Epilepsy & Behavior 31 (2014) 267–275

Contents lists available at ScienceDirect

Epilepsy & Behavior

journal homepage: www.elsevier.com/locate/yebeh

Behavioral impairments in rats with chronic epilepsy suggest comorbidity between epilepsy and attention deficit/hyperactivity disorder

Eduardo Pineda [a], J. David Jentsch [b,c], Don Shin [a], Grace Griesbach [d], Raman Sankar [a,e,f], Andrey Mazarati [a,f,*]

[a] Department of Pediatrics, David Geffen School of Medicine at UCLA, USA
[b] Department of Psychology, David Geffen School of Medicine at UCLA, USA
[c] Department of Psychiatry and Biobehavioral Sciences, David Geffen School of Medicine at UCLA, USA
[d] Department of Neurosurgery, David Geffen School of Medicine at UCLA, USA
[e] Department of Neurology, David Geffen School of Medicine at UCLA, USA
[f] UCLA Children's Discovery and Innovation Institute, USA

ARTICLE INFO

Article history:
Received 3 September 2013
Revised 29 September 2013
Accepted 2 October 2013
Available online 18 November 2013

Keywords:
Epilepsy
Attention deficit/hyperactivity disorder
Depression
Norepinephrine
Serotonin
Lateralized reaction-time task

ABSTRACT

Attention deficit/hyperactivity disorder (ADHD) is encountered among patients with epilepsy at a significantly higher rate than in the general population. Mechanisms of epilepsy–ADHD comorbidity remain largely unknown. We investigated whether a model of chronic epilepsy in rats produces signs of ADHD, and thus, whether it can be used for studying mechanisms of this comorbidity. Epilepsy was induced in male Wistar rats via pilocarpine status epilepticus. Half of the animals exhibited chronic ADHD-like abnormalities, particularly increased impulsivity and diminished attention in the lateralized reaction-time task. These impairments correlated with the suppressed noradrenergic transmission in locus coeruleus outputs. The other half of animals exhibited depressive behavior in the forced swimming test congruently with the diminished serotonergic transmission in raphe nucleus outputs. Attention deficit/hyperactivity disorder and depressive behavior appeared mutually exclusive. Therefore, the pilocarpine model of epilepsy affords a system for reproducing and studying mechanisms of comorbidity between epilepsy and both ADHD and/or depression.

1. Introduction

Attention deficit/hyperactivity disorder (ADHD) represents one of the most common comorbidities of epilepsy: its prevalence among patients with epilepsy is >20% as opposed to 5% in the general population [1–4]. Although an epidemiological connection between epilepsy and ADHD is well established, mechanisms of the comorbidity (as well as mechanisms of ADHD as a stand-alone disease) remain poorly understood. Clinical studies of the ADHD–epilepsy connection are complicated because of its bidirectional nature [4,5], and thus by difficulties with separating causes from consequences. With this regard, animal models may be useful, as they afford reproducible systems in which either epilepsy or a neurobehavioral disorder of interest represents an unequivocal and an on-demand primary pathology; furthermore, epilepsy comorbidities can be examined in the absence of iatrogenic neurobehavioral abnormalities, the latter being attributed to some antiepileptic drugs, such as phenobarbital [6,7], gabapentin [8,9], valproate [10,11], and topiramate [12,13]. There has been growing evidence that rodent models of acquired chronic epilepsy are not only characterized by spontaneous recurrent seizures but also produce a spectrum of neurobehavioral impairments, some of which have been validated as experimental equivalents of neurobehavioral comorbidities of epilepsy [14–19].

The present work originated from our findings that rats with chronic epilepsy develop specific behavioral, biochemical, and neuroendocrine impairments indicative of depression [20–23]. Further analysis of animals' behavior suggested that some animals exhibited elements of impulsivity instead of depressive behavior. This led us to employ a specific ADHD-relevant assay [24–26] in order to explore whether these animals indeed develop ADHD-like abnormalities. Furthermore, considering that central noradrenergic dysfunction has been implicated in mechanisms of both ADHD [27–30] and depression [31–34], we explored whether epileptic animals, along with/instead of the already established suppression of serotonin (5-HT) transmission in the raphe nucleus-forebrain ascending pathway [20,23], also exhibit dysfunction in the ascending norepinephrine (NE) pathway.

Abbreviations: 5-HT, serotonin; ADHD, attention deficit/hyperactivity disorder; EPMT, elevated plus maze test; FCV, fast cyclic voltammetry; FST, forced swimming test; LC, locus coeruleus; LRTT, lateralized reaction-time task; NE, norepinephrine; PFC, prefrontal cortex; RN, raphe nucleus; SE, status epilepticus; TLE, temporal lobe epilepsy.

* Corresponding author at: Dept. of Pediatrics, Neurology Division, D. Geffen School of Medicine at UCLA, Box 951752, 22-474 MDCC, Los Angeles, CA 90095-1752, USA. Fax: +1 310 825 5834.
E-mail address: mazarati@ucla.edu (A. Mazarati).

1525-5050/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yebeh.2013.10.004

2. Methods

2.1. Subjects

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

2.2. Induction of chronic epilepsy

Animals received an intraperitoneal injection of LiCl (128 mg/kg, Sigma, St. Louis, MO) and 24 h later, a subcutaneous injection of pilocarpine HCl (40 mg/kg, Sigma). The resulting status epilepticus (SE) was characterized by continuous secondarily generalized clonic and clonic–tonic seizures starting from 10 to 15 min after pilocarpine injection. One, four, and eight hours after seizure onset, rats were injected with diazepam (10 mg/kg) and phenytoin (50 mg/kg) in order to limit neuronal injury and to mitigate subsequent chronic epilepsy [20,22]. In control animals, pilocarpine was substituted with saline.

Beginning from the fourth week after SE, animals underwent four weeks of continuous video monitoring in order to confirm the presence of chronic epilepsy and to select subjects for further studies. Animals were held individually in their cages with free access to food and water (until the commencement of the ADHD test) and 12-hour light-12 h dark cycle (during the latter, LED light was used as a light source). Video was acquired using PC33CHR-4G digital cameras connected to a DMR41DVD Linux-based computer used for data storage. Video was analyzed offline for the presence of secondarily generalized clonic–tonic seizures, corresponding to stages 4–5 on the Racine scale [35]. Only those animals which showed between 1 and 5 seizures per week were used for behavioral assays. This insured the presence of epilepsy but at the same time limited seizure frequency to a level that rendered those animals amenable to further behavioral tests [20,22].

2.3. Forced swimming test (FST)

Forced swimming test is used as a test for hopelessness/despair (which is a key symptom of depression), whereby the animal's ability to effectively cope with an inescapable stressful situation is quantified [36–38]. The test was conducted at the end of video monitoring. Forced swimming test consisted of a single five-minute swimming session in a tank filled with water at 22–25 °C [20,37,39]. Swimming behavior was videotaped and analyzed offline. Three types of behavior were analyzed (Supplementary data video): (i) active swimming, representing attempts to escape from the tank: swimming along the walls, climbing on the walls of the tank (effective coping); (ii) immobility: movements were limited to maintaining head above the water, without attempts to escape (no coping); and (iii) noncued struggle: actively treading water away from the walls, without attempts to escape (ineffective coping). Swimming sessions were videotaped; cumulative duration of immobility and noncued struggle was calculated by two independent observers. Based on our earlier report, the increase of immobility time in epileptic animals was designated as either moderate, when it did not exceed 100 s (i.e., no more than 30% of total test duration) or severe, when its cumulative duration was 100 s or more [23]. For each parameter, the average duration from the two observations was used. Cumulative duration of active swimming duration was derived by subtracting the sum of immobility and struggling from 300 s (i.e., total test duration).

2.4. Lateralized reaction-time task (LRTT)

Lateralized reaction-time task was used to examine animals' impulsivity and attention [24–26]. The test started within one week after the FST. Prior to the inception of testing, ad libitum feeding was ended; instead, food was provided in limited amount to the rats once per day. The amount that was fed to each subject was individualized in order to reduce their weights to 80–85% of their initial, ad libitum feeding weights and to maintain it at this level through the period of testing. Once testing began, this daily feeding was provided 1–3 h after the completion of testing.

2.4.1. Behavioral testing apparatus

Standard extra tall aluminum and Plexiglas operant conditioning chambers with a curved panel fitted with a horizontal array of five nose poke apertures on one side and a photocell-equipped pellet receptacle on the other side (Med Associates, Mt. Vernon, VT, USA) were used. The boxes were housed inside a sound-attenuating cubicle with ambient white noise (85 dB) broadcast to mask external noise; the environment was illuminated with a house light diffuser that was positioned outside the testing chamber, providing indirect illumination of the testing environment.

2.4.2. Pretraining

All rats were first trained in a single session in which the house light was continuously illuminated, and single pellets (45-mg Dustless Precision Pellets; Bio-Serv Inc., Frenchtown, NJ) were delivered into an illuminated magazine on a fixed time 30-s schedule over a 45-min period. One day after this session, the rats were trained to make a sustained nose poke at the center aperture in three consecutive daily sessions. On the first day, the session began with illumination of the house light; a variable-duration nose poke of 0.01, 0.2, 0.4, or 0.6 s was required in the illuminated center aperture to trigger a pellet to be dispensed within the head entry magazine on the back wall (the nose poke duration requirements were varied randomly from trial to trial). When the rat successfully responded for the duration of the hold period, the head entry magazine was illuminated, and a pellet was dispensed. After the rat retrieved the pellet, the magazine light was extinguished, and 3 s later, the center aperture was illuminated to signal the initiation of another trial. The session terminated after 60 min passed or the rat earned 100 pellets, whichever occurred first. On the second and third days, the procedure was identical except that the rat was required to sustain 0.01, 0.2, 0.5, or 0.7-s nose pokes or 0.2, 0.5, 0.7, or 1.0-s nose pokes, respectively.

2.4.3. Acquisition of the task

After being trained to make the sustained nose poke, rats began daily testing on the LRTT; in the first four sessions, a target stimulus of fixed duration was presented for all trials in a session (which terminated after 60 min or 128 trials, whichever came first). The task began with the illumination of the house light and the rats retrieving a single pellet from the magazine. The center aperture on the opposite wall was illuminated 3 s later. The rat was then required to make a sustained, variable-duration nose poke (0.2, 0.5, 0.7, or 1.0 s) in the center aperture. After the observing response was completed, the far left or far right aperture was illuminated for a fixed period (30, 5, 2.5, or 1 s). During target presentation, a nose poke response at that aperture resulted in a pellet being delivered at the magazine, and a “correct” choice was scored. A limited hold period also applied on days 3 and 4; a response within 5 s of onset of target illumination was reinforced. Three seconds after the pellet was retrieved, the center aperture was illuminated to signal the onset of another trial. When a rat responded at a location that was not that of the target during target presentation or within the limited hold period, all lights in the box were extinguished, and the rat was given a 3-s “time-out” period in complete darkness; in this case, an “incorrect choice” was scored. In addition, if the rat made no response within target presentation or the limited hold period, the rat received a 3-s “time-out” period in darkness, and an “omission” was recorded. In both cases, the time-out period was immediately followed by illumination of the house light diffuser and the onset of another trial. An additional contingency was in place to discourage premature responses. If a rat responded to either of the possible target locations before