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# Elevated plasma corticosterone level and depressive behavior in experimental temporal lobe epilepsy

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## Abstract

Depression is frequently reported in epilepsy patients; however, mechanisms of co-morbidity between epilepsy and depression are poorly understood. An important mechanism of depression is disinhibition within the hypothalamo-pituitary-adrenocortical (HPA) axis. We examined the functional state of the HPA axis in a rat model of co-morbidity between temporal lobe epilepsy and depression. Epilepsy was accompanied by the interictal elevation of plasma corticosterone, and by the positive combined dexamethasone/corticotropin releasing hormone test. The extent of the HPA hyperactivity was independent of recurrent seizures, but positively correlated with the severity of depressive behavior. We suggest that the observed hyperactivity of the HPA axis may underlie co-morbidity between epilepsy and depression.

### Keywords

Epilepsy; depression; co-morbidity; hypothalamo-pituitary-adrenocortical axis; chronic stress

## Introduction

Depression is one of the most common co-morbidities of epilepsy, including temporal lobe epilepsy (TLE), and has a high negative impact on the quality of life in epilepsy patients (Hermann, et al., 2000; Kanner and Balabanov, 2002; Kondziella, et al., 2007). While psychosocial factors may contribute to depression in epilepsy, there is growing evidence that this condition has a neurobiological basis (Kanner, 2005; Kondziella, et al., 2007). Nevertheless, mechanisms that underlie depression in epilepsy are poorly understood, and its effective therapies are lacking.

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Validation of animal models of co-morbidity between TLE and depression is instrumental for understanding mechanisms of this condition and for the development of its rational therapies. In the pursuit of such an animal model, we previously established that LiCl and pilocarpine status epilepticus (SE) in rats, along with chronic epilepsy, led to behavioral (despair and anhedonia) and biochemical (deficit of the raphe-hippocampal serotonergic transmission) hallmarks of depression (Mazarati, et al., 2008). However, since depression in epilepsy is a multifactorial disorder (Kanner et al., 2003; Kondziella et al., 2007), the model validation process should include the identification of other contributing physiological, biochemical and endocrine factors.

One core feature of major depression is chronic stress (Chaouloff, 2000; El Yacoubi et al., 2003; Yu, et al., 2008) which is manifested as the paucity of the negative feedback inhibition within the hypothalamo-pituitary-adrenocortical (HPA) axis: under conditions of chronic stress, cortisol fails to inhibit the production of corticotropin releasing hormone (CRH) and of adrenocorticotropic hormone (ACTH), thus resulting in a high sustained level of circulating cortisol (Herman and Cullinan, 1997). The laboratory diagnosis of depression involves the dexamethasone (DEX)/CRH test, which is purposed to reveal the described HPA dysregulation: the test is considered positive, that is diagnostic of depression, if DEX fails to decrease the level of plasma cortisol, and if exogenously administered CRH leads to the exacerbated increase of the plasma stress hormone (Watson, et al., 2006).

While the described dysregulation of the HPA axis has been established in major depression, the role of this neuroendocrine impairment in depression associated with epilepsy has not been sufficiently examined. Nevertheless, several reports suggested that epilepsy may be indeed accompanied by chronic, interictal hyperactivity of the HPA axis evident as the increased content of plasma cortisol (Gallagher, 1987) and positive DEX/CRH test (Zobel et al., 2004); the latter study suggested that such chronic HPA dysfunction may be a factor contributing to depression in TLE.

In animal epilepsy models, several reports showed ictal or early postictal HPA hyperactivity (Daniels, et al., 1990; Lai, et al., 2006; Szafarczyk, et al., 1986); however, the interictal state of the HPA axis has not been examined, and thus it is not known whether chronic experimental epilepsy is accompanied by the depressive endocrine abnormalities.

The present study continues our efforts in the validating an animal model of co-morbidity between TLE and depression and focuses on the establishing of endocrine hallmark of depression in the post-SE model. Specifically, we examined whether chronic epilepsy in rats is accompanied by the interictal hyperactivity of the HPA axis, and if it does, whether the extent of such dysregulation correlates with the severity of depressive behavior.

## Materials and methods

### Experimental subjects

The experiments were performed in male Wistar rats (Charles River, Wilmington, MA), 45-55 days old at the beginning of the study, in accordance with the policies of the National Institutes of Health.

Study design is outlined in Fig. 1.

### Status epilepticus and spontaneous seizure monitoring

SE was induced by LiCl (128 mg/kg, dissolved in deionized water, injected intraperitoneally, i.p. at a volume of 1 ml/kg) and pilocarpine (40 mg/kg, dissolved in 0.9% NaCl, injected subcutaneously, s.c. at a volume of 1 ml/kg; both from Sigma, St. Louis, MO) (Mazarati, et

al., 2008). Only those animals, which had exhibited continuous generalized clonic-tonic seizures lasted for 2 hours or more, were used for further studies. To alleviate seizure severity, 2 and 6 hours after SE onset, animals received i.p. injections of diazepam (10 mg/kg, diluted in 0.9%NaCl, injected at a volume of 2 ml/kg) and phenytoin (50 mg/kg, dissolved in 0.1M NaOH, injected at a volume of 1 ml/kg) (Mazarati, et al., 2008). Control animals were injected with LiCl, saline instead of pilocarpine, and the antiepileptic drugs. Four weeks after SE, animals underwent ten-week long video monitoring for detecting spontaneous seizures. Two seizure types were considered: focal seizures (motor arrest, facial twitches and mastication), and generalized clonic or clonic-tonic seizures (all body clonus, rearing or rearing and falling) (Cavalheiro, et al., 2006). For detecting spontaneous seizures, we only relied on video monitoring, in order to avoid likely effects of surgical implantation of recording electrodes on the functional state of the HPA axis. Throughout the experiments, animals' weight was checked once a week; the weight gain dynamics was statistically similar between control and post-SE rats (Table 1).

### Corticosterone (CORT) radioimmunoassay (RIA)

Basal concentration of plasma CORT (a major glucocorticoid in rodents, (Steimer, et al., 2007) was measured in plasma samples obtained 3-7 days before, and 6-8 weeks after SE. (It should be noted that since the level of circulating glucocorticoids depends significantly on a variety of external and internal stimuli, in the context of the present paper "basal" refers merely to the plasma CORT content prior to the DEX/CRH test). To attenuate effects of handling and of restraint on CORT level, the animals had been handled for 3 days prior to blood collection (animals were removed from their cages and were held in hands for 1 minute twice a day), and the procedure was performed under the isoflurane anesthesia. Between 8:00 AM and 10:00 AM (during the nadir of circadian plasma CORT concentration, Leitch et al, 2003; Steimer et al., 2007), 50 - 100 μl of blood was collected from the tail vein into the EDTA – coated tubes. In addition, 6-8 weeks after SE, animals were subjected to the DEX/CRH test (Johnson, et al., 2006; Steimer, et al., 2007). After collecting blood for basal CORT detection, animals were injected into the tail vein with DEX (Sigma; 0.03 mg/kg, dissolved in 10% dymethyl sulfoxide, injected at a volume of 0.1 ml). Six hours later, blood was collected again, and animals were injected into the tail vein with CRH (Sigma; 50 ng/kg, dissolved in 0.9% NaCl, injected at a volume of 0.1 ml); two blood samples were taken 30 and 60 min after CRH injection. To avoid possible immediate effects of spontaneous seizures on CORT level, blood was only collected upon verification (by reviewing video recordings) that animals had not developed seizures for at least 6 hours prior. In different post-SE animals, time between spontaneous seizure and blood collection was 6-48 hours. The minimal period of 6 hours was selected based on pilot studies, which showed that at this time basal CORT levels were in the same range as at time points separated from the preceding spontaneous seizures by longer periods (up to 4 days). Blood samples collected less than 6 hours after the last spontaneous seizure showed significant (up to two ranks of order) increase or decrease of plasma CORT levels, as compared with time points used for the present study.

Plasma was separated by centrifuging. CORT was detected in 10 μl samples, using Immuchem™ Double Antibody Corticosterone 125I RIA kit (MP Biomedicals, Orangeburg, NY), following procedure recommended by the manufacturer.

### Forced swim test (FST)

FST is commonly used in experimental studies of depression, and relies on the innate ability of rodents to adopt active strategies in an inescapable stressful situation; failure to do so is indicative of despair associated with depression (Mazarati, et al., 2008; Shumake and Gonzalez-Lima, 2003; Zangen, et al., 2001). The experiment was performed one-two weeks after blood collection for CORT RIA, upon verification that animals had not developed

spontaneous seizures for at least six hours prior to the test, between 8:00AM and 10:00AM. To avoid a possible influence of motor abnormalities on the swimming behavior, the animals were selected for the FST based on their performance in the Rotarod test on the prior day. Rats were placed on the rotating Rotarod (Ugo Basile, Italy); the rotation speed started at 5 revolutions per minute (rpm), with 5 rpm increments until the 40 rpm was reached. The cut-off time was set at 5 min. The animals were set to be used in the FST only if they maintained balance throughout the test.

For the FST, the animals were placed for 5 minutes in a cylinder (50 cm high, 30 cm wide), filled with water at 20-22°C. During the FST, animals show two alternating behaviors: active swimming, which reflects escape strategies (trying to escape from the tank; swimming along the wall, across the tank, diving), and immobility (moving limbs and tail only enough for the head to stay above water) (Mazarati, et al., 2008; Zangen, et al., 2001). The increase of the cumulative immobility time reflects the state of despair, which represents a core symptom of depression (Overstreet, et al., 2005). Animals' behavior was videotaped and reviewed off-line by two "blinded" investigators; cumulative time spent immobile was calculated.

### Afterdischarge properties

We previously showed that LiCl and pilocarpine SE led to the sustained neuronal hyperexcitability, which was independent of spontaneous seizures and was evident as the decrease of the hippocampal afterdischarge threshold and the prolonged afterdischarge duration (Mazarati, et al., 2008). Thus, hippocampal afterdischarge test can be used as a surrogate marker of epileptogenicity, as it allows to reveal the enhanced predisposition to seizures even in those post-SE animals in which spontaneous seizures are either extremely rare or (as the presents results will show) are not documented at all.

Afterdischarge properties were examined at the end of the studies, so that to avoid possible effects of surgery and brain stimulation on the outcomes of endocrine and behavioral tests. On the next day after FST, some animals were anesthetized with isoflurane, and were stereotaxically implanted with a recording/stimulating electrode (Plastics 1, Roanoke, VA), into the hippocampus (4.8 mm posterior, 5.3 mm left, 6.5 mm ventral from Bregma) (Mazarati, et al., 2008). One week after surgery the animals were connected to the DS8000 electrical stimulator (World Precision Instruments, Sarasota, FL), and to the MP100/EEG100B acquisition system (BIOPAC, Santa Barbara, CA). Afterdischarge threshold and afterdischarge duration were examined by applying electrical stimuli to, and recording from the hippocampal electrode; stimulation parameters were: 10 s train, 50 ms peak interval, 1 ms pulse duration, square wave biphasic waveform, starting with 0.1 mA, with 0.05 mA increments, delivered every 10 minutes (Mazarati, et al., 2008). Afterdischarge test was performed upon verification that animals had not developed spontaneous seizures for at least 6 hours.

### Data analysis

Data were analyzed using Prizm 4 software (GraphPad, San Diego, CA). Statistical tests are described in respective figure legends.

## Results

### Spontaneous seizures and hippocampal afterdischarge properties

Out of 16 animals which had developed SE and were used for further studies, spontaneous seizures were observed in 10 rats. The animals exhibited both focal and generalized seizures. For both types of seizures combined, weekly minimal/maximal/median count was 1/8/3.5. No rats exhibited seizures consisting of exclusively a single pattern (i.e. only focal or only generalized). At the same time, post-SE animals, both with and without documented recurrent

seizures, showed a statistically similar increase of hippocampal excitability, which was evident as lower afterdischarge threshold and longer afterdischarge duration, as compared with controls (Fig. 2); furthermore, unlike control animals, all post-SE rats developed behavioral seizures in response to the threshold stimulation; on the Racine (1972) scale, Mean+SEM seizure score was 2.9±0.3; minimal/maximal/median seizure score was 2/4/3. Such hyperexcitability of the hippocampus after SE, which was independent of spontaneous seizures, was in agreement with our earlier studies (Mazarati, et al., 2008), and suggested that even those animals which exhibited rare, or no documented recurrent seizures, developed the enhanced seizure predisposition as a result of SE.

### Functional state of the HPA axis

In post-SE animals (n=16), basal plasma CORT level was significantly higher as compared both with the pre-SE concentration in the same subjects, and with that in the age-matched controls (n=8, Fig. 3A, ii). DEX induced significant (65%) decrease of plasma CORT concentration in control rats, but did not affect the hormone level in post-SE animals (Fig. 3A, iii). Thirty minutes after CRH injection, plasma CORT was elevated in both post-SE and control subjects; however, the extent of this increase was larger in post-SE rats (7-fold vs. pre-CRH value), than in controls (4-fold, Fig. 3A, iv). Sixty minutes after CRH injection, plasma CORT concentration returned to the pre-CRH level in control animals, but remained elevated in post-SE rats, in which it was statistically similar to the level detected 30 minutes after CRH administration (6 fold Fig. 3A, v).

In order to examine possible contribution of recurrent seizures into the observed hyperactivity of the HPA axis, plasma CORT levels were analyzed separately in post-SE animals with and without documented spontaneous seizures. No differences were found between the two subsets in both basal CORT levels, and in responses to DEX/CRH challenge (Fig. 3B).

### Correlation between the functional state of the HPA axis and depressive behavior

To establish a connection between the observed hyperactivity of the HPA axis and depression after SE, we performed statistical correlation analysis between endocrine impairments and behavior in the FST. None of control and post-SE animals failed in the Rotarod test. In agreement with our previous studies (Mazarati, et al., 2008), SE led to a significant increase of the immobility time in the FST; the severity of behavioral deficit was similar in animals with and without spontaneous seizures (Fig. 4 A). Statistical analysis revealed no correlation between the severity of depressive behavior and either basal CORT levels (Fig. 4 B), or those after DEX injection (not shown); however, positive correlation was observed between the increase of the immobility time and the increase of CORT concentrations both at 30 minutes (Fig. 4 B) and at 60 minutes (not shown) after CRH injection.

## Discussion

The main finding of this study is that SE led to hyperactivity of the HPA axis, which was evident as the increased basal plasma CORT level and a positive DEX/CRH test. This endocrine abnormality was observed both in animals with documented clinical seizures during the interictal period, and in those post-SE rats, in which clinical seizures were not detected. Furthermore, the endocrine response to CRH positively correlated with the severity of depressive behavior.

While several studies showed the hyperactivity of the stress hormone axis in animal epilepsy models, such changes were observed either during seizures, or postictally (Daniels, et al., 1990; Lai, et al., 2006; Szafarczyk, et al., 1986). To our knowledge, our report provides the

first evidence that experimental TLE is accompanied by the interictal HPA dysregulation, particularly by the positive DEX/CRH test.

The functional state of the HPA axis in human epilepsy has received little attention. Several findings showed postictal increase of plasma glucocorticoids in epilepsy patients (Pritchard, 1991; Takeshita, et al., 1986); however, these changes likely reflected an acute stress response to seizures. In contrast, reports on the interictal HPA hyperactivity, independently of seizure frequency (Gallagher, 1987; Zobel, et al., 2004), suggested chronic HPA dysfunction associated with the epileptic state. Such independence of the HPA hyperactivity from seizures observed both in the present study and in clinical reports, indicated that chronic stress in epilepsy reflects chronic alterations in respective neuro-endocrine circuits. With this regard, our earlier studies showed that the severity of depressive behavior in post-SE rats positively correlated with the interictal hyperexcitability of the hippocampus, but not with the frequency of recurrent seizures (Mazarati, et al., 2008). Furthermore, in the kindling model of epileptogenesis, in the absence of spontaneous seizures, rats exhibited depressive behavior, the severity of which directly correlated with the decrease of the hippocampal afterdischarge threshold (Mazarati, et al., 2007). Similarly, in the present experiments, post-SE animals showed the enhanced interictal hippocampal excitability independently of the presence of clinical seizures, as judged by changes in properties of hippocampal afterdischarge. These observations suggest that SE consistently leads to hippocampal hyperexcitability, which may or may not coincide with the development of recurrent seizures; thus, conceivably, even those animals in which spontaneous seizures were not detected, could be characterized as being in the epileptic state (similar to the kindling model of epilepsy which is characterized by the permanent increase of seizure susceptibility, but frequently without spontaneous seizures, McIntyre, 2006). At the same time, it should be noted, that the fact that no spontaneous seizures were observed in some of post-SE animals at the time of the examination, did not mean that these animals would have not developed spontaneous seizures at a later time after SE, or had not developed seizures prior to the beginning of video monitoring. It is also likely that post-SE rats developed non-convulsive or sub-clinical epileptiform impairments, such as high-frequency oscillations, or interictal spikes (Bragin, et al., 2004; Mazarati, et al., 2002), which conceivably could affect functional state of the HPA axis. However, it is important that within the study window, the reported hippocampal hyperexcitability, endocrine dysfunction and behavioral impairments were recorded in all post-SE rats, whether or not overt clinical seizures had been documented. Considering hippocampal control over the HPA axis (Herman and Cullinan, 1997), it is conceivable that chronic hippocampal impairment following SE leads to the HPA dysregulation, which in turn may contribute to the development of depression. Indeed, clinical findings suggest that in TLE patients, the extent of hippocampal dysfunction may be a more important factor of depression, than frequency of seizures (Gilliam, et al., 2007).

The reported endocrine and behavioral impairments may be precipitated by the SE – induced hippocampal neuronal injury; this issue requires further detailed studies. However, our previous experiments showed that rapid kindling epileptogenesis, which induced no neuronal injury, led to depressive behavioral deficits similar to those observed in the post-SE model of epilepsy (Mazarati et al., 2007). Furthermore, the fact that in the present studies, endocrine and behavioral alterations were similar between the subsets of animals with and without recurrent seizures, along with the assumption that the latter reflect the extent of neurodegeneration, suggests that neuronal injury is not a decisive factor contributing to the development of depression after SE.

The mechanistic link between the stress-associated dysregulation of the HPA axis and depression is well established; pathways through which chronic stress leads to depression can involve hippocampal hypometabolism, suppression of synthesis of brain-derived neurotrophic factor, impaired neurogenesis, compromised serotonergic transmission in the raphe-

hippocampal pathway (Chaouloff, 2000; Kondziella, et al., 2007; Yu, et al., 2008). Thus, sustained hyperactivity of the HPA axis, should one develop in chronic epilepsy, could explain the high incidence of co-morbidity between epilepsy and depression.

It should be mentioned, that while the present studies focus on depression as a consequence of chronic HPA dysfunction, the observed neuroendocrine impairments are likely to have other important ramifications in the epileptic brain. For example, in patients with major depression, the hypersecretion of glucocorticoids leads to hippocampal atrophy (Lee et al., 2002; Sheline et al., 1996). Consequently, the hyperactivity of the HPA axis observed in patients with TLE (Zobel et al., 2004) as well as in our experimental studies may be a factor, which along with glutamate toxicity, contributes to the epilepsy-associated hippocampal neurodegeneration. Furthermore, chronic stress results in the loss of dendritic spines of pyramidal cells in the hippocampus (Chen, et al., 2008) - an event which may contribute to hippocampal dysfunction and cognitive impairments, frequently observed in TLE. Moreover, chronic stress, which is triggered by the epileptic process, may lead to a variety of central nervous system and somatic impairments (Herman and Cullinan, 1997).

In conclusion, while intervention experiments are required to directly prove the connection between the HPA dysregulation and the epilepsy-associated depression, the observed positive correlation between endocrine and behavioral impairments, along with the well-known connection between chronic stress and depression, suggests that the HPA hyperactivity may contribute to the development of depression in the LiCl and pilocarpine model of TLE. Together with previously identified behavioral and serotonergic abnormalities (Mazarati et al., 2008), the present data further validate the LiCl and pilocarpine model as a model of co-morbidity between TLE and depression, which can be used both for studying mechanisms of this condition and as a screening platform for the development of respective therapeutic strategies.

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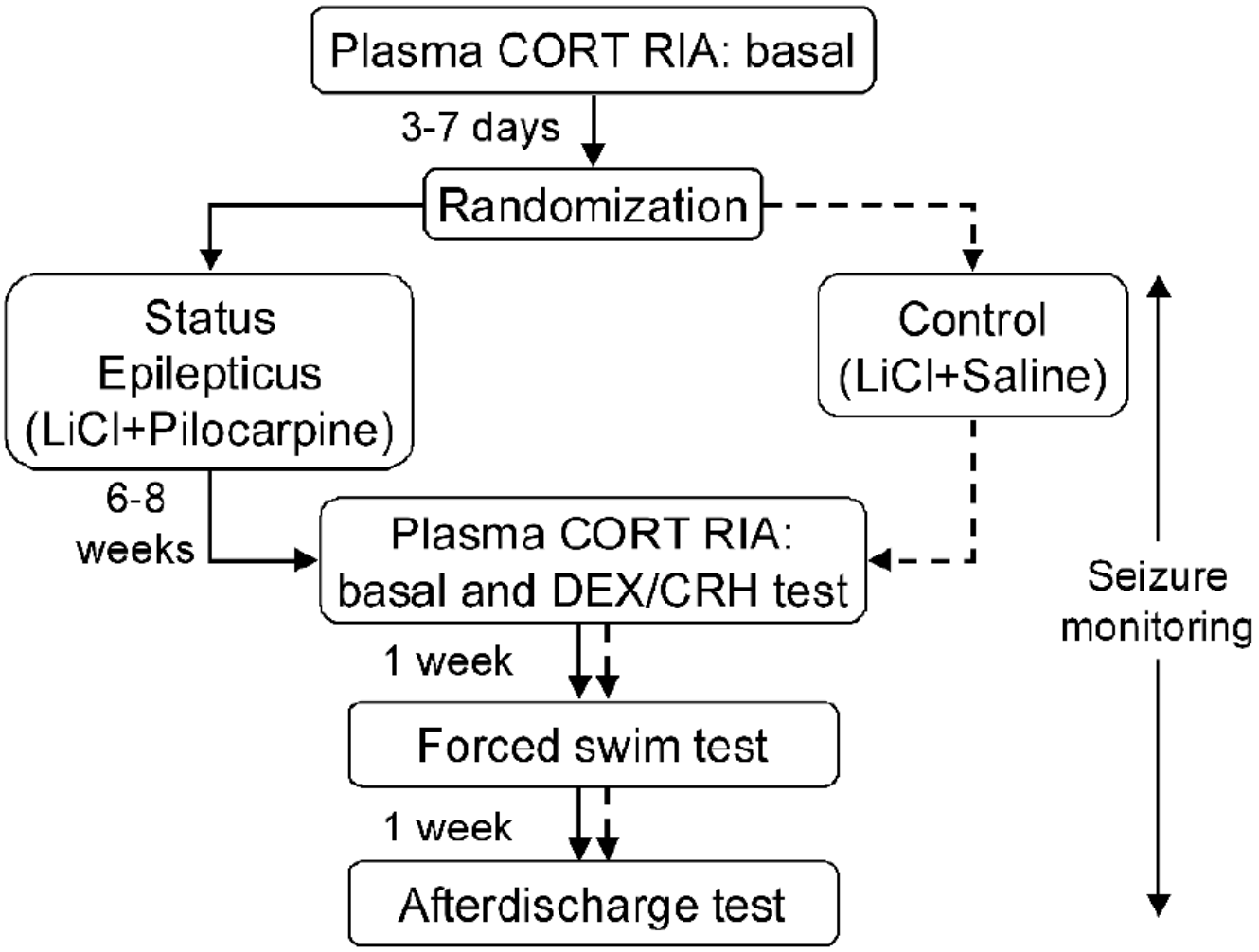


**Fig. 1. Study design**

Prior to the induction of SE, samples of venous blood were collected for detecting of basal plasma CORT level. Three-seven days later, the animals were randomized and subjected to either LiCl or pilocarpine SE, or to control treatment (LiCl and saline instead of pilocarpine). Six-eight weeks after SE, blood was collected for detecting plasma CORT level under basal conditions and in response to the DEX/CRH test. One week after the DEX/CRH test, the animals' behavior was examined in the forced swim test. After endocrine and behavioral assays were completed, the animals were implanted with stimulating/recording hippocampal electrode and the properties of hippocampal afterdischarge (threshold and duration) were examined. Animals were under continuous video monitoring for detecting clinical spontaneous seizures starting from four weeks after SE and until the end of the experiments. Abbreviations: CORT- corticosterone; RIA- radioimmunoassay; DEX- dexamethasone; CRH- corticotropin releasing hormone.

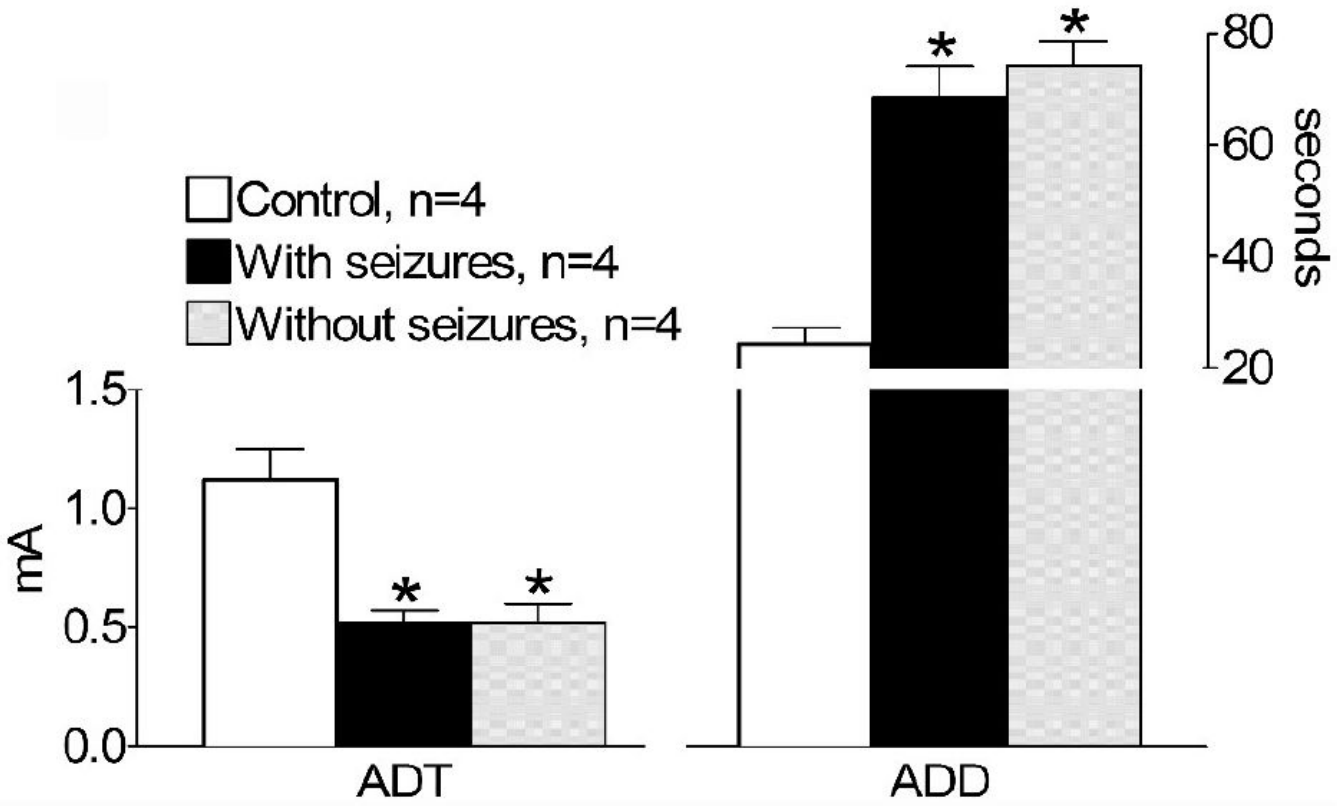


**Fig. 2. Hippocampal afterdischarge properties after SE**

Post-SE rats, both with, and without documented recurrent seizures displayed decreased afterdischarge threshold (ADT) and increased afterdischarge duration (ADD), as compared with control. Data are presented as Mean±SEM. *- $p<0.05$ vs. Control (One-Way ANOVA + Neuman-Keuls).

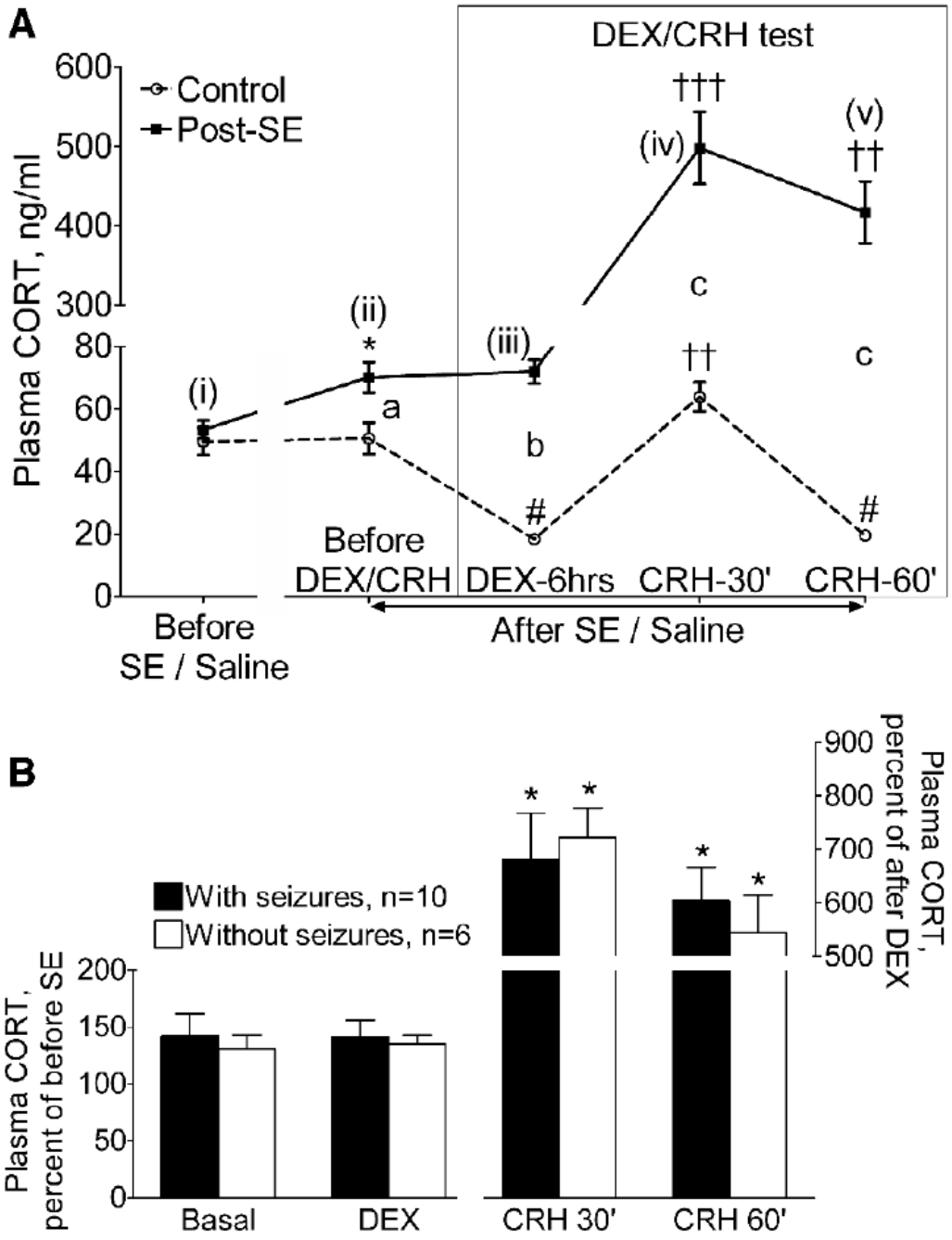


**Fig. 3. Plasma corticosterone (CORT) levels in control and post-SE rats under basal conditions and in response to the combined DEX/CRH test**

**A**. (i)- basal CORT levels before SE (saline in control rats); (ii)- basal CORT levels 6-8 weeks after SE; (iii) CORT levels 6 hours after DEX injection; (iv) and (v) – CORT levels 30 and 60 minutes after CRH injection (and 6.5 and 7 hours respectively after DEX injection). Post-SE animals showed the increase of basal CORT level, loss of a response to DEX, and an exacerbated and longer-lasting response to CRH. Data are presented as Mean±SEM. *- $p<0.05$ vs. “Before SE/Saline”; #- $p<0.01$ vs. “Before DEX/CRH”; ††- $p<0.01$ and †††- $p<0.001$ vs. “DEX-6 hrs” (repeated measures ANOVA+Tukey test). a- $p<0.05$; b- $p<0.01$; c- $p<0.001$ for SE vs. Control (Student t-test). **B**. No statistical differences were observed between the subsets

of post-SE animals with and without documented spontaneous seizures, for both basal CORT concentrations and in response to the combined DEX/CRH test. ($p>0.05$, Student t-test). *-$p<0.05$ vs. both “Basal” and “DEX” (repeated measures ANOVA+Tukey test). Data are presented as Mean±SEM.

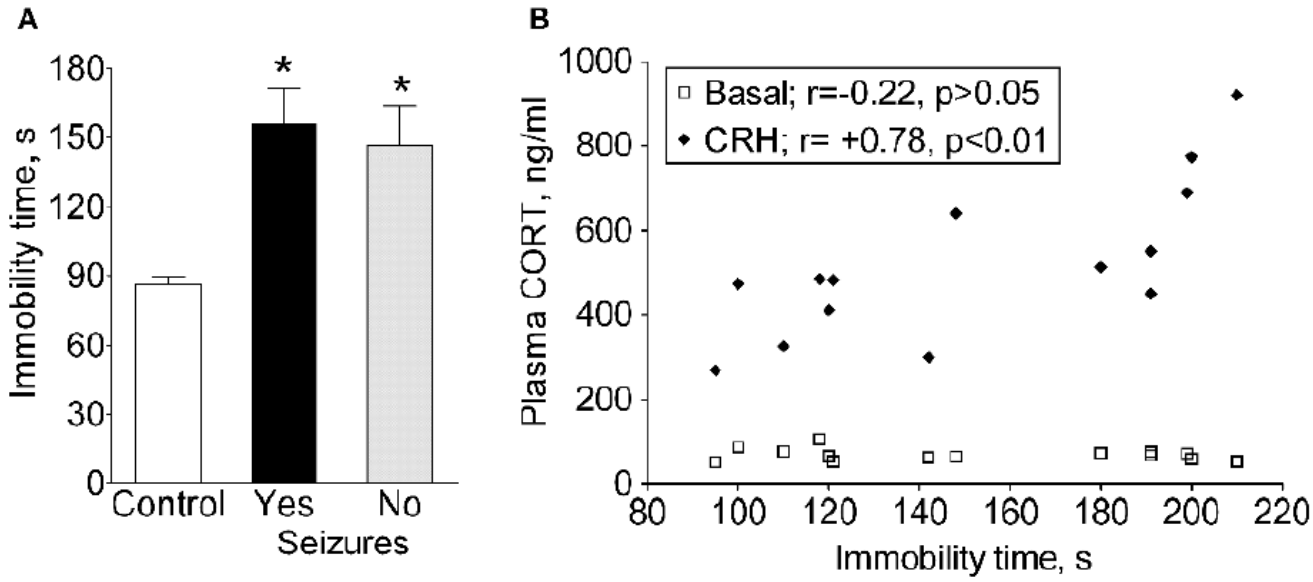


**Fig. 4. Correlation analysis between behavioral impairments in the forced swim test (FST) and plasma CORT levels in post-SE animals**

A. Immobility time in the FST in control animals (n=8, open bar), in post-SE animals with (n=8, black bar) and without (n=6, gray bar) documented spontaneous seizures. Data are presented as Mean±SEM. *- $p<0.05$ vs. Control (One-Way ANOVA + Neuman-Keuls). B. In individual animals, the immobility time is plotted against previously obtained basal CORT concentration (open squares) and CORT concentration 30 min after CRH injection (black diamonds). No correlation was observed between the severity of behavioral deficit and either basal CORT concentration ($r=-0.22$, $p>0.05$, Pearson correlation), or that after DEX injection ($r=0.14$, $p>0.05$, not shown); however, the severity of depressive behavior positively correlated with CORT concentration 30 min after CRH injection ($r=+0.78$, $p<0.01$, Pearson correlation) and 60 min after CRH injection ($r=+0.61$, $p<0.05$, not shown).

**Table 1**

Changes in body weight during the course of the study.

| Experimental group, sample size | Before SE (saline in controls) | Six weeks after SE (saline in controls) | Ten weeks after SE (saline in controls) |
|---|---|---|---|
| Status epilepticus, n=16 | 245±3.9 g | 484±4.9 g | 501±3.7 g |
| Control (saline) n=8 | 241±3.6 g | 489±4.3 g | 503±3.2 g |

Data are presented as Mean±SEM. SE- status epilepticus.