Epilepsy & Behavior 31 (2014) 263–266

Contents lists available at ScienceDirect

Epilepsy & Behavior

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

Effect of lithium-pilocarpine-induced *status epilepticus* on ultrasonic vocalizations in the infant rat pup

Maria-Leonor López-Meraz *, Jesus-Servando Medel-Matus, Consuelo Morgado-Valle, Luis Beltrán-Parrazal, César Pérez-Estudillo, Jorge Manzo

Centro de Investigaciones Cerebrales, Universidad Veracruzana, Xalapa, Av. Luis Castelazo s/n Carr. Xalapa-Veracruz, Km. 3.5 Col. Industrial-Animas, C.P. 91190 Xalapa, Veracruz, Mexico

ARTICLE INFO

Article history:
Received 12 August 2013
Revised 13 September 2013
Accepted 3 October 2013
Available online 12 November 2013

Keywords:
Status epilepticus
Developing rat
Ultrasonic vocalizations

ABSTRACT

Evidence shows that febrile convulsions induced in rat pups increase ultrasonic vocalizations (USVs); however, the effect of *status epilepticus* (SE) induced in developing rats on USVs has not been fully investigated. The goal of this study was to analyze USVs following lithium-pilocarpine-induced SE in fourteen-day-old (P14) rat pups. The rat pups were given 3-mEq/kg lithium chloride i.p. on the day before the induction of SE, which was carried out at P14 by subcutaneous injection of 100-mg/kg pilocarpine hydrochloride; control animals were given an equal volume of lithium chloride and saline on P13 and P14, respectively. Ultrasonic vocalizations were monitored at P15, P16, and P21 with a Mini 3 Bat Detector Ultra Sound Advice (15 kHz–160 kHz) set at 40 ± 4 kHz and digitally recorded in WAV format using the Audacity 1.3 beta software. A clear box (60 × 40 × 30 cm) split down the middle with a holed wall was used; each pup was placed alone in one compartment, whereas its dam was placed on the other cage side at room temperature. Vocalizations were recorded over a 5-minute period, converted to sonograms and spectrograms, and analyzed using the Raven software. Parameters evaluated were as follows: USV frequency, latency to the first USV, and mean USV duration. There was a significant decrease in the latency (35.5 ± 6.9 s) and duration (50.8 ± 8.6 s) of USVs after SE compared with the control group (81.9 ± 10.8 s and 78.1 ± 9.9 s, respectively). *Status epilepticus* affected male and female rats differentially.

1. Introduction

Epidemiologic studies indicate that *status epilepticus* (SE) is more common in young children [1–3]. *Status epilepticus* is a nonself-limited type of epileptic seizure characterized by an enduring epileptic state during which seizures are unremitting and tend to be self-perpetuating [4,5]. *Status epilepticus* can be induced experimentally in rats during the fourteenth postnatal day (P14) using the lithium-pilocarpine model, which reproduces motor seizure manifestations and causes extensive neuronal injury in several brain areas [6–8]. This model has been extensively used to study consequences of SE, such as neuronal damage [6–8], epileptogenesis [9–11], as well as cognitive and mnemonic deficits associated with epilepsy [12,13].

Rodent pups interact with their dams through ultrasonic vocalizations (USVs), which are important for maternal response including searching, retrieving, and anogenital licking [14,15]. Ultrasonic vocalizations strongly contribute to the survival of the pup and also to its physiological and behavioral development [15]. In developing rats, the sonographic characteristics of USVs change with maturity, and the number of USVs decreases during the third week of life [16]. The effect of seizures on USV and its possible consequences in the maternal care or the pup viability have been insufficiently evaluated. Keller and collaborators [17] demonstrated that febrile convulsions induced at P7 significantly increased the number of USVs during P10–P12, an effect that did not affect the mother–pup interaction. However, the consequences of SE induced in developing rats on USVs have not been studied. The goal of this study was to investigate whether SE induced at P14 modifies USVs induced by maternal separation at P16, P20, and P21.

2. Material and methods

2.1. Animals

The animals used in this study were Wistar rats derived from animals obtained from Rismart Mexico. The animals were born in our colony (Centro de Investigaciones Cerebrales, Universidad Veracruzana, Mexico); at the time of weaning on day 21 of life, the offspring were housed in same-sex groups of 4–6 animals in clear Plexiglas cages (20 × 30 × 50 cm). After mating and throughout lactation, adult females and litters were housed singly in clear Plexiglas cages (15 × 24 × 37). The animals were maintained on a 12-h light–12-h dark cycle (0800–2000), and food (Rismart Mexico) and water were provided ad libitum. The

* Corresponding author.
E-mail addresses: leonorlopez@uv.mx (M.-L. López-Meraz), jservandomm@hotmail.com (J.-S. Medel-Matus), comorgado@uv.mx (C. Morgado-Valle), lubeltran@uv.mx (L. Beltrán-Parrazal), cesperez@uv.mx (C. Pérez-Estudillo), jmanzo@uv.mx (J. Manzo).

1525-5050/$ – see front matter © 2013 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.yebeh.2013.10.006

animals underwent routine cage maintenance (aspen chip bedding from Rismart Mexico).

Four litters were used in the study [pups of both sexes, n = 36 (20 male and 16 female rats)]. The day of birth was considered day 0. The pups were housed with their dams in cages containing aspen chip bedding (Rismart Mexico), with 12-h light–12-h dark cycles (lights on at 0800) and had free access to food (Rismart Mexico) and water. All experiments were conducted during the light period. Experiments were carried out with the goal of minimizing the number of animals used and their suffering. Studies were conducted in accordance with Mexican guidelines on the care and use of laboratory animals (NOM-062-ZOO-1999) and the Guide for the Care and Use of Laboratory Animals (eighth edition, National Research Council, 2011).

2.2. Induction of SE

Postnatal day 13 (P13) rat pups were given intraperitoneal injections of lithium chloride (3 mEq/kg; #L-0505, Sigma), and 20 h later, during P14, SE was induced with a subcutaneous injection of pilocarpine hydrochloride (n = 18, 8 female and 10 male rats) (60 mg/kg; #P6503, Sigma) as described previously [6]. Control rats (n = 18, 8 female and 10 male rats) were given an equal volume of lithium chloride and saline instead of the convulsant drug. Behavioral motor seizures were carefully monitored by an experienced analyst and scored according to a slightly modified Racine scale [18]: (0 = behavioral arrest; 1 = face clonus; 2 = head nodding; 3 = forelimb clonus; 4 = forelimb clonus and rearing; 5 = forelimb clonus with rearing and falling). Only animals reaching SE, defined as near continuous seizure activity lasting over 30 min [19], were included in the study. After SE, the pups received 1-ml isotonic 5% dextrose in saline solution subcutaneously to avoid dehydration without stressing the cardiovascular system. After the cessation of seizures, the pups were placed back with their mothers (approximately 6 h to avoid cannibalism); time of separation from the mother was strictly controlled and was similar in the control group and in the group with SE [8]. Considering that the P14 rats tolerated well the pilocarpine dose given, which was effective to induce convulsive SE and neuronal damage as reported previously in literature without mortality [6–8], any anticonvulsant drug was applied to the rat pups.

2.3. Ultrasonic vocalizations induced by maternal isolation

After SE or control conditions, on P15, P16, and P21 (between 1200 and 1400), USVs were monitored with a Mini 3 Bat Detector Ultra Sound Advice (15 kHz–160 kHz) set at 40 ± 4 kHz (rat pups emit a 40-kHz vocalization when they are separated from their mothers) [20] and digitally recorded in WAV format using the Audacity 1.3 beta software. We used a clear box (60 × 40 × 30 cm) split down the middle with a holed wall; each pup was placed alone in one compartment, whereas its dam was placed on the other cage side at room temperature. The litter was allowed to adapt to their mother's absence for 5 min in the home cage. Then, each pup was picked up and tested individually in arbitrary turns. Vocalizations were recorded over a 5-minute period, converted to sonograms and spectrograms, and inspected manually using the Raven software to ensure that all USVs detected were legitimate calls. Parameters evaluated were as follows: USV frequency, i.e., the number of isolated calls during 5 min; latency to the first USV (s); and mean USV duration (s). The investigators conducting USV analyses were blind to the experimental condition and sex of the pups.

2.4. Statistical analysis

The number of animals displaying USVs per group was analyzed with a chi-square test (Sigma Stat version 3.5 (Systat Software, Inc.) was used for this statistical analysis). Data for latency, duration, and frequency of USVs were normalized [Log 10 (data + 0.5)] and analyzed with a three-way repeated measures ANOVA [Factor A: treatment (control vs SE), Factor B: litter (1–4), and Factor C: age (P15, P16, P21)] (Statistica version 7.0 (Stat Soft, Inc.) was used for this statistical analysis). Data are presented as the mean ± S.E.M. of the raw data. The level of significance for all comparisons was $p < 0.05$.

3. Results

3.1. Convulsions

All animals injected with pilocarpine developed generalized motor seizures scored as stage 4.1 ± 0.1 and SE. Latency to SE was 10.7 ± 0.5 min, and duration of behavioral SE was around 5 h. Thus, all the rats in the group with SE were included in the analysis of USVs.

3.2. Effect of SE on ultrasonic vocalizations in the rat pups

The chi-square test failed to detect changes in the number of rats (considering males and females) displaying USVs at P15 (15/18 vs 8/18), P16 (16/18 vs 10/18), or P21 (12/18 vs 10/18) between the control group and the group with SE [χ^2 (2, 36) = 0.202, p = 0.904]. The three-way repeated measures ANOVA detected a significant decrease (57%) in the latency to the first USV after SE when compared to the control animals [F(1,56) = 11.27; p = 0.003]. Similarly, a reduction (35%) was observed in the average duration of USVs after SE when compared to the control animals [F(1,56) = 6.08; p = 0.02]. No differences in the number of vocalizations were observed after the treatment [F(1,56) = 4.24; p = 0.05] (Fig. 1). Significant differences in the latency, duration, and number of USVs were observed neither between litters or ages nor between the interactions treatment × age, treatment × litter, or treatment × age × litter (Table 1).

With regard to males, the chi-square test revealed no differences in the number of rats displaying USVs at P15 (9/10 vs 5/10), P16 (10/10 vs 6/10), or P21 (7/10 vs 6/10) between the control group and the group with SE [χ^2 (2, 20) = 0.351, p = 0.839]. Table 1 summarizes the results from the three-way repeated measures ANOVA for ultrasonic vocalizations when the male and female rats were analyzed separately. The

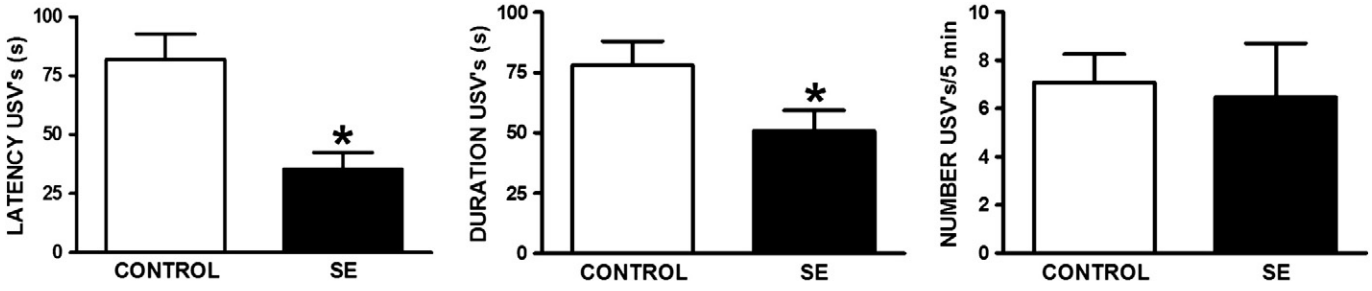

Fig. 1. Effect of *status epilepticus* (SE) on the latency, duration, and number of ultrasonic vocalizations (USVs) in rat pups monitored between P15 and P21. Data are presented as the mean ± S.E.M. (n = 18 per group) and were analyzed with a three-way repeated measures ANOVA. *$p < 0.05$.