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# Imipramine counteracts corticosterone-induced enhancement of glutamatergic transmission and impairment of long-term potentiation in the rat frontal cortex

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**Abstract:**
The effects of corticosterone administration lasting for 7 and 21 days were studied *ex vivo* in rat frontal cortex slices prepared 48 h after the last dose of the hormone. In slices originating from corticosterone-treated animals, the amplitude of extracellular field potentials recorded in cortical layer II/III was increased. Corticosterone administration also resulted in an increase of the mean frequency, but not the mean amplitude, of spontaneous excitatory postsynaptic currents (sEPSCs) in layer II/III pyramidal neurons. These effects were accompanied by a reduced magnitude of long-term potentiation (LTP) of field potentials. In a separate set of experiments, rats were treated with corticosterone for 21 days and additionally with a tricyclic antidepressant, imipramine, beginning on the eighth day of corticosterone administration. In this experimental group, the amplitude of field potentials, the mean frequency of sEPSCs and the magnitude of LTP were not different from the control, indicating that corticosterone-induced modifications of basal glutamatergic transmission and synaptic plasticity were reversed by the antidepressant.

**Key words:**
brain slices, model of stress, neocortex, tricyclic antidepressant

**Abbreviations:** aCSF – artificial cerebrospinal fluid, AMPA – α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, LTP – long-term potentiation, NMDA – N-methyl-D-aspartate, sEPSC – spontaneous excitatory postsynaptic current, TBS – theta burst stimulation

## Introduction

In recent years, the pathophysiology of mood disorders has been linked to abnormal glutamatergic transmission within the brain (reviewed in: [18, 24, 26, 43]). It has been shown that depression is accompanied by alterations in AMPA and NMDA receptor-mediated synaptic transmission (reviewed in: [3, 28]), and in major depression, levels of metabotropic glutamate receptors are changed as well [9]. Moreover, small clinical trials indicate that treatments with drugs inhibiting the release of glutamate, lamotrigine and riluzole, as well as with the NMDA receptor antagonist, ketamine, may alleviate symptoms of at least some forms of depression [1, 5, 43].

An important factor contributing to the development of depressive disorders is a hyperactivation of the hypothalamic–pituitary–adrenal (HPA) axis resulting from chronic stress [6, 20] (reviewed in: [23,

25]). Adrenal glucocorticoids, secreted into the circulatory system due to the activation of the HPA axis, interact with nerve cells through binding to two types of intracellular receptors: the high-affinity mineralocorticoid receptors (MRs) and the lower-affinity glucocorticoid receptors (GRs), whose activation alters expression of numerous genes [8, 15, 22]. In addition to the genomic mechanisms, corticosteroids may exert rapid, non-genomic effects *via* cell membrane-associated receptors (reviewed in: [12]).

While the effects of acute stress on glutamatergic transmission and neuronal activity are relatively well understood, the available data regarding the effects of chronic stress and prolonged elevation of the corticosterone level, which exert pro-depressive influence, are scarce (reviewed in: [23]). The repeated administration of corticosterone in rats represents one animal model studying the effects of chronic stress [11]. Although this model simulates only one aspect of the influence of stress on the organism, namely an elevated level of corticosterone, several groups of investigators have shown that the prolonged treatment of rats with corticosterone produces effects that resemble many of the symptoms and underlying neurobiological changes associated with human depression (reviewed in: [35]). However, the effects of repeated corticosterone injections on the function of glutamatergic synapses and their plasticity in the frontal cortex have not yet been studied. Thus, we sought to determine the effects of corticosterone treatments lasting for 7 and 21 days on glutamate-mediated field potentials and spontaneous excitatory postsynaptic currents (sEPSCs), as well as on long-term potentiation. Previously, our electrophysiological studies showed that repeated administration of imipramine, a tricyclic antidepressant which blocks 5-HT and norepinephrine reuptake, attenuated glutamatergic transmission in the rat frontal cortex [4, 37]. Therefore, in the present study we also aimed to determine whether the effects of corticosterone on synaptic transmission and plasticity could be reversed by concurrent treatment with imipramine.

## Materials and Methods

### Animals and treatment

The experimental procedures were approved by the Animal Care and Use Committee at the Institute of Pharmacology, Polish Academy of Sciences, and were carried out in accordance with the European Community guidelines for the use of experimental animals and the national law. Male Wistar rats, weighing approximately 120 g at the beginning of experiments, were used. They were housed in groups on a controlled light/dark cycle (light: 7.00–19.00). Standard food and tap water were available *ad libitum*.

In the first experimental group (termed: Cort 7x), corticosterone (Sigma Aldrich), suspended in 1% Tween 80 (Sigma Aldrich), was injected subcutaneously (dose: 10 mg/kg, volume: 1 ml/kg) twice daily for 7 days. Rats in the control group (termed: Tw 7x) received just the vehicle (1% Tween 80) for 7 days. The second experimental group (termed: Cort 21x) received corticosterone twice daily for 21 days. The third experimental group (termed: Cort 21x + Imi 14d) received corticosterone for 21 days and, beginning on the eighth day of corticosterone treatment, also received imipramine (Sigma Aldrich) for 14 days. Imipramine was dissolved in water and administered *per os* (dose: 20 mg/kg, volume: 2 ml/kg) twice daily. This amount of imipramine was chosen based on pilot experiments. Control rats for treatments lasting 21 days (termed: Tw 21x) received 1% Tween 80 and water. All control animals were handled identically and were investigated concurrently with the corticosterone and corticosterone plus imipramine-treated animals.

### Slice preparation

Brain slices were prepared 2 days after the last substance administration. Rats were anesthetized with isoflurane (Aerrane, Baxter) and decapitated. Then, their brains were rapidly removed and immersed in an ice-cold artificial cerebrospinal fluid (aCSF) containing (in mM): 130 NaCl, 5 KCl, 2.5 $CaCl_2$, 1.3 $MgSO_4$, 1.25 $KH_2PO_4$, 26 $NaHCO_3$, 10 D-glucose and bubbled with a mixture of 95% $O_2$ and 5% $CO_2$. Frontal cortical slices (420 μm thick) were cut in the coronal plane using a vibrating microtome (Leica VT1000), and they were stored submerged in aCSF at 32 ± 0.5°C.

### Field potential recording and LTP induction

A slice was placed in the recording chamber of an interface type and superfused (2.5 ml/min) with warm (32 ± 0.5°C), modified aCSF of the following composition (mM): 132 NaCl, 2 KCl, 1.25 $KH_2PO_4$, 26

$NaHCO_3$, 1.3 $MgSO_4$, 2.5 $CaCl_2$, and 10 D-glucose 10, bubbled with 95% $O_2$ and 5% $CO_2$ [3, 35]. A bipolar stimulating electrode (FHC, USA) was placed approximately 2 mm lateral to the midline and approximately 1.5 mm below the pial surface (layer V). Stimuli (duration: 0.2 ms) were applied at 0.033 Hz using a constant-current stimulus isolation unit (WPI, Germany). Field potentials were recorded using glass micropipettes filled with aCSF (2–5 MΩ), which were placed approximately 0.3 mm below the cortical surface (layer II/III). Responses were amplified (EXT 10-2F amplifer, NPI, Germany), filtered (1 Hz–1 kHz), A/D converted (10 kHz sampling rate), and stored using the Micro1401 interface and Signal 2 software (CED, UK).

After the determination of a stimulus-response (input-output) curve, stimulation intensity was adjusted to evoke a response of 30% of the maximum amplitude. For the induction of LTP, a high frequency protocol of theta burst stimulation (TBS) was used [30]. The TBS consisted of ten trains of stimuli at 5 Hz, each composed of five pulses at 100 Hz, repeated 5 times every 15 s. During TBS, the pulse duration was increased to 0.3 ms. The amount of LTP was assessed in terms of an average increase in the amplitude of responses recorded between 60–75 min after the last burst, relative to baseline.

### Whole-cell recording

A slice was placed in the recording chamber of a submerged type and superfused (3 ml/min) with warm (32 ± 0.5°C), modified aCSF, as in the field potentials experiments. Neurons were visualized using a Zeiss Axioskop 2FS upright microscope with Nomarski optics, a 40× water immersion lens and an infrared camera [37, 39]. Patch pipettes were pulled (P-97 puller, Sutter Instrument, Novato, CA, USA) from borosilicate glass capillaries (Clark Electromedical Instruments, UK). The pipette solution contained (in mM): 130 K-gluconate, 5 NaCl, 0.3 $CaCl_2$, 2 $MgCl_2$, 10 HEPES, 5 $Na_2$-ATP, 0.4 Na-GTP, and 1 EGTA. The osmolarity and the pH were adjusted to 290 mOsm and 7.2, respectively. Pipettes had an open tip resistance of approximately 6 MΩ. Signals were recorded using the SEC 05 LX amplifier (NPI, Germany), filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440 interface and Clampex 9.2 software (Molecular Devices, USA).

Layer II/III neurons were sampled from sites located approximately 2 mm lateral to the midline and approximately 0.3 mm below the pial surface. Pyramidal cells were identified by the established morphological criteria and electrophysiological characteristics in the current clamp mode (for details, see: [37]). Next, cells were voltage-clamped at –76 mV for a period of 8 min to record spontaneous excitatory postsynaptic currents (sEPSCs), which were then detected off-line and analyzed using the Mini Analysis software (Synaptosoft, USA). Recordings were accepted for analysis if the access resistance ranged between 15–18 MΩ and if it was stable (25% change) during the recording. In part of the experiments, 200 nM kynurenic acid (Sigma Aldrich) was added to the aCSF.

### Data analysis

The stimulus-response curves obtained for each slice were fit with the Boltzmann equation: $V_i = V_{max} / (1 + \exp((u - u_h)/ - S))$, where $V_{max}$ is the maximum field potential amplitude; u is the stimulation intensity; $u_h$ is the stimulation intensity evoking a field potential of half-maximum amplitude; S is the factor proportional to the slope of the curve (for details, see: [42]). The threshold stimulation was determined as the stimulus intensity necessary to evoke a field potential of approximately 0.1 mV in amplitude. The results are expressed as the means ± SEM. Statistical analyses were carried out using a one-way analysis of variance (ANOVA) followed by the Tukey's *post-hoc* test or the Student's *t*-test.

## Results

### Effects of treatment on field potentials

Analyses of field potentials (FPs) recorded from slices obtained from rats receiving corticosterone for 7 days (Fig. 1A) and for 21 days (Fig. 1B) revealed a marked increase in the relationship between stimulus intensity and FP amplitude (input-output curve) compared with slices obtained from rats receiving the vehicle. Representative examples of FPs are shown in Figure 3. The effects of corticosterone treatment on parameters characterizing stimulus-response curves

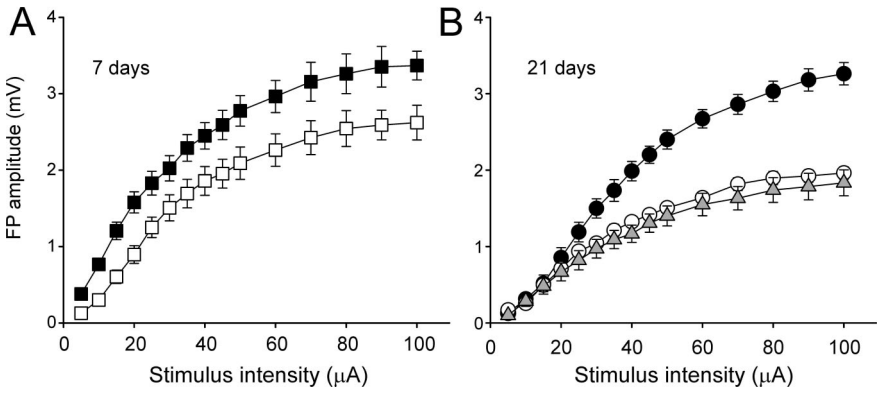


**Fig. 1.** The relationship between stimulus intensity and the amplitude of field potentials (FPs) recorded in cortical layer II/III. Graphs represent plots of the mean FP amplitude (± SEM). (**A**) The effect of treatment with corticosterone for 7 days (filled squares, n = 17) compared with that of control preparations obtained from rats receiving vehicle (open squares, n = 21). (**B**) The effect of treatment with corticosterone for 21 days (black circles, n = 20) and of treatment with corticosterone for 21 days plus imipramine for 14 days (grey triangles, n = 16), compared with control preparations (open circles, n = 17)

of FPs, calculated using the Boltzmann fits, are summarized in Table 1.

No differences were evident between the input-output curves of the slices prepared from animals treated concurrently with corticosterone (21 days) and imipramine (14 days, beginning on the 8th day of corticosterone administration) and the control group (Fig. 1B), apart from a small increase in the threshold value (Tab. 1).

### Effects of treatment on excitatory postsynaptic currents

All cells included in the analysis of sEPSCs exhibited a regular spiking firing pattern in response to a depolarizing current pulse (not shown, cf. [37]). There were no significant differences between control neurons and cells in slices obtained from corticosterone-treated rats (7 and 21 days) either in the resting mem-

**Tab. 1.** Effects of corticosterone and imipramine treatments on parameters characterizing stimulus-response curves of field potentials calculated using the Boltzmann fits

| | Threshold (μA) | $V_{max}$ (mV) | $U_h$ (μA) | S | n |
|---|---|---|---|---|---|
| Tw 7x | 6.73 ± 0.5 | 2.33 ± 0.17 | 28.39 ± 1.33 | 11.16 ± 0.49 | 21 |
| Cort 7x | 5.9 ± 0.44 | 3.18 ± 0.21* | 27.31 ± 1.42 | 10.85 ± 0.6 | 17 |
| Tw 21x | 7.05 ± 0.74 | 1.93 ± 0.09 | 27.59 ± 1.69 | 11.61 ± 8.85 | 17 |
| Cort 21x | 7.72 ± 0.6 | 3.11 ± 0.14*** | 32.83 ± 1.76** | 11.16 ± 0.58 | 20 |
| Cort 21x + Imi 14x | 11.76 ± 1.48** | 1.75 ± 0.16 | 23.65 ± 1.75 | 10.75 ± 1.06 | 16 |

Data are presented as the mean ± SEM. $V_{max}$ – maximum field potential amplitude; $u_h$ – half maximum stimulation, S – factor proportional to the slope of the curve; n – number of slices. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$; ANOVA followed by Tukey's *post-hoc* test. The threshold was determined as the stimulus intensity necessary to evoke a field potential of approximately 0.1 mV in amplitude

**Tab. 2.** Basic membrane properties of recorded pyramidal neurons

| | $V_m$ (mV) | $R_m$ (MΩ) | n |
|---|---|---|---|
| Tw 7x | –70.3 ± 1.1 | 61.8 ± 7.5 | 14 |
| Cort 7x | –69.0 ± 1.3 | 62.9 ± 8.4 | 16 |
| Tw 21x | –69.5 ± 1.3 | 66.2 ± 8.1 | 8 |
| Cort 21x | –71.3 ± 1.2 | 53.9 ± 5.6 | 19 |
| Cort 21x + Imi 14x | –70.3 ± 1.1 | 68.9 ± 6.6 | 13 |

Data are presented as the mean ± SEM. $V_m$ – resting membrane potential; $R_m$ – input resistance. Differences between values for neurons in each group are not significant ($p > 0.05$)

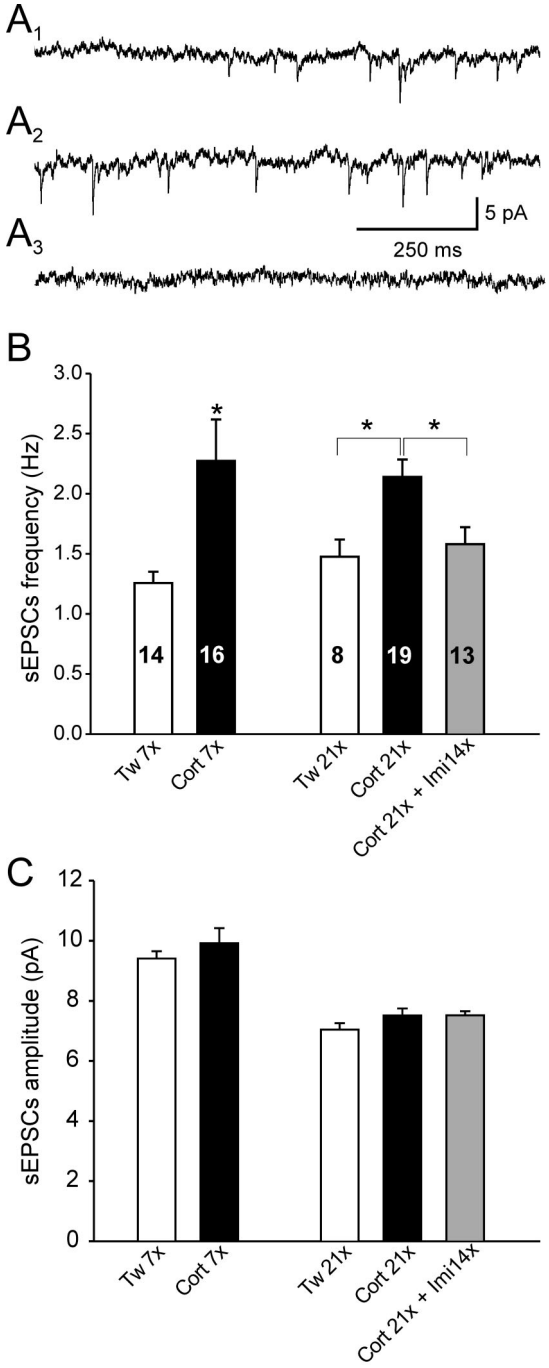


**Fig. 2.** Spontaneous EPSCs in layer II/III pyramidal cells. Traces in (**$A_1$** – **$A_2$**) show representative recordings of sEPSCs from individual neurons in slices prepared from control animals receiving the vehicle and animals receiving corticosterone (7 days), respectively. (**$A_3$**) A representative example of a recording in the presence of 200 nM kynurenic acid. (**B**) Mean frequency (± SEM) of sEPSCs after 7 days of administration of corticosterone, (Cort 7x) and vehicle (Tw 7x) and after 21 days of administration of corticosterone (Cort 21x), vehicle (Tw 21x) and corticosterone for 21 days plus imipramine for 14 days (Cort 21x + Imi 14x). The numbers on the bars indicate the numbers of neurons in each group. (**C**) Mean amplitude (± SEM) of sEPSCs after 7 days of administration of corticosterone (Cort 7x), vehicle (Tw 7x) and after 21 days of administration of corticosterone (Cort 21x), vehicle (Tw 21x) and corticosterone for 21 days plus imipramine for 14 days (Cort 21x + Imi 14x). * $p < 0.05$

brane potential or in the input resistance (Tab. 2). At a membrane potential of –76 mV, sEPSCs were recorded as inward currents (Fig. 2$A_1$, $A_2$).

As illustrated in Figure 2B, in cells prepared from rats treated with corticosterone for 7 days, the mean frequency of sEPSCs (2.28 ± 0.34 Hz) was markedly higher than that in cells originating from control animals (1.26 ± 0.09 Hz, $p < 0.05$, $t = –2.71$, $df = 28$). A similar effect has been found in neurons after 21 days of corticosterone treatment compared with control neurons (2.14 ± 0.15 *vs.* 1.47 ± 0.14 Hz, respectively, $p < 0.05$, $t = –2.71$, $df = 25$). In contrast, in slices prepared from animals treated concurrently with corticosterone and imipramine, the mean frequency of sEPSCs (1.58 ± 0.14 Hz) was not different from that recorded in slices taken from control rats ($p = 0.61$, $t = –0.52$, $df = 19$).

Neither corticosterone administered for 7 days nor that administered for 21 days alone or in combination with imipramine affected the mean amplitude of sEPSCs (Fig. 2C). We note that sEPSC amplitudes in slices prepared after 21 days of treatment were generally smaller; however, this effect appears to be unrelated to corticosterone administration.

### Effects of treatment on long-term potentiation

In slices prepared from animals receiving the vehicle for 7 days, the mean amplitude of FPs, measured between 60 and 75 min after TBS application, was 132 ± 2% of baseline (Fig. 3A). A LTP of a similar magnitude was induced in slices originating form rats receiving the vehicle for 21 days (139 ± 3%; Fig. 3B). The LTP was significantly attenuated in slices prepared from animals treated with corticosterone for 7 days (102 ± 1%, $p < 0.001$) and for 21 days (106 ± 4%, $p < 0.001$). However, in slices prepared from animals treated concurrently with corticosterone and imipramine, the magnitude of the LTP was not different from that recorded in slices originating from control rats ($p = 0.082$, Fig. 3B, C).

## Discussion

The major finding of this study is that imipramine administered concurrently with corticosterone normalizes the enhanced basal excitatory synaptic transmis-

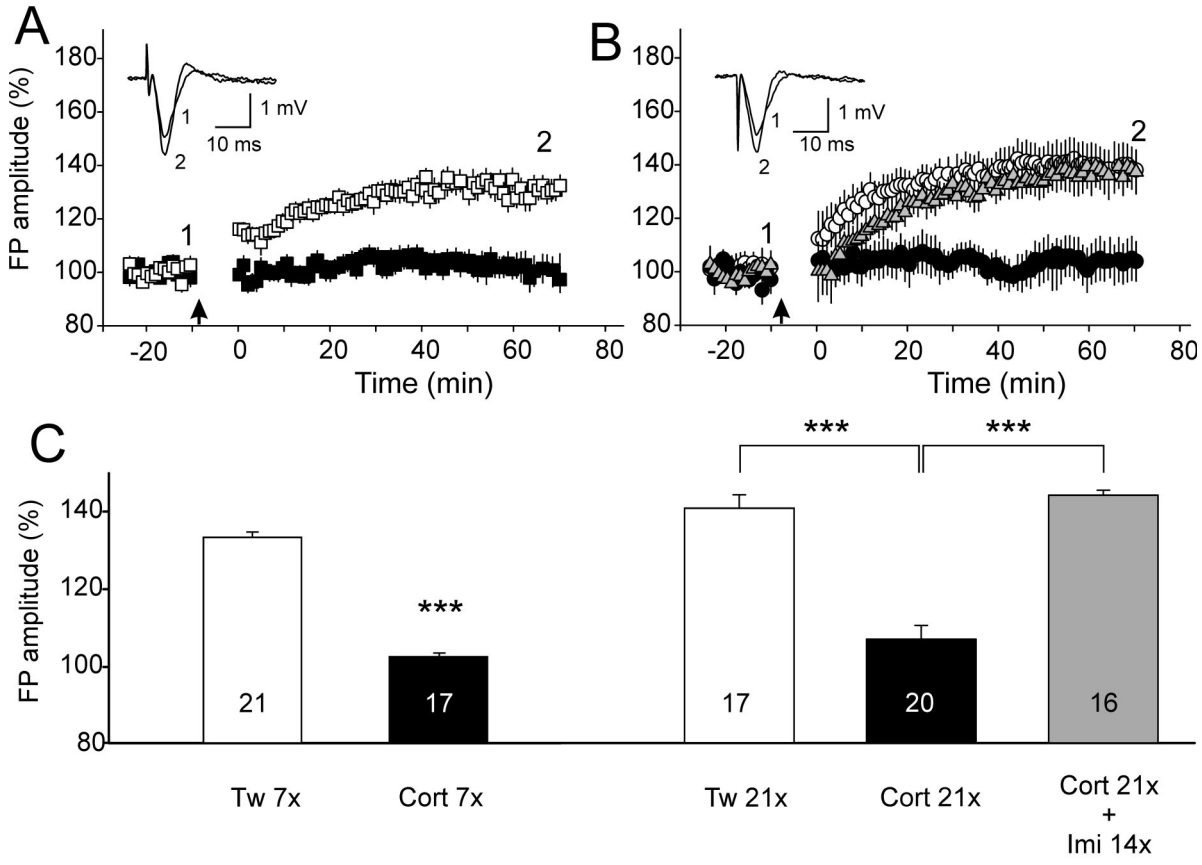


**Fig. 3.** Long-term potentiation in layer II/III. (**A**) Plot of the amplitude of FPs (the mean ± SEM) recorded in the course of the control experiments (open squares) and in slices obtained from rats treated with corticosterone for 7 days (filled squares). Arrow denotes the time of the beginning of theta-burst stimulation (TBS). Inset shows superposition of averaged FPs (n = 4) recorded in a representative control experiment at times indicated by numbers. (**B**) Plot of the amplitude of FPs (the mean ± SEM) recorded in slices obtained from control rats (open circles), from rats treated with corticosterone for 21 days (filled circles) and from rats treated with corticosterone for 21 days plus imipramine for 14 days (grey triangles). Arrow denotes the time of the beginning of TBS. Inset shows superposition of averaged FPs (n = 4) recorded at times indicated by numbers from a representative slice obtained from an animal treated with corticosterone for 21 days plus imipramine for 14 days (**C**) Mean amplitude of FPs recorded between 60–75 min after TBS in slices prepared after 7 days of corticosterone (Cort 7x), vehicle (Tw 7x) administration and after 21 days of corticosterone (Cort 21x), vehicle (Tw 21x) administration and treatment with corticosterone for 21 days plus imipramine for 14 days (Cort 21x + Imi 14x). The numbers on the bars indicate the numbers of slices in each group. *** $p < 0.001$

sion and reduced potential for long-term synaptic plasticity in the frontal cortex brought about by the repetitive administration of corticosterone.

The effects of chronic stress on the function of glutamatergic synapses in the rodent brain have not been investigated extensively so far. In a few studies, different forms of stress were employed and their effects were usually measured in the hippocampal formation. It had been reported that restraint stress repeated for 40 days increased basal and $K^+$-stimulated glutamate release from hippocampal synaptosomes [10]. These effects were accompanied by increased neuronal presynaptic [$^3$H]-glutamate uptake and decreased binding to hippocampal membranes. Shorter-lasting chronic restraint stress (21 days) resulted in an enhancement of glutamate release in the hippocampus that became evident after a subsequent single stress challenge [40]. Three weeks of unpredictable stress resulted in an enhanced glutamatergic transmission and suppression of synaptic plasticity in the dentate gyrus [14]. Using electrophysiological approaches, we investigated the effects of repeated daily neck restraint sessions lasting 10 min on glutamatergic field potentials in the dentate gyrus of mice. We found no changes after up to 21 successive daily neck restraint sessions [34]. In accordance with an earlier study employing chronic restraint stress [27], the data showed that repeated neck restraint stress also did not influence the paired-pulse facilitation ratio in the dentate gyrus [34].

The diverse outcomes of these studies most likely result from a variability in the strength of stressful stimuli imposed by different procedures and from individual differences in the response to stressors resulting in a variable level of the stress-induced corticosterone surge from the adrenal glands.

Repeated corticosterone injections mimic only one of the effects of stress. Nevertheless, this procedure allows experimenters to examine the direct influence of the hormone on the development of depressive symptomatology [7, 11, 35]. In our experiments, animals received 20 mg/kg of corticosterone per day. This amount of hormone, when administered repetitively for 20 days, has been found to increase the immobility of rats in the forced swim test, commonly regarded as a depression-like behavior in rats [13]. Other investigators confirmed those findings [11], although it should be noted that a higher dose of corticosterone (40 mg/kg) is more effective in inducing depression-like behavior [16].

In our previous study, we showed that repeated corticosterone administration (20 mg/kg/day) lasting for 7 days induced no change in the amplitude of field potentials in the hippocampal CA1 area. However, treatment lasting for 21 days resulted in a decrease of population spikes evoked in the CA1 area by stimuli of small and intermediate intensity without a change in the maximum amplitude of responses [42]. Additionally, it has been reported that corticosterone injections at a dose of 10 mg/animal/day did not result in a reduction in the maximum amplitude of CA1 population spikes [17]. We demonstrate in the present paper that corticosterone administration for 7 or 21 days results in an increase in the amplitude of extracellular field potentials in slices of the frontal cortex over a wide range of stimulation intensities. Thus, the effects of corticosterone treatment on basal excitatory synaptic transmission in the hippocampus and in the frontal cortex seem to differ.

We have found that an increase in the amplitude of FPs, related to corticosterone administration, was accompanied by an increased frequency of spontaneous EPSCs. We have previously shown that the blockade of $Na^+$ channels did not significantly modify the mean frequency of sEPSCs in layer II/III pyramidal cells of the rat frontal cortex *in vitro*, indicating that a majority of recorded spontaneous events correspond to miniature EPSCs whose occurrence is not related to the spiking activity of presynaptic neurons [36] (see also: [33]). Changes in the frequencies of sEPSCs and miniature EPSCs are indicative of changes in the probability of glutamate release and/or changes in the number of release sites (e.g., [19]). Thus, corticosterone-induced increases in the mean frequency of sEPSCs and in the mean amplitude of FPs observed in the present study are consistent with an enhanced glutamate release from presynaptic terminals. In the neocortex, synaptic connections with greater initial strengths are less likely to undergo plasticity, whereas weak connections tend to potentiate by presynaptic mechanisms (e.g., [31], see also: [32]). Thus, a reduced possibility of the induction of LTP after corticosterone treatment resembles the occlusion of LTP occurring after learning, resulting in an enhanced transmission in learning-activated synapses and a saturation of long-term potentiation mechanisms [21, 30]. Alterations in the regulation of glutamate release by glycogen synthase kinase (GSK-3β) have been implicated in the mechanisms of the effects of stress and corticosterone on the function of frontal cortical neurons [36].

We have previously shown that the repeated administration of 20 mg/kg/day of imipramine results in a decrease of field potentials recorded in layer II/III of the rat frontal cortex and a reduction of the frequency of sEPSCs in layer II/III pyramidal neurons [4, 37]. The results of the present study extend those findings by showing that imipramine treatment lasting for 14 days, during which the antidepressant was administered concurrently with corticosterone, reverses the effects of the latter. In the present study, we used an increased dose of imipramine, 40 mg/kg/day, as a lower dose of the antidepressant used in preliminary experiments was ineffective. This dose is higher than those employed by other investigators, which usually do not exceed 30 mg/kg/day (e.g., [29]). Nevertheless, in our study we observed no obvious detrimental effects of imipramine during repeated administration and, moreover, the obtained data indicate a lack of such effects at the cellular level. It has been shown that the oral procedure of drug administration *per se* does not influence the outcome of experiments [2].

Our previous work has shown that repeated imipramine administration for 14 days (dose: 20 mg/kg/day) reverses corticosterone-induced functional modifications in the reactivity of 5-$HT_{1A}$ and 5-$HT_2$ receptors in the rat frontal cortex [41]. We have also demonstrated that imipramine ameliorates corticosterone-related modifications in the effects of 5-$HT_{1A}$, 5-$HT_4$ and 5-$HT_7$ receptor activation in the hippocam-

pus [38, 42]. Together with the present results, these data indicate the potential of imipramine to counteract chronic stress-related adverse modifications of the function of serotonergic and glutamatergic systems within the brain.

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