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Behavioral and cognitive alterations, spontaneous seizures, and neuropathology developing after a pilocarpine-induced status epilepticus in C57BL/6 mice

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ABSTRACT

Many patients with epilepsy suffer from psychiatric comorbidities including depression, anxiety, psychotic disorders, cognitive, and personality changes, but the mechanisms underlying the association between epilepsy and psychopathology are only incompletely understood. Animal models of epilepsy, such as the pilocarpine model of acquired temporal lobe epilepsy (TLE), are useful to study the relationship between epilepsy and behavioral dysfunctions. In the present study, we examined behavioral and cognitive alterations, spontaneous seizures, and neuropathology developing after a pilocarpine-induced status epilepticus in the C57BL/6 (B6) inbred strain of mice, which is commonly used as background strain for genetically modified mice. For this study, we used the same pilocarpine ramping-up dosing protocol and behavioral test battery than in a previous study in NMRI mice, thus allowing direct comparison between these two mouse strains. All B6 mice that survived SE developed epilepsy with spontaneous recurrent seizures. Epileptic B6 mice exhibited significant increases of anxiety-related behavior in the open field and light–dark box, increased locomotor activity in the open field, elevated plus maze, hole board, and novel object exploration tests, and decreased immobility in the forced swimming and tail suspension tests. Furthermore, spatial learning and memory were severely impaired in the Morris water maze, although hippocampal damage was much less severe than previously determined in NMRI mice. B6 mice in which pilocarpine did not induce SE but only single seizures did not exhibit any detectable neurodegeneration, but differed behaviorally from sham controls in several tests of the test battery used. Our data indicate that the pilocarpine model of TLE in B6 mice is ideally suited to study the neurobiological mechanisms underlying the association between seizures, brain damage and psychopathology.

Introduction

In recent years, the pilocarpine model of temporal lobe epilepsy (TLE) has become the most popular and widely used rodent model of this common and difficult-to-treat type of epilepsy (Cavalheiro et al., 2006; Curia et al., 2008). In this model, the cholinomimetic convulsant pilocarpine is used to induce a status epilepticus (SE), which is followed by hippocampal damage and development of spontaneous recurrent seizures (SRS). The model has initially been described in rats, but is increasingly used in mice (Cavalheiro et al., 2006; Curia et al., 2008). Surprisingly, although the acute convulsive and neurodegenerative effects of pilocarpine in mice were first reported in 1984 (Turski et al., 1984) and SRS following a pilocarpine-induced SE in mice in 1996 (Cavalheiro et al., 1996), to our knowledge the long-term behavioral and cognitive alterations occurring in this model have not been fully characterized in this species since then. In patients, TLE is often associated with behavioral alterations, such as depression, anxiety and psychosis, and impaired cognitive performance (Boro and Haut, 2003; Swinkels et al., 2005; Cornaggia et al., 2006; Hoppe et al., 2007; Marcangelo and Ovsiew, 2007). Memory impairment in patients with TLE is thought to be a consequence of the hippocampal alterations that are associated with TLE, whereas the neurobiological mechanisms of the relationship between TLE and psychiatric disorders are poorly understood (Devinsky, 2003; Motamedi and Meador, 2003; Swinkels et al., 2005; Hoppe et al., 2007). Animal models of epilepsy may help to enhance our understanding of causal mechanisms underlying the association between epilepsy and behavioral abnormalities (Post, 2004; Majak and Pitkanen, 2004; Heinrichs and Seyfried, 2006).

In the NMRI outbred strain of mice, we have recently reported that pilocarpine-induced epilepsy is associated with significant increases of anxiety-related behavior and impairment of learning and memory, thus indicating that pilocarpine-treated mice reflect several of the behavioral and cognitive disturbances that are associated with TLE in humans (Gröticke et al., 2007). In the present study, we examined development of SRS, neurodegeneration and behavioral and cognitive alterations after a pilocarpine-induced SE in the C57BL/6 (B6) inbred

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strain of mice, using the same pilocarpine dosing protocol and behavioral test battery than in our previous study in NMRI mice (Gröticke et al., 2007). Pilocarpine has previously been used to induce SE and subsequent SRS in B6 mice (Shibley and Smith, 2002; Borges et al., 2003; Peng et al., 2004; Houser et al., 2008), but, to our knowledge, behavioral alterations developing after SE have not been described in this strain, except for one study in which pilocarpine-treated B6 mice were evaluated in an open field and water maze shortly after SE (Mohajeri et al., 2004). Our goal was to directly compare the various long-term consequences of pilocarpine-induced SE in B6 mice with those previously determined by us in NMRI mice, thus allowing to determine the impact of genetic background in this model of TLE.

Materials and methods

Animals

Mice (C57BL/6NCrl) were obtained from Charles River (Sulzfeld, Germany) at an age of about 5–6 weeks. Female mice were used to allow comparison with previous studies of our group on pilocarpine in mice (Gröticke et al., 2007; Müller et al., in press) and to ease housing in groups for the long period needed to perform experiments in models of epilepsy. After arriving at our Department, animals were housed in groups under controlled conditions (temperature: 21 ± 0.5 °C; humidity: 55 ± 5%), under a 12-h light–dark cycle with lights on at 6.00 a.m. and food and water *ad libitum*. The animals were allowed to adapt to laboratory conditions for at least 1 week before starting the experiments. All experiments were performed between 7.00 a.m. and 1.00 p.m., i.e., during the light phase of the light–dark cycle. All possible steps were taken to avoid animals' suffering at each stage of the experiment. The procedures used in this study had the approval of the Institutional Animal Care and Use Committee and were carried out in accordance with the European Council Directive of November 24th, 1986 (86/609/EEC).

Induction of status epilepticus

Based on previous experiments of our group with pilocarpine in different mouse strains (Gröticke et al., 2007; Müller et al., in press), a dosing protocol with repeated low-dose treatment by i.p. application of 100 mg/kg pilocarpine every 20 min until onset of SE was used. Compared to injection of one high single dose of pilocarpine, this ramping-up protocol allowed a more individual dosing of the convulsant, resulting in a higher percentage of mice developing SE and reduced mortality (Gröticke et al., 2007; Müller et al., in press). It usually took three injections to a first seizure (typically a score 4 or 5 seizure according to the Racine scale (Racine, 1972)), but for development of SE with continuous seizure activity (see Results) it was important to continue the injections of pilocarpine after the occurrence of this first seizure, until SE started. Typically, 1–2 additional injections were needed after the first seizure to induce SE. If SE was not induced after 7–8 injections in an individual animal, usually additional injections failed to induce SE, but mice died in individual convulsive (tonic–clonic) seizures, due to respiratory arrest. Thus, the maximum number of repeated pilocarpine injections was restricted to about 14. In order to avoid peripheral cholinergic effects, methylscopolamine (1 mg/kg) was administered 30 min before the application of pilocarpine. SE was defined as continuous limbic seizure activity (see Results for more detailed description), which typically lasted for several hours if not terminated earlier by diazepam

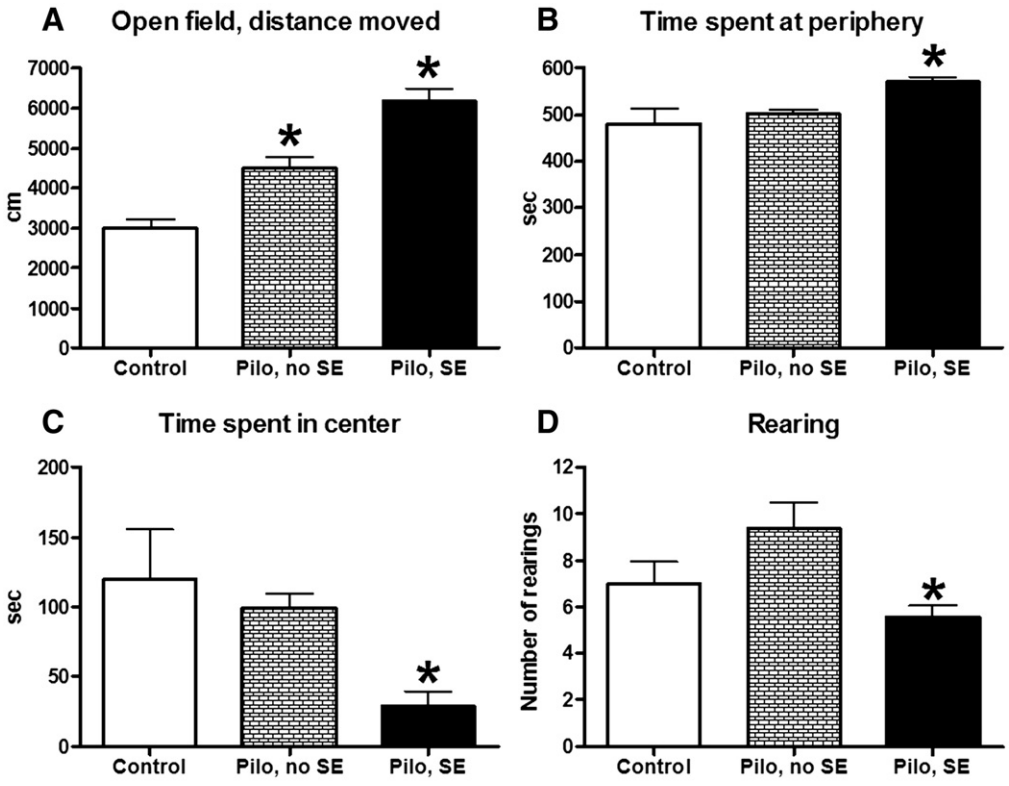

Fig. 1. Behavior of epileptic mice and nonepileptic controls in the open field. Data are shown as means ± SEM of 12 sham controls, 5 mice that received pilocarpine but did not develop SE, and 14 mice that developed SE after administration of pilocarpine, respectively. All mice with SE had developed epilepsy with spontaneous recurrent seizures. Behavioral testing in the open field was performed 9–12 weeks after SE. A illustrates the total distance that the mice moved during the 10 min of the open field test. B illustrates the time that mice spent in the periphery of the open field. C illustrates the time that mice spent in the aversive center of the open field. D illustrates the number of rearings in the open field. Analysis of data by ANOVA indicated significant inter-group differences for the data shown in A–D. Individual differences vs. sham controls are indicated by asterisk ($P<0.05$) except for D in which the asterisk indicates significant difference to pilocarpine-treated mice without SE ($P<0.05$).