Epilepsy & Behavior 77 (2017) 33–38

Contents lists available at ScienceDirect

Epilepsy & Behavior

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

Kindling epileptogenesis and panic-like behavior: Their bidirectional connection and contribution to epilepsy-associated depression

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ARTICLE INFO

Article history:
Received 11 August 2017
Revised 28 September 2017
Accepted 1 October 2017
Available online xxxx

Keywords:
Epilepsy
Panic disorder
Depression
Comorbidity

ABSTRACT

Anxiety is one of the most common comorbidities of epilepsy, which has major detrimental effects on the quality of life. Generalized anxiety disorder (GAD) associated with epilepsy has been receiving most attention. However, several other forms of anxiety reportedly present in patients with epilepsy, including panic disorder (PD). In this study, using an animal model of limbic epilepsy, we examined the interplay between epilepsy and panic-like behavior (PLB). Further, considering the high degree of comorbidity between depression on the one hand, and both epilepsy and PD on the other hand, we studied whether and how the presence of PLB in animals with epilepsy would affect their performance in depression-relevant tests. Fifty-day-old male Wistar rats were subjected to repeated alternating electrical stimulations of the basolateral amygdala (BLA) to induce kindling of limbic seizures, and the dorsal periaqueductal gray (DPAG) to induce panic-like episodes. Seizure susceptibility and panic reaction threshold were examined before the first and 24 h after the last stimulation. At the end of the stimulations, the rats were examined in depression-relevant tests: saccharin preference test (SPT) for anhedonia and forced swimming test (FST) for despair/hopelessness. With regard to kindling, BLA + DPAG stimulation induced more profound increase of seizure susceptibility than BLA stimulation alone (evident as the reduction of the afterdischarge threshold and the increase of the afterdischarge duration). With regard to PLB, the BLA + DPAG stimulation exacerbated the severity of panic-like episodes, as compared with the DPAG stimulation alone. Basolateral amygdala stimulation alone had no effects on panic-like reactions, and DPAG stimulation alone did not modify kindling epileptogenesis. Combined stimulation of BLA and DPAG induced depressive-like behavioral impairments. This is the first experimental study showing bidirectional, mutually exacerbating effect of epilepsy and PLB, and the precipitation of depressive-like state by the epilepsy–PLB comorbidity.

1. Introduction

Anxiety disorders have been reported in 20% of people with epilepsy vs. 10% in people without epilepsy [1,2]. In patients with epilepsy, anxiety contributes to further deterioration of quality of life (QoL) [3,4]. The anxiety disorder spectrum includes several types of disorders with different mechanisms and symptoms [5]. In the laboratory, the most commonly examined type is generalized anxiety disorder (GAD), while other types have received scarce if any attention. At the same time, patients with epilepsy present with various types of anxiety. Particularly, panic disorder (PD) is one of the most common types, and may have even higher prevalence (5%) than GAD (3%) [1,6]. Panic disorder is characterized by recurrent presence of panic attacks (PA), which are the hallmark of its diagnosis [7].

In rodents, phobic avoidance, a characteristic manifestation of the PD shares neural substrates that generate innate fear-induced reactions, such as freezing [8], running, and jumping behaviors [9,10]. Panic reactions are related to proximal threat and recruit brainstem structures [9], such as the dorsal raphe nucleus (DR) that contains organized subgroups of serotonergic neurons, which are involved in mechanisms of anxiety. Neurons in the dorsal region of DR (DRD) project into the forebrain and facilitate anxiety-related behaviors, including PA; while the neurons in the dorsal raphe nucleus ventrolateral (DRVL)/ventrolateral periaqueductal gray (VLPAG) are involved in the inhibition of panic reactions [11,12]. Furthermore, the role of the dorsal periaqueductal gray (DPAG) in the expression of unconditioned defensive reactions and panic is well established [8,13]. This process implicates ascending

Abbreviations: ADD, afterdischarge duration; ADT, afterdischarge threshold; BLA, basolateral amygdala; DPAG, dorsal periaqueductal gray; DR, dorsal raphe nucleus; DRD, dorsal region of DR; DRVL, DR ventrolateral; EEG, electroencephalography; FST, forced swimming test; GAD, generalized anxiety disorder; PA, panic attacks; PD, panic disorder; PLB, panic-like behavior(s); QoL, quality of life; SEM, standard error of mean; SPT, saccharin preference test; VLPAG, ventrolateral periaqueductal gray.

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https://doi.org/10.1016/j.yebeh.2017.10.001
1525-5050/

connections from DPAG to the prosencephalic centers, such as the amygdala, through the medial forebrain bundle, which allows the animal to assess the aversive situation and helps in the recognition of the frightening stimuli [8,13]. In research setting, exogenous activation of the DPAG has been proposed as a rodent model of PD. Both chemical and electrical stimulation of DPAG produces panic-like behaviors (PLB) in rodents, similar to the responses exhibited by the animals in the presence of predators [8,14]. In patients, the electrical stimulation of DPAG generates physiological changes that are similar to those observed during PA, such as autonomic alterations and unpleasant feelings [8,15].

Aside from the PD–epilepsy connection, each of the two conditions has a high degree of comorbidity with depression [16–19]. Cooccurrence of anxiety and depression in people with epilepsy has worse impact on their QoL than each of the disorders in isolation [3,20]. In patients with epilepsy with comorbid depression, the presence of PD decreased the likelihood of remission of the depressive disorder [21].

Considering the discussed clinical aspects of PD–epilepsy–depression comorbidity, as well as the lack of experimental studies on this subject, we pursued to reproduce this comorbidity in the animal system, and to examine their interactions between seizures and panic-like and depressive-like impairments.

2. Material and methods

Experimental design is shown on Fig. 1A. The study was performed in a controlled randomized blinded fashion. Control animals were subjected to sham procedures. Randomization occurred during the assignment of the animals to various experimental and control groups. For the performance of all behavioral tests, the experimenter was unaware of the preceding procedures, to which the animals had been subjected. For the off-line data analysis, persons analyzing the data were blinded vis-à-vis all the procedures.

2.1. Animals

Male Wistar rats, 50 days old at the beginning of the study were maintained under controlled conditions (room temperature 20–26 °C, humidity 30–70%, 12 h light–dark cycles, food and water ad libitum). All experiments adhered to NIH regulations and were approved by the UCLA Animal Research Committee.

2.2. Surgery

Under isoflurane anesthesia, rats were stereotaxically implanted with two bipolar stimulating electrodes (Plastics One, Roanoke, VA), one in the left basolateral amygdala (BLA, from bregma: 2.5 mm posterior, 4.8 mm lateral, 8.5 mm ventral), and another in the DPAG (from bregma: 7.3 mm posterior, midline, 4.5 mm ventral) [22]. A tripolar electrode for electroencephalographic (EEG) recordings was placed 3 mm anterior to the bregma with the ground connected to a screw on the nasal bone. The implantation sites were identified by histology using Nissl staining (Fig. 1B–C).

2.3. Electrical stimulations

Subjects were randomly assigned to one of four groups: control (BLA sham + DPAG sham, n = 8); kindling + PLB (BLA stimulation + DPAG stimulation, n = 8); kindling (BLA stimulation + DPAG sham, n = 8), and PLB (BLA sham + DPAG stimulation, n = 8). Kindling and PLB were induced in the same animals through intermittent stimulations of BLA and DPAG as described below (Fig. 1A).

2.4. Afterdischarge properties and kindling

As a model of epilepsy, we have chosen BLA kindling, as it affords graded limbic seizures, which are induced on demand [23,24]. Seven days after surgery, the animals were connected to the DS8000 electrical stimulator via DSI100 stimulus isolators (World Precision Instruments,

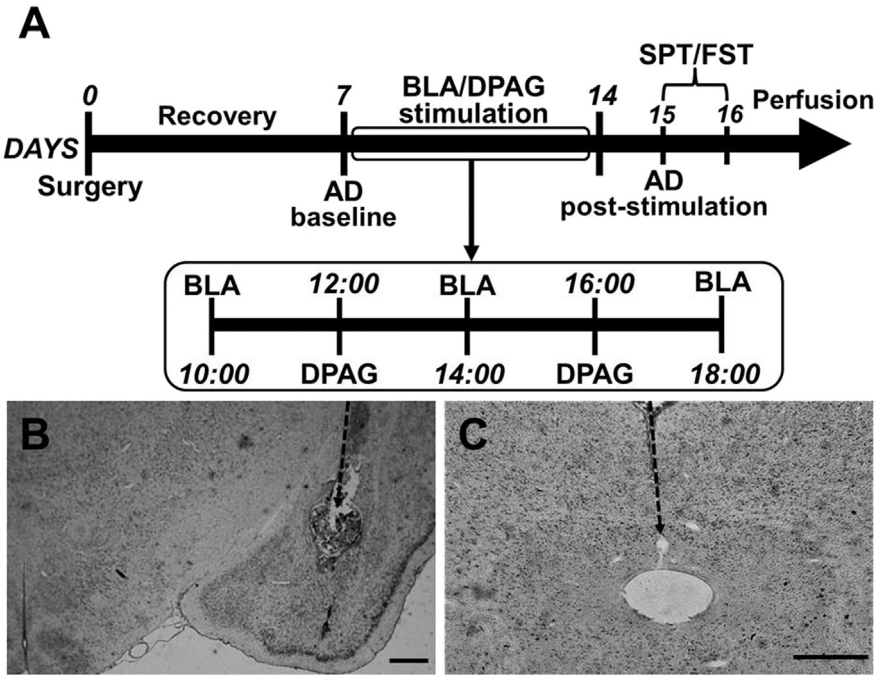

Fig. 1. Experimental protocol. A. Day 0, stereotaxic surgery for the implantation of the electrodes, followed by seven days of recovery. The afterdischarge tests were performed before (day 7) and 24 h after (day 15) the electrical stimulations. BLA and DPAG stimulations were induced over seven consecutive days (the box shows the daily stimulation protocol). After completion of electrical stimulations (during days 15 and 16), the behavioral tests (SPT and FST) were carried out. Photomicrographs indicate the trajectory (dashed line) and the implantation site (arrowhead) of the electrodes, which were located in the BLA (B) and DPAG (C) as was expected. This was achieved in 100% of the experimental subjects. Scale bar = 500 μm.