

## Early deficits in social behavior and cortical rhythms in pilocarpine-induced mouse model of temporal lobe epilepsy

Jinsoo Seo<sup>a,1</sup>, Seungmoon Jung<sup>a,1</sup>, So-Young Lee<sup>a</sup>, Hyunwoo Yang<sup>a</sup>, Byung Sun Kim<sup>a</sup>, Jiye Choi<sup>a</sup>, Minji Bang<sup>a</sup>, Hee-Sup Shin<sup>b</sup>, Daejong Jeon<sup>a,\*</sup>

<sup>a</sup> Laboratory for Brain Behavior and Therapeutics, Department of Bio and Brain Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon, Republic of Korea

<sup>b</sup> Center for Cognition and Sociality, Institute of Basic Science, Seoul, Republic of Korea

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### ABSTRACT

Many patients with epilepsy are afflicted with psychiatric comorbidities including social dysfunction. However, although social deficits have been a major concern in epilepsy treatment, the relationship between social behavioral pathogenesis and the time course of epileptogenesis is not well defined. To address this, we investigated social behavioral alterations and cortical rhythms during two distinct periods in a mouse model of temporal lobe epilepsy (TLE): 1) a seizure-free, latent period after status epilepticus and 2) the subsequent, chronic period characterized by spontaneous recurrent seizures (SRSs). We found that severe social impairments, such as reduced sociability/social novelty preference, social interaction, social learning, and enhanced defensiveness, appeared during the latent period in mice with TLE. The social dysfunctions in the latent-period mice were nearly comparable to those in the chronic-period mice. We also found that both the latent- and chronic-period mice showed similar aberrant neural activities. They showed enhanced delta-band (1–4 Hz) activity and reduced alpha- (8.5–12 Hz) and gamma-band (30–55 Hz) activity during baseline behavior. Interestingly, concomitant increases in alpha- and gamma-band activities during social behavior, which were characteristic in control mice, were not observed in either latent- or chronic-period mice. Our results indicate that social deficits and abnormal neural activities appear at an earlier stage in epileptogenesis regardless of SRS occurrence. These findings may help to understand behavioral pathogenesis in patients with TLE and at-risk patients with initial insults that develop into TLE.

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### Introduction

Epilepsy is one of the most common neurological diseases. It is characterized by episodic abnormal electrical activity, cell loss or neurodegeneration, and inflammation in the brain (Fabene et al., 2008; Vezzani and Granata, 2005). Epilepsy can be caused by various precipitating events, such as initial/acute prolonged seizure (status epilepticus, SE), stroke, head trauma, or cerebral infections (Herman, 2002). After a latent period (seizure free) spontaneous recurrent seizures (SRSs) develop and lead to a chronic period of epilepsy. Many patients with epilepsy suffer from psychiatric comorbidities, including social dysfunctions, cognitive impairment, anxiety disorders, and psychosis (Cornaggia et al., 2006; Motamedi and Meador, 2003; Schwartz and Marsh, 2000; Swinkels et al., 2005). Abnormal social

behaviors are closely associated with limited social opportunities, family dysfunction, and poor self-esteem and have a considerable impact on the medical management and quality of life of patients with epilepsy (Devinsky, 2003; Jacoby et al., 1996; Suurmeijer et al., 2001). Despite the high prevalence and adverse effects of social dysfunction in patients with epilepsy, the neural mechanisms underlying the alterations in social behavior are poorly understood, and the onset of social behavioral dysfunction in epileptogenesis is still an unresolved issue.

Experimental models of temporal lobe epilepsy (TLE) such as the pilocarpine model result in abnormal behaviors that are similar to behavioral comorbidities in patients with TLE and thus are useful to study the relationship between epilepsy and behavioral comorbidities (Groticke et al., 2007; Heinrichs and Bromfield, 2008; Muller et al., 2009). The pilocarpine model of TLE exhibits a seizure-free, latent period after pilocarpine-induced SE, and thereafter, SRSs develop throughout the animal's life (chronic period) (Cavalheiro et al., 1991; Curia et al., 2008; Turski et al., 1983; White, 2002). Some studies that have considered the relevance of social behavior and epilepsy in animal models of TLE have reported behavioral alterations during social interaction or discrimination and aggression tasks (Franke and Kittner, 2001; Holmes et al., 1988; Krsek et al., 2004; Letty et al., 1995; Marin et al., 2008; Mellanby et al., 1981). However,

*Abbreviations:* TLE, temporal lobe epilepsy; SRS, spontaneous recurrent seizure; EEG, electroencephalography; SE, status epilepticus.

\* 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. Fax: +82 42 350 4310.

E-mail address: [clark@kaist.ac.kr](mailto:clark@kaist.ac.kr) (D. Jeon).

<sup>1</sup> The first two authors equally contributed to this work.

these social behavioral studies were conducted only during the chronic period of epilepsy. Furthermore, alteration or modification in cortical rhythms, which can influence or be related to social behavior (Rybak et al., 2006; Wang, 2010; Yizhar et al., 2011), has not been well examined in animal models of TLE. Therefore, it is necessary to clarify and perform a thorough investigation of social behavioral alterations with a time course after SE and to examine the neural activity related to the social dysfunction.

In this study, we investigated behavioral pathogenesis using many different social behavioral tasks in a pilocarpine-induced TLE mouse model during the latent and chronic epileptic periods. We also simultaneously performed a social behavioral task with electroencephalographic (EEG) measurements to find changes in cortical rhythms of epileptic mice during social behavior.

## Material and methods

### Epilepsy model

Young male C57BL/6 mice (22–25 g) were used. The epilepsy animal model was generated by a single injection of pilocarpine (330 mg/kg, intraperitoneally, Sigma, St. Louis, MO, USA) (Jeon et al., 2011). To minimize peripheral muscarinic effects, mice received methyl-scopolamine (1 mg/kg, intraperitoneally, Sigma) 30 min before the pilocarpine injection. Diazepam (5 mg/kg, intraperitoneally) was administered to mice 40 min after the onset of SE (the time point showing continuous and prolonged tonic-clonic behavioral seizures) to interrupt the prolonged seizures (i.e., SE). After SE, all animals were given food soaked in a 5% glucose solution for 2 days until they started to eat normal food pellets. Sham control mice received a saline injection instead of pilocarpine. Different mice were used in each of the behavioral tasks.

Mice were housed under a 12-h light/dark cycle and had ad libitum access to food and water. Animal care and handling were carried out according to the guidelines of the Institutional Animal Care and Use Committee at the Korea Advanced Institute of Science and Technology.

### In vivo electrophysiology and analysis of seizure

To identify the time course of SRS occurrence, mice underwent EEG surgery 2 weeks before the injection of pilocarpine, following previous studies (Jeon et al., 2010, 2011). Mice were anesthetized by intraperitoneal injection of 1% ketamine (30 mg/kg) and xylazine hydrochloride (4 mg/kg). Surgery was performed using a stereotaxic apparatus (Kopf Instruments, Tujunga, CA, USA). EEG recordings were obtained using tungsten electrodes (0.005 in., 2 M $\Omega$ ), which were positioned in the left hemisphere at AP  $-1.8$  mm, L 2.1 mm, and DV 2.1 mm (hippocampal CA3) and the right hemisphere at AP  $-1.8$  mm, L 2.1 mm, and DV 0.8–1.0 mm (cortex) from the bregma with grounding over the cerebellum. Electrical activities were recorded after being amplified ( $\times 1200$ ), bandpass-filtered at 0.1–70 Hz, and digitized with a 400-Hz sampling rate using a digital EEG system (Comet XL, Astro-Med, Warwick, RI, USA).

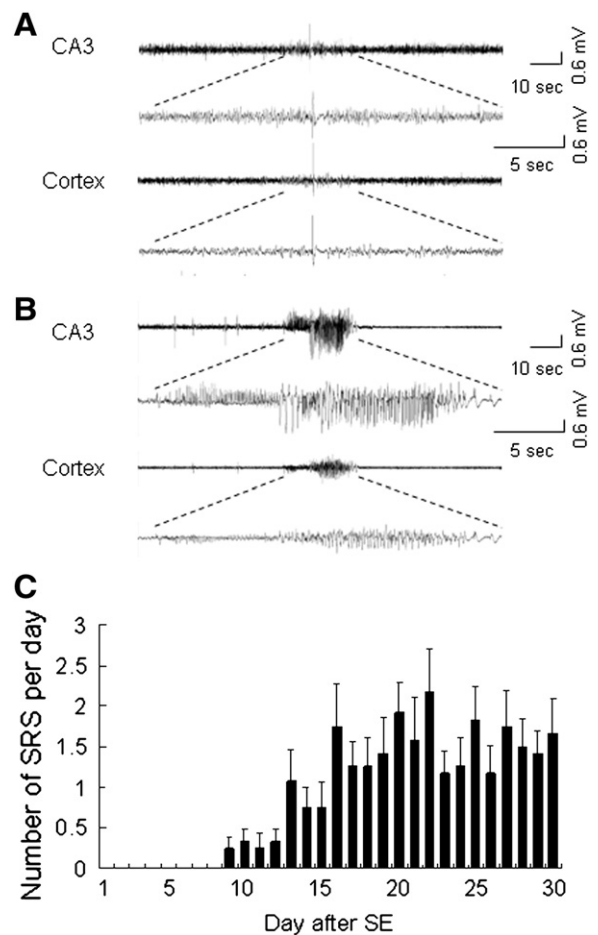
Continuous EEG recordings were combined with video monitoring, and the video-EEG signals were continuously recorded 24 hr per day from days 1 to 30 after SE. Electroclinical seizures were examined, and the frequency of SRS occurrence was analyzed offline using PSG Twin 4.2 (Astro-Med). Behavioral seizures were examined according to Racine's scale (Racine, 1972): stage 1: immobility and rigid posture; stage 2: mouth movements, head nodding, and repetitive movements; stage 3: forelimb clonus; stage 4: severe seizures with rearing and falling; stage 5: severe seizures with loss of posture or jumping; and stage 6: tonic-clonic seizures. SRS was defined as convulsive seizures (stages 4–6) following stage 1, 2, or 3. The continuous video-EEG monitoring revealed that the first SRS occurred at

9–14 days after SE (Fig. 1). Thus, mice from days 5 to 7 after SE were used as epileptic mice in the latent period (latent-period mice), and mice from days 30 to 40 after SE were used as epileptic mice in the chronic period (chronic-period mice).

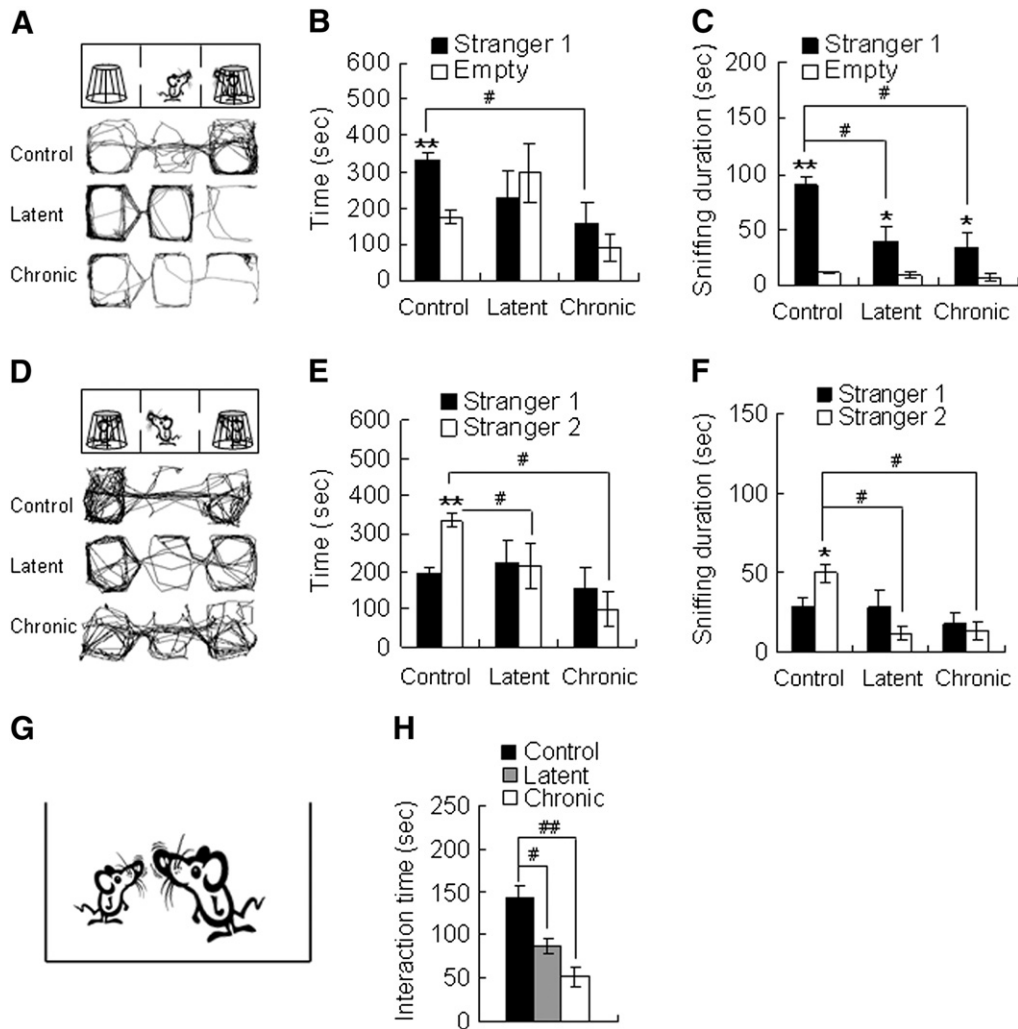
### Social behavioral tasks

All behavioral procedures were video recorded. Except for the EEG recording experiment during social behavior (Fig. 4), all mice performed behavioral tasks without EEG surgery.

**Sociability/social novelty preference task:** This experiment was performed in a three-room chamber as described previously (Moy et al., 2004; Nadler et al., 2004) (illustration in Fig. 2A). The apparatus used was a black rectangular three-chambered plastic box (60 $\times$ 30 $\times$ 22 cm), with the chambers divided by clear Plexiglas and small doorways (5 $\times$ 8 cm) allowing access into each chamber. To house strangers, inverted wire cage (wire cups; diameter, 7.7 cm; height, 10 cm) were placed in each side chambers. For habituation, subject mice (epileptic and control mice) were first placed in the middle chamber and allowed to explore for 10 min. Following a 10-min habituation, for the sociability test, a novel mouse (stranger 1, age-matched male C57BL/6 mice) was enclosed in one of the wire cages and placed in one of the side chambers, and then the subject mice were allowed to explore for 10 min. The social novelty preference test was performed immediately after the sociability test. Another novel mouse (stranger 2, age-matched male C57BL/6 mice) was enclosed in the other empty wire cage, and the subject mice then



**Fig. 1.** Development of spontaneous recurrent seizures (SRSs) after the pilocarpine-induced status epilepticus (SE). (A) Representative original traces of electroencephalogram (EEG) recordings in the CA3 (upper)/cortex (lower) at day 9 after SE. (B) The average number of SRSs per day.



**Fig. 2.** Impaired sociability/social novelty preference and social interaction during the latent and chronic period of epilepsy. (A–C) Sociability task. (A) Diagram of the apparatus for sociability task. This paradigm (top) tests sociability for spending time with a conspecific mouse (stranger 1). Sample mouse trajectories from control, latent-period, and chronic-period mice are shown in the lower panels. (B) The control mice spent significantly more time in the side chamber with the cage containing the stranger 1 mouse than in that with an empty cage. However, both the latent- and chronic-period mice spent similar amounts of time in the two side chambers.  $**p < 0.01$ , two-tailed *t*-test. (C) Both the latent- and chronic-period mice spent less time sniffing the stranger 1 cage than the control mice did.  $**p < 0.01$ , two-tailed *t*-test.  $*p < 0.05$ ,  $##p < 0.01$ , Scheffe's post hoc test. (D–F) Social novelty preference task. (D) This paradigm (top) tests preference for spending time with a familiar mouse (stranger 1) vs. an unfamiliar mouse (stranger 2). Sample mouse trajectories from control, latent-period, and chronic-period mice are shown in the lower panels. (E) Control mice spent significantly more time in the side chamber with the cage containing the stranger 2 mouse than in that with the cage containing the stranger 1 mouse. Similarly, the duration of sniffing the stranger 2 cage in control mice was longer than that sniffing the stranger 1 cage (F). However, both the latent- and chronic-period mice spent similar amounts of time in the two side chambers (E) and sniffing the two stranger cages (F).  $*p < 0.05$ ,  $**p < 0.01$ , two-tailed *t*-test.  $*p < 0.05$ ,  $##p < 0.01$ , Scheffe's post hoc test. (G–H) Social interaction task (G). Mice in the latent or chronic period showed a significant decrease in social interaction with an unfamiliar juvenile mouse (H).  $*p < 0.05$ ,  $##p < 0.001$ , Scheffe's post hoc test.

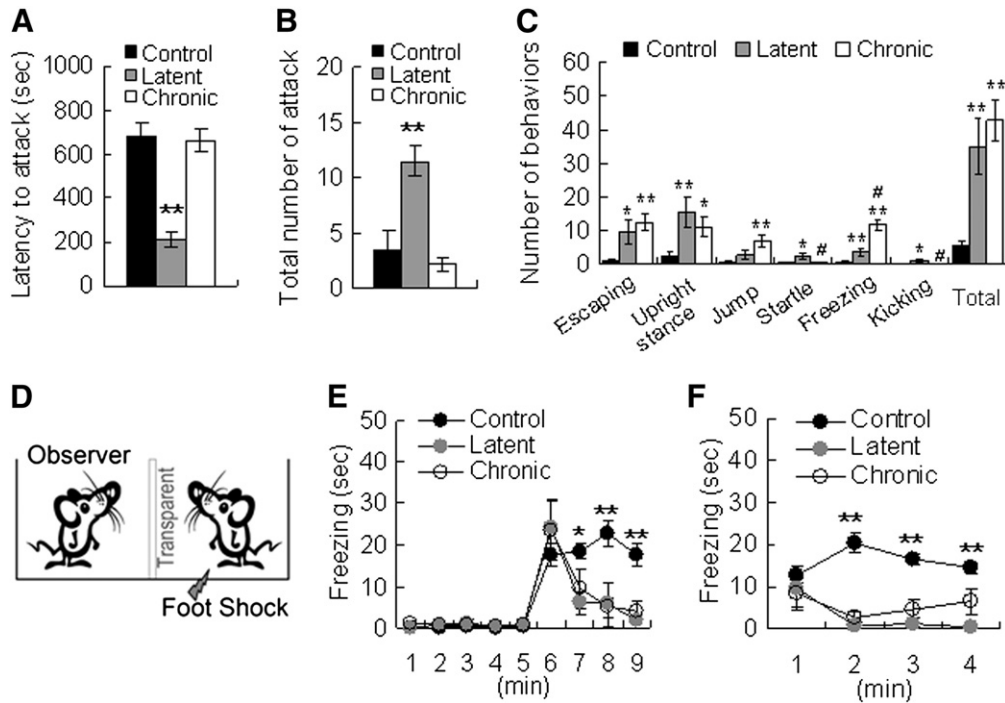
were allowed to explore the two strangers for 10 min. The time spent in sniffing each wire cage and in each of the side chambers was measured. A mouse was considered to be sniffing the wire cage when its head was facing the cage within 1 in.

**Social interaction with a juvenile mouse:** A single mouse in the home cage was allowed to roam freely for 10 min (habituation). A novel juvenile (3–4 weeks old) male C57BL/6 mouse was introduced to the home cage of the resident male subject and then allowed to roam freely for 5 min (test session). The total of the interactions, including sniffing, allogrooming, chasing, biting, and mounting between the two mice was quantified, scoring social interaction as the time during which the resident mouse actively explored the juvenile mouse.

**Resident-intruder test:** This experiment was conducted as described previously (Kim et al., 2009). Resident mice were housed in isolation for 7 days without a bedding change before testing. The isolation-induced resident-intruder aggression test was performed by introducing intruder mice into the home cages of resident mice.

For the offensive behavior experiment, epileptic or control mice were used as resident mice, and aged-matched naïve male C57BL/6 mice were used as intruder mice. For the defensive behavior experiment, aged-matched naïve male C57BL/6 mice were used as resident mice, and epileptic or control 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 bite attack, the latency to the first attack was recorded as 900 s (test duration), and all other attack scores were recorded as zero. In the experiment on defensive behavior, the number of escapes, upright stances, jumps, startles, freezes (immobility with vigilance), and kicks were measured for 15 min.

**Social observational fear conditioning:** Observational fear conditioning, which is empathy-related behavior, was performed as described previously (Jeon and Shin, 2011; Jeon et al., 2010) (illustration in Fig. 3D). The apparatus for observational fear conditioning consisted of two identical chambers (each, 21×17.5×25 cm) containing a



**Fig. 3.** Altered aggressive behaviors and reduced social-fear learning during the latent and chronic period of epilepsy. (A–C) Resident-intruder task. When the latent- and chronic-period mice were used as a resident for offensive behaviors (A, B), only the latent period epileptic mice exhibited a shorter latency to the first attack (A) and a greater number of attacks (B) than did those of the control and chronic-period mice. \*\* $p < 0.01$  by Scheffe's post hoc test between the control and the latent- or chronic-period mice. (C) When the latent- and chronic-period mice were used as intruders for defensive behaviors, the total number of defensive behaviors was greatly increased in both. \* $p < 0.05$ , \*\* $p < 0.01$  by Scheffe's post hoc test between the control and latent- or chronic-period mice. # $p < 0.05$  by Scheffe's post hoc test between the latent- and chronic-period mice. (D–F) Observational fear conditioning. (D) Diagram of the apparatus for observational fear conditioning. When the observer mouse observed that another mouse was receiving a foot shock, the observer showed a freezing response. This freezing response occurred when the observer mouse was returned to the conditioning chamber 24 hr later. (E) Both the latent- and chronic-period mice exhibited significantly reduced freezing responses compared with control mice during training on the first day (E) and on the memory test during the second day (F). \* $p < 0.01$ , \*\* $p < 0.001$ , one-way ANOVA.

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, epileptic and control mice; demonstrator, age-matched naïve male C57BL/6 mice) were individually placed in the apparatus chambers for 5 min, and a 2-sec 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.

*In vivo electrophysiology for cortical rhythms during social behavior*

Except for the recording site on the brain, EEG surgery and recording were the same as described in *In vivo electrophysiology for seizure section*. An electrode was implanted at the frontal cortex (AP 1.8 mm, L 0.4 mm, and DV 1.5 mm), with grounding over the cerebellum, for EEG recordings during social interactions with juvenile mice. Video-EEG recordings were performed simultaneously during a juvenile interaction task. EEG power of baseline behavior was determined across a 30-s period during a habituation session, immediately before introducing a juvenile mouse for the social interaction task. For each 30-s recording, we computed the power as a function of frequency and time, with frequency varying between 1 and 70 Hz. The relative normalized power at each individual frequency was presented as a fraction of the sum of powers at all frequencies of delta (1–4 Hz), theta (4.5–8 Hz), alpha (8.5–12 Hz), beta (12.5–30 Hz), and gamma (30–55 Hz) (Goffin et al., 2011; Llinas et al., 2007; Marrosu et al.,

2006). The concomitant changes in EEG power during social behavior were expressed as a ratio of EEG power from the social interaction (30 s, immediately after introducing a juvenile mouse) over that from habituation (30 s, immediately before introducing a juvenile mouse) in individual animals. To avoid disturbance in the power spectra, EEG signals without interictal or ictal spikes during social behavior were used for analysis. If animals exhibited interictal or ictal spikes during social behavior, the animals were removed for analysis. The electrophysiological data were analyzed offline using PSG Twin 4.2 (Astro-Med) and pClampfit 10.2 (Axon Instruments, Union City, CA, USA).

*Statistical analysis*

Statistical analyses were conducted using SPSS software (SPSS, Chicago, IL, USA) and R (Software Foundation, Boston, MA, USA). Data were analyzed by analysis of variance (ANOVA) followed by post hoc comparisons. Scheffe's post hoc test, a one-way ANOVA, or a two-tailed *t*-test was used to identify main effects. A *p*-value  $< 0.05$  was considered as statistically significant. All experiments were performed in a blind manner with respect to the animal status, and the data were quantified by an experimenter blind to the conditions.

**Results**

*Development of SRSs in epileptic mice*

To investigate the impact of the epileptic periods on development of behavioral dysfunction, we first determined the latent and chronic

periods by detecting SRSs in video-EEG monitoring. The time course of the occurrence of SRSs was examined in mice ( $n = 12$ ) after SE was induced with pilocarpine (Fig. 1). As described in **Material and methods**, mice from days 5 to 7 after SE were used as latent-period mice, and mice from days 30 to 40 after SE were used as chronic-period mice in behavioral tests. During the latent period, only interictal spikes infrequently occurred without ictal spikes (Fig. 1A). In addition, neither convulsive nor other behavioral seizures were observed in the latent period. The first SRS was observed on day 9 following the SE in two of 12 mice (Fig. 1B). The first SRS in the others (10 of 12 mice) occurred between days 10 and 14 after the SE. The number of SRSs appeared to slowly increase as time progressed (Fig. 1C).

#### *Impaired sociability and social novelty preference in latent- and chronic-period mice*

We first subjected the epileptic mice to a sociability/social novelty preference task (Fig. 2A). A difference was observed in the amount of time spent on each side of the chambers ( $F_{2,58} = 5.25$ ,  $p < 0.01$ , two-way ANOVA, Fig. 2B). A post hoc test revealed that the control mice ( $n = 11$ ) spent more time on the side of the chamber with a cage containing stranger 1 ( $334 \pm 17.93$  s) than time on the other side of the chamber with an empty cage ( $175 \pm 17.54$  s) ( $p < 0.01$ , two-tailed t-test) (Fig. 2B). However, both the latent- ( $n = 9$ ) and chronic-period mice ( $n = 12$ ) showed no significant difference in the time spent in the side chambers (Fig. 2B). When sniffing duration was measured, all mice spent more time sniffing stranger 1 than sniffing the empty cage ( $F_{2,58} = 46.86$ ,  $p < 0.001$ , two-way ANOVA, Fig. 2C). However, both the latent- and chronic-period mice spent less time sniffing stranger 1 than did control mice ( $F_{2,29} = 8.56$ ,  $p < 0.001$ , two-way ANOVA).

In the social novelty preference task (Fig. 2D), in which stranger 2 was placed in the empty wire cage, a difference in the amount of time spent in each of the side chambers was observed among control, latent-, and chronic-period mice ( $F_{2,29} = 4.66$ ,  $p < 0.05$ , two-way ANOVA). Control mice spent more time in the chamber containing stranger 2 ( $334 \pm 19.38$  s) than in the chamber with the familiar stranger 1 ( $193 \pm 17.17$  s) ( $p < 0.01$ , two-tailed t-test) (Fig. 2E). In contrast, the latent- and chronic-period mice spent similar amounts of time in both of the side chambers. Sniffing duration was also longer with stranger 2 than that with familiar stranger 1 in control mice ( $p < 0.01$ , two-tailed t-test) (Fig. 2F). However, the latent- and the chronic-period mice spent less time sniffing stranger 2 than did control mice ( $F_{2,29} = 7.63$ ,  $p < 0.001$ , two-way ANOVA, Fig. 2F). These results indicate that sociability and social novelty preference were significantly impaired not only in chronic-period but also in latent-period mice.

#### *Reduced social interaction in latent- and chronic-period mice*

Next, we subjected the epileptic mice to a social interaction task with a juvenile mouse (Fig. 2G). During the 5-min test session, both the latent- ( $n = 8$ ,  $87.01 \pm 8.54$  s) and chronic-period mice ( $n = 12$ ,  $51.46 \pm 11.53$  s) showed a significant reduction in social interaction time compared with that spent by the control mice ( $n = 12$ ,  $142.21 \pm 13.22$  s) ( $F_{2,29} = 16.21$ ,  $p < 0.001$ , one-way ANOVA, Fig. 2H). No significant difference was observed in the interaction time between the latent- and chronic-period mice. This result indicates that social behavior related to social interaction was altered during the latent and chronic periods of epilepsy.

#### *Altered aggression in latent- and chronic-period mice*

We further investigated social interaction using a resident–intruder test related to aggression. When the chronic-period mice were used as

residents for offensive behaviors, they ( $n = 11$ ) showed a similar latency before the first attack (Fig. 3A) and number of attacks (Fig. 3B) to those of control mice ( $n = 15$ ). However, the latent-period mice ( $n = 11$ ) exhibited a shorter latency before the first attack ( $F_{2,34} = 10.76$ ,  $p < 0.01$ , one-way ANOVA, Fig. 3A) and a greater number of attacks ( $F_{2,34} = 9.66$ ,  $p < 0.01$ , one-way ANOVA, Fig. 3B) than did the control and chronic-period mice, indicating a high level of offensive behaviors in the latent-period mice. To assess defensive behavior, naïve latent- and chronic-period mice were subjected to a resident–intruder experiment as intruders. Compared with those in the control mice ( $n = 15$ ), the total number of defensive behaviors increased substantially in both the latent- ( $n = 12$ ) and chronic-period mice ( $n = 14$ ) ( $F_{2,38} = 11.56$ ,  $p < 0.001$ , one-way ANOVA, Fig. 3C). Although a subtle difference was observed in some defensive behaviors such as startle, freezing, and kicking behaviors, between the latent- and chronic-period mice, no difference was seen in the total number of defensive behaviors between the two groups. These results demonstrate that abnormal aggressiveness appeared only during the latent period, and that highly defensive behaviors occurred during both the latent and chronic periods.

#### *Impaired social fear learning in latent- and chronic-period mice*

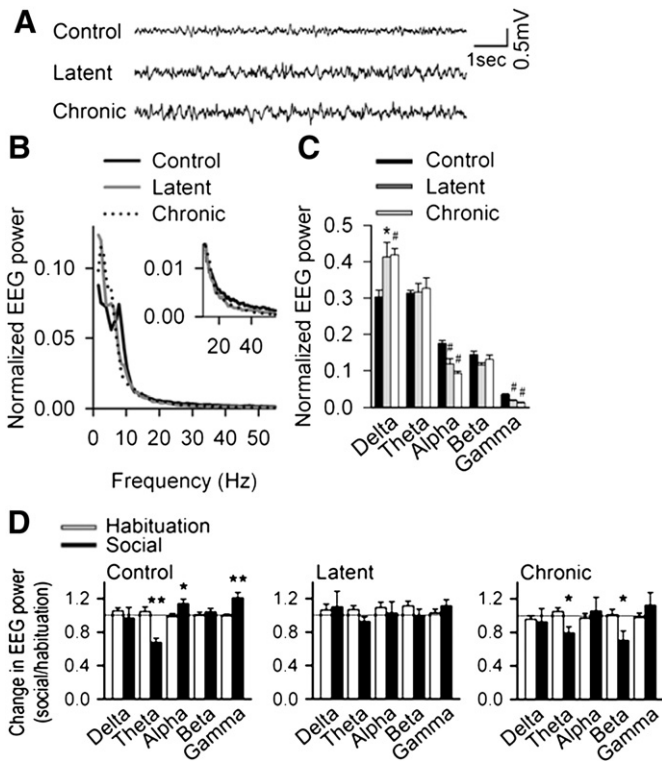
We used an observational fear learning system to examine social fear learning (Fig. 3D). Both the latent- ( $n = 9$ ) and chronic-period mice ( $n = 9$ ) exhibited reduced freezing levels compared with those in control mice ( $n = 13$ ) on training day ( $F_{2,28} = 5.06$ ,  $p < 0.05$ , two-way repeated ANOVA, Fig. 3E). The impairment also appeared when the epileptic mice were returned to the conditioning chamber 24 h later for the contextual memory test ( $F_{2,28} = 23.18$ ,  $p < 0.001$ , two-way repeated ANOVA, Fig. 3F). The amount of freezing in the latent-period mice was not different from that in the chronic-period mice (Figs. 3E–F), indicating that latent-period mice had a social fear learning deficit comparable to that in chronic-period mice.

#### *Altered cortical rhythms in latent- and chronic-period mice*

To examine oscillatory activity in the brain, we performed EEG recordings from the frontal cortex simultaneously with a social behavioral test (social interaction with a juvenile mouse). The EEG recordings during the baseline behavior (i.e., during the habituation period before a social interaction with a juvenile mouse) revealed a significant enhancement in the delta-band power (1–4 Hz) in the latent- ( $n = 8$ ) and chronic-period mice ( $n = 8$ ) compared with that in control mice ( $n = 10$ ) ( $p < 0.05$ , one-way ANOVA) (Figs. 4A–C). We also found a reduction in the power of the alpha (8.5–12 Hz) and gamma bands (30–55 Hz) in the latent- and the chronic-period mice ( $p < 0.05$ , one-way ANOVA) (Fig. 4C). This result indicates that neural activities in latent-period mice were altered during baseline behavior and that they were comparable to those of chronic period mice.

Next, we further examined the concomitant changes in neural activities during social behavior. Concomitant reduction in theta-band frequency ( $0.68 \pm 0.05$ ,  $p < 0.01$ ) and the concomitant enhancement of alpha- ( $1.14 \pm 0.05$ ,  $p < 0.05$ ) and gamma-band frequency ( $1.21 \pm 0.01$ ,  $p < 0.01$ ) were observed in control mice when the EEG power during a social interaction was compared with that in the habituation session (Fig. 4D, left). However, the latent-period mice did not exhibit any concomitant changes in power at each frequency (Fig. 4D, middle). Although the chronic-period mice showed concomitant reductions in theta- ( $0.79 \pm 0.07$ ,  $p < 0.05$ ) and beta-band ( $0.70 \pm 0.11$ ,  $p < 0.05$ ) power (Fig. 4D, right), they did not show concomitant changes in alpha- and gamma-band frequency.

Together, these results indicate that both the latent- and chronic-period mice had similar abnormal cortical rhythms during social behavior.



**Fig. 4.** Altered cortical rhythms in the latent- and the chronic-period mice. (A–C) EEG analysis during baseline behavior (habituation in a social behavior task). (A) Representative electroencephalogram (EEG) traces from control (top), latent- (middle), and chronic-period (bottom) mice during habituation. (B) Normalized EEG power spectra of the habituation period. EEG-power values of both the latent- and chronic-period mice decreased at the alpha- and gamma-band frequencies, and the value increased at the delta-band frequencies. \* $p < 0.05$ , # $p < 0.01$  by Scheffe's post hoc test between the control and latent- or chronic-period mice. (D) Concomitant changes in EEG power of control (left) and latent- (middle) and chronic-period (right) mice during social behavior. Data are presented as ratios (social, immediately after introducing a juvenile mouse/habituation, immediately before introducing a juvenile mouse). \* $p < 0.05$ , \*\* $p < 0.01$  by two-tailed *t*-test.

**Discussion**

Although many nonsocial behavioral dysfunctions of epileptic mice in the chronic period are known, their behavioral alterations in social behaviors are not well documented. Moreover, it is not known when the behavioral dysfunctions develop during epileptogenesis. In this study, we investigated social behavioral pathogenesis and neural activities during two distinct epileptogenic stages in an experimental TLE model. Social behavior was severely impaired in latent-period and chronic-period mice. Except for abnormal offensive behavior in the latent-period mice, social deficits in the latent-period mice were almost comparable to those in the chronic-period mice. The latent- and chronic-period mice exhibited increased exploratory behavior toward an inanimate object, and they showed normal responses in acoustic startle reflex (Supplemental data). Thus the social dysfunctions in the latent- and chronic-period mice might not be attributed to reduced general interest in novelty or locomotor activity and abnormal sensorimotor function regarding the acoustic startle reflex. Simultaneously measuring EEG during social interaction revealed that brain activity was already altered during the latent period before the chronic period, which might have corresponded with the behavioral results. We suggest that social deficits and aberrant neural activities develop at the earlier epileptogenic stage before SRSs develop.

Epileptogenesis is a process by which a normal brain develops epilepsy, a chronic condition in which SRSs occur. After a brain injury or insult occurs, there is a “silent” or “latent period” in which seizures

do not occur. A number of physiological and structural changes can occur in the brain during the latent and chronic period of epilepsy (Cavalheiro et al., 1991; Dudek et al., 2002; Herman, 2002; Pitkanen et al., 2002; Scharfman, 2007; Sloviter, 2008). In fact, cell damage is known to appear at 24 hr after the pilocarpine-induced SE, and cell loss was observed in earlier stage (1 week after SE) during epileptogenesis (Groticke et al., 2007; Hendriksen et al., 2001; Navarro Mora et al., 2009). Some animal studies have demonstrated alterations in the levels of  $\gamma$ -aminobutyric acid, glutamate, and monoamines, as well as the expression of *N*-methyl-D-aspartate (NMDA) receptors (Cavalheiro et al., 1994; Di Maio et al.). Particularly, NMDA receptors are known to be involved in cortical gamma rhythms (Kocsis, 2012; Yizhar et al., 2011). Thus, it is likely that alterations on neurotransmitters/receptors, cell damage, and the consequent imbalance in neural excitation/inhibition in the circuits during latent period are sufficient to evoke behavioral alterations and aberrant neural activities (Fritschy, 2008).

Diverse social behaviors involve perception, interpretation, and production of signals that influence individual behavior in a manner that depends on social context (Robinson et al., 2008). Given the complexity of social behavior, complex brain network activity would be expected to contribute to this behavior. A key component of social behavior is social attention, and attentive behavior is associated with enhanced gamma rhythms in the cortex (Harris and Thiele, 2011; Wang, 2010). It has been suggested that altered gamma activity could also impair social behavior, which might be associated with an imbalance in neocortical excitation/inhibition (Yizhar et al., 2011). Additionally, abnormal gamma activity is also featured in some neurological diseases characterized by social deficits, such as autism spectrum disorders (Goffin et al., 2011; Gogolla et al., 2009; Orekhova et al., 2007). Along with gamma-band frequency, alpha-band frequency is also involved in social behavior (Naem et al., 2012; Rybak et al., 2006). Although we cannot prove that the concomitant increases in alpha and gamma frequencies were correlated with social behavior, the social deficits shown in the latent- and chronic-period mice may be associated with altered alpha and gamma frequencies, which might be driven by physiological and structural changes during epileptogenesis.

In conclusion, a novel finding of this study was that social dysfunction and aberrant neural activities developed during the earlier epileptogenic period (i.e., latent period before the occurrence of SRSs) in a mouse pilocarpine model of TLE. These findings may help to understand behavioral pathogenesis of patients with TLE and the pathophysiology of TLE. Our results may also help in understanding the relationship between epilepsy and other neurological diseases or brain disorders, such as autism, which is characterized by social dysfunction and frequently has epilepsy as a comorbid condition.

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Authors declare no conflict of interest.

**Appendix A. Supplementary data**

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.expneurol.2012.11.024>.

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