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A single high dose of dexamethasone affects the phosphorylation state of glutamate AMPA receptors in the human limbic system

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Glucocorticoids (GC) released during stress response exert feedforward effects in the whole brain, but particularly in the limbic circuits that modulate cognition, emotion and behavior. GC are the most commonly prescribed anti-inflammatory and immunosuppressant medication worldwide and pharmacological GC treatment has been paralleled by the high incidence of acute and chronic neuropsychiatric side effects, which reinforces the brain sensitivity for GC. Synapses can be bi-directionally modifiable via potentiation (long-term potentiation, LTP) or depotentiation (long-term depression, LTD) of synaptic transmission efficacy, and the phosphorylation state of Ser831 and Ser845 sites, in the GluA1 subunit of the glutamate AMPA receptors, are a critical event for these synaptic neuroplasticity events. Through a quasi-randomized controlled study, we show that a single high dexamethasone dose significantly reduces in a dose-dependent manner the levels of GluA1-Ser831 phosphorylation in the amygdala resected during surgery for temporal lobe epilepsy. This is the first report demonstrating GC effects on key markers of synaptic neuroplasticity in the human limbic system. The results contribute to understanding how GC affects the human brain under physiologic and pharmacologic conditions.

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INTRODUCTION

Stress responses initiated by environmental threats promote autonomic, endocrine, and behavioral changes that help self-preservation. The prefrontal cortex (PFC), amygdala (AMY) and hippocampus (HIP) are the key brain structures of the feedforward and feedback networks that mediate states of stress and fear. Glucocorticoids (GC) released during stress responses have feedforward effects in the whole brain, with a particular importance in limbic structures. During non-stress conditions, the PFC exerts top–down regulation of limbic structures including the AMY, but in acute stress bottom–up processes prevail and behavior changes from slower, highly flexible responses to faster, stereotyped reaction. Stress can be helpful or harmful depending on its intensity, duration and personal features. In predisposed individuals acute and intense stress has been associated with post-traumatic stress disorder, and chronic and repetitive stress with depression, mania and anxiety. Dependence on the applied stimulation frequency, active synapses are bi-directionally modifiable in mammalian brain regions such as the AMY, HIP and neocortex. The long-lasting increase of synaptic transmission, called long-term potentiation (LTP), is induced by high-frequency neuronal stimulation. Decreases in synaptic efficacy are also needed to reset the synapses, and are accounted for by low-term depression (LTD), after low-frequency stimulation. Pharmacological evidence ‘in vivo’ suggests an association between LTP and one-trial inhibitory avoidance, a fear associative memory task that induces LTP in the HIP. The fear conditioning, another fear associative memory task, can be inactivated and reactivated by LTD and LTP in the AMY.
supporting a causal link between these synaptic processes and memory. Memory consolidation for both these tasks also can be modulated by GC.

AMPAs (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) receptors (AMPARs) are heterotetrameric assemblies of GluA1–GluA4 subunits, usually permeable to Na⁺ and K⁺. Expression of Ca²⁺-permeable AMPARs, lacking GluA2 subunits (that is, GluA1 homomers) exist especially in the extrasynaptic and intracellular locations but can be recruited to synapses during neuroplasticity. Phosphorylation and dephosphorylation states of distinct sites of the GluA1 subunit of the AMPAR regulates the channel conductance and GluA1 synaptic membrane insertion is involved in the LTP and LTD induction. Two major sites of GluA1 phosphorylation of AMPAR are studied regarding their role in neuroplasticity: (1) the Ser831, phosphorylated by Ca²⁺/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC), which increases the channel conductance; (2) the Ser845, phosphorylated by cAMP-dependent protein kinase (PKA) that affects the open-channel probability of the receptor and phosphorylated by cAMP-dependent protein kinase II (CaMKII) and protein kinase C (PKC). A study has revealed that LTD induction dephosphorylates Ser845, although in potentiated synapses the Ser831 is dephosphorylated by LTD induction. Conversely, LTD induction in naive synapses increases phosphorylation of GluA1-Ser831, whereas in depressed synapses phosphorylation of GluA1-Ser845 was enhanced. Serine 845 and 831 are dephosphorylated by low-frequency stimulation (LTD) in a manner dependent of protein phosphatase activity including PP1, PP2A and PP2B. Mice generated with knockin mutations in the GluA1 phosphorylation sites that shows deficits in LTD and LTP in the CA1 region of Hippocampus and has memory deficits in spatial learning tasks. These results demonstrate that phosphorylation of GluA1 is critical for LTD and LTP expression and is involved in memory processing. Therefore, GluA1 phosphorylation of AMPARs sites at Ser831 and Ser845 sites are critical for ‘in vivo’ synaptic plasticity being an emerging focus as a major target for stress and GC in the limbic system.

The GC effects on the phosphorylation state of GluA1-AMP, has never been investigated in the human limbic system. Mesial temporal lobe epilepsy related to hippocampal sclerosis (MTLE-HS) is the most common surgically remediable epileptic syndrome. Because surgery involves a ‘standard’ resection of the neocortical and mesial temporal lobe structures, it offers an ethical opportunity to obtain human samples of AMY and HIP under well-controlled conditions to investigate phosphorylation state of proteins. Here we present a quasi-randomized controlled study showing the effect of a single high dose of dexamethasone (DEXA), on the phosphorylation levels of GluA1-Ser845 and GluA1-Ser831 in the human AMY, head of HIP and middle temporal neocortex gyrus (CX) of surgical patients with drug resistant MTLE-HS.

**Materials and Methods**

Patients

We included 31 adult drug-resistant MTLE-HS patients treated surgically between May 2009 and December 2012 at the Centro de Epilepsia de Santa Catarina. They participated in a prospective study about synaptic plasticity markers in MTLE-HS. All patients had seizures that impaired awareness at least once a month (mean 7.5 per month), despite adequate treatment with at least two antiepileptic drugs (AEDs) in monotherapy. Their medical history, seizure semiology, neurological examination, psychiatric and neuropsychological evaluation, interictal and ictal surface video-EEG, and magnetic resonance imaging findings were consistent with unilateral MTLE-HS. Controlled clinical variables included gender, race, side of HS, age, disease duration, age of recurrent seizures onset, psychiatric comorbidities and quality of life. The AEDs used were carbamazepine, phenobarbital, diphenylhydantoin, valproic acid, lamotrigine or topiramate, associated or not with benzodiazepines (clazocam or clonazepam). The protocol was approved by the Ethics Committee for Human Research of Universidade Federal de Santa Catarina (365-FR304969). Written informed consent was obtained from all patients.

Anesthesia protocol

The anesthetic protocol, except the DEXA treatment, were the same for all patients. Anesthesia started between 0730 hours to 0830 hours with intravenous (i:v) bolus of propofol (2 mg kg⁻¹), fentanyl (2 μg kg⁻¹) and rocuronium (0.9 mg kg⁻¹), followed by i.v. remifentanil infusion (0.1 to 0.2 μg kg⁻¹ min) and isoflurane inhalation (0.5 to 0.6 M.A.C.). Hydration was done with isotonic saline (1.2 ml kg⁻¹ h⁻¹) plus the half volume of diuresis. Cephaprole (30 mg kg⁻¹) was given 30 min before the anesthesi. Oral AEDs were maintained until the day of surgery (0600 hours). Patients received 20 mg kg⁻¹ of phenytoin i.v. 12 h before the surgery and 5 mg kg⁻¹ i.v. after the brain samples were collected. Patients under phenytoin at home received only their morning oral dose and the intraoperative dose after the brain samples were collected.

Dexamethasone treatment and study design

After the first 11 patients were included in the study, the anesthesiology team decided to use DEXA (10 mg i.v. bolus) immediately after intubation as an adjuvantive anti-inflammatory and anti-emetic therapy. The DEXA infusion was not based on any clinical data. This change in the anesthesia protocol gave us the opportunity to design this quasi-randomized controlled study. The DEXA dose was calculated dividing 10 mg by the patient weight. The mean (s.e.) DEXA dose was 0.1575 (0.006) mg kg⁻¹ (range 0.11 to 0.2). Although this selective GR agonist differs from endogenous cortisol in many aspects of its transcriptional activity, this dose results in at least 27 times the effect of a daily adult human secretion of cortisol.

Surgery, intraoperative variables and brain tissue sampling

The surgeries and tissue sampling were done by the same neurosurgeon (MNL) and the principal investigator (RW) as previously described. The samples came from the brain tissue removed during a standard anterior and temporal lobectomy (ATL) procedure and were immediately frozen in liquid nitrogen and stored in −80 °C freezer until the analyses. The sampling course is presented in Supplementary Figure 1. The temporal lobe resection included the middle and inferior temporal gyr extended up to 4 cm posterior from the temporal pole. Prior to the cortical resection, a 1 cm³ sample of the CX localized 3 cm posterior to the temporal pole was gently dissected from the white matter. After assessing the mesial temporal region, 2/3 of the AMY were collected including its basal and lateral nucleus. Both AMY and CX were collected without previous thermoacoagulation. After AMY resection, the HIP was removed ‘en bloc’ and the bone and part of the body were quickly dissected on ice-refrigerated glass. The time of HIP manipulation since the electrocoagulation of its vascular supply start until its resection was controlled. Arterial blood gases, electrolytes, hematocrit, hemoglobin, acid-basic, mean arterial pressure heart and respiratory rate parameters during the AMY/HIP sampling were controlled. The anesthesia duration until each brain sample was controlled as well. The hemodynamic and respiratory parameters remained stable and no surgical complication was reported.

Biochemical analysis

Biochemical analysis was blinded for all clinical data. All samples were homogenized by the same researchers (MWL) in the same day, placed in liquid nitrogen, and storage at −80 °C until the analysis. The quantification of phosphorylation levels and total amount of the target proteins were performed by western blot as we described previously. Protein content was determined by Peterson’s method. Proteins were detected after overnight incubation with specific antibodies diluted in TBS with tween with 2% bovine serum albumin in a 1:10000 dilution (anti- phospho-GluA1-Ser831 (Sigma-Aldrich, St Louis, MO, USA, A4352); anti-phospho-GluA1-Ser845 (Sigma-Aldrich, A4477); anti-total-GluA1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA, sc-13152); anti-EAAT1 (Cell Signaling, Beverly, MA, USA, #5684); anti-EAAT2 (Cell Signaling,#8338); anti-Pp1α (Santa Cruz Biotechnology,s.c-7482); anti-phospho-CaMKII (Cell Signaling, #9321); anti-total-CaMKII (Cell Signaling, #9323); anti-phospho-AKT (Cell Signaling, #3670) and anti-total-AKT (Cell Signaling, #9272); 1:2000 (anti-phospho-ERK1/2 (Sigma-Aldrich, M8159); anti-phospho-AKT (Sigma-Aldrich, P4112); anti-phospho-PKA substrates (Cell Signaling, #9624) and anti-phospho-PKC substrates (Cell Signaling, #2261)); 1:5000 (anti-phospho-JNK p54/46
Table 1. Clinical, demographic, neuroradiological, neurophysiological and surgical variables of patients with mesial temporal lobe epilepsy related to hippocampal sclerosis according to dexamethasone treatment

| Variables                        | All cases, n = 31 | No dexamethasone, n = 11 | Dexamethasone, n = 20 | P-value |
|----------------------------------|-------------------|--------------------------|------------------------|---------|
| **Gender**                       |                   |                          |                        |         |
| Female                           | 18 (58.1)         | 4 (36.4)                 | 14 (70.0)              |         |
| Male                             | 13 (41.9)         | 7 (63.6)                 | 6 (30.0)               | **0.13**|
| **Race**                         |                   |                          |                        |         |
| Caucasian                        | 27 (87.1)         | 9 (81.8)                 | 18 (90.0)              |         |
| Others                           | 04 (12.9)         | 2 (16.2)                 | 2 (10.0)               | **0.60**|
| **Marital status**               |                   |                          |                        |         |
| Single                           | 17 (54.8)         | 8 (72.7)                 | 9 (45.0)               |         |
| Married                          | 10 (32.3)         | 2 (18.2)                 | 8 (40.0)               |         |
| Divorced or widower              | 4 (12.9)          | 1 (9.1)                  | 3 (15.0)               | **0.33**|
| **Current work activity**        |                   |                          |                        |         |
| Working                          | 11 (35.5)         | 4 (36.4)                 | 7 (35.0)               |         |
| Health insurance                 | 4 (12.9)          | 7 (63.6)                 | 9 (45.0)               |         |
| Not working                      | 16 (51.6)         | 0 (0.0)                  | 4 (20.0)               | **0.26**|
| **History of initial precipitant injury** |           |                          |                        |         |
| No                               | 7 (22.6)          | 3 (27.3)                 | 4 (20.0)               |         |
| Yes                              | 24 (77.4)         | 8 (72.7)                 | 16 (80.0)              | **0.68**|
| **Family history of epilepsy**   |                   |                          |                        |         |
| No                               | 12 (38.7)         | 4 (36.4)                 | 8 (40.0)               |         |
| Second-degree or distant         | 10 (32.3)         | 2 (18.2)                 | 8 (40.0)               |         |
| First- degree                    | 6 (19.4)          | 3 (27.3)                 | 3 (15.0)               |         |
| Unknown                          | 3 (9.7)           | 2 (18.2)                 | 1 (5.9)                | **0.41**|
| **MRI hippocampal sclerosis**    |                   |                          |                        |         |
| Right side                       | 16 (51.6)         | 4 (36.4)                 | 12 (60.0)              |         |
| Left side                        | 15 (48.4)         | 7 (63.6)                 | 8 (40.0)               | **0.27**|
| **Antiepileptic drugs regimen**  |                   |                          |                        |         |
| Monotherapy                      | 9 (29.0)          | 6 (54.5)                 | 3 (15.0)               | **0.04**|
| Two or more drugs                | 23 (71.0)         | 5 (45.5)                 | 17 (85.0)              |         |
| **Benzodiazepines**              |                   |                          |                        |         |
| Yes                              | 15 (48.4)         | 7 (63.6)                 | 9 (45.0)               |         |
| No                               | 16 (51.6)         | 4 (36.4)                 | 11 (55.0)              | **0.46**|
| **Antiepileptic drugs**          |                   |                          |                        |         |
| Carbamazepine                    |                   |                          |                        |         |
| No                               | 6 (19.4)          | 4 (36.4)                 | 2 (10.0)               |         |
| Yes                              | 29 (80.6)         | 7 (63.3)                 | 18 (90.0)              | **0.16**|
| Phenobarbital                    |                   |                          |                        |         |
| No                               | 19 (61.3)         | 9 (81.2)                 | 10 (50.0)              |         |
| Yes                              | 12 (38.7)         | 2 (18.2)                 | 10 (50.0)              | **0.13**|
| Diphenilhydantoin                |                   |                          |                        |         |
| No                               | 28 (90.3)         | 11 (100.0)               | 17 (85.0)              |         |
| Yes                              | 3 (9.7)           | 0 (0.0)                  | 03 (15.0)              | **0.54**|
| Valproic acid                    |                   |                          |                        |         |
| No                               | 29 (93.5)         | 9 (81.8)                 | 18 (90.0)              |         |
| Yes                              | 4 (12.9)          | 2 (18.2)                 | 2 (10.0)               | **0.60**|
| Lamotrigin                       |                   |                          |                        |         |
| No                               | 27 (87.1)         | 9 (81.8)                 | 18 (90.0)              |         |
| Yes                              | 4 (12.9)          | 2 (18.2)                 | 2 (10.0)               | **0.60**|
| Topiramate                       |                   |                          |                        |         |
| No                               | 29 (93.5)         | 11 (100.0)               | 18 (90.0)              |         |
| Yes                              | 2 (6.5)           | 0 (0.0)                  | 2 (10.0)               | **0.53**|
| **Hand dominance**               |                   |                          |                        |         |
| Right                            | 27 (87.1)         | 9 (81.8)                 | 18 (90.0)              |         |
| Non-right                        | 4 (12.9)          | 2 (18.2)                 | 2 (10.0)               | **0.60**|
| **Psychiatric comorbidities**    |                   |                          |                        |         |
| No diagnosis                     | 14 (45.2)         | 4 (36.3)                 | 10 (50.0)              |         |
| Depressive disorder              | 8 (25.8)          | 2 (18.2)                 | 6 (30.0)               |         |
| Anxiety disorder                 | 3 (9.7)           | 2 (18.2)                 | 1 (5.0)                |         |
| Other psychiatric conditionsc    | 6 (19.4)          | 3 (27.3)                 | 3 (15.0)               | **0.48**|
Table 1. (Continued)

| Variables                  | All cases, n = 31 | No dexamethasone, n = 11 | Dexamethasone, n = 20 | P-value |
|----------------------------|-------------------|---------------------------|-----------------------|---------|
| **Mean (s.e.)**            |                   |                           |                       |         |
| Age (years)                | 36.4 (2.2)        | 34.6 (3.8)                | 37.3 (2.7)            | 0.57    |
| Education (years)          | 6.6 (0.5)         | 6 (1.0)                   | 6.9 (0.6)             | 0.43    |
| Disease duration (years)   | 24.3 (2.0)        | 25.8 (3.1)                | 23.5 (2.6)            | 0.60    |
| Monthly seizure frequency  | 7.5 (0.9)         | 4.9 (0.9)                 | 8.5 (1.1)             | 0.04    |
| QOLIE-31                   | 35.2 (2.7)        | 38.2 (3.8)                | 33.5 (3.7)            | 0.43    |
| **Intraoperative parameters** |                  |                           |                       |         |
| Mean arterial pressure     | 67.5 (1.7)        | 64 (3.0)                  | 68.8 (2.1)            | 0.30    |
| Heart rate                 | 73.7 (2.2)        | 70 (4.1)                  | 75 (2.7)              | 0.30    |
| Respiratory frequency      | 11.6 (0.3)        | 11.7 (0.5)                | 11.5 (0.4)            | 0.88    |
| **Biochemical analysis of the blood** |                  |                           |                       |         |
| pH                         | 7.41 (0.07)       | 7.41 (0.01)               | 7.42 (0.01)           | 0.66    |
| PCO₂                        | 28 (0.8)          | 29.6 (1.5)                | 28.1 (0.9)            | 0.35    |
| HCO₃                        | 20.0 (0.3)        | 20.7 (0.6)                | 19.6 (0.3)            | 0.20    |
| PO₂                         | 229 (11.0)        | 214.6 (25.1)              | 237.8 (10.3)          | 0.32    |
| O₂ saturation              | 99.7 (0.04)       | 99.6 (0.08)               | 99.7 (0.05)           | 0.90    |
| Hematocrit                 | 35.0 (0.7)        | 33.9 (1.4)                | 35.6 (0.8)            | 0.27    |
| Hemoglobin                 | 12.5 (0.9)        | 14.9 (2.9)                | 11.7 (0.3)            | 0.26    |
| Glucose                    | 116.3 (4.9)       | 103.4 (6.4)               | 121 (6.0)             | 0.10    |
| Sodium                     | 138.2 (3.9)       | 136.9 (1.2)               | 139 (1.7)             | 0.20    |
| Potassium                  | 4.1 (0.1)         | 4.2 (0.2)                 | 4.2 (0.1)             | 0.63    |
| Ionic calcium              | 4.2 (0.1)         | 4.1 (0.1)                 | 4.2 (0.2)             | 0.95    |
| Magnesium                  | 0.4 (0.08)        | 0.4 (0.02)                | 0.5 (0.01)            | 0.15    |
| Lactic acid                | 2.1 (0.2)         | 1.7 (0.3)                 | 2.3 (0.3)             | 0.22    |
| Storage time of samples (months) | 24 (1.5) | 28.2 (2.9) | 21.5 (1.7) | 0.05 |
| Time since last seizure (hours) | 225 (82) | 200 (63) | 239 (122) | 0.08 |
| Time for CX sampling (min) | 188.3 (7.1) | 184.8 (10.3) | 190 (9.7) | 0.71 |
| Time for AMY/HIP sampling (min) | 260.1 (10.0) | 254.6 (16.9) | 262.8 (12.8) | 0.78 |
| Time of HIF manipulation (min) | 11.2 (0.9) | 11.7 (1.3) | 11.1 (1.2) | 0.70 |

**Abbreviations:** AMY, amygdala; CX, middle temporal neocortex gyrus; HIP, hippocampus; MRI, magnetic resonance imaging; QOLIE-31, Quality of Life in Epilepsy Inventory-31. 1Time course since brain tissue sampling and the homogenization for neurochemical evaluation (range 8 to 39 months). 2Anxiety disorders: generalized anxiety disorder (two patients in the non-dexamethasone group), social phobia (one patient in non-DEX group). 3Other psychiatric conditions: three patients with dysphoric disorder of epilepsy (one in the non-DEXA group), two patients with postictal psychosis (in the non-DEXA group), one patient with postictal anxiety (in the DEXA group). 4Time course since the last seizure attack occurrence and brain tissue sampling (range 12 to 590 h). 5Time course since anesthesia induction until CX tissue sampling (range 119 to 255 min). 6Time course since anesthesia induction until AMY/HIP tissue sampling (range 160 to 360 min). 7Time course since HIF vessels thermo-coagulation started until the complete resection of the HIP (range 5 to 25 min). Variables showing a P level of significance < 0.15 are in bold.

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Supplementary Figure 2b). This analysis confirmed the treatment-dependent decrease of P-GluA1-Ser831 levels in the AMY by DEXA also was performed only with patients who received DEXA (P = 0.0002). Because the non-normal distribution of DEXA dose was not affected by the order of surgery (P = 0.79), reducing the possibility of non-identified confounders related to tissue sampling of non-DEXA group (data not shown).

To exclude confounding biases resulting from imbalances in the distribution of gender, AEDs regimen, phenobarbital use, frequency of seizures, intraoperative serum glucose and storage time of samples, the association between the AMY levels of P-GluA1-Ser831 and these variables were analyzed together with DEXA treatment. There were trends for association between AMY levels of P-GluA1-Ser831 and gender (P = 0.02) and serum glucose (P = 0.002). The observed association was not affected by the order of surgery (P = 0.79), reducing the possibility of non-identified confounders related to tissue sampling of non-DEXA group (data not shown).

The Supplementary Figure 2a shows a dose-dependent effect of DEXA on P-GluA1-Ser831 levels in the AMY (r = 0.69; r² = 0.48; P = 0.0002). Because the non-normal distribution of DEXA dose was not affected by the order of surgery (P = 0.79), reducing the possibility of non-identified confounders related to tissue sampling of non-DEXA group (data not shown).

| Variables | All cases, n = 31, mean (s.e.) | No dexamethasone, n = 11, mean (s.e.) | Dexamethasone, n = 20, Mean (s.e.) | P-value |
|-----------|-------------------------------|--------------------------------------|----------------------------------|---------|
| Middle temporal neocortex | | | | |
| P-GluA1-Ser831 | 118.6 (3.2) | 126.9 (6.3) | 114.3 (3.2) | 0.06a |
| P-GluA1-Ser845 | 112.4 (3.0) | 103.1 (4.1) | 117.3 (3.6) | 0.02b |
| Amygdala | | | | |
| P-GluA1-Ser831 | 109.3 (3.0) | 122.8 (4.6) | 101.9 (2.8) | 0.0003c |
| P-GluA1-Ser845 | 108.1 (3.3) | 108.2 (6.0) | 108.0 (4.1) | 0.98 |
| Hippocampus | | | | |
| P-GluA1-Ser831 | 97.0 (3.7) | 105.2 (7.2) | 92.5 (4.0) | 0.10d |
| P-GluA1-Ser845 | 104.2 (3.6) | 109.5 (7.8) | 106.3 (3.7) | 0.46 |

*Non-significant trend of 12.6% decrease in the neocortex levels of P-GluA1-Ser831; **Non-significant trend of 14.2% decrease in the neocortex levels of P-GluA1-Ser845; "Non-significant decrease of 20.9% in the amygdala levels of P-GluA1-Ser831; "Non-significant trend of 12.7% decrease in the hippocampus levels of P-GluA1-Ser831.

DISCUSSION

To the best of our knowledge, this is the first report showing the GC effects on synaptic neuroplasticity biomarkers in the human limbic system. We show that 4 h after a high dose of DEXA the AMY levels of P-GluA1-Ser831, but not P-GluA1-Ser845, decrease significantly in MTLE-HS patients. These findings indicate that DEXA treatment shifts the serine 831 residue of the GluA1-AMPAR to a dephosphorylated state in the AMY. Notably, in the same structure we also observed a reduction in the levels of P-CaMKII (Thr286), indicating a reduction in the autonomous activity of CaMKII activity.32,58,59 DEXA did not affect the PKC activity or PP1ca levels in AMY.

The effects could not be attributed to imbalances in the distribution of demographic, clinical, radiological, intraoperative and neurochemical variables of our patients. A trend for lower levels of P-GluA1-Ser831 was also observed in the HIP and CX, but the possibility of a false negative result related to the small sample size cannot be completely ruled out.

The AMY is particularly sensitive to rapid responses to GC both in animals60,61 and in man.62 Furthermore, in an ex vivo rodent study, using lower concentrations of exogenous GC than in the current study, we have shown that both a stressor or the administration of exogenous GC affects both the physiology of AMPARs and neuroplasticity.63 Brief restraint or DEXA administration to rats increases the surface expression of GluA1 (but not the GluA2 subunit) and the magnitude of electrically induced LTP in the HIP. Furthermore, 60 min after the restraint stress or slice incubation with corticosterone or DEXA for 30 min the serine 845 residue phosphorylation levels of the of GluA1 subunit increases in the HIP. The effect is dependent on GC receptors and PKA, but independent of NMDARs.40 Corticosterone increases GluA2-AMPAR surface mobility in a time-dependent manner (peak in 15 min), thereby conditioning the extent to which chemical LTP stimuli effectively increase GluA2 synaptic content during synaptic potentiation in the HIP.39 In addition, corticosterone also increases GluA1-AMPAR surface trafficking.39 The phosphorylation state of Ser831 and Ser845 of GluA1-AMPAR was variable according to different studies, depending on the brain region, stress type applied or duration and sampling time after stress.45

Karst et al.64 described that the exposure to two pulses of corticosterone (10 to 20 min pulse duration with 1 to 3 h pulse interval) enhances the miniature excitatory postsynaptic currents (mEPSC) frequency in CA1 pyramidal cells of mice. By contrast, basolateral amygdala (BLA) neurons responded to the first pulse with increased mEPSC frequency, but with a decreased mEPSC frequency to a second pulse. Furthermore, in BLA cells from mice exposed to restraint stress before slice preparation, corticosterone...
Table 3. Variables associated with amygdala levels of phospho-GluA1-Ser831

| Variables                        | Amygdala levels of phospho-GluA1-Ser831 | P-value |
|----------------------------------|----------------------------------------|---------|
|                                  | Mean (s.e.)                             |         |
| Gender                           |                                        |         |
| Female                           | 103 (3.6)                               |         |
| Male                             | 117.4 (4.5)                             | 0.02a   |
| AEDs regimen                     |                                        |         |
| Monotherapy                      | 114 (5.0)                               |         |
| Two AEDs or more                 | 109 (3.9)                               | 0.51a   |
| Phenobarbital treatment          |                                        |         |
| No                               | 111.7 (3.8)                             |         |
| Yes                              | 105.4 (4.9)                             | 0.32a   |

**Univariate linear regressions**

| Monthly seizure frequency       | −0.71                                  | 0.21    | 0.05    | 0.26b |
| Serum glucose                   | −0.19                                  | 0.32    | 0.10    | 0.12b |
| Storage time of samples (months) | 0.36                                   | 0.19    | 0.04    | 0.30b |
| Dexamethasone dose (mg kg⁻¹)    | −145.6                                 | 0.69    | 0.48    | 0.00002b |

**Multiple linear regression**

| B     | r    | r²   | P-value |
|-------|------|------|---------|
| 134.8 | < 0.00001 |      |         |
| Female| −0.90| 0.85 |         |
| Serum glucose| −0.10  | 0.33 |         |
| Dexamethasone dose (mg kg⁻¹) | −131.1 | 0.73 | 0.001c |

Abbreviation: AEDs, antiepileptic drugs. aUnivariate analysis by Student’s t-test; bUnivariate analysis by linear regression; cLevel of significance for the multiple linear regression model.

raptulately decreased mEPSC frequency, an effect that is dependent on the non-genomic activation of mineralocorticoid receptors. Although cross-species implications can be problematical, it is certainly worth pointing out that our subjects would undoubtedly have had some degree of pre-surgical hospitalization stress prior to receiving DEXA with subsequent reduction of the AMY levels of P-GluA1-Ser831. This is a neurochemical marker which has been associated with the synaptic depotentiation to the naïve state from a potentiated state. Interestingly, compatible with a rapid non-transcriptional effect, Lovallo et al. showed a reduction of the BOLD signals in both the AMY and HIP 15–18 min after the injection of a small hydrocortisone dose. The relationship between this GC-related effect on BOLD signal and the synaptic potentiation or AMPA phosphorylation state in the limbic system is unknown. The AMY is a central structure in the processing of emotional components of memory, coding social and biological meanings of events. Clinical findings suggest that the AMY is necessary for modulating negative and positive arousing stimuli during encoding, indicating its involvement in processing biologically relevant stimuli independently of their valence. These hypotheses received support by recent findings showing the BLA is a site of divergence for circuits mediating positive and negative emotional or motivational valence. The role of GC on the synaptic plasticity of neurons participating in the positive and negative valence-circuits deserve further investigation.

The results help not only to understand the mechanisms involved in the human brain modulation by the stress hormones, but also the common side effects related to a worldwide frequently prescribed class of pharmaceuticals. The DEXA dose used in our patients (or an equipotent dose of other GC) is commonly used in clinical practice. A large study examining the effects of oral GC treatment (n = 786 868 courses) showed an overall incidence of 15.7 per 100 person-years at risk of adverse neuropsychiatric outcomes, and 22.2 per 100 person-years at risk for patients on their first course of GC. The outcomes included depression, delirium, mania, panic disorder and suicidal behavior. Considering the critical role of the GluA1 phosphorylation at Ser831 and Ser845 sites for in vivo synaptic plasticity, we believe the observed effect of DEXA in the limbic system of our patients may be, at least in part, related to the high incidence of adverse neuropsychiatric side-effects during GC treatments. This hypothesis is in agreement with a previous study showing the association between the GRIA1 gene, that encode the GluR1 AMPAR, and psychiatric disorders. A combined linkage analysis of 60 families from National Institute of Mental Health Bipolar Genetics Initiative (NIMH-BPGI) suggested an association between a SNP in the second intron on the GRIA1 gene and psychotic bipolar disorder. A case–control study showed that two specific polymorphisms for the GRA1 were associated with schizophrenia in Italians. Recently the role of the dually phosphorylated GluR1 AMPAR at S831 and S845 on synaptic plasticity was questioned by Hosokawa et al. Using Phos-tag SDS-PAGE they found the majority of synapses did not contain any phosphorylated AMPAR and the amount of phosphorylated GluA1 was very low. Although the neuronal stimulation (chemical LTP and learning (inhibitory avoidance) increased phosphorylation, the proportion also was still low. In contrast, Diering et al. using a variety of measurement methods, showed a large fraction of synapses positive for phosphorylated GluA1-containing AMPARs, were highly responsive to numerous physiologically relevant ‘in vivo’ and ‘in vitro’ stimuli. Their results support the large body of research indicating a prominent role of GluA1 phosphorylation in synaptic plasticity. This controversy has no implications for the analysis of our results because we demonstrated significant changes in the percentage of the GluA1 subunit phosphorylation that was
To summarize, a single high dose of i.v. DEXA reduces significantly the levels of P-CaMKII and P-GluA1Ser831 in the AMY of MTLE-HS patients in a dose-dependent manner. These effects on the signal transduction molecules and synaptic neuroplasticity in the limbic system contribute to a better understanding the GC effects in the human brain under physiologic and pharmacologic conditions.

CONFLICT OF INTEREST
The authors declare no conflict of interest.

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