Depression after status epilepticus: behavioural and biochemical deficits and effects of fluoxetine

Andréy Mazarati,1 Prabha Siddarth,2 Roger A. Baldwin,3 Don Shin,1 Rochelle Caplan1,2 and Raman Sankar1,4

1Department of Pediatrics, 2Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, 3Research, West Los Angeles Veterans Administration Medical Center and 4Department of Neurology, David Geffen School of Medicine at UCLA, Los Angeles, CA, USA

Correspondence to: Andrey Mazarati, MD, PhD, David Geffen School of Medicine at UCLA, Room 22-474 MDCC (17527), Los Angeles, CA 90095-1752, USA
E-mail: mazarati@ucla.edu

Depression represents one of the most common comorbidities in patients with epilepsy. However, the mechanisms of depression in epilepsy patients are poorly understood. Establishment of animal models of this comorbidity is critical for both understanding the mechanisms of the condition, and for preclinical development of effective therapies. The current study examined whether a commonly used animal model of temporal lobe epilepsy (TLE) is characterized by behavioural and biochemical alterations involved in depression. Male Wistar rats were subjected to LiCl and pilocarpine status epilepticus (SE). The development of chronic epileptic state was confirmed by the presence of spontaneous seizures and by enhanced brain excitability. Post-SE animals exhibited increased immobility time under conditions of forced swim test (FST) which was indicative of despair-like state, and loss of taste preference in saccharin solution consumption test which pointed to the symptomatic equivalence of anhedonia. biochemical studies revealed compromised serotonergic transmission in the raphe-hippocampal serotonergic pathway: decrease of serotonin (5-HT) concentration and turnover in the hippocampus, measured by high performance liquid chromatography, and decrease of 5-HT release from the hippocampus in response to raphe stimulation, measured by fast cyclic voltammetry. Administration of fluoxetine (FLX, 20 mg/kg/day for 10 days) to naive animals significantly shortened immobility time under conditions of FST, and inhibited 5-HT turnover in the hippocampus. In post-SE rats FLX treatment led to a further decrease of hippocampal 5-HT turnover; however, performance in FST was not improved. At the same time, FLX reversed SE-induced increase in brain excitability. In summary, our studies provide initial evidence that post-SE model of TLE might serve as a model of the comorbidity of epilepsy and depression. The finding that behavioural equivalents of depression were resistant to an antidepressant medication suggested that depression in epilepsy might have distinct underlying mechanisms beyond alterations in serotonergic pathways.

Keywords: comorbidity; depression; epilepsy; hippocampus; serotonin

Abbreviations: 5-HT = serotonin; 5-HIAA = 5-hydroxyindolacetic acid; ADT = afterdischarge threshold; ADD = afterdischarge duration; FCV = fast cyclicvoltammetry; FLX = fluoxetine; FST = forced swim test; HPLC = high performance liquid chromatography; PCA = parachloroamphetamine; SE = status epilepticus; SERT = serotonin transporter; SSRI = selective serotonin reuptake inhibitors; TLE = temporal lobe epilepsy.


Introduction

Depression represents one of the most common comorbidities in patients with epilepsy (Kanner and Balabanov, 2002; Harden, 2002). While psychosocial aspects of being in the epileptic state may contribute to depression associated with epilepsy, there is a growing consensus that this condition has neurobiological basis (Kanner, 2005). For example, both clinical and experimental evidence suggest that the imbalances in such neurotransmitters as GABA, glutamate, norepinephrine and serotonin, which are commonly observed in epilepsy patients, may concurrently contribute to the evolvement of depression (Kanner and Balabanov, 2002; Jobe, 2003; Kanner, 2005; Kondziella et al., 2007). One clinical study emphasized comparable impairments in the functioning of hypothalamic pituitary adrenocortical system in patients with epilepsy and those with depression.
(Zobel et al., 2004). A number of clinical reports have implicated hippocampal neurodegeneration (e.g. Cascino et al., 1991; Sheline et al., 1996), or dysfunction (Gilliam et al., 2007) in the development of depression in epilepsy patients, although few studies have suggested otherwise (Briellmann et al., 2007; Richardson et al., 2007).

Establishment and validation of animal models of comorbidity between epilepsy and depression is instrumental for both understanding the mechanisms of the condition, and for preclinical development of effective therapies. For example, depression has been described in Wag-Rij inbred rats, which exhibit spontaneous absence seizures (Sarkisova et al., 2003), and in genetic absence epilepsy rats from Strasbourg (GAERS, Jones et al., 2008). However reports on depression-like disorders associated with experimental temporal lobe epilepsy (TLE) are scarce and controversial. Thus, Mortazavi et al. (2005) reported increased behavioural despair under conditions of forced swim test (FST) in pentylenetetrazole-kindled rats. We recently described anhedonia and despair in animals that had been subjected to rapid kindling in young age (Mazarati et al., 2007). However, Ma and Leung (2004) found no changes in forced swimming behaviour after amygdala kindling. Adamec et al. (2004) and Wintink et al. (2003) failed to reveal any changes in taste preference in animals which had been subjected to kindling. Koh et al. (2007) reported changes in FST in juvenile rats following kainic acid induced status epilepticus (SE). In contrast, Groticke et al. (2007) found an improved performance under conditions of FST in post-pilocarpine SE model of epilepsy in mice, as compared to non-epileptic controls. Clearly, more efforts are required in the pursuit of validation of available models of epilepsy as a comorbidity between the two states.

The current study had several goals. First, we examined whether depression developed under conditions of a commonly used model of TLE, namely post-SE chronic epilepsy in the rat. Second, in addition to characterizing the behavioural symptoms of depression, we studied some neurochemical correlates of the comorbidity. Among multiple candidate mechanisms we selected serotonergic transmission as a target for initial experiments. On the one hand, serotonin (5-HT) deficiency is not only a mechanism of depression (Jobe et al., 1999; Kondziella et al., 2007), but is also a defining approach in the treatment of the disease through the use of selective serotonin reuptake inhibitors (SSRI). On the other hand, dysfunction of serotonergic transmission has been reported under conditions of TLE (Theodore, 2003). It was suggested that impairments in serotonergic transmission represent a pathophysiological link between epilepsy and depression (Jobe et al., 1999). Finally, we examined whether treatment with a clinically available and widely used antidepressant—SSRI fluoxetine (FLX), would improve both behavioural and biochemical impairments indicative of depression in post-SE animals.

Materials and Methods

The study design is shown in Fig. 1.

Animals

The experiments were performed in male Wistar rats (Charles River, Wilmington, MA, USA), 34 days of age at the beginning of the study. The experiments were done in accordance with the policies of the National Institutes of Health and the UCLA Office for the Protection of Research Subjects.

Status epilepticus

Animals received an intraperitoneal (i.p.) injection of LiCl (130 mg/kg, Sigma, St. Louis, MO, USA). On the next day (i.e. at the age of 35 days) animals were injected subcutaneously (s.c.) with atropine methyl bromide (10 mg/kg, Sigma) to alleviate peripheral effects of pilocarpine, and 30 min later were injected s.c. with pilocarpine hydrochloride (40 mg/kg, Sigma). SE was characterized by continuous limbic seizures which started 10–15 min after pilocarpine injection. At their peak, seizure behaviour consisted of rearing or rearing and falling, which corresponded to stages 4–5 on the Racine (1972) scale. Those animals that failed to develop stage 4–5 seizures were excluded from further studies. After 3 and 8 h of seizure onset, rats were injected i.p. with diazepam (5 mg/kg) and phenytoin (50 mg/kg), in order to alleviate further seizures and to increase survival. Control animals received injections of LiCl, atropine, diazepam and phenytoin, and saline in lieu of pilocarpine.

Surgery

After 21–24 days of SE (or after atropine and antiepileptic drugs injections in control rats), under isoflurane anaesthesia animals received injections of LiCl, atropine, diazepam and phenytoin, and saline in lieu of pilocarpine.

Fig. 1 Study design. Veh = vehicle.
were stereotaxically implanted with a bipolar stimulating electrode (Plastics one, Roanoke, VA, USA) into the ventral hippocampus (4.8 mm posterior and 5.3 left from Bregma, 6.5 mm down from brain surface; Paxinos and Watson, 1986). A bipolar recording electrode (Plastics one Inc.) was wrapped around skull screws using the nasal bone as the ground. The electrodes were fixed to the skull with Cerebond adhesive (MyNeurolab.com, St. Louis, MO, USA). Animals were allowed to recover between 3 and 7 days before further studies.

**Recording and analysis of spontaneous seizures**

Four weeks after SE animals were placed in the observation chambers with free access to food and water (Instech Solomon, Plymouth Meeting, PA, USA). Recording electrodes were connected to MP100/EEG100C acquisition system (BIOPAC Systems Inc., Santa Barbara, CA, USA), via low-torque commutator swivels (Plastics One). EEG was continuously acquired for a period of one week using AcqKnowledge 3.82 software (BIOPAC). Incidence and frequency of spontaneous seizures were analysed off-line.

**Behavioural tests**

Starting from the next day after the end of seizure monitoring in post-SE animals, or on the congruent day in the control group, we evaluated depressive behaviour using two consecutive tests: taste preference to examine a behavioural correlate of anhedonia (i.e., inability to experience pleasure), and FST to study the ability to adapt active strategy in an inescapable stressful situation.

The study of taste preference was performed using the saccharin solution consumption test (Pucilowski et al., 1993; Moreau, 1997; Mazarati et al., 2007). The animals had free access to a standard rodent diet. On the first day (habituation), each cage was supplied with two identical graduated water bottles, each containing 250 ml of water. On the next day (test), regular water in one of the bottles was replaced with 0.1% saccharin (Sigma, St. Louis, MO, USA) diluted in tap water. The test was performed starting from 5:00 PM and ran for 24 h. Taste preference was expressed as percent of the volume of saccharin solution of a total volume of fluid (saccharin plus regular water) consumed over 24 h.

We employed a modified version of the FST in which the animals underwent a single 5-min trial. This modification of the classic Porsolt test (1979) was shown to be relevant for examining depressive-like state and for screening antidepressant agents (Pucilowski and Overstreet 1993; Overstreet et al., 1995; Mazarati et al., 2007).

The test was performed the day after the taste preference test. In each trial, the animal was placed for 5 min in a plastic container (60 cm in height and 30 cm in width) filled with tap water to a height of 45 cm, and maintained at 22–25°C. After each trial, the container was washed and refilled with fresh water. Swimming behaviour was videotaped and analysed offline by an investigator blinded to treatment. Total immobility time was calculated. Immobility was defined as moving the limbs just enough to stay above water, as opposed to escaping or exploring behaviour.

**Afterdischarge properties**

After the end of the behavioural tests, afterdischarge properties were examined in both post-SE and control animals. A stimulating electrode was connected to a DS8000 stimulator via a DLS100 stimulus isolator [World Precision Instruments Inc. (WPI), Sarasota, FL, USA]; a recording electrode was connected to the EEG acquisition system (BIOPAC) as described earlier. Afterdischarge threshold (ADT) and duration (ADD) were detected by applying electrical stimuli consisting of 10 s, 20 Hz train, 1 ms pulse duration, square wave monophasic stimuli, starting at 0.2 mA, at 0.1 mA increments, delivered every 10 min. In addition, the occurrence and the severity of seizures in response to the threshold stimulation was evaluated using the Racine (1972) scale: (i) Motor arrest and twitching vibrissae; (ii) chewing, head bobbing; (iii) forelimb clonus; (iv) forelimb clonus and rearing and (v) rearing and falling.

**FLX treatment**

After the studies of seizures, depressive behaviour, and afterdischarge properties, both naive and post-SE animals underwent FLX treatment. FLX (Spectrum Chemical, Gardena, CA, USA) was injected once a day for 10 consecutive days, i.p. at a dose of 20 mg/kg (Frankfurt et al., 1994; Hernandez et al., 2002; Thompson et al., 2004). Control treatments consisted in daily injections of vehicle (0.9% NaCl). One to two days after the end of FLX administration, the animals underwent EEG monitoring, as well as the behavioural and afterdischarge tests described earlier (Fig. 1).

**Fast cyclic voltammetry**

One week after the end of second afterdischarge test, 5-HT release from the hippocampus was electrochemically detected in vivo using fast cyclic voltammetry (FCV) (Kennett and Joseph, 1982; Jackson et al., 1995). Equipment included a scanning potentiostat POT500 (WPI), MP100 amplifier/stimulator (BIOPAC) and a computer equipped with AcqKnowledge 3.82 software (BIOPAC). The three-electrode system consisted of working Nafion-coated carbon fibre electrode (250 μ length, 10 μ diameter), Dri-Ref reference electrode, and a platinum auxiliary electrode (all from WPI). FCV was performed, as described elsewhere (Jackson et al., 1995; Iravani and Kruk, 1997; Chuma et al., 2002), modified according to the goal of the experiments, equipment and WPI application notes.

Prior to each experiment, the carbon fibre electrode had been sensitized to 5-HT. All three electrodes were immersed in phosphate buffered saline (pH 7.4). Electrochemical treatment consisted of triangular wave which was generated by MP100 stimulator function: 0 to +3.0 V at 70 Hz for 20 s, 0 to +2.5 V at 20 Hz for 20 s, 0 to +1.0 V at 70 Hz for 20 s (WPI application notes for carbon fibre electrodes). Sensitivity towards 5-HT was confirmed by acquiring voltamograms from solutions of 5-HT, norepinephrine or dopamine (Sigma) of known concentrations (Fig. 2A). The voltammetric waveform consisted of a rest potential 0.2 V scanned to 1 V, then to −0.1 V and then back to 0.2 V, at a rate of 1000 V/s (Fig. 2, inset a), generated by MP100 stimulator function, fed into the external input of the potentiostat (Jackson et al., 1995).

5-HT release was examined in rats anaesthetized by urethane (1.25 mg/kg s.c, Sigma) and placed in stereotaxic device. Carbon fibre electrode was inserted into CA1 (4.3 mm posterior to Bregma, 3 mm lateral from midline, 3.6 mm down from dura), or CA3 (4.1 mm posterior to Bregma, 4.2 mm lateral from midline, 4 mm down from dura; Paxinos and Watson, 1986). Auxiliary and reference electrodes were positioned on the surface.
of the dura and on the parietal area of the cranial bone, respectively. Bipolar stimulating electrode (Plastics One) was positioned into the dorsal raphe (7.8 mm posterior from Bregma, midline, 6.7 mm ventral from dura; Paxinos and Watson, 1986; Fig. 2B), a major source of serotonergic innervation of the hippocampus (Azmitia and Segal, 1978). After acquisition of background current (in the absence of raphe stimulation), 5-HT release was induced by an electrical train applied to the stimulating electrode using DS8000 stimulator (WPI): bipolar square wave pulses, 100 Hz, 200 ms, 0.35 mA (Jackson et al., 1995). One second after the end of electrical stimulation, five consecutive voltammograms were acquired with the intervals of 100 ms. The stimulation/acquisition cycle was repeated five times with 2.5 min intervals. The release of 5-HT was expressed as a peak amplitude of Faradaic current, which represented the difference between stimulation-induced and baseline current values (Iravani and Kruk, 1997). Each voltammogram used for the analysis represented an average of five consecutive Faradaic currents acquired during single acquisition cycle.

Validation of FCV in vivo was performed in a separate group of rats in which 5-HT in the brain had been depleted by a selective 5-HT neurotoxin parachloroamphetamine (PCA, Sigma). We previously showed that PCA treatment (60 mg/kg i.p.) led to nearly complete disappearance of 5-HT in the hippocampus (Mazarati et al., 2005). In the present study, this method was used to confirm the validity of FCV in vivo for detecting 5-HT release. Four rats were injected with PCA (60 mg/kg); FCV was performed 10 days after PCA administration.

**High performance liquid chromatography**

The 5-HT concentration and turnover in the hippocampus was measured as described earlier (Mazarati et al., 2005). Immediately after the end of FCV, animals were decapitated, brains removed and hippocampi dissected on ice. Tissue samples were homogenized in the solution containing 0.09 mol/l perchloric acid, 0.04 mmol/l EDTA and 5 mmol/l Sodium Bisulfite (1 ml per100 mg of wet tissue), and centrifuged at 4°C and 16000 G; supernatant is stored at −70°C. Fifty aliquots, 50 µl each, were loaded onto the column. High performance liquid chromatography (HPLC) was performed using with L-ECD-6A electrochemical detector (Shimadzu, Kyoto, Japan) and HR-80 column, 4.6 × 8 × 3 µm (ESA Biosciences, Chelmsford, MA, USA). The separation was done in isocratic elution mode using CAT-A-Phase II mobile phase (ESA Biosciences). The measurements were done at an electrode potential of +0.7 V. Data were analysed using EZ Start software (Shimadzu); areas under peaks were calculated and plotted against the areas for the known concentration of the standards. Two peaks were identified: 5-HT, and serotonin metabolite 5-hydroxyindolacetic acid (5-HIAA). 5-HT turnover was expressed as 5-HIAA/5-HT ratio (Zangen et al., 1997).

**Data analysis**

Data were analysed using Prizm 4 statistical software (GraphPad, San Diego, CA, USA). The number of animals in experimental groups and statistical tests used are indicated in the respective results sections. For samples that had passed D’Agostino and Pearson omnibus normality test, paired and unpaired t-tests, as well as one-way analysis of variance (ANOVA) followed by post hoc Bonferroni test were used were appropriate. For samples that failed normality test, Mann–Whitney or Wilcoxon tests were employed. \( P<0.05 \) was accepted as an index of statistically significant differences. Correlation analysis was performed using Spearman correlation test.

**Results**

**Spontaneous seizures**

Four weeks after SE, prior to FLX treatment, 16 animals were randomly divided into two groups (\( n=8 \) per group), and underwent seizure monitoring for 1 week. In each of the groups, seizures were observed in five animals. The minimal/maximal/median seizure count over 1 week, was 0/5/1.5 in group 1 and 0/4/1.0 in group 2 (\( P>0.05 \) between the groups). After vehicle (group 1) and FLX (group 2) treatment (8 weeks after SE), seizure incidence and
frequency were within the same statistical range as during the first monitoring session (Fig. 3B), although there was a trend toward a decrease in seizure frequency in the FLX-treated animals. In the vehicle treated group, seven animals exhibited seizures, but after FLX, seizures were recorded in all eight rats. The minimal/maximal/median seizure frequency was 0/15/5 in vehicle-treated rats and 1/5/2 after FLX administration (P > 0.05). During the two observation periods combined, each of the animals exhibited at least one seizure.

Afterdischarge properties
During the first test prior to FLX treatment, ADT was significantly lower and ADD—significantly longer in post-SE animals (two groups, n = 8 each), as compared to controls (two groups, n = 7 each; Fig. 4A–D). Furthermore, in contrast to naïve rats, post-SE animals developed behavioural seizures in response to threshold stimulation (Fig. 4D).

In naïve rats, treatment with FLX significantly elevated ADT (from 1.1 ± 0.14 mA to 1.8 ± 0.12 mA, n = 7, P < 0.05); ADD was not affected by FLX treatment (Fig. 4C). Administration of FLX to post-SE animals (n = 8) reversed the increase of excitability which had been induced by SE. Thus, ADT increased from 0.53 ± 0.1 mA before treatment to 1.6 ± 0.14 mA after FLX; ADD shortened from 64 ± 14.1 s to 24.1 ± 5.6 s (P < 0.05 for both parameters, Fig. 4D). Further, the severity of convulsions in response to threshold stimulation decreased from 2.5 ± 0.6 to 1.2 ± 0.4 (P < 0.05, Fig. 4D).

Behavioural deficits
During the first test, prior to FLX treatment, the naïve animals exhibited a strong preference towards saccharin solution over tap water (two groups, n = 7 each; Fig. 5A). In contrast to controls, post-SE animals consumed statistically equal amounts of regular water and saccharin solution (two groups, n = 8 each; Fig. 5A). The introduction of saccharin did not alter total volume of consumed fluid (saccharin + regular water) in both control and post-SE rats (data not shown). The administration of FLX did not modify saccharin consumption in both the naïve and post-SE animals (Fig. 5A) nor change the total volume of consumed fluid (data not shown).

Prior to FLX (n = 7) or vehicle (n = 7) treatment, total immobility time in the naïve rats was between 75 s and 110 s. In post-SE animals (two groups, n = 8 each) immobility time was between 90 s and 210 s. The increase in immobility time after SE was statistically significant (P < 0.05, Fig. 5B). Administration of FLX to naïve rats resulted in significant shortening of immobility time under conditions of FST (from 80 ± 4.6 s to 48 ± 3.5 s P < 0.05, Fig. 5B). However, similar treatment with FLX, did not improve forced swim behaviour in post-SE animals: in this group immobility time was 148 ± 26 s before, and 147 ± 22 s after FLX administration (Fig. 5B).

5-HT release from the hippocampus
Pre-treatment with PCA (n = 4) led to a 7-fold reduction of 5-HT release from the rat hippocampus, as compared to naïve rats (Fig. 6A and B), thus confirming the validity of the used technique for detecting 5-HT release in vivo. In the hippocampus of vehicle-treated post-SE animals (n = 7) the release of 5-HT in response to raphe stimulation was significantly diminished as compared to vehicle-treated controls (n = 7). Chronic treatment with FLX did not affect 5-HT release in both control (n = 7) and post-SE (n = 7) animals (Fig. 6A and B).

5-HT concentration and turnover in the hippocampus
In hippocampal tissue of vehicle-treated post-SE animals (n = 6), both the concentration of 5-HT and its turnover were significantly lower as compared to control rats (n = 7; Fig. 7). Chronic treatment with FLX did not alter 5-HT concentration in hippocampi of both the naïve (n = 7) and post-SE (n = 7) rats. At the same time, FLX administration to controls significantly inhibited 5-HT turnover. Treatment of post-SE animals with FLX resulted in a further decrease of the 5-HIAA/5-HT ratio, which was lower than both the FLX-free post-SE group and the FLX-treated control subjects (Fig. 7).
Correlation analysis

In the absence of FLX treatment, no correlation was found between the frequency of seizures and any of other parameters (Table 1). At the same time, animals with more profound changes in afterdischarge properties exhibited longer immobility time in the FST and lower preference towards saccharin (Table 1). Furthermore, longer immobility time during FST was observed in animals with slower 5-HT turnover in, and release from the hippocampus. However, parameters of serotonergic transmission were not associated with changes in taste preference. Likewise, no correlation was found between the extent of behavioural changes in forced swim and taste preference tests. Finally, no statistical association was revealed between the changes in either of the two of afterdischarge indices and the extent of serotonergic deficits (Table 1).

After FLX treatment, no correlation was observed between both seizure frequency and afterdischarge properties on the one hand and behavioural indices on the other hand ($-0.06 < r > +0.065$, $P > 0.05$ for all pairs of variables). Correlation between 5-HT turnover and behavioural alterations in FST also disappeared after FLX administration ($P > 0.05$); at the same time, correlation between 5-HT release from the hippocampus and the extent of forced swim behavioural deficits was statistically significant as it had been without FLX treatment ($r = -0.81$, $P < 0.05$).

Discussion

Our experiments showed that SE led to behavioural and biochemical impairments which were congruent with depression. The behavioural impairments included increased immobility time under conditions of FST, which represented a model equivalent of despair, and the loss of taste preference under conditions of saccharin consumption test,
which suggested the development of anhedonia-like symptomatology. The decrease in 5-HT concentration, turnover and release in the hippocampus suggested compromised serotonergic transmission. Treatment with FLX using a regiment which effectively prolonged active swimming behaviour in naive animals and inhibited 5-HT metabolism in both the naive and post-SE rats, reversed the SE-induced increase in brain excitability, but failed to improve the behavioural deficits associated with SE.

**Status epilepticus leads to depression-like behavioural impairments**

Increased immobility time under conditions of FST has been well established under conditions of animal models of depression. The specified behavioural deficit was observed in genetic models of depression both in rats (Zangen et al., 1997) and mice (El Yacoubi et al., 2003). Increased immobility time in FST was found in animals in which depression had been induced by maternal separation (Lee et al., 2007), and chronic food restriction (Jahng et al., 2007). The increased immobility time in our studies suggests that SE does indeed lead to the development of a condition which can be interpreted as an experimental correlate of a state of despair.

Anhedonia is one of key symptoms in depressed individuals (Overstreet et al., 2005). In rats, examination of anhedonia is based on the inherent preference towards sweets (e.g. saccharin or sucrose solutions). Loss of taste preference was observed in a mouse genetic model of depression (El Yacoubi et al., 2003), as well as under conditions of depression induced by chronic mild stress (Willner, 1997). At the same time, Flinders Sensitive Line rats (a genetic model of depression) did not exhibit loss of taste preference (Overstreet et al., 2005).

In animal models of epilepsy, symptoms equivalent to anhedonia have been reported only in few studies.
Thus, reduced sucrose preference was reported in Wag-Rij rats, a model of absence epilepsy (Sarkisova et al., 2003). We recently reported the loss of taste preference in kindled animals (Mazarati et al., 2007). The loss of taste preference observed in post-SE rats represents another behavioural symptom of depression in animals during chronic epileptic state.

**Deficits of serotonergic transmission and depression**

Dysfunction in serotonergic transmission, including that in the hippocampus, has been regarded as factor contributing in depression (Theodore, 2003). However, there is no agreement as to the direction in which 5-HT concentration, turnover and release are altered both in patients with depression and under conditions of animal models.

In the laboratory setting, increased 5-HT and 5-HIAA concentration, and unaltered 5-HIAA/5-HT ratio in the hippocampus was reported in Finders Sensitive Line rats (Zangen et al., 1997). 5-HT concentration (Gronli et al., 2007) and release (Bianchi et al., 2003) appeared to be intact under conditions of depression induced by stress. At the same time, a mouse genetic model of depression was characterized by the inhibition of 5-HT turnover in the hippocampus, as well as by the reduced firing of serotonergic neurons in raphe nucleus (El Yacoubi et al., 2003). Decline of 5-HT concentration in the rat hippocampus was

![Fig. 7 5-HT concentration and turnover in the hippocampus, and the effects of FLX treatment. Data are shown as mean ± SEM for control and post-SE groups, both vehicle and FLX-treated. 5-HT concentration is plotted on the left, and turnover—on the right Y-axis. *P < 0.05 versus respective group as indicated by connecting line (one-way ANOVA + Bonferroni test).](https://academic.oup.com/brain/article-abstract/131/8/2071/266688)

**Table 1 Correlation analysis between spontaneous seizures, brain excitability, behavioural and biochemical impairments after status epilepticus**

<table>
<thead>
<tr>
<th>Seizure count (1 week)</th>
<th>Afterdischarge threshold/duration</th>
<th>5-HIAA/5-HT (HPLC)</th>
<th>5-HT release (FCV), CA1±CA3/2</th>
<th>FST-immobility time</th>
<th>Per cent saccharin intake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seizure count (1 week)</td>
<td>r = +0.55</td>
<td>r = -0.59</td>
<td>r = +0.46</td>
<td>r = -0.62</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td></td>
</tr>
<tr>
<td>Afterdischarge threshold/duration</td>
<td>r(t) = +0.64</td>
<td>r(t) = +0.63</td>
<td>r(t) = +0.82</td>
<td>r(t) = -0.72</td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td>P &gt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td></td>
</tr>
<tr>
<td>5-HIAA/5-HT (HPLC)</td>
<td>r = +0.84</td>
<td>r = -0.89</td>
<td>r = +0.69</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5-HT release (FCV)</td>
<td>r = -0.85</td>
<td>r = +0.68</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &lt; 0.05</td>
<td>P &lt; 0.05</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FST-immobility time</td>
<td>r = +0.69</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>P &gt; 0.05</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

All parameters were evaluated in the group of animals (n = 6) in the absence of FLX treatment. Data on FLX-treated animals are discussed in Results. Coefficient of correlation (+r’ positive; ‘–r’ negative correlation) and its statistical significance were calculated using Spearman correlation. In the afterdischarge threshold/duration row, r(t) = afterdischarge threshold, r(d) = afterdischarge duration. For FCV, averages of CA1 and CA3 values were used, since values of the two indices were statistically similar (Fig. 6). Statistically significant correlation is indicated by bold italic font.
reported under conditions of depression induced by maternal separation (Lee et al., 2007), olfactory bulbectomy (Xu et al., 2005) and chronic food restriction (Jahng et al., 2007). In addition, inhibition of both 5-HT turnover and release in the hippocampus was reported under conditions of depression induced by forced swimming (Adell et al., 1997; Kirby et al., 1997) and olfactory bulbectomy (Xu et al., 2005).

In human studies, no differences were reported in hippocampal 5-HT, 5-HIAA and 5-HIAA/5-HT ratio between depressed suicide victims and age-matched controls (Cheetham et al., 1989). At the same time, the reduction of 5-HT, 5-HIAA and 5-HT turnover in the brainstem of depressed suicide patients has been well established (Mann et al., 1989). Since raphe serotonergic neurons represent a major source of hippocampal 5-HT (Azmitia and Segal, 1978), deficient serotonergic transmission in raphe suggests that hippocampal serotonergic innervation might be compromised. With this regard, reduced 5-HT release from the hippocampus in response to raphe stimulation observed in our post-SE animals is in line with the discussed human findings. Furthermore, because serotonin is not synthesized in the hippocampus, but rather represents axonal pool of 5-HT neurons located in raphe nuclei (Azmitia and Segal, 1978), the observed reduction of 5-HT concentration in post-SE animals likely reflects compromised neurotransmitter signalling along the raphe-hippocampal serotonergic pathway, which was demonstrated in our experiments by means of the FCV technique.

Deficits of serotonergic transmission and epilepsy

There are some clinical and experimental data available as to the 5-HT concentration and metabolism under conditions of epilepsy. Thus, the increase of 5-HT metabolism was shown in actively spiking hippocampal and cortical samples from TLE patients (Louw et al., 1989). At the same time, patients with mesial TLE had decreased 5-HT release from the hippocampus, as compared with patients with neocortical temporal lobe epilepsy (Broderick et al., 2000). No changes in hippocampal 5-HT concentration were revealed in the rat chronic epilepsy model induced by pilocarpine SE (Cavalheiro et al., 1994; Szyndler et al., 2005). However, chronic epileptic state induced by kindling was associated with the inhibition of hippocampal 5-HT metabolism (Szyndler et al., 2002a). The discrepancy between the decrease of 5-HT concentration and metabolism observed in our experiments, and the lack of changes reported in other studies, as well as differences observed for two different epilepsy models (Szyndler et al., 2002a; 2005), are difficult to address, given overall limited experimental data. One noticeable difference between our and earlier studies are different ages at the time of precipitating insult: while Szyndler et al., (2005) and Cavalheiro et al. (1994) used adult rats, we used animals of peri-adolescent age (postnatal day 35) at the time of SE.

Mechanisms of the observed inhibition of 5-HT turnover (5-HIAA/5-HT ratio) in post-SE hippocampus require further exploration. Several studies found reduced expression of serotonin reuptake transporter (SERT) in raphe nuclei of animals with induced depression (Jahng et al., 2007; Lee et al., 2007). The data on the alterations of SERT in the hippocampus under conditions of TLE are limited. Thus, decrease of SERT binding was observed in the hippocampus after amygdala (Clark et al., 1993) and pentylenetetrazole (Szyndler et al., 2002b) kindling. Rocha et al. (2007) found reduced SERT binding in the neocortex of patients with TLE. The association between the polymorphism of SERT gene and TLE has been discussed (Manna et al., 2007). The inhibition of 5-HT reuptake due to the downregulation of hippocampal SERT would explain the decrease of 5-HT turnover: indeed, the reduction of intracellular pool of transmitter amenable to the monoamine oxidase would limit the formation of 5-hydroxy-indoleacetaldehyde and subsequent oxidation of the latter to 5-HIAA.

Lack of the effects of fluoxetine on depressive behaviour following status epilepticus

The increase in active swimming behaviour in response to FLX treatment was reported both under conditions of classic (Page et al., 1999), and modified (Pucilowski and Overstreet, 1993; Overstreet et al., 1995) Porsolt tests. In our experiments, the validity of FST was confirmed in naive animals, which exhibited significant reduction of the immobility time after FLX treatment. The fact that post-SE rats did not show an improvement in forced swim behaviour after similar FLX administration implies that depression in epileptic animals is mechanistically different from behaviourally similar condition which is regulated by SSRI.

FLX reduced intake of saccharin solutions both in naive Long–Evans (Leander, 1987) and in alcohol-preferring FH/WJD rats (Kampov-Polevoy and Rezvani, 1997), possibly as a consequence of curbing the appetite. In line with such suggestion, Overstreet et al. (2007) found that FLX in a dose that reduced immobility time under conditions of FST did not affect alcohol consumption in FH/WJD rats, thus implying that the drug did not affect reward pathways. At the same time, Muscat et al. (1992) reported attenuation of stress-induced anhedonia by FLX in Lister rats. It might be problematic to dissociate between appetite- and pleasure-related effects of saccharin solution in rats. In fact, we previously reported the dissociation between calorie-free saccharin and calorie-containing sucrose intake in kindled Wistar animals (Mazarati et al., 2007). It is possible that in the Wistar strain saccharin consumption predominantly tests pleasure experience instead of appetite. If the latter is true, FLX could be expected to increase saccharin intake; however, because of high baseline saccharin preference in naive animals, such an effect might be difficult to detect.
**Regulation of serotonergic transmission by fluoxetine**

In line with previous reports (Thompson et al., 2004), in our studies FLX inhibited 5-HT turnover in the hippocampus of naive animals without affecting 5-HT concentration. Reduced 5-HIAA concentration after FLX treatment is due to the inhibition of 5-HT reuptake and, as discussed earlier, subsequent decrease of the intracellular fraction of transmitter available for monoamine oxidase-mediated metabolism of 5-HT. Furthermore, in post-SE animals FLX further inhibited already reduced 5-HT turnover. Such additive effect of SE and FLX treatment imply a common underlying mechanism, thus supporting our suggestion that reduction of 5-HIAA/5-HT ratio in animals with epilepsy might result from the inhibition 5-HT reuptake.

**Effects of fluoxetine on seizures and neuronal excitability**

There is some evidence that FLX might exert anticonvulsant effects. Thus, Albano et al. (2006) reported reduction of frequency of complex partial seizures in patients after introduction of FLX to conventional anti-epilepsy drug treatment. In the laboratory setting, Hernandez et al. (2002) reported inhibition of spontaneous recurrent seizures by FLX in post-SE animals. In our studies, we did not find statistically significant effect of FLX treatment on seizure frequency. However, in contrast to Hernandez et al. (2002), we did not examine the direct effect of FLX administration on seizures; instead seizure frequency was examined after completion of FLX therapy. Nevertheless, our treatment regimen resulted in a post-treatment decrease of brain excitability as measured by AD properties. This finding agrees with the data of Wada et al. (1995), that the anticonvulsant effects of FLX on seizures induced by hippocampal stimulation were observed only after chronic treatment, and persisted for 1 week after the end of FLX administration. Another possible reason as to why FLX administration failed to affect spontaneous seizures is that baseline seizures frequency in our study was relatively low, which could prohibit revealing modifying effects of therapeutic interventions. This low frequency of spontaneous seizures might be attributed to the younger age at which our animals were subjected to SE. Indeed, a positive correlation between the age of the precipitating insult and the subsequent frequency of spontaneous seizures has been reported (Sankar et al., 2000).

**Insights from correlation analysis**

Since statistical correlation does not imply causative relationship between the observed phenomena, the results of correlation analysis should be interpreted with caution. Nevertheless, our observations allow drawing several conclusions.

First, the frequency of spontaneous seizures translated into neither behavioural, nor serotonergic deficits. This finding was congruent with several clinical observations, in which alleviation of seizures through surgical resection of epileptic foci did not improve symptomatology of depression in epilepsy patients (Spencer et al., 2003; Wrench et al., 2004). Second, the increase of brain excitability which was revealed in afterdischarge tests, may contribute to depression-like behavioural impairments. Similar correlation was reported under conditions of kindling model of epilepsy (Mazarati et al., 2007). Third, correlation between biochemical alterations and forced swim behaviour suggests that hippocampal serotonergic dysfunction may also contribute to state of helplessness associated with depression. At the same time, lack of correlation between changes in serotonergic transmission in the hippocampus and the reduction of taste preference suggests the involvement of extrahippocampal and/or non-serotonergic mechanisms in modulating such behaviour (e.g. mesolimbic system and hypothalamus, Nestler and Carlezon, 2006). Fourth, statistical dissociation between afterdischarge properties and serotonergic transmission implies that these two changes may independently evolve and lead to the same set of depressive behavioural alterations. The latter suggestion emphasizes the complexity and multiplicity of the mechanisms of depression associated with epileptic state. Such complexity is further stressed by the absence of correlation between changes in forced swim and taste preference behaviours.

Assuming that the discussed statistical correlations indeed reflect several independent mechanistic links between changes in hippocampal excitability and behavioural impairments, and concurrently between serotonergic deficiency and behavioural disorders, the question remains whether altered afterdischarge properties and changes in serotonergic transmission have a common denominator. One important issue not explored in our study is a possible contribution of post-SE neuronal injury (as well as of an ongoing injury resulting from recurrent seizures) in the development of the reported physiological, biochemical and behavioural phenomena. Our previous studies using rapid kindling model (Mazarati et al., 2007) proved that behavioural symptoms of depression may develop in the absence of neuronal injury, since the latter is not found under conditions of rapid kindling (Mazarati, unpublished data). This observation, however does not exclude the possibility that in post-SE models, mechanisms of depression-like behaviour might differ from those in non-lesional model(s) of epileptic state. In the present study we did not examine spatial distribution of neuronal injury after SE, since brain tissue was used for biochemical assays. However, it is well established that pilocarpine SE leads to a wide-spread injury to the hippocampus, as well as to extrahippocampal and extralimbic areas (Cavalheiro et al., 2006), some of which are likely involved in the regulation of mood and mechanisms of depression.
Depression after status epilepticus

(Shumake and Gonzalez-Lima, 2003; Kanner, 2005; Kondziella et al., 2007). The contribution of neuronal injury in both epilepsy-associated depressive behaviour and deficient serotonergic transmission is quite possible; particularly, the failure of FLX to improve forced swim behaviour in post-SE animals may be due to the fact that this symptom is a result of neurodegeneration. However, the complexity of the mentioned connection requires separate line of experiments which should employ detailed histopathological (and/or functional) mapping.

Concluding remarks
Validation of animal models of comorbidity of TLE and depression is complicated by several factors. Both behavioural and biochemical correlates of depression are diverse and multifaceted; therefore selecting the best hallmarks is difficult, particularly because many of the putative signalling pathways are involved in both depression and epilepsy. Furthermore, clinical relevance of behavioural indicators of mood in experimental animals should be approached with caution. Thus, validation of animal models of comorbidity ideally should include comprehensive analysis of complex behaviours (e.g. sleep, reward, appetite, etc.), as well studies of various signalling candidates (e.g. hypothalamic–pituitary–adrenal axis, other monoamines, peptides, etc.). As an initial step in this direction, our study provides evidence that the post-SE model of TLE leads to behavioural (equivalents of despair and anhedonia) and biochemical (compromised 5-HT transmission) alterations suggestive of depression. Furthermore, resistance of symptoms of depression in epileptic animals to an antidepressant medication implies distinct mechanisms of depression in epilepsy, beyond alterations in serotonergic pathways. Finally, correlation analysis suggests that depression associated with epilepsy has multiple underlying mechanisms, which if proven true, should be considered in the development of therapeutic interventions.

Acknowledgements
This work was supported by the National Institutes of Health grants NS043409 and NS059505 (A.M.); NS046516 (R.S.), and the DAPA Foundation (R.S.).

References


