Behavioural Brain Research 236 (2013) 23–29

Contents lists available at SciVerse ScienceDirect

Behavioural Brain Research

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

Research report

Social deficits in the AY-9944 mouse model of atypical absence epilepsy

Seungmoon Jung[a,1], Jin Soo Seo[a,1], Byung Sun Kim[a], Doheon Lee[a], Keun-Hwa Jung[b], Kon Chu[b,c], Sang Kun Lee[b], Daejong Jeon[a,*]

[a] Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, 305-701, Republic of Korea
[b] Department of Neurology, Comprehensive Epilepsy Center, Biomedical Research Institute, Seoul National University Hospital, College of Medicine, Seoul National University, Seoul, 110-744, Republic of Korea
[c] Department of Premedicine, College of Medicine, Seoul National University, Seoul, 110-744, Republic of Korea

HIGHLIGHTS

- Psychiatric comorbidities are a major concern in atypical absence epilepsy (AAE).
- Administration of AY-9944 can produce a mouse model of AAE.
- AY-treated mice show spontaneous and recurrent spike-and-wave discharge (4–5 Hz).
- Severe social deficits, but normal anxiety, were found in the AY model of AAE.

ARTICLE INFO

Article history:
Received 11 June 2012
Received in revised form 17 August 2012
Accepted 18 August 2012
Available online 24 August 2012

Keywords:
Atypical absence epilepsy
Behavioral comorbidities
Social behavior
AY-9944
Lennox–Gastaut syndrome

ABSTRACT

Atypical absence epilepsy (AAE) showing slow spike-and-wave discharges (SWD) is characterized by severely abnormal cognition and neurodevelopmental or neurological outcomes in humans. However, despite the severe behavioral outcomes in AAE, the relationship between AAE and social-behavioral dysfunctions has not defined well, either experimentally or in patients with AAE. Experimentally, AAE can be produced by administering AY-9944 (AY), a cholesterol biosynthesis inhibitor. In this study, we characterized social behavior in the AY mouse model of AAE. AAE in the mouse was induced by repeated postnatal administration of AY every 6 days from postnatal day (P) 2 to P20. AY-treated mice exhibited spontaneous, recurrent, and synchronous SWD (4–5 Hz) in electroencephalographic recordings. AY-treated mice performed tasks involving sociability/social novelty preference, social interaction with a juvenile conspecific, observational fear, and resident–intruder aggression. They showed behavioral dysfunction in social interactions with a juvenile conspecific and sociability/social novelty preference tasks. They also exhibited reduced social fear learning in observational fear conditioning. Interestingly, they showed increased levels of offensive behaviors in a resident–intruder task. However, AY-treated mice displayed normal levels of anxiety in light/dark transition and the elevated plus maze tasks, and showed slightly increased locomotor activity in an open-field task. These results demonstrate social dysfunction in the AY-induced AAE model. Our study of social behavior can also provide valuable information about Lennox–Gastaut syndrome, in which AAE is a component. Thus, our findings may help to understand behavioral pathogenesis or characteristics of patients with AAE.

1. Introduction

Absence epilepsy is characterized by bilaterally synchronous spike-and-wave discharges (SWD) in electroencephalogram (EEG), and two types of absence epilepsy are observed both clinically in children and experimentally in animals [1–4]. In humans, typical absence epilepsy is characterized by a distinct SWD at a frequency of 3 Hz that is associated with paroxysmal loss of consciousness or decrease in consciousness; in experimental models of typical absence epilepsy, a frequency of 7–9 Hz is observed [4–6]. Experimentally and clinically, typical absence epilepsy does not involve the hippocampus, is constrained within the thalamocortical circuitry [7,8], and usually has a benign outcome [9]. In contrast, atypical absence epilepsy (AAE) is clinically distinct from the typical form in terms of EEG manifestations, ictal behavior, and behavioral outcomes [9–11]. The epileptic activity in AAE involves limbic

* Corresponding author at: Laboratory for Brain Behavior and Therapeutics, Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), 291 Daehak-ro, Yuseong, Daejeon, 305-701, Republic of Korea. Tel.: +82 42 350 4328; fax: +82 42 350 4310.
E-mail address: clark@kaist.ac.kr (D. Jeon).
[1] S.J. and J.S.S. contributed equally to this work.

0166-4328/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.bbr.2012.08.029

circuitry, including the hippocampus, as well as thalamocortical circuitry [1,12,13]. The frequency of SWD in AAE is slower than 3 Hz [1,9,14]. Although during the ictus, voluntary movement and partial consciousness are maintained in AAE, severe cognitive and neurodevelopmental impairments have been reported [2,14,15].

Experimentally, an animal model of AAE can be developed with a single or repeated postnatal administration of AY-9944 (AY), a cholesterol biosynthesis inhibitor, which inhibits the reduction of 7-dehydrocholesterol to cholesterol and induces recurrent atypical absence seizures through the animal's life [1,16–19]. However, the neural mechanism of seizure development as a result of AY administration during postnatal development remains largely unknown. Nevertheless, the AY model has been shown to be a valid model of AAE. Based on EEG features (slow SWD at a frequency of 5–6 Hz in rats and 4–5 Hz in mice) and ictal behaviors as well as on pharmacological, cognitive, and developmental profiles [1,20,21], this model has a similar phenotype to human AAE. AY-treated rats can move or exhibit voluntary behavior during bursts of slow SWD derived from both thalamocortical and hippocampal circuitries [1,13,20,22]. Thus, the AY model appears to be appropriate for the study of AAE.

Clinically, AAE is often found as a major component of Lennox–Gastaut syndrome, a malignant childhood epileptic disorder [15,23–26]. Lennox–Gastaut syndrome is also associated with mental retardation and severe behavioral outcomes, including hyperactivity, inattention, aggressiveness, and autistic tendencies [23,27–29]. Worse, it is largely refractory to antiepileptic medications [2,14,23,24].

Thus, efforts have been made to understand AAE. However, social behaviors, such as social interactions, social learning, and aggression, have rarely been examined in the AY model or in patients with AAE. In this study, we investigated behavioral dysfunction in several different social-behavioral tasks in the AY mouse model of AAE to characterize social behaviors in AAE.

2. Materials and methods

2.1. AY model generation

Male B6/129 F1 mice, obtained from breeding two inbred mice, C57BL/6J and 129S4/SvJae were used. To induce AAE, mice pups were treated with a subcutaneous dose of the cholesterol synthesis inhibitor AY-9944 (7.5 mg/kg, Tocris), every 6 days from postnatal day (P) 2 to P20 (P2, P8, P14, P20), as described previously [1,20,21]. Age-matched control mice were given an equivalent volume of 0.9% saline.

Mice were housed with a 12/12-h light/dark cycle and ad libitum access to food and water. Animal care and experimental handlings were approved by animal ethics committee and were carried out according to the guidelines from the Institutional Animal Care and Use Committee at the Korea Advanced Institute of Science and Technology (KAIST).

2.2. Electrode implantation and in vivo electrophysiology

After repeated postnatal administration of AY, EEG surgery was performed on P45–P50, according to previous reports [30,31]. Mice were housed in groups of 3–4 mice per cage (polycarbonate, 20 cm × 33 cm × 14 cm) before EEG surgery, and mice were singly housed after EEG surgery. Mice (AY-treated, $n = 8$; control, $n = 7$) were anesthetized with 2,2,2 tribromoethanol (0.2 mL/10 g of a 20 mg/mL solution), and surgery was performed using a stereotaxic apparatus (Kopf Instruments, Tujunga, CA). EEG recordings were obtained using blunt Teflon-coated tungsten electrodes (0.005 in., 2 MΩ), which were positioned in the right hemisphere at AP 1.0 mm, L 0.7 mm, and DV 1.0 mm (frontal cortex, Fr) and at AP −1.8 mm, L 1.6 mm, and DV 1.5 mm (hippocampal CA1, Hi) or at AP −2.0 mm, L 2.0 mm, and DV 1.0 mm (parietal cortex, Pa) from bregma, with grounding over the cerebellum [1,22]. Electrodes were fixed to the skull using dental acrylic. Electrical signals were recorded after being amplified (×1200), bandpass-filtered from 0.1 to 70 Hz, and digitized with a 400-Hz sampling rate (AS 40) using a digital EEG system (Comet XL, Astro-Med, Warwick, RI). EEG signals were recorded continuously with video for 24 h/day from P60 to P63. Electrophysiological data were analyzed offline using PSG Twin 4.2 (Astro-Med, Inc.) and pClampfit 10.2 (Axon Instruments, CA). According to previous reports [18,32], SWD was considered as a characteristic epileptic activity at 4–5 Hz with three-fold higher amplitude compared with baseline. Only when SWD appeared simultaneously in all three regions (Fr, Pa, Hi), they were recognized as such and included in the data for further statistical analyses. The duration and the number of SWD were measured and analyzed. To obtain power spectra, EEG waveforms were filtered from 1 to 70 Hz. Power spectra were calculated and drawn with Fourier transformations of 2-s window sizes.

2.3. Behavioral tasks

Mice treated with AY or saline without EEG surgery were subjected to behavioral tasks on P60–P70, all behavioral procedures were video-recorded, and data were quantified by an experimenter blind to the conditions. Different mice were used in each of the behavioral tasks.

2.3.1. Sociability/social novelty preference

This experiment was based on a previously described protocol [33,34]. The apparatus is a black Plexiglas rectangular, three-chambered box (60 cm × 30 cm × 22 cm). The middle chamber (17 cm × 30 cm × 22 cm) was slightly smaller than the side chambers (each 21.5 cm × 30 cm × 22 cm). Dividing partitions were made from clear Plexiglas, with small doorways (5 cm × 8 cm) allowing access to each chamber. To house strangers, inverted wire cages (wire cups: diameter, 7.7 cm; height, 10 cm) were placed in each side chamber. Stranger was placed on the left/right (counterbalanced) side. For habituation, subject mice (AY-treated and control mice) were first placed in the middle chamber and allowed to explore for 10 min. During habituation, each of the two side chambers contained an empty wire cage. Following habituation, for the sociability test, a novel mouse (stranger 1, age-matched male B6/129 F1 mouse) was enclosed in one of the wire cages and placed in one of the side chambers; the subject mice were then allowed to explore for 10 min. The social novelty preference test was performed immediately after the sociability test. Following the sociability test, another novel mouse (stranger 2, age-matched male B6/129 F1 mouse) was enclosed in the other wire cage, and the subject mice then were allowed to explore the two strangers for 10 min. The time spent sniffing each wire cage and in each of the side chambers was measured. A mouse was considered to be sniffing the wire cage when its head was facing the cage from a distance of within 1 in. Only when all four paws crossed from the middle chamber into a side chamber, was it counted as an entry.

2.3.2. Social interaction with a juvenile mouse

Male juvenile mice were used instead of adults to exclude any effect of mutual aggression [35]. A single mouse (male subject, AY-treated or control mouse) in a new cage was allowed to roam freely for 10 min (habituation). The cages used were identical to those in which the mice were normally housed. A novel juvenile (3–4 weeks old) male B6/129 F1 mouse was introduced to the cage and then allowed to roam freely for 5 min (test session). Behaviors that were scored as social interaction included the following: nose-to-nose sniffing, direct contact (pushing the snout or head underneath the juvenile's body and crawling over or under the juvenile's body), and close following (within <1 cm) [36]. The total time of social interaction behaviors was quantified.

2.3.3. Social observational fear conditioning

Observational fear conditioning, which is related to empathetic behavior, was performed as described previously [31,37]. Briefly, the apparatus for observational fear conditioning consisted of two identical chambers (each, 21 cm × 17.5 cm × 25 cm) containing a transparent Plexiglas partition in the middle and a stainless steel rod floor (modified passive avoidance cages: 5-mm diameter rods, spaced 1 cm apart, Med Associates, Albans, VT). For observational fear conditioning, mice (observer, AY-treated or control mice; demonstrator, age-matched naïve male B6/129 F1 mice) were individually placed in the apparatus chambers for 5 min, and a 2-s foot shock (1 mA) was then delivered to the demonstrator every 10 s for 4 min via a computer-controlled animal shocker (Med Associates). To assess contextual memory, the observers were placed back into the training context 24 h after training, and freezing behavior was observed for 4 min. The length of the time during which an animal showed freezing behavior (a fear response) was measured.

2.3.4. Resident-intruder test

The experiment was conducted as described previously [38]. Resident mice were housed in isolation for 7 days without a change of bedding before testing. The isolation-induced resident–intruder aggression test was performed by introducing intruder mice into the home-cages of resident mice. AY-treated or control mice were used as resident mice, and naïve male B6/129 F1 mice were used as intruder mice. The offensive behaviors of the resident mouse were measured by determining the latency to the first attack and the total number of bite attacks by the isolated resident mouse for 15 min. If an animal did not make a bite attack, the latency to the first attack was recorded as 900 s (test duration), and all the other attack scores were recorded as zero.

2.3.5. Light/dark transition test

For assessment of anxiety, light/dark transition task was performed as described previously [30,31]. The light/dark box (30 cm × 45 cm × 27 cm) was made of plastic and had a dark compartment (one-third of the total area) and a light compartment with a hole in the middle. The light compartment was illuminated at 400 lx. Both the elapsed time to entry into the light compartment (latency), and the amount of