Glucocorticoid modulation of synaptic plasticity in the human temporal cortex of epilepsy patients: Does chronic stress contribute to memory impairment?

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Abstract
Objective: Memory impairment is common in patients with temporal lobe epilepsy and seriously affects life quality. Chronic stress is a recognized cofactor in epilepsy and can also impair memory function. Furthermore, increased cortisol levels have been reported in epilepsy patients. Animal models have suggested that aggravating effects of stress on memory and synaptic plasticity were mediated via glucocorticoids. The aim of this study was, therefore, to investigate the effect of glucocorticoid receptor (GR) modulation on synaptic plasticity in the human cortex of epilepsy patients.

Methods: We performed field potential recordings in acute slices from the temporal neocortex of patients who underwent surgery for drug-resistant temporal lobe epilepsy. Synaptic plasticity was investigated by a theta-burst stimulation (TBS) protocol for induction of long-term potentiation (LTP) in the presence of GR modulators.

Results: LTP was impaired in temporal cortex from epilepsy patients. Pretreatment of the slices with the GR antagonist mifepristone (RU486) improved LTP induction, suggesting that LTP impairment was due to baseline GR activation in the human cortex. The highly potent GR agonist dexamethasone...
INTRODUCTION

Memory is often impaired in patients with chronic epilepsy. In the case of temporal lobe epilepsy (TLE), the most common drug-resistant type of epilepsy, neurodegenerative alterations of the mesial temporal lobe structures may directly contribute to memory disturbance. However, memory impairment is not limited to epilepsies of temporal origin, and good seizure control after surgical resection usually leads to recovery of cognitive functions despite resection of the mesial temporal lobe structures. Whereas it is not surprising that disruption of the neuronal network through recurrent seizures may impair normal function, it is less clear why memory modalities corresponding to areas unrelated to the seizure focus are affected. A more generalized mechanism might be involved.

Besides being electrical events, from a psychological perspective, seizures are also stressful events. Following seizures, serum cortisol levels rise. Glucocorticoids and stress show dual effects on memory function. Acute stress potentiates memory encoding, leading to extremely lucid memories of stressful events, whereas chronic stress is associated with memory impairment. Chronically elevated cortisol levels in patients with Cushing disease also show detrimental effects on memory. Furthermore, there is increasing evidence that chronic stress promotes the development of neurodegenerative diseases such as Alzheimer disease. In epilepsy patients, besides elevated cortisol levels, increased expression of glucocorticoid receptors (GRs) has been reported. Therefore, stress-related mechanisms may contribute to memory impairment in epilepsy. In an animal epilepsy model, concomitant stress compromised hippocampal memory function and elevated cortisol levels in epilepsy patients correlated with poor memory performance. However, the direct effects of glucocorticoids on memory-related processes in epileptic patients remain to be clarified.

Key Points

• Long-term potentiation of synaptic plasticity, a biomarker of memory function, was impaired in the perilesional cortex of temporal lobe epilepsy patients
• Poor plasticity was additionally aggravated by glucocorticoid receptor activation in an activity-dependent manner
• Blockade of glucocorticoid receptors rescued induction of long-term potentiation of synaptic plasticity
• Chronic stress may therefore contribute to memory impairment in epilepsy patients

Significance: Our results show a direct negative glucocorticoid effect on synaptic potentiation in the human cortex and imply chronic activation of GRs. Chronic stress may therefore contribute to memory impairment in patients with temporal lobe epilepsy. Furthermore, the activity-dependent acute inhibitory effect of dexamethasone suggests a mechanism of synaptic downscaling by which postictally increased cortisol levels may prevent pathologic plasticity upon seizures.

KEYWORDS
dexamethasone, epilepsy, human cortex, long-term potentiation, stress

Ever since the pioneer experiments of Bliss and Lomo showing that repetitive electrical stimulation modifies synaptic strength, evidence has accumulated that activity-dependent synaptic plasticity underlies memory formation. In line with Donald O. Hebb’s prediction in 1949 that neurons that “fire together, wire together,” repetitive high-frequency stimulation leads to long-term potentiation (LTP) of synaptic plasticity, whereas irregular or low-frequency stimulation causes persistent weakening of synaptic strength, known as long-term depression (LTD). In animal models, stress and increased corticosterone levels have shown effects on synaptic plasticity that correspond well to the effects on memory. LTP was enhanced by acute stress and glucocorticoids in rodents, whereas chronic exposure to glucocorticoids or chronic stress disrupted LTP induction. Less is known about human synaptic plasticity. Activity-dependent synaptic plasticity has been successfully induced in human cortex and hippocampus specimens obtained from epilepsy surgery. Furthermore, LTP was impaired in the region of seizure origin. Regarding glucocorticoid effects,
dexamethasone causes decrease of LTP mediators, namely calcium-calmodulin kinase and phosphorylated α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPA-Rs), in the human amygdala of epileptic patients. However, the direct functional implications of GR activation on human synaptic plasticity remain unknown.

To delineate a possible contribution of stress and glucocorticoid exposure to memory impairment in chronic epilepsy, we investigated the effects of GR modulation on synaptic plasticity in temporal cortex specimens from patients undergoing surgery for drug-resistant TLE. We used the highly potent, highly selective GR agonist dexamethasone, which in animal models mimics the lasting detrimental effects of stress on synaptic plasticity. We could demonstrate that LTP induction was compromised in the cortex of epileptic patients and was additionally aggravated by dexamethasone in an activity-dependent manner, whereas dexamethasone showed no negative effect on LTP in a similar setting in the cortex of naïve mice. Furthermore, pretreatment with the GR antagonist mifepristone (RU486) promoted LTP induction, implying that in epilepsy patients, chronic activation of GRs may contribute to memory impairment.

2 | MATERIALS AND METHODS

2.1 | Patients

The study was performed in accordance with the principles of the Helsinki Declaration and was approved by the local ethics committee (Vote Nr. 193-18B, Friedrich Alexander University Ethics Committee). Patients were enrolled in the study after written informed consent was obtained. All patients suffered from drug-resistant TLE and underwent an anterotemporal resection (Figure 1A) in terms of an extended lesionectomy or combined with an amygdalohippocampectomy. Patient data are listed in Table 1. Memory performance was evaluated preoperatively via the Berliner Amnesietest (see Methods S1).

2.2 | Acute slice preparation

Upon surgical resection, temporal (middle and inferior; Figure 1A) cortex specimens were immediately immersed into ice-cold artificial cerebrospinal fluid (aCSF) containing (in mmol·L⁻¹): NaCl 129.0, KCl 3.0, CaCl₂ 1.6, MgSO₄ 1.8, NaH₂PO₄ 1.25, NaHCO₃ 21.0, and glucose 10.0, saturated with carbogen (95% O₂/5% CO₂) and transferred to the pathology department. After visual inspection by an experienced pathologist, a small block of extralosional temporal cortex (see Methods S1) was selected for electrophysiological experiments before proceeding with standard neuropathologic workup. Overall transfer time to the laboratory was approximately 10 min. Cortical slices (400 µm) were prepared using a Vibratome (Leica) and incubated at 32°C in an interface-type recording chamber (Charité Berlin) perfused with carbogenated aCSF (flow rate = 1.8 ml/min, pH 7.4, osmolarity = 300 ± 3 mOsm/L). At least 120 min were allowed for slice equilibration. For mouse experiments, horizontal ventral temporohippocampal slices were prepared in an analogous manner (for details, see Methods S1).

2.3 | Field potential recordings

Field potential recordings were performed using chlorided silver wire glass electrodes (filled with aCSF, tip diameter = 2–3 µm, resistance 2–4 MΩ). Signals were recorded with an extracellular differential amplifier (npi electronic), filtered at 3 kHz, and digitized with a sampling frequency of 10 kHz (Micro1401, CED). Stimulation was performed with bipolar tungsten electrodes (Microprobes for Life Science, tip separation = 75 µm, impedance = .1 MΩ) via an isolated pulse stimulator (Model 2100, A-M Systems). Field postsynaptic potentials (fPSPs) were recorded from the cortical layer II/III while stimulating in layer IV (Figure 1B), thus activating direct projections from layer IV neurons and ascending en route fibers to layer II/III pyramidal neurons, as well as recurrent collaterals of layer II/III neurons. After initial input–output curves, the baseline stimulation strength was adjusted to 40%–60% of the maximal fPSP amplitude. For assessing baseline fPSPs, single or paired pulses (interspike interval = 40 ms, spike duration = .1 ms) were administered every 30 s. Only slices with a response > .2 mV were accepted for analysis. For LTP induction, after at least 20 min of stable baseline recordings, a theta-burst stimulation (TBS) protocol consisting of 10 stimulus trains at 100 Hz (10 pulses per train, intertrain interval = 200 ms) repeated four times with a 10-s interval was applied.

2.4 | Drugs

Drugs were purchased from Sigma-Aldrich. Dexamethasone and RU486 were dissolved in dimethylsulfoxide (DMSO).

2.5 | Serum cortisol measurements

Blood samples for cortisol measurements were drawn in citrate tubes during epilepsy surgery at the time of tissue
resection as well as in a time-matched manner from control patients undergoing brain surgery for other lesions. After centrifugation, the supernatant was stored at −80°C until processing. Cortisol was measured with an immunochemiluminometric assay (Immulite 2000 XPI Cortisol, Siemens Healthcare; normal range = 5–25 µg/dl). Patients treated with dexamethasone before or during surgery were excluded from analysis.

2.6 | Data analysis

Off-line analysis of field potential recordings was performed in MATLAB (MathWorks) using self-written scripts. Unless stated otherwise, all data are presented as mean ± SEM. Statistical analyses were performed in Prism (GraphPad Software) and SigmaPlot (Systat Software). The statistical tests performed are stated in the text. The test selection was based on whether the data were normally distributed and whether groups showed equal variance. Significance was defined as p < .05. Individual brain slices were considered in the statistical analyses of LTP experiments. For correlation to clinical parameters, whenever more than one slice from the same patient was used for the same protocol, averages were calculated. If not specified otherwise, the number of slices is given first, followed by the number of patients. The fPSP slope was estimated between 20%
### TABLE 1  Clinical patient data

| Hemisphere | Sex | Age at onset, years | Epilepsy duration, years | AED                          | Memory, z-score | Pathology                  | Serum cortisol, µg/dl |
|------------|-----|---------------------|--------------------------|-----------------------------|-----------------|---------------------------|-----------------------|
|            |     |                     |                          |                             | Naming          | Resection type            | Neocortex            | Mesial                           |
|            |     |                     |                          |                             | Figural         |                           |                       |                                |
|            |     |                     |                          |                             | Resection type   |                           |                       |                                |
|            |     |                     |                          |                             | Pathology        |                           |                       |                                |
|            |     |                     |                          |                             | Serum cortisol   |                           |                       |                                |
|            |     |                     |                          |                             |                 |                           |                       |                                |

#### Note: The last four patients listed were only included in clinical data and cortisol level analysis; no long-term potentiation experiments were performed in these patients.

Abbreviations: AED, antiepileptic drug; AHE, amygdalohippocampectomy; ATLR, anterior temporal lobe resection; BRI, brivaracetam; CLA, clonazepam; CLB, clobazam; ESC, eslicarbazepine acetate; HS, hippocampal sclerosis according to International League Against Epilepsy type; LAC, lacosamide; LE, levetiracetam; LTG, lamotrigine; mMCD, mild malformation of cortical development; np, no pathology; OP, oxcarbazepine; PER, perampanel; PGA, pregabalin; RG, reactive gliosis; RG*, reactive gliosis after deep electroencephalography; SCI, escitalopram; TLI, temporomesial lymphocytic infiltrates; TO, topiramate; VEN, venlafaxine; WHO, World Health Organization; ZON, zonisamide.
and 80% of the amplitude of the fPSP. The mean of all slope values recorded 10 min prior to TBS was defined as 100% (baseline). LTP induction in individual slices was defined as 10% increase of the mean of 10 fPSP slope values recorded 25–30 min after TBS, whereas LTD was defined as at least 10% decrease. For LTP and drug effects, the means of 5-min baseline and 5 min at the time points given in the results were compared.

3 | RESULTS

3.1 | LTP is impaired in temporal cortex of epilepsy patients

To evaluate synaptic plasticity, we performed field potential recordings in acute slices from temporal cortex specimens obtained during epilepsy surgery. A schematic of the extent of cortical resection is shown in Figure 1A. fPSPs were recorded from cortical layers II/III upon electrical stimulation in layer IV (Figure 1B). The fPSPs had an average amplitude of 0.62 ± 0.04 mV (n, slices/patients = 27/14). Paired-pulse stimulation was used to evaluate short-term plasticity (Figure 1C,D). Double pulses were delivered at increasing interstimulus intervals (ISIs) between 10 ms and 500 ms, to discriminate effects of early and late inhibition from those of presynaptic short-term potentiation. At 10-ms ISI, the paired-pulse ratio (PPR = amplitude fPSP2/amplitude fPSP1) was <1, indicative of early recurrent inhibition. At longer ISIs, paired-pulse facilitation was observed, with a maximum at 25 ms (PPR = 1.13 ± .3, n = 47/12), indicating regular short-term synaptic plasticity and low glutamate release probability of the synapses. Next, after obtaining stable baseline recordings for at least 20 min, we applied TBS to check the ability of the slices to express LTP. Altogether, there was no significant increase in the fPSPs recorded during 30 min after TBS (105.4% ± 5.7% of baseline, p = .82, Wilcoxon signed-rank test [WSR], n = 25/13, Figure 1E). However, in individual experiments, fPSP slopes were often altered. Interestingly, both decrease and increase in fPSP slope were observed. We defined an increase or decrease of 10% from baseline as LTD and LTP, respectively. Thus, LTP was induced in seven slices, whereas in six slices TBS caused LTD and in 12 slices it had no effect (Figure 1F,G). We further assessed the duration of baseline fPSPs as a measure of network hyperexcitability and checked whether there was a relationship between duration and LTP expression. The fPSP duration was significantly shorter in slices where LTP was successfully induced (5.1 ± 1.4 ms, n = 7) compared to slices showing no LTP (10.9 ± 2.1 ms, n = 18, p = .03, t-test with Welch correction; Figure 1H), implying that hyperexcitability of the network disrupts synaptic plasticity.

To check whether LTP induction depended on clinical parameters (Table 1), for each patient the fPSP slope values obtained from individual slices were averaged. Spearman correlation analysis showed no dependence between the fPSP slope change 30 min after TBS and patient age (Spearman rank [r] = .44, p = .12), epilepsy duration (r = .26, p = .39), or age at epilepsy onset (r = −.16, p = .61, n = 13 patients). Nor did fPSP slopes depend on patient gender or the location of resection (left vs. right hemisphere, temporal vs. temporohippocampal resection, paired t-tests, p > .05, n = 13 patients). We also failed to observe a correlation between the LTP slopes and hemisphere-specific memory performance, namely, naming for left TLE (r = −.6, p = .1, n = 8) and figural memory for right TLE (r = −.3, p = .62, n = 5). However, considering the small number of patients, these results should be interpreted with care.

3.2 | Dexamethasone impairs activity-dependent synaptic depression

To delineate a possible role of stress neuromodulators in LTP impairment, we investigated the effect of the glucocorticoid dexamethasone on synaptic plasticity. First, we tested whether dexamethasone affected baseline synaptic transmission by looking at the fPSP slope and amplitude. Because dexamethasone was dissolved in DMSO, which may have negative effects on membrane permeability, we added DMSO (.01%) to the aCSF from the beginning of the experiments, to correct for possible effects of the solvent. Compared to control experiments (aCSF only), DMSO did not influence the fPSP slope (aCSF: −447 ± 69 V/s, n = 27/14; aCSFDMSO: −444 ± 68 V/s, n = 25/10; p = .91, Mann–Whitney test [MW]; Figure 2A) or amplitude (aCSF: −.62 ± .04 V/s, n = 27/14; aCSFDMSO: −.67 ± .06, n = 25/10; p = .5, MW). Adding 5 μmol·L−1 dexamethasone to the perfusion solution did not affect the fPSP slope upon 20 min of application (aCSFDMSO: −267 ± 46 V/s; dexamethasone: −278 ± 55 V/s; i.e., 103.7% ± 7%, n = 11/6, paired t-test, p = .62; Figure 2B). The average fPSP amplitude also remained stable after dexamethasone (99.9% ± 5.6% of the initial values, n = 11/6, paired t-test, p = .61). Next, we applied TBS to the slices perfused with dexamethasone for 20 min. As in control slices, TBS failed to induce reliable LTP in this setting. The fPSP slopes 30 min after TBS were 99.5% ± 9.1% of the baseline values (n = 12/7, p = .91, paired t-test; Figure 2C). In individual experiments, LTP was induced in three of 12 slices and LTD in five slices, which was similar to the control experiments (Figure 2D; chi-squared test, p = .53).
**FIGURE 2** Dexamethasone (DEX) induces field postsynaptic potential (fPSP) slope depression in an activity-dependent manner. (A) Slopes of fPSPs recorded under control conditions (artificial cerebrospinal fluid [aCSF], \( n_{slices/patients} = 27/14 \)) and in the presence of .01% dimethylsulfoxide (DMSO; \( n_{slices/patients} = 25/10 \)), which was later used as a solvent for DEX and RU486. DMSO did not affect the fPSP slope. Each point represents the average of 10 fPSPs from one slice. (B) Slopes of fPSPs recorded 15–20 min upon perfusion with .5 µmol·L⁻¹ DEX. The average of 10 values is normalized to the average of 10 fPSP slopes recorded just before perfusion with DEX. Each point represents one slice, \( n_{slices/patients} = 11/6 \). (C) Long-term potentiation (LTP) induced by theta-burst stimulation (TBS) upon 20-min perfusion with .5 µmol·L⁻¹ DEX (green trace), compared to control conditions (black trace, aCSF). fPSPs are induced every 30 s, and slope averages are estimated every 5 min. Slope values are normalized to 5-min baseline (before TBS and before DEX perfusion, respectively) averages. As in the control group, there was no significant LTP induction in slices prewashed with DEX (\( p = .91 \), paired t-test, \( n_{slices/patients} = 12/7 \)). Gray window indicates the values used for analysis (25–30 min after TBS). Example fPSPs from a slice perfused with DEX before and 30 min after TBS are shown on top. (D) Quantitative distribution of slices treated with DEX undergoing at least 10% LTP or long-term depression (LTD) and slices with <10% change in the fPSP slope (no effect) 30 min after TBS. (E) LTP maintenance during DEX application 30 min upon TBS stimulation (green trace, green arrow indicates perfusion start, \( n_{slices/patients} = 14/10 \)). The black trace represents a time-matched control (\( n_{slices/patients} = 10/8 \)). Note the significant reduction of fPSP slopes 25–30 min after DEX treatment (green window) compared to values just before DEX perfusion was started (25–30 min after TBS, gray window, \( p = .009 \), Wilcoxon signed-rank test). No such reduction was observed in the control group at the corresponding timepoint (55–60 min after TBS, \( p = .11 \), Wilcoxon signed-rank test). The asterisk (*) indicates the significantly different fPSP slopes in the two groups (aCSF and DEX) 55–60 min after TBS (\( p = .01 \) Kruskal-Wallis-test). Example fPSPs from a slice perfused with DEX before and 30 min after perfusion are shown. (F) LTP maintenance in individual slices between 30 and 60 min after TBS. Left panel: Control slices (aCSF). Right panel: Slices treated with DEX 30 min after TBS. fPSP slope values are normalized to 5-min baseline (before TBS) averages. Each pair represents one slice. Slices with >10% change in fPSP slope between the two time points (30 and 60 min post-TBS) are color-coded in red (>110%) or blue (<90%), whereas slices in which the fPSP slope stayed in the range of ±10% are plotted in black.
To simulate the clinical situation, in which high-frequency stimulation during an epileptic seizure is followed by a stress response (i.e., cortisol release), in a subset of slices we applied dexamethasone 30 min after TBS. In this setting, dexamethasone already significantly reduced the fPSP slope after 30 min of application (86.2% ± 9.4% of the baseline slope vs. 100.3% ± 0.72% 30 min after TBS, n = 14/10; p = .009, WSR). This slope reduction was significantly different from slices without dexamethasone, where the fPSP slope remained stable with a trend of increase during recordings of the same duration (116.1% ± 10.2%, n = 10/8; p = .01, Kruskal–Wallis test). Looking at individual experiments, dexamethasone induced at least 10% reduction of the fPSP slope compared to the values 30 min post-TBS in seven of 14 slices (Figure 2F). In time-matched control slices, we never observed such reduction between 30 min and 60 min after TBS (Figure 2E). There was no correlation between the effect of dexamethasone and patient age (r_s = −.06, p = .87), epilepsy duration (r_s = .3, p = .39), age at epilepsy onset (r_s = −.6, p = .07), or hemisphere-specific memory performance, namely, naming for left TLE (r_s = .5, p = .25, n = 7) and figural memory for right TLE (r_s = −.5, p = .67, n = 3). Nor did the fPSP slopes depend on gender or the resection side (paired t-tests, p > .05, n = 10 patients).

To test whether the effect was specific to the epileptic human tissue, we performed recordings from the perirhinal cortex of naïve mice (Figure 3A). In the mouse perirhinal cortex, TBS successfully induced LTP. The fPSP slope potentiation at 30 min was 153% ± 17% of the baseline response (n = 10 slices from four mice) and remained stable 60 min after TBS (149% ± 16%; Figure 3C). In slices perfused with dexamethasone 30 min after TBS, LTP maintenance was unaffected (at 30 min: 160 ± 24, 60 min: 157% ± 23%; n = 9 slices from four mice; Figure 3C) and did not differ significantly from the control group (two-way repeated measures analysis of variance, p = .02 for LTP induction, p = .97 for drug effect).
3.3 | GR blockage unmaskst LTP in the human cortex

To confirm that the effect of dexamethasone was due to GR activation, we repeated the experiments in the presence of the GR blocker RU486. When 10 µmol·L⁻¹ RU486 was added to the perfusion solution from the beginning, dexamethasone failed to induce LTD after 30 min of application (124% ± 10.7% of the baseline slope vs. 130% ± 14.6% 30 min after TBS, n = 12/6, p = .42, WSR; Figure 4A).

Interestingly, in slices pretreated with RU486, we observed much higher LTP induction than in the control slices, which prompted us to perform further analysis. RU486 (10 µmol·L⁻¹) showed no effect on baseline transmission 20 min after application, as there was no significant change in the fPSP slope upon drug application (aCSF,DMSO: −583 ± 102 V/s, RU486: −645 ± 109 V/s; i.e., 111.8% ± 6.8%, n = 14/6, p = .19, paired t-test; Figure 4B). The fPSP amplitudes were also stable (106.6% ± 7.2% of the initial values, n = 14/6, paired t-test, p = .33; Figure 4B). However, in slices pretreated with the GR blocker, TBS resulted in significant LTP induction (125.7% ± 12.76%, n = 14/6, p = .016, WSR). Analysis of the single experiments revealed LTP induction in eight of 14 slices, whereas LTD was observed in only one slice (Figure 4D). Thus, GR inhibition unmasked successful LTP induction in the slices, suggesting that baseline GR activation in the tissue might be preventing LTP under control conditions.

3.4 | Perioperative cortisol levels are not increased in patients with TLE

Perioperative stress may be associated with acute rise in cortisol values,³⁰ which in turn may activate GRs and thus interfere with synaptic plasticity. To test this hypothesis, we determined cortisol levels in blood samples obtained from the patients during the surgical resection. The cortisol levels were on average 5.9 ± 1.5 µg/dl (n = 18) and were within the expected low normal range for the time of day.³¹ Moreover, the values did not differ from those of patients who had undergone brain surgery for a different pathology without history of epilepsy (5.3 ± 1.2 µg/dl, n = 12, p = .66, MW). Therefore, it seemed unlikely that the GR-dependent LTP modulation was due to acute perioperative stress effects.

4 | DISCUSSION

We demonstrated that LTP was disrupted in the temporal cortex of epilepsy patients and was occasionally replaced by LTD. Synthetic depression was also induced by the GR agonist dexamethasone applied after TBS. By contrast, dexamethasone perfusion before TBS did not additionally aggravate LTP induction, whereas the GR inhibitor RU486 unmasked LTP induction, implying baseline activation of GRs in the tissue. These data, together with normal perioperative cortisol levels, suggest that chronic activation of GRs in the temporal neocortex, such as by recurrent postictal cortisol release, disrupts synaptic plasticity in patients with epilepsy.

Repetitive electrical stimulation can induce LTP or LTD, depending on the stimulus frequency, resulting in different intracellular calcium levels.³² Similar to rodents, in human tissue LTP involves N-methyl-D-aspartate receptor (NMDA-R) activation.²²,²³ Because of the voltage-dependent Mg²⁺ block of the NMDA-R at resting membrane potential, only prolonged depolarization or precisely timed presynaptic/postsynaptic depolarization will activate NMDA-Rs, causing calcium influx into the cell with consecutive activation of downstream kinases and trafficking of AMPA-Rs to the membrane.³⁴ Conversely, more moderate calcium increase by low-frequency stimulation seems to activate predominantly LTD-driving phosphatases.³² The paradoxical LTD induction in our experiments may be due to decreased calcium trafficking through the NMDA-R. LTP was successfully induced in temporal cortex by others using either lower Mg²⁺ concentrations in the perfusion solution²⁵ or parallel pre-/postsynaptic stimulation,³⁵,³⁶ thus bypassing the depolarization required to remove the Mg²⁺ block.

Alternatively, a mismatch between calcium-sensing kinases and phosphatases may cause paradoxical LTD. Such mismatch was associated with LTP disruption in chronically stressed rats,³⁷ and we also observed stimulation-dependent LTD induction by dexamethasone, a GR agonist that mimics the effects of stress on synaptic plasticity. Chronic stress and glucocorticoid exposure have been repeatedly associated with poor LTP induction.¹⁹,²⁰,³⁷,³⁸ Besides structural reorganization of dendritic spine length and density,³⁹ GR activation may cause altered expression of AMPA-Rs⁴⁰ or increased inhibitory drive.⁴¹ In our study, application of dexamethasone showed no effect on baseline transmission or LTP induction. However, LTP was already impaired in the human cortex. Furthermore, GR blockage by RU486 recovered LTP induction, indicating that activated GRs in the epileptic cortex might be preventing LTP. Because perioperative cortisol levels of the patients were not increased, chronic GR activation seemed likely. Non-GR effects of RU486 include inhibition of progesterone receptors⁴² and neuroprotective antioxidant properties.⁴³ However, in our study RU486 also omitted activity-dependent synaptic depression by dexamethasone, which is in accordance with a GR-dependent
effect on synaptic plasticity. Furthermore, baseline GR activation would explain why addition of dexamethasone before TBS did not affect LTP induction.

The idea of chronic GR activation in epilepsy is not new. Increased cortisol levels in epilepsy have been repeatedly reported. A review of 38 studies on basal and
postictal cortisol levels in epilepsy patients concluded that epilepsy can be considered a model of chronic stress. In rats, concomitant effects of a single stress stimulus and chronic epilepsy have also been described as resulting in permanent cortisol level increase, distorted paired-pulse facilitation, and LTP disruption. Moreover, increased GR expression and brain-derived neurotrophic factor (BDNF)-dependent increase in GR occupancy in the epileptic brain may promote baseline GR activation. 

Apart from chronic stress, an interaction between antiepileptic drugs (AEDs) and GRs should be considered, especially because adrenocorticotropic hormone (ACTH) secretion depends on the release of excitatory amino acids, a common target of AEDs. Thus, lamotrigine stabilizes ACTH/cortisol secretion and felbamate was found to inhibit stress-induced corticosterone release in mice. In addition, carbamazepine and valproate are known to affect GR expression. To challenge the relationship between GR modulation, AEDs, and memory performance, a much bigger patient cohort would be necessary.

In addition to AEDs, the influence of other clinical factors might explain the highly heterogeneous LTP in the patients. Although we did not observe any significant correlation between clinical parameters and LTP levels, results need to be confirmed in a larger patient cohort. Thus, sex differences have been reported both for synaptic plasticity and for stress effects. Sex hormones alter synaptic spine density and progesterone and testosterone can inhibit LTP, whereas estrogen can induce LTP. In women, heterogeneity in synaptic plasticity may therefore depend on cyclic hormonal fluctuations. In addition, deficits in BDNF signaling in response to stress can be sex-dependent.

Although dexamethasone treatment prior to TBS did not affect the already impaired LTP induction, we observed fPSP slope depression by dexamethasone when the drug was applied after TBS, implying involvement of a stimulus-activated agent. The exact mechanism remains to be revealed; however, possible candidates are calcium-sensing proteins, such as calcium-calmodulin kinase, protein kinase C (PKC), and the LTD-driving phosphatase calcineurin. Fast nongenomic GR activation causes a PKC-dependent increase of γ-aminobutyric acid type A synaptic currents and anticonvulsive effects in epileptic mice. Our results indicate that besides chronically impairing synaptic plasticity, cortisol increase, when precisely timed upon repetitive stimulation may interfere with LTP maintenance. This is unusual, because acute stress or GR agonists are predominantly associated with enhancing effects on synaptic plasticity and excitability. Nor could we reproduce the inhibitory action of dexamethasone in the cortex of naïve mice within 30 min of application. Therefore, other than slow GR-mediated effects observed by others in animal models, the rapid inhibitory effect in the human cortex is likely a consequence of epileptic reorganization of the tissue. Multiple structural and functional alterations take place in chronic epilepsy. Some of these, such as increased GR expression and sensitivity, are overtly related to GR signaling, whereas others, such as network reorganization, altered inhibitory drive, and changes in glutamate and potassium homeostasis, may affect GR signaling indirectly. Furthermore, chronic inflammation is a hallmark of epileptogenesis, and dexamethasone as a potent anti-inflammatory agent may reduce hyperexcitability by inhibiting the transcription of proexcitatory cytokines.

Beyond the possible impact on LTP and memory, a clinical significance of the inhibitory dexamethasone action emerges from the finding that cortisol levels rise upon seizures, with a peak at 30 min following seizure onset. Increased cortisol may affect synaptic plasticity in a similar manner as application of dexamethasone after high-frequency stimulation. Provided that repetitive neuronal firing during seizures leads to pathologic entrainment of the epileptic circuit, LTD induction via GRs may counteract this pathologic plasticity. In the epileptic brain, compensational mechanisms known as homeostatic plasticity commonly provide resilience toward hyperexcitability. Thus, LTD induced by postictal cortisol release may represent a putative mechanism for postictal synaptic down-scaling and homeostatic plasticity.

In summary, we propose that LTP induction is impaired in the chronically epileptic human cortex in a GR-dependent manner, which might involve chronic baseline activation of GRs due to disease-associated stress, recurrent seizures, or AEDs. Although chronic GR activation is likely to be associated with impaired memory, synaptic depression via postictal cortisol peaks may represent a useful neuroprotective mechanism against pathologic plasticity.

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CONFLICT OF INTEREST
None of the authors has any conflict of interest to disclose. We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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