCK release. As activation of CCK_2 receptors massively increases the activity of PV+ cells (Földy *et al*, 2007), the endogenous CCK should also contribute to the facilitating effect of DEX on GABA release (Figure 3b). This interpretation is in line with recent data showing that stress increases cortical CCK release (Becker *et al*, 2008).

Stress Increases GABAergic Transmission in the Hippocampus

Here, we show for the first time that exposing animals to acute stress modulates the GABAergic input to CA1 pyramidal neurons. The effect of acute stress on the occurrence of sIPSCs was partially mimicked by DEX application to control slices. Both acute stress and DEX increased the frequency of sIPSCs to a similar extent, whereas the amplitude of sIPSC was affected only by DEX. In addition, in our *ex vivo* experimental condition, the DEX-induced facilitation of sIPSCs appeared to be relatively brief and started to dwindle already $\sim$10 min after initial exposure to the agonist, whereas the animals in the acute stress group were subjected to restraint for 30 min. The similarity and diversity between the effect of DEX and acute stress are not surprising. On the one hand, corticosterone is the main stress hormone in rodents; on the other, a stress response involves the release of various other 'stress mediators' that act in concert along with corticosterone. In addition, together with the data by Maggio and Segal (2009), it is possible that an acute stress response collectively involves fast nongenomic GR action as elucidated in this study, as well as genomic GR actions that emerge after $\sim$30 min of stress exposure.

Furthermore, we provide the first electrophysiological evidence showing that the altered hippocampal GABAergic transmission sustains following a prolonged period of stress. Three weeks of daily restraint stress induced an elevation in Ca^{2+}-dependent sIPSC frequency, and the membrane-permeable Ca^{2+} chelator EGTA-AM was able to compensate this stress effect. This is in line with previous findings that chronic stress and glucocorticoid treatments regulate hippocampal GAD expression, which presumably leads to enhanced GABA synthesis and hippocampal GABA receptor expression (Bowers *et al*, 1998; Stone *et al*, 2001). One may argue that the increased interneuron activity observed in the stressed animals may be due to an increased excitatory drive to interneurons rather than an effect on interneurons themselves, as enhanced excitatory activity evoked by chronic stress has long been reported (Joëls *et al*, 2007; Joëls, 2008). However, this should not take credit for the observations under our experimental condition in which glutamatergic transmission was blocked by CNQX and APV in the bath to isolate inhibitory postsynaptic currents. In addition, the increased sIPSC frequency in chronically stressed animals may indicate an increased discharge of interneurons or an enhanced release probability at their terminals.

Importantly, the same concentration of DEX that elicited rapid facilitation of sIPSCs in the control hippocampal slices failed to induce any further increase in sIPSCs in the slices from the chronically stressed animals. This result can be explained by the following two possibilities: (i) The rapid nongenomic DEX-NO signaling pathway is continually activated during the entire period of chronic stress, which may contribute to the Ca^{2+}-dependent enhancement of sIPSC frequency observed in the hippocampus of the stressed animals. It is likely that the ligand-binding domains of all the membrane-bound GRs are saturated because of a sustained increase in GC levels, and therefore the bath application of DEX failed to induce any further effect; or alternatively (ii), the DEX-NO signaling is destructed or desensitized after a certain period of stress exposure. However, during the time when this pathway is still active, sustained hyperactivity and enhanced Ca^{2+} influx into the interneurons might induce activity-dependent gene transcription through mechanisms such as epigenetics and progressively result in adaptive alterations in the expression levels or properties of proteins that are associated with GABA metabolism or transmission (eg, GAD) and/or ion channels that regulate the firing activity of the interneurons (eg, voltage-gated calcium channel). These secondary changes may underlie the observed chronic stress-induced elevation in hippocampal GABAergic transmission.

Chronic Stress Results in a Functional Deficit of the PV+ Neuronal Network, whereas CCK+ Neurons Remain Largely Unchanged

According to the theory of the functional dichotomy of PV+ and CCK+ neurons proposed by Freund and Katona (2003), one may assume that the functional integrity of CCK+ neurons should be more affected in the chronically stressed animals. However, as discussed above, endogenous CCK is released in response to GC exposure. As CCK specifically stimulates PV+ cells (Földy *et al*, 2007), it appears that during stress, stimulatory effects converge on the PV+ cells in that they are affected by both NO and CCK. In this hub site, PV+ cells appear to be in a more vulnerable position compared with CCK+ cells. In support of this hypothesis, an earlier morphometric study suggested that PV+ neurons are the ones vulnerable to stress (Czéh *et al*, 2005). Consistently, our present results show that after 3 weeks of restraint stress, the number of PV-immunoreactive cells was significantly reduced in all hippocampal subfields. Furthermore, the PV interneuron network of stressed animals exhibited a functional deficit in that it

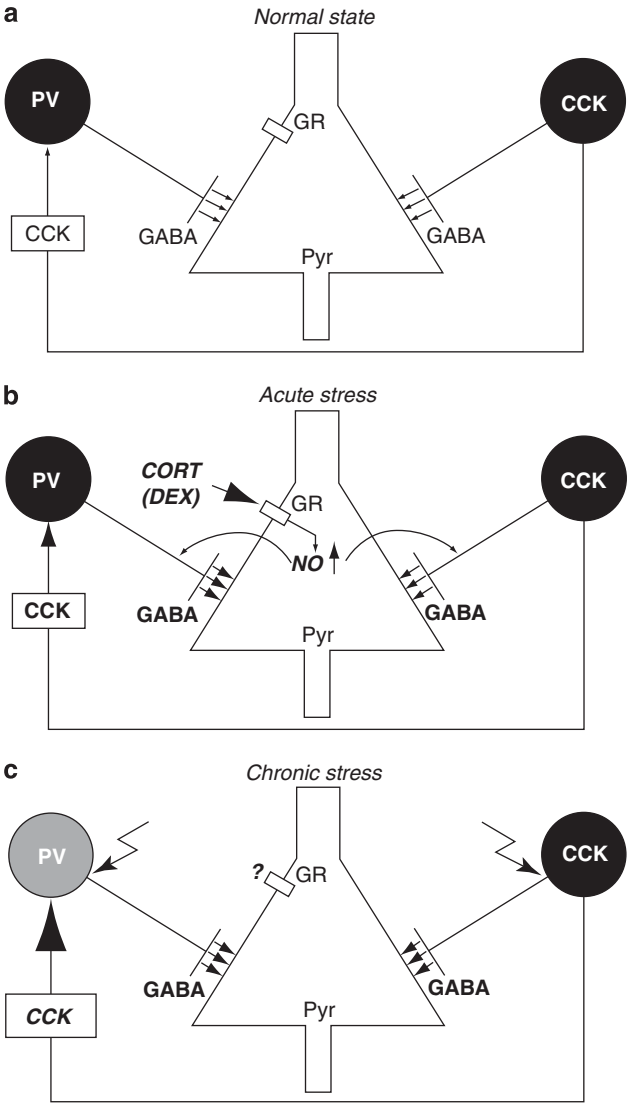

Figure 10 Theoretical summary of the present findings. (a) Under control conditions, PV+ and CCK+ cells provide two parallel, functionally distinct sources of perisomatic GABAergic inhibition to the pyramidal cells. In addition, CCK, presumably released from the CCK+ cells, stimulates the PV+ cells. (b) In response to acute stress, the glucocorticoid surge rapidly activates membrane-bound GRs on pyramidal cells, which results in NO release. Retrograde NO signaling stimulates GABA release from the GABAergic terminals. In addition, NO induces release of CCK, which in turn stimulates PV+ cells. (c) During chronic stress, the above mechanisms are continuously or repetitively activated, which results in sustained elevation of Ca^{2+} influx and GABA release. In this situation, the PV+ cells are in a more vulnerable position as they receive stimulatory effects also from the CCK+ cells. The persistently elevated Ca^{2+} level in the PV+ cells is detrimental to their cellular integrity, and their capacity to generate rhythmic oscillations is impaired. Thus, chronic stress causes functional and structural deficits in PV+ cells. Secondary adaptive alterations of proteins associated with GABAergic transmission are probably induced to facilitate sustained hyperactivity of interneurons. CCK, cholecystokinin; GR, glucocorticoid receptor; NO, nitric oxide; PV, parvalbumin; pyr, pyramidal neuron.

failed to generate rhythmic sIPSCs in response to the CCK analog.

This functional deficit of the PV interneuron network may reflect a dysfunction of CCK_2 receptors. At this point, we cannot rule out this possibility. However, the bath application of CCK induced a massive increase in sIPSCs in both the control and chronic stress groups. It was only the rhythmicity of the CCK-induced sIPSCs that was impaired by chronic stress. On the other hand, application of EGTA-AM could normalize chronic stress-induced elevation in the hippocampal sIPSC frequency, suggesting that the stress-stimulated GABA transmission comes along with excessive intracellular free Ca^{2+}. As reported earlier, an elaborately regulated Ca^{2+} signaling contributes to the synchronous release of GABA quanta from hippocampal PV+ neurons (Caillard *et al*, 2000; Hefft and Jonas, 2005). Therefore, we hypothesize that during chronic stress, the prolonged elevation of corticosterone results in sustained high activity of PV+ cells, which in turn leads to increased Ca^{2+} influx that is beyond the buffering capacity of the calcium binding protein PV expressed in those neurons. As a consequence, the high presynaptic Ca^{2+} level boosts GABA release from the PV+ cells and results in inaccurately timed response patterns of these neurons.

Conclusions

The summary of our findings and a theoretical explanation are presented in Figure 10. Our data show that short-term exposure to DEX increases activity of hippocampal interneurons through retrograde signaling of NO and CCK (downstream from NO) by activating membrane-bound GRs in the pyramidal neurons. We suggest that the outlined nongenomic action of DEX provides a potential pathogenic mechanism that underlies the chronic stress-induced effects reported in this study, although the cellular mechanisms of acute glucocorticoid action and the chronic stress effects are likely to differ. We hypothesize that the continual activation of nongenomic GRs contributes to the development of the chronic stress-induced Ca^{2+}-dependent elevation in GABAergic transmission. Furthermore, as hippocampal PV interneurons are affected by both NO and CCK, their excessive excitation may eventually develop into a cellular and functional deficit. Considering the vital role of PV+ cells in the regulation of network oscillations (Somogyi and Klausberger, 2005; Sohal *et al*, 2009), the stress-induced deficits of these cells may underlie the altered oscillation patterns that are implicated in cognitive impairments, which are common in patients with stress-related psychiatric illnesses.

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DISCLOSURE

The authors declare that, except for income received from primary employer, no financial support or compensation has been received from any individual or corporate entity over the past 3 years for research or professional service and that there are no personal financial holdings that could be perceived as constituting a potential conflict of interest.

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