Altered taste preference and loss of limbic-projecting serotonergic neurons in the dorsal raphe nucleus of chronically epileptic rats

Gisela H. Maia\textsuperscript{a,b,d,e}, Joana I. Soares\textsuperscript{a,b,d}, Pedro A. Andrade\textsuperscript{a,b,d}, Juliana F. Leite\textsuperscript{e}, Liliana L. Luz\textsuperscript{a,b}, José P. Andrade\textsuperscript{c}, Nikolai V. Lukoyanov\textsuperscript{a,b,c,e,*}

\textsuperscript{a} Instituto de Investigação e Inovação em Saúde, Universidade do Porto, Portugal
\textsuperscript{b} Instituto de Biologia Molecular e Celular da Universidade do Porto, Porto, Portugal
\textsuperscript{c} Departamento de Anatomia, Faculdade de Medicina da Universidade do Porto, Porto, Portugal
\textsuperscript{d} Programa Doutoral em Neurociências, Universidade do Porto, Porto, Portugal
\textsuperscript{e} Medibrain—Centro de Estudos Neurofisiológicos, Vila do Conde, Portugal

\textbf{HIGHLIGHTS}

- Kainate-induced epilepsy in rats is associated with anhedonia.
- Epileptic rats show 5-HT cells loss in interfascicular part of the dorsal raphe.
- Depression in epilepsy may be related to loss of 5-HT neurons in the dorsal raphe.

\textbf{A R T I C L E  I N F O}

Article history:
Received 31 July 2015
Received in revised form 30 September 2015
Accepted 3 October 2015
Available online 9 October 2015

Keywords:
Epilepsy
Depression
Interfascicular nucleus
5-Hydroxytryptophan
Kainic acid
Stereology

\textbf{A B S T R A C T}

Mood disorders and major depression are frequently comorbid with epilepsy. While the nature of this comorbidity is not fully understood, multiple lines of evidence suggest that changes in serotonin (5-HT) neurotransmission may be an underlying mechanism. In this study, we tested the hypothesis that chronic epilepsy in rats can be associated with loss of 5-HT neurons in the dorsal raphe (DR) nucleus complex, the main source of 5-HT projections to the cerebral cortex, which would help to explain respective behavioral deficits. Epilepsy was induced using the kainate model of status epilepticus in adult Wistar rats. After a 3-month recovery period, all kainate-treated rats that had experienced status epilepticus showed spontaneous seizures and reduced sucrose preference (anhedonia), a core symptom of depression. No changes in the forced swim test were detected. The total numbers of 5-HT immunoreactive cells were estimated in all DR subdivisions of control and epileptic rats. Interestingly, epilepsy-related loss of 5-HT neurons (approximately 35\%) was observed only in the interfascicular part of the DR complex, which is known to innervate brain regions involved in depression. These findings support the notion that mental health impairments observed in epilepsy may be related to loss of a specific population of the DR 5-HT neurons projecting to limbic brain areas.

© 2015 Elsevier B.V. All rights reserved.

1. Introduction

Clinical evidence indicates that epilepsy can be accompanied by comorbid psychiatric disorders, such as anxiety, depression, and mood impairments [1–3]. In particular, it has been reported that the prevalence rates for major depression disorder (MDD) in patients with temporal lobe epilepsy (TLE) may reach 30–35\% and prevalence rates of mood disorders range between 24 and 72\% [for review, see Ref. 4]. Furthermore, a growing number of experimental studies have confirmed the presence of anxiety and depression-like behaviors in epileptic animals [5,6]. It has been hypothesized that seizure disorders and affective symptomatology can be interrelated via common neurobiological mechanisms, which include dysregulation of the hypothalamo–pituitary–adrenal (HPA) axis, changes in monoamine neurotransmission, neuroinflammation or neurodegenerative processes in specific brain regions [7,8]. However, the precise mechanisms underlying the comorbidity of epilepsy with mental health impairments remain to be clarified.

It has long been known that dysfunction of ascending serotonergic pathways is crucially implicated in psychiatric disorders, such as panic, depression, and suicide. Several lines of evidence...
support this viewpoint, including that (1) low levels of serotonin (5-hydroxytryptophan; 5-HT) metabolites were found in depressed suicides [9], (2) depletion of 5-HT in volunteers triggers relapse of depressive episodes [10], (3) affective disorders appear to be linked to changes in the activity of serotonin transporter (SERT) [11], and (4) treatment with 5-HT reuptake inhibitors mitigates depressive symptoms, at least in a subpopulation of patients [12]. In addition, in depressed patients a number of structural defects were observed in the dorsal raphe nucleus (DRN), the brainstem region which provides the majority of cortical serotonergic fibers. They include an overall neuron number deficit [13,14], reduced cross-sectional area of DRN in MDD, but increased in depressed suicides [15], loss of SERT-expressing cells [16], increased [17] or unaltered [18] number of neurons immunoreactive to TPH (tryptophan hydroxylase, enzyme required for 5-HT synthesis), and changes in binding properties of the presynaptic serotonin autoreceptor 5-HT1A [19,20].

Finally, that depressive disorders are characterized by respective changes in the postsynaptic 5-HT receptors located in target corticolimbic brain areas implicated in affective functions has been also reported [21,22].

It is perhaps not surprising that many of the above-mentioned serotonergic abnormalities, particularly changes in 5-HT receptor binding, have also been found in epilepsy patients with comorbid depression and mood disorders [1,23,24]. With respect to animal studies, Mazarati et al. reported that the induction of epilepsy in rats, in addition to producing depression-like behaviors, results in compromised neurotransmission in the DRN—hippocampal serotonergic pathway [6]. However, whether or not epilepsy-related depression is likewise attributable to structural alterations in the DRN is still an open question. In this study, we hypothesized that chronic epilepsy in rats can be associated with loss of serotonin-producing neurons in the DRN, which would help to explain the compromised raphe-hippocampal transmission as well as respective behavioral impairments. To address this issue, we estimated the total number of 5-HT-immunoreactive neurons in all subdivisions of the DRN of control and chronically epileptic rats. Epilepsy was induced using the kainate model of status epilepticus. Behavioral changes were assessed using two common tests for depression in rodents, the forced swim test and the sucrose preference test.

2. Material and methods

2.1. Ethical statement

The handling and care of the animals were conducted according to the “Principles of laboratory animal care” (NIH publication No. 86–23, revised 1985) and Directive 2010/63/EU of the European Parliament and of the Council of 22 September 2010 on the protection of animals used for scientific purposes. The experimental protocol has been approved by the Ethics Committee of the Faculty of Medicine of Porto and the General Veterinary Direction (03.04.2012) for the FCT application grant PTDC/SAU-NSC/115506/2009. All efforts were made to minimize the number of animals used and their suffering.

2.2. Animals and treatments

Male Wistar rats, maintained individually under standard laboratory conditions, were used in this study. At 10 weeks of age, they were randomly divided into two groups: KA group (n = 10) and control group (n = 8). In the first group, the rats were injected with 9.5 mg/kg of KA (i.p., Sigma) to induce convulsive SE, which was defined as the appearance of behavioral symptoms corresponding to stage 3, 4 or 5 seizures on the Racine scale [25], i.e., bilateral forelimb clonus, rearing, and rearing with falling. Rats in this group initially demonstrated numerous wet-dog-shake seizures, which culminated in SE lasting 3–6 h. The animals were periodically injected with saline (s.c.) during the first 48 h of the recovery period. On the following days, the rat diet was supplemented with apples that were sliced and left at the bottom of the cage. The rats that refused to eat or drink were hand-fed using a plastic syringe. Rats in the control group were injected with saline alone.

2.3. Surgery, behavioral monitoring and electroencephalographic (EEG) recording

Following the treatments, all animals were given a 3-month recovery period. In the beginning of the third month, the rats received stereotaxic surgery conducted under isoflurane anesthesia. Rats were placed in a Kopf stereotaxic apparatus and the scalp was incised along the midline and retracted to the side. Three epidural stainless steel electrodes (E363/20 Plastics One Inc., Roanoke, VA, USA) were implanted above the right prefrontal cortex (3 mm anterior to bregma, 2 mm lateral to midline), left parietal cortex overlaying the hippocampus (4.3 mm posterior to bregma, 2.0 mm lateral to midline) and right occipital cortex (1.0 mm anterior to lambda, 3.5 mm lateral to midline). Two additional screw electrodes were placed over the cerebellum to serve as a reference electrode and as a ground. All of the electrodes were connected to a plastic pedestal (Plastics One, Inc.) that was cemented to the skull using dental acrylic.

Starting from the sixth week following the treatments, the rats were daily (except weekends) observed for spontaneous behavioral seizures during 2 h intervals between 09:00 h and 11:00 h by a person blind to treatment groups.

Video-EEG recording was performed in the last two weeks of the recovery period in order to confirm the presence of electrographic seizures in KA-treated rats. Recordings were simultaneously performed in pairs of rats randomly selected from each group and were 24 h in duration. For this purpose, rats were placed in Plexiglas cages where they could move freely (one rat per cage). EEG activity was continuously registered from the epidural electrodes using the Truscan-32 acquisition system (Deymed Diagnostic, Hronov, Czech Republic) connected to computer via a universal serial bus port amplifier (Deymed Diagnostic). Recordings were sampled at 256 Hz, high- and low-pass-filtered at 1 Hz and 100 Hz, respectively, and stored on the computer disk for offline seizure review using the TruScan Explorer software (Deymed Diagnostic). The behavior of the animals was simultaneously recorded using a digital video camera Sony DCR-SR58E (Sony Corporation, Japan), which was positioned above the cages. The video-EEG recordings were analyzed by a study-blinded clinical neurophysiologist. Electrographic seizures were defined by the presence of sustained spike and poly-spike activity longer than 3 s. Behavioral seizures were defined according to the Racine scale.

2.4. Behavioral testing

Three months after induction of SE, the animals were handled 3 min per day during 5 days and subjected to the forced swim test (FST) and sucrose preference test (SPT). The rats were counterbalanced so that, in each group, half received the FST first and the other half received the SPT first. There was a 7-day interval between the two tests.

The procedure for the FST was essentially as described by Porsolt et al. [26]. The apparatus used in this task consisted of a transparent glass cylinder, 20 cm in internal diameter and 50 cm in height. It was filled with tap water (25°C) to a depth of 30 cm. On the first day, the rats were forced to swim in the apparatus for 15 min. During this session, animals progressively reduce their attempts to escape from the water and spend more time without movement.
This immobility is believed to reflect a learned behavioral despair, which is characteristic of depression. The following day, rats were retested for 5 min under identical conditions and their behavior was recorded using the digital video camera Sony DCR-SR58E positioned above the cylinder. The video files were then analyzed by a person unaware of the treatment condition using the VLC media player (VideoLAN, France). Immobility (defined as the absence of any movement other than that required for keeping the head and nose above the water) was scored if the rat remained inactive for at least 3 s. The percentage of accumulated time spent inactive during the 5 min testing session was calculated.

In the SPT, rats were tested for a decreased sensitivity to reward (anhedonia), a core aspect of depression. The entire procedure was performed over 6 days, during which rats were given the choice to drink a liquid from one of two bottles placed side-by-side into their home cages. The animals were not deprived of food or water before the experiment. During the first days of the procedure, rats were habituated to the two-bottle configuration of their home cages and to the taste of sucrose in order to minimize the effects of novelty in the results. Thus, on the first day (starting at 08:00 h), each cage was supplied with two identical bottles, each containing 200 ml of regular tap water. The following day (24 h later), water was replaced in one of the bottles with a 0.75% sucrose (Sigma–Aldrich) solution. On the third and fourth days, both bottles contained 0.75% sucrose solution, which was replaced with tap water in the beginning of the fifth day. On day 6 (testing day), rats were given the choice to drink either water or a 1% sucrose solution. Throughout the entire 6-day period, all bottles were weighed at 12 h intervals in order to estimate the amount of liquid consumed by rats. To prevent possible effects of side preference in drinking behavior, the position of the bottles in the cages was interchanged every 6 h. Fresh solutions were prepared every 24 h. The sucrose preference index was calculated as a percentage of the sucrose solution intake relative to the total volume of fluid intake.

2.5. Tissue preparation

Following the completion of the behavioral experiments, animals were deeply anesthetized with pentobarbital (90 mg/kg) and injected intracardially with 0.1 ml of a heparin solution, followed by 1 ml of 1% sodium nitrite in saline. Then, they were perfused transcardially with 150 ml of 0.1 M phosphate buffer (pH 7.4) for vascular rinse, followed by 250 ml of a fixative solution containing 4% paraformaldehyde in phosphate buffer. The brains were removed from the skulls, immersed for 2 h in the fixative, and infiltrated during 36 h in 10% sucrose solution at 4 °C. After the forebrain regions were trimmed away, the remaining tissue blocks were mounted on a vibratome and sectioned in the coronal plane at 40 μm. Every second section cut through the midbrain–hindbrain region was collected using a systematic random sampling procedure [27] and stored until use at −20 °C in cryoprotectant (30% sucrose, 30% ethylene glycol, 0.25 mM polyvinylpyrrolidone in PBS).

2.6. Immunostaining for 5-HT

Sections were washed twice in PBS, treated with 3.5% H2O2 for 7 min to inactivate endogenous peroxidase and incubated during 72 h at 4 °C with the polyclonal rabbit antibody against 5-HT (Neuromark Scientific, United Kingdom; 1:15,000 dilution in PBS). Thereafter, the sections were washed twice and incubated with biotinylated anti-rabbit antibody (Vector Laboratories, Burlingame, CA, USA; 1:400 dilution in PBS). Sections were then treated with avidin–biotin peroxidase complex (Vectastain Elite ABC kit, Vector Laboratories; 1:800 dilution in PBS). In the two last steps, the incubation was carried out for at least 1 h at room temperature. Following treatment with the peroxidase complex, sections were incubated for 10 min in 0.05% diaminobenzidine (Sigma) solution to which H2O2 (0.01%) was added. Sections were rinsed with PBS for at least 15 min between each step. To increase the tissue penetration, Triton X-100 (0.05%) was added to PBS that was used in all immunoreactions and washes. Specificity of the immune reactions was controlled by omitting the incubation step with primary antisera. All immunochemical reactions and washings described above were carried out simultaneously in four 12-well tissue culture plates, 6 sections in each well, to assure that staining of the sections from all animals analyzed was performed under identical conditions. Following termination of the staining procedures, sections were mounted on gelatin-coated slides and air-dried. They were then dehydrated in a series of ethanol solutions (70%, 90%, 96% and 100%), cleared with undiluted xylene, and coverslipped using Histomount (National Diagnostics, Atlanta, GA, USA).

2.7. Anatomical definition of dorsal raphe nucleus complex

The dorsal raphe (DR) nucleus complex belongs to the rostral group of serotonergic raphe nuclei (B6–B7 groups according to the original classification of Dahlström and Fuxe [28]) and is located in ventral portion of the mesencephalic periaqueductal gray matter and rostral pons [29]. It consists of five cytoarchitectonically distinct subdivisions, the dorsal (DRD), ventral (DRV), ventrolateral (DRVL), interfascicular (DRI) and caudal (DRC) nuclei [30,31]. The boundaries between the nuclei can be delineated in the brainstem sections immunostained for 5-HT markers (Fig. 1) using previously described cytoarchitectonic criteria [29,32]. Thus, DRV nucleus is composed of relatively small, round, and densely packed cells [32]. It is continuous with more dorsally located DRD nucleus, whose cells are less densely packed, and borders ventrally the DRI nucleus, which is composed of larger, spindle-shaped neurons with vertically oriented dendritic arbors [29,32]. The DRI nucleus, in turn, borders dorsosagittally the DRC nucleus containing small and medium-sized round neurons [32]. In our material, immunostained cells located in the outmost ventral (interfascicular) part of the rostral DRV nucleus had neuronal morphology similar to those of the DRI nucleus and distinct from those of the remaining part of DRV. Therefore, for the sake of morphological consistency, the interfascicular part of the DRV was considered as a rostral extension of the DRI nucleus [29]. Finally, the two laterally located and wing-shaped DRVL nuclei are populated with very large multipolar neurons [31,32]. These cells are intermingled with the serotonin neurons of the ventrolateral periaqueductal grey (VLPG; Fig. 1, [33]). Therefore, for the purposes of this study, all 5-HT-labelled cells of the VLPG region were counted together with DRVL neurons. The stereotaxic coordinates of the DR subdivisions with reference to a standard rat brain atlas of Paxinos and Watson [34] have been previously determined [31,35]. In this study, we estimated the rostrocaudal boundaries of the five DR nucleus at approximately the following coordinates (relative to bregma): DRD and DRV, from −7.37 to −8.45; DRVL, from −7.64 to −8.27; DRI, from −7.64 to −8.54; DRC, from −8.45 to −9.26.

2.8. Morphological analysis

The brainstem sections immunostained for 5-HT were visualized using an Olympus BX-53 microscope equipped with a color digital camera and a computer-controlled motorized stage system (MBF Bioscience, Williston, USA). The boundaries of each region of interest were consistently defined at all levels along the rostrocaudal axis of the brainstem with a 40× objective lens and using the anatomical criteria described above. The total number of neurons in each DR nucleus was estimated using the optical fractionator probe [36] of the Stereo Investigator software (MBF Bioscience). Cell
counting was carried out with a 100× oil-immersion lens. Beginning at a random starting position within the region of interest, counting frames were systematically sampled using a raster pattern procedure. Tissue thickness was estimated at each counting frame and guard zones of 2 μm were implemented. The nucleus of the neurons was used as the counting unit. The coefficients of error (CE) were calculated according to Gundersen et al. [27]. The stereological parameters used in the estimations of neuron numbers are summarized in Table 1.

2.9. Statistical analysis

Before conducting statistical comparisons, data were tested for normality using the Shapiro–Wilks W test. Because all data samples satisfied the assumption of normality (p > 0.05), they were analyzed for statistical significance using the parametric statistics tests. To correct for multiple comparisons, the neuron numbers in the DR subdivisions were analyzed using MANOVA and the Rao’s R parameter was calculated. When appropriate, the Tukey HSD test was then applied. The remaining data were analyzed using Student’s t test. Data derived from the FST and SPT, which were presented in a counterbalanced manner, were collapsed across order of presentation. Differences were considered as significant at the p < 0.05 level.

3. Results

3.1. Monitoring during the recovery period

Two rats have died within 24 h following the treatment with KA and 1 rat has died during the first month of the recovery period.

No. sections—mean number of sections per animal, h—height of the optical dissector, ΣQ—total number of neurons counted in each nucleus, CE (N)—mean coefficients of error.

Table 1
Summary of the stereological parameters used in the estimation of total neuron numbers.

<table>
<thead>
<tr>
<th>DRD</th>
<th>12</th>
<th>10</th>
<th>187</th>
<th>0.09</th>
</tr>
</thead>
<tbody>
<tr>
<td>DRV</td>
<td>12</td>
<td>10</td>
<td>212</td>
<td>0.08</td>
</tr>
<tr>
<td>DRVL (left)</td>
<td>8</td>
<td>10</td>
<td>195</td>
<td>0.08</td>
</tr>
<tr>
<td>DRVL (right)</td>
<td>8</td>
<td>10</td>
<td>198</td>
<td>0.07</td>
</tr>
<tr>
<td>DRI</td>
<td>12</td>
<td>10</td>
<td>224</td>
<td>0.07</td>
</tr>
<tr>
<td>DRC</td>
<td>11</td>
<td>10</td>
<td>175</td>
<td>0.09</td>
</tr>
</tbody>
</table>

One control rat died during surgery. Thus, the final group sizes for the KA-treated rats and sham-treated controls were n = 7. No signs of behavioral seizures were detected in KA group during the first 6 weeks of the recovery period. However, in the second half of the observation period, all rats from this group showed spontaneous stage 1–2 seizures on the Racine scale, i.e. repeated mouth and facial movements, and mild (unilateral) forelimb clonus. At least 2 spontaneous generalized behavioral seizures of stage 3–5 on the Racine scale were observed in a total of 4 KA-treated rats. Offline

Fig. 1. Representative photomicrographs of coronal sections from the midbrain and rostral pons of a control rat showing main subdivisions of the dorsal raphe nuclear complex at 4 different rostrocaudal levels. The sections were immunostained using a primary antibody for 5-HT. The boundaries of the dorsal raphe nuclei, shown by dotted lines, were delineated on the basis of previously established cytoarchitectonic criteria and neuronal morphology. In each panel, the numbers indicate approximate distances (in mm) from bregma. The total number of immunolabelled cells was counted in each of the dorsal raphe nuclei: the dorsal, DRD; ventral, DRV; ventrolateral, DRVL; interfascicular, DRI; caudal, DRC. Other abbreviations: fourth ventricle, 4V; cerebral aqueduct, aq; medial longitudinal fasciculus, mlf; median raphe nucleus, MnR; decussation of superior cerebellar peduncle, scp. Scale bar = 200 μm.

Fig. 2. Representative EEG recording of an awake kainate–treated rat approximately 3 months post status epilepticus. The recording electrodes were located over the left parietal cortex overlaying the hippocampus (Ch1), right prefrontal cortex (Ch2) and right occipital cortex (Ch3). Inset (A) depicts the beginning of a spontaneous seizure with a high-amplitude spike (*). Inset (B) shows high-frequency spiking activity (14–19 Hz) with increased amplitude of EEG signals. The seizure lasted for 19 s and ended with the onset of a post-ictal period characterized by a decrease in the amplitude of EEG signals (C). Electrographic seizures of this type were typically accompanied by the stage 2–4 behavioral seizures on the Racine scale, that is, unilateral and bilateral forelimb clonus or forelimb clonus with rearing.
review of the video-EEG recordings, which were performed during the last week of the recovery period, revealed the presence of seizure-like activity in all KA-treated rats (Fig. 2). The electrographic seizures were often associated with behavioral seizure manifestations, such as stereotypic orofacial movements and periods of sudden immobility. In some cases, they were coincident with the onset of generalized motor seizures, including bilateral forelimb clonus, rearing and tonic-clonic convulsions. No behavioral or EEG seizures were observed in the control group.

3.2. Behavioral changes in rats with KA-induced epilepsy

The results obtained on the second day of the FST are shown in Fig. 3. Somewhat unexpectedly, KA-treated rats spent less time immobile than control rats. However, this effect was not statistically significant (p > 0.05, n = 7 in each group). Similarly, latencies to become immobile in KA-treated rats were not significantly different from those of control rats (data not shown). Thus, the present results do not provide evidence that epileptic rats used in this study displayed increased behavioral despair in the FST.

On the testing day of the SPT, both control sham-operated rats and KA-treated rats ingested more sweetened water than regular water (Fig. 3). However, the preference for sucrose solution shown by rats in epilepsy group was inferior to that of sham-treated rats (63% vs. 80%, respectively). The difference in the taste preference index between the two groups was statistically significant (p < 0.01), suggesting that induction of chronic epilepsy with KA was accompanied by the development of anhedonic-like behaviors.

3.3. Total neuron numbers in the dorsal raphe nuclei

Visual examination of the 5-HT-stained sections revealed no gross neuroanatomical abnormalities in the DR nuclear complex of epileptic rats. However, the cell density in the DRI region, populated by dorso-ventrally oriented spindle-shaped neurons (Fig. 4A), appeared to be smaller in KA-treated rats when compared to control rats (Fig. 4B,C). The stereological estimates of the total numbers of neurons in all DR subdivisions are shown graphically in Fig. 5. MANOVA of these data yielded a significant main effect of treatment (RaoR8 = 3.82, p < 0.05). However, post-hoc tests for multiple comparisons did not reveal significant effect of treatment on the number of 5-HT positive neurons in the DRD, DRV, DRVL and DRC nuclei (p > 0.05). In contrast, post-hoc tests detected a significant group effect on the number of 5-HT neurons in the DRI nuclei (p < 0.01). In fact, epileptic rats had, in the average, approximately 35% less 5-HT-stained cells in DRI region when compared to control rats. However, the total numbers of 5-HT containing cells in the DR nuclear complex (summed across all nuclei) did not significantly differ between the two groups (p > 0.05, Student’s t test). There were no significant differences in the numbers of 5-HT cells between the left and right subdivisions of the DRVL nucleus.

4. Discussion

The most frequent psychiatric disorders that can be comorbid with epilepsy are mood impairments and major depression [7]. Neuroimaging studies in TLE patients have demonstrated noticeable changes in binding properties of 5-HT receptors in the hippocampal, prefrontal and DR regions [1,23,24], which fits with the serotonergic hypothesis of depression. Furthermore, studies in epileptic rats showed compromised serotonergic neurotransmission between the DR and the hippocampus [6]. The present study yielded a new important finding related to this hypothesis, namely, that chronic epilepsy in rats is associated with significant loss of 5-HT-containing neurons in the DR nuclear complex. This finding was obtained in animals in which behavioral assessments revealed reduced reward sensitivity (anhedonia), one of the core symptoms of depression. Interestingly, the loss of 5-HT neurons was region/modality-specific, that is, it was observed in only one of the DR subdivisions, the DRI nucleus, known to innervate brain regions involved in depression [for review, see Refs. 31,37,38].

There is some degree of inconsistency in the literature regarding the presence of depression-like symptoms in epileptic animals. In the FST, in particular, some studies revealed increased immobility of rats with spontaneous seizures [6,39–41], whereas reports from other groups, including the present report, failed to confirm this effect [42–45]. The behavioral data obtained with the SPT are more consistent between studies from different labs, with all showing reduced taste preference in epileptic animals [5,6,41]. In the present study, reduced responsiveness to reward was observed in all rats with confirmed spontaneous seizures. Behavioral despair, presumably underlying the immobility of rodents in the Porsolt FST, is related, partly at least, to cognitive aspects of depression [46]. KA model of epilepsy is associated with large lesions of the brain regions implicated in cognitive processes [47], which may thus account for the seemingly unimpaired performance of epileptic rats in this test. This explanation [43,44] predicts that the Porsolt test is more likely to reveal depression-like behaviors in animal models characterized by relatively small lesions of the amygdalohippocampal area, which is probably the case for the studies demonstrating increased forced swim immobility in epileptic animals [6,39–41]. Reward processing is mainly mediated by the striato– pallidal system, even though other brain areas, such as orbital and medial prefrontal cortices are also involved [48]. Therefore, detection of anhedonia in subjects with severe damage to the hippocampus is likely to be less of a problem. Considered together, the current data strengthen the evidence that induction of chronic epileptic state in animals results in development of behavioral symptomatology compatible with human depression disorders.

Following behavioral testing, we compared the total numbers of the DR serotonergic neurons between the control group and the epilepsy group. The results of pilot studies performed in our lab surprisingly revealed no apparent difference between the two groups when cell counts were done in the DR nuclear complex as a whole. However, neurons of different DR subdivisions project to and receive inputs from very distinct brain areas [49,50,51, for review, see Refs. 31,52], raising the possibility that they may be differentially affected in epilepsy. Therefore, in this study, we compared the 5-HT neuron numbers estimated separately in each of the DR nuclei. We have found that, indeed, epileptic condition is associated with an approximately 35% loss of 5-HT-containing neurons in the DRI nucleus, whereas no epilepsy-related changes were detected in the other DR subdivisions. The fact that the total numbers of 5-HT cells of the DR nuclear complex as a whole again did not differ between the two groups is, perhaps, not surprising, taking into consideration that DRI neurons, according to our estimates, constitute only approximately 15% of all DR neurons. Yet, despite this small contribution of DRI 5-HT cells to the overall DR cell population, their loss may be of considerable importance to neuropathology of affective disorders. Indeed, previous neuroanatomical studies have shown that DRI neurons directly project to various limbic regions known for their involvement in emotion processing, notably the hippocampal formation, amygdala, ventromedial prefrontal cortex and medial thalamus [31,37,38]. It has been previously reported that stimulation of the DR area in close proximity to DRI nucleus evokes 5-HT release in the hippocampus and that this response is compromised in epileptic rats [6]. Thus, our histological results offer a plausible explanation for the depressive-like behavior of epileptic animals observed in this and other [5,39–41] studies and for its physiological correlate, that is, the compromised raphe-hippocampal neurotransmission [6].
It has been previously proposed that the mesolimbic serotonergic projection system centered in the DRI nucleus is dysfunctional in patients suffering from MDD and that the beneficial effects of the serotonin reuptake inhibitors may be related to their capacity to increase neurotransmission of DRI neurons [37]. This idea is based not only on the existence of direct, often reciprocal anatomical connections between the DRI nucleus and the array of limbic structures implicated in the physiopathology of depression, but also on the findings that DRI neurons are strongly activated by warm or cold temperature signals [53,54] and by a peripheral immune challenge [55], the latter being associated with an increase in serotonin metabolism in the prefrontal cortex and with antidepressant-like behavioral effects [56]. Also consistent with this idea, DRI nucleus appears to play an important role in an extended DR neuronal circuit, additionally involving the DRV and DRVL nuclei, which is responsible for the inhibition of panic- and anxiety-like responses [57]. Nevertheless, further experiments are required to validate this approach in other models. In this respect, our study provides the first evidence that depressive-like symptoms in epileptic rats can be directly related to selective loss of serotonin neurons in the DRI nucleus.

Although the levels of neurotrophic factors can be locally increased in epileptic tissue as a result of ongoing seizure activity, both hippocampal sclerosis in TLE and severe damage to the temporal lobe structures in epilepsy models create conditions in which the overall production of neurotrophins is likely to decline in areas

Fig. 3. Behavioral changes shown by KA-treated rats on the forced swim test (FST, left panel) and sucrose preference test (SPT, right panel). Although epileptic rats spent somewhat less time immobile than control rats in the FST, this effect was not statistically significant (p > 0.05). In the SPT, the sucrose preference index in epilepsy group was significantly lower when compared to control group (p < 0.01). Columns represent means, and vertical bars represent 1 SD. n = 7 in each group.

Fig. 4. Representative high-power images of coronal sections through the dorsal raphe interfascicular region (DRI) of a control rat (A, B) and of an epileptic rat (C). The sections were immunostained using a primary antibody for 5-HT. Arrows in (A) point to large and densely stained spindle-shaped cells characteristic of serotonergic neurons of this area. Note that the density of the 5-HT-immunoreactive cells appears to be lower in DRI of the epileptic rat (C) when compared with the control rat (B). Scale bar = 25 μm (A) and 40 μm (B, C).
targeted by DRI afferents. Shrinkage of the hippocampal volume [58,59] and reduced brain-derived neurotrophic factor (BDNF) levels [60,61] have also been reported in patients with MDD. BDNF is capable of promoting the survival of serotonin neurons and of stimulating the sprouting of their axons [62,63]. Postnatal ablation of BDNF in conditional mutant mice results in a significant decrease in 5-HT levels and dramatic deficits in 5-HT2A-mediated neurotransmission in the DR [64]. However, no serotonin cell loss was noticed under this condition. Another mechanism, which may also contribute to functional deficits in 5-HT neurons, is dysregulation of the HPA axis associated with chronic stress exposure. Chronic stress is a major risk factor for anxiety and depression disorders [65], and HPA axis dysregulation, characterized by elevated circulating levels of glucocorticoids, is well documented in patients with TLE and MDD [2,7], aside from respective animal models [8,39]. Chronic stress and glucocorticoids are also known for their ability to reduce BDNF levels in the cerebral cortex, including the hippocampus [66,67]. Further, given that glucocorticoids are capable of modulating the functional state of the presynaptic 5-HT1A autoreceptor [40,68], it has been proposed that epilepsy-related perturbations in the HPA axis can decrease DR serotonin synthesis thus rendering the entire raphe-hippocampal pathway dysfunctional [8]. Interestingly, it has been reported that corticotropin-releasing factor (CRF), a key mediator of the stress response and regulator of the HPA axis [69], selectively activates a subset of 5-HT neurons predominantly located in the DRI nucleus and ventral part of the DRV nucleus [70], suggesting that these cells can be particularly sensitive to stress-related stimuli [71,72]. However, whether and what changes in DRI neurons may occur due to chronically increased CRF signaling is unknown. Altogether, it appears that stress-related factors combined with lack of sufficient trophic support negatively impact functionally-specific populations of DR neurons, which can lead to strong inhibition or, perhaps, permanent disabling of serotonin synthesis in a portion of them. Because the prevalence rates of depression and mood disorders are extremely high in both patients with epilepsy and in the general population, additional studies of the mechanisms underlying these effects are urgently needed in order to ascertain reliable molecular targets for future therapeutic treatments.

Acknowledgments

This work was supported by FEDER Funds through the Programa Operacional Factores de Competitividade—COMPETE and National Funds through FCT—Fundaçao para a Ciencia e a Tecnologia within the scope of the Project PTDC/SAU-NSC/115506/2009 (FCOMP-01-0124-FEDER-015919).

References

model temporal sampling
nucleus, people, M.W.
M.J. H.W.M.
R.D. M.
M.D.
dorsomedial sensitive
Psychopharmacol.
receptor
Mann, C.A.
Price, M.A.
Biol.
neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neurons, neuron...


