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Antimuscarinic-induced convulsions in fasted mice after food intake: no evidence of spontaneous seizures, behavioral changes or neuronal damage

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Prolonged or repeated seizures have been shown to cause spontaneous recurrent seizures, increased anxiety-related behavior, locomotor hyperactivity, impaired functions of learning and memory, and neuronal damage in the hippocampus and other brain regions in animals. Mice and rats treated with antimuscarinic drugs after fasting for two days or less develop convulsions after being allowed to eat *ad libitum*. To address whether such behavioral and neuroanatomic changes occur following these convulsions, mice treated i.p. with saline (control) or 2.4 mg/kg atropine and given food after 24 h of fasting were grouped according to seizure scores for behavioral and histological analysis. Following convulsions, the occurrence of spontaneous recurrent seizures was observed for 30 days. Motor activity and grooming behavior were assessed in the open field, and memory was assessed using the novel object recognition test 4 and 7 days after onset of convulsions, respectively. Animals allocated for the histological analysis were decapitated 7 days after onset of convulsions and hippocampal slices were evaluated for the percentage of degenerating neurons stained with Fluoro-Jade C. Spontaneous recurrent seizures, locomotor alterations, anxiety-related behavior, memory impairment, and neuronal loss in the granular layer of the dentate gyrus were not detected in the animals with seizure score 1–2 or 3–5. These results are in accordance with those related to the absence of behavioral changes, cognitive deficits, and hippocampal neuronal damage after single brief seizures in animals and patients with epilepsy.

Key words: atropine-induced convulsions, memory, motor activity, grooming, neuronal damage, fasting

INTRODUCTION

In a series of studies, we have shown that mice and rats treated with scopolamine, atropine or biperiden after fasting for two days or less develop convulsions soon after being allowed to eat *ad libitum* (Nurten and Enginar 2006, Enginar and Nurten 2010). Convulsions appear in the form of single or repetitive seizures lasting for seconds or minutes during the observation period of 30 min. Food deprivation itself, but not its hypoglycemic consequence, seems to be critical in the development of seizures because glucose intake during fasting increases plasma levels almost to fed levels, but could not produce any preventive effect (Enginar et al. 2005). Deprivation of food produced significant changes in the kinetics of [^{3}H]glutamate binding in the brain (Enginar et al. 2003) and increased expression of M_1 muscarinic receptors in the frontal cortex and expression of M_2 muscarinic receptors in the hippocampus (unpublished data), implying that neuroadaptative changes occur in animals during fasting. Development of convulsions only after solid food intake, but not slurry or fluid feeding, suggests that all of the complicated acts that occur during eating (e.g. chewing and swallowing movements, smelling and tasting), and stimulation of the amygdala by repetitive oral and masticator movements are the triggering and underlying factors (Nurten et al. 2009). Most of the major antiepileptic drugs in clinical use were ineffective in suppressing these convulsions (Enginar et al. 2003, Nurten and Enginar 2006, Büget et al. 2016). Bearing some similarities in triggering factors and manifestations of seizures in patients with eating epilepsy, convulsions in fasted animals may provide insight into the mechanism of this rare and partially controlled form of reflex epilepsy (Senanayake 1994, Striano et al. 2012).

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Repetitive limbic seizures and *status epilepticus* developed in rodents administered with the muscarinic receptor agonist pilocarpine have been shown to induce spontaneous recurrent seizures, behavioral and cognitive alterations, as well as neuronal damage in the hippocampus and other brain regions after a latent period (Turski et al. 1983, Akula et al. 2007, Müller et al. 2009a, 2009b, Cardoso et al. 2011, Karoly et al. 2015). Similar findings were also obtained in animals that had chemically-induced *status epilepticus* (Gröticke et al. 2008, Furtado et al. 2010, Aniol et al. 2013, Schultz et al. 2014, Rojas et al. 2016) or brief seizures after treatment with electroshock kindled seizures (Cavazos et al. 1994, Bengzon et al. 1997, Cardoso et al. 2011). Consistent with these results, it has been shown that recurrent seizures in patients with epilepsy are associated with progressive atrophic changes in some brain regions (Bernhardt et al. 2009) which are suggested to cause behavioral alterations and impaired cognitive performance (Jokeit and Ebner 2002, Vingerhoets 2006). Nevertheless, there are some contrasting data that only prolonged seizures, but not repeated brief seizures, can cause brain damage (Stula et al. 2003). It has been shown that specific populations of hippocampal neurons are highly susceptible to damage from seizures evoked by a wide range of convulsants (Olney et al. 1986, Cavazos et al. 1994, Haas et al. 2001, Müller et al. 2009a, 2009b, Cardoso et al. 2011). Damage in the hippocampus, a structure implicated in both memory acquisition and seizure expression, seems to be an important contributor to seizure-induced effects (Haas et al. 2001).

In view of the facts mentioned above, the present study evaluated whether spontaneous recurrent seizures, behavioral and cognitive alterations, and histological changes in the hippocampus occur following convulsions induced by antimuscarinic treatment and food intake in mice fasted for 24 h.

METHODS

Animals

Inbred male BALB/c mice weighing 25–30 g were used in the study. They were housed in groups under a regular 12-h light/dark cycle with lights on at 07:00 AM and at 21±3°C for at least 1 week prior to experimentation and were allowed free access to both food and water. All studies were approved by the Istanbul University Local Ethics Committee on Animal Experiments (2012/180) and were in accordance with the *EU Directive 2010/63/EU* on the protection of animals used for scientific purposes.

Induction of convulsions in fasted animals

After being weighed, animals were moved to clean cages with fresh bedding. They were deprived of food for 24 h. Water was freely available during the fasting period. At the time of testing, fasted animals were re-weighed and treated intraperitoneally (i.p.) with 2.4 mg/kg atropine (Sigma, St Louis, MO) or saline (control) and then were individually placed in wire mesh observation cages. Twenty minutes later, the animals were given food pellets and allowed to eat *ad libitum*. All animals were observed for 30 min for the incidence and onset of convulsions. Using a modified Racine's scale (1972), seizure activity was quantified as: (0) no difference; (1) immobility; (2) forelimb clonus; (3) forelimb clonus with rearing; (4) forelimb clonus with rearing and falling down; (5) generalized convulsions with rearing, falling down, and jumping. A convulsive response was assessed as forelimb clonus with rearing. Onset of convulsions was defined as the time passed before an animal displayed forelimb clonus with rearing after starting to eat. Incidence of convulsions was expressed as the percentage of animals displaying either stage 3, 4 or 5 activity in each group.

Soon after the observation period, mice with seizure activity 1 or 2 (seizure score 1–2; n=9) and animals with seizure activity 3, 4 or 5 (seizure score 3–5; n=14) were pooled for behavioral and histological analysis. These two groups and the control group (n=9) were housed under standard laboratory conditions with free access to both food and water.

Monitoring for spontaneous recurrent seizures

Starting 24 h after convulsions, the animals with seizure score 1–2 or 3–5 were observed for the development of spontaneous recurrent seizures for a period of 30 days. Observations were repeated 5 days per week for a minimum of 2 h at random times between 08:00 and 20:00 each day. Control mice were also observed for comparison. All animals were tested for motor activity and novelty-induced grooming behavior, and for novel object recognition 4 and 7 days after convulsions, respectively.

Assessment of motor activity and novelty-induced grooming in the open field

A wooden box (width 35 cm, length 50 cm, height 20 cm) with the floor divided into 12 quadrants of equal size and a Plexiglas cover with holes for air entrance was used to assess motor activity and grooming be-

havior. Mice were individually placed in the apparatus. Following a 1-min habituation period, the animals were observed for locomotor activity and rearing for 10 min and for grooming activity for 5 min. Locomotor activity was measured by counting the number of times the animals crossed from one square to another (ambulation). The number of rearing events, standing on hind legs, was counted. Grooming activity was considered as scratching of body and face, licking of body fur, tail, limbs, and genital area. Latency to grooming and total time spent grooming were measured.

Novel object recognition test

Novel object recognition test used in the present study was modified from previously described methods (Yuede et al. 2009, Zhang et al. 2012). A box with a wooden floor and opaque sides (width 35 cm, length 50 cm, height 35 cm) was used for the test. Soon after motor activity and grooming assessments performed 4 days after convulsions, mice were individually habituated to the box for 3 consecutive days for a period of 5 min per day. Training and test trials were performed 7 days after convulsions. During the training trial (familiarization phase), the animals were allowed for a period of 15 min to freely explore two identical objects placed on the central line of the floor 10 cm apart from the shorter sides of the box. Two hours later, they were placed back in the testing chamber with the familiar object and a new object for a period of 5 min. During the testing trial, the animals were evaluated for their ability to remember the familiar object. Objects were constructed using commercial plastic blocks (Lego) with same or different heights, colors and shapes. Exploration was defined as the animal directing the nose within 2 cm of the object while looking at, sniffing or touching it. Sitting or standing on the object was not scored as object exploration. The time spent exploring the familiar (TF) and the novel (TN) object was recorded. A recognition index (RI) was calculated for each animal with the following formula: [RI=TN/(TF+TN)] (Oliveira et al. 2015) At the end of the test trials, animals were housed again for the monitoring of spontaneous recurrent seizures.

All behavioral experiments were carried out between 08:30 AM and 13:30 PM in a temperature controlled (21±2°C) quiet room under dim light. Mice were acclimatized to the experimental room 1 h prior to experimentation. Observers were blind to the treatments. The boxes and the objects were thoroughly and vigorously wiped with a damp cloth and then dried with a dry cloth between animals, and between training and testing trials.

Histological procedures

The animals in the control (n=7), seizure score 1–2 (n=6), and seizure score 3–5 (n=11) groups were decapitated 7 days after convulsions. The brains were removed immediately. After overnight fixation in 4% paraformaldehyde, dehydration with ethanol solution and keeping in xylene, the tissues were embedded in paraffin. Using online Allen Mouse Brain Atlas as a reference guide, hippocampal region was found and sectioned coronal plane at a nominal thickness of 10 µm using a rotary microtome (Leica RM2155). Every fifth section was collected using a systematic random sampling procedure. The slices were placed in distilled water for 2 sec for rehydration, followed by boiling with citrate buffer (pH 6.0, abcam ab64236) for 15 min in a microwave for unmasking of epitopes. After cooling, the slices were washed with phosphate buffered saline (PBS) (pH 7.6, abcam ab64246) and then incubated with 2.5% goat serum (96000 mg/ml, pH 6.0, abcam ab7481) for 30 min at 37°C for blocking nonspecific staining. The slices were then rewashed with PBS (pH 7.6), followed by incubation with primary antibody (1/50, Rabbit polyclonal, IgG, ab124455) at room temperature overnight. After washing with PBS, the slices were incubated with secondary antibody (1/1000, Anti-Rabbit IgG-H&L (TRITC), pre-adsorbed (abcam ab6011), for 1 h at 37°C. Following re-washing with PBS and distilled water, the tissues were treated with 0.06% potassium permanganate for 1 min and then were stained with 0.0001% Fluoro-Jade C (MERCK Millipore, USA) and 0.0001% 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) (Sigma-Aldrich Co-32670) solutions for

Table I. Development of convulsions in fasted mice after atropine treatment and food intake[a]

Groups (n)	Convulsions		Number of deaths[c]
	Incidence (%)	Time of onset (min) (mean±SE)[b]	
Control (16)	0	–	–
Atropine (49)	67.3*	3.10±0.50	8

[a] Mice fasted for 24 h were injected i.p. with saline or 2.4 mg/kg atropine and were given free access to food 20 min later; [b] Calculated from seizing animals; [c] Caused by generalized convulsions (stage 5); (n): number of animals; * $p<0.001$, significantly different from control (saline) group.

20 min (Schmued et al. 1997, 2005). Finally, the slices washed with distilled water were mounted with permount and observed under a fluorescence microscope (Olympus BX51) with adapted fluorescein isothiocyanate (FITC), tetramethylrhodamine isothiocyanate (TRITC) and DAPI filters (Cardoso et al. 2011, Müller et al. 2009a, 2009b). The total number of neurons' nuclei stained by DAPI and the number of degenerating neurons stained by Fluoro-Jade C were counted in 2 randomly selected fields in the granular layer of the dorsal dentate gyrus. The percentage of Fluoro-Jade C positive neurons was calculated.

Statistical analysis

Statistical analyses were performed using SPSS Statistics (version 22.0). Motor activity, latency to grooming, and Fluoro-Jade C positive neuron count data were evaluated using one-way ANOVA. Grooming duration and recognition index data were evaluated using the Kruskal-Wallis test. Fisher's exact test ($n<20$) was used for the evaluation of the frequency of the incidence of convulsions. Significance was accepted at $P<0.05$. Data are shown as mean with standard error of the mean (± SEM).

RESULTS

Convulsions in fasted mice

The body weights of the mice fell to approximately 85% of the starting body weights after being deprived of food for 24 h. Atropine treatment caused convulsions in fasted animals after food intake. This effect was statistically significant when compared with the saline given control animals ($P<0.001$). Onset and incidence of convulsions, and number of animals in each seizure stage are shown in Table I and Table II, respectively. During the 30 min observation period, the animals exhibited short single or repetitive (2–4 times) seizures with stage 3, 4 or 5 activity. Single seizure duration was 20-50 sec in animals with seizure score 1–2 and 20–180 sec for seizure score 3–5. Some animals exhibited continuous (5–10 min) stage 2 or 3 *status epilepticus*-like activity.

Spontaneous recurrent seizures after convulsions

The observation of animals daily for 2 h for 30 days following convulsions revealed that neither animals with seizure score 3–5 nor 1–2 experienced spontaneous recurrent seizures during this period.

Motor activity and novel environment-induced grooming behavior

Table III shows the motor activity and novelty-induced grooming behavior of the control animals and animals with seizure score 1–2 or 3–5 evaluated 4 days after convulsions. One-way ANOVA revealed no significant differences between groups in the number of squares crossed [$F_{(2,29)}=0.233$; $P=0.794$], number of rearing [$F_{(2,29)}=0.364$; $P=0.698$], and latency to grooming [$F_{(2,29)}=0.709$; $P=0.501$]. The Kruskal-Wallis test also revealed that the duration of grooming [$\chi^2(2,29)=0.343$; $P=0.837$] did not differ between the control and seizure groups.

Novel object recognition memory

Table III shows the novel object recognition index data of the control animals and animals with seizure score 1–2 or 3–5 measured 7 days after convulsions. The Kruskal-Wallis test revealed no significant differences between the control and seizure groups in the total time spent exploring both the novel and the familiar objects [χ^2 (2,29)=1.846; $P=0.397$].

Neuron counts

Fig. 1 presents representative photographs of Fluoro-Jade C-stained hippocampal sections obtained from control animals and animals with seizure score 1–2 or 3–5 decapitated 7 days after convulsions. The quantitative analysis of the neuronal density in the granular layer of the dentate gyrus of each group is illustrated in Fig. 1 also. Kruskal-Wallis test yielded no significant group differences in the percentage of Fluoro-Jade C positive neurons [$F_{(2,21)}= 0.335$; $P=0.719$].

Table II. Number of animals showing seizure stages in saline (control) and atropine groups

Groups (n)	Stage 0	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
Control (16)	16	0	0	0	0	0
Atropine (49)	1	3	12	13	10	10

(n): number of animals.

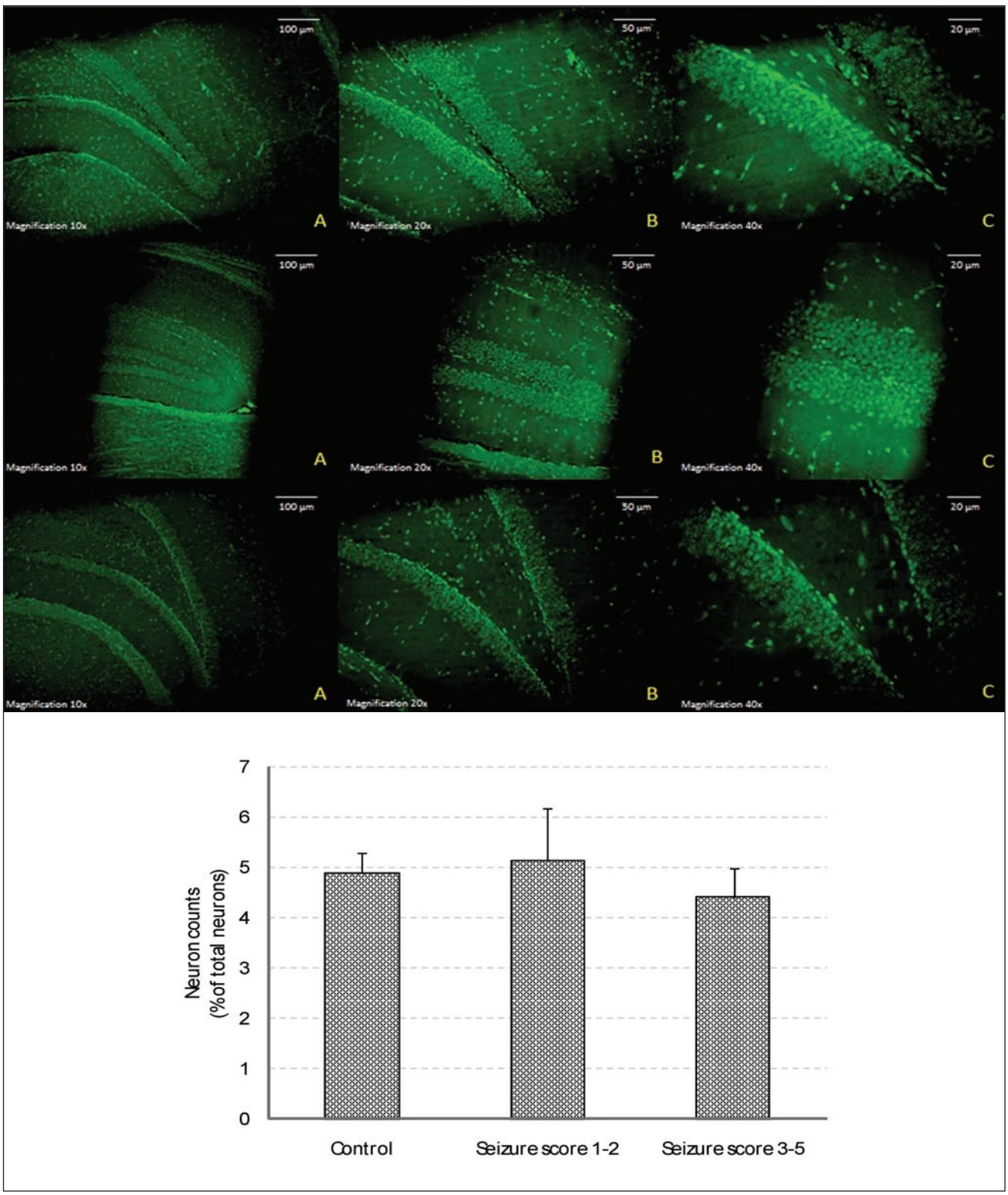

Fig. 1. Photomicrographs of representative Fluoro-Jade C-stained (green) coronal sections of the granular layer of the dentate gyrus from a control mouse (top row), and from mice belonging to seizure score 1–2 (middle row) and seizure score 3–5 (bottom row) groups. Animals were decapitated 7 days after convulsions. Photomicrographs displayed in "B" and "C" are magnifications of photomicrographs illustrated in "A". Scale bars are 100 µm in Panel A, 50 µm in Panel B and 20 µm in Panel C. Panel D illustrates the quantitive analysis of the neuronal density in each group. Bars denote to the degenerating neurons stained with Fluoro-Jade C (% of total neurons).

DISCUSSION

As demonstrated in our previous studies, atropine treatment and refeeding caused convulsions in mice after 24 h of fasting. This study was designed to group animals with respect to the duration and recurrence, as well as the severity of seizures displayed by each mouse. Unfortunately, the total number of mice showing repetitive or prolonged seizures did not produce adequate sample sizes for grouping. Therefore, for the assessment of the influence of seizure severity on the measured parameters, the animals were pooled into two groups according to their seizure scores. Animals with seizure score 1–2 or 3–5 and those in the control group were observed for the occurrence of spontaneous recurrent seizures for 2 h per day for a period of 30 days, starting 24 h after the convulsions. During this period, no episodes of seizure activity were visually observed in any animals. In other words, we could not observe spontaneous recurrent seizures. But some subclinical seizures may be present in the animals because electroencephalographic recordings were not carried out (Drexel et al. 2012). The absence of spontaneous recurrent seizures in the present study confirms previous studies that no spontaneous recurrent seizures were observed in rats after short lasting recurrent seizures elicited by electroshock (Cardoso et al. 2011) or pentylenetetrazole (Aniol et al. 2013). On the other hand, observation of animals intermittently or continuously after severe seizures induced by pilocarpine (Gröticke et al. 2007, Müller et al. 2009a, 2009b, Bortel et al. 2010, Cardoso et al. 2011), kainate (Szyndler et al. 2006, Gröticke et al. 2008) or electrical stimulation (Brandt et al. 2003) showed that spontaneous recurrent seizures developed in most animals. In the pilocarpine model, the duration of *status epilepticus* was 30–120 min (Bortel et al. 2010) and the interval between the induction and the first observed spontaneous recurrent seizure was 3-30 days (Arida et al. 1999). With respect to all these findings, the severity and/or duration of seizures in fasted animals might not be sufficient to induce recurrent spontaneous seizures. However, pilocarpine injected rats that did not develop *status epilepticus* exhibited spontaneous recurrent seizures almost 8 (±2) months after injection (Navarro Mora et al. 2009) suggests the need for long-term monitoring of the animals following the initial seizures.

Using open field and novelty-induced grooming tests, the presence of behavioral alterations were assessed in all animals 4 days after convulsions. Square crossing and rearing in the open field were measures of anxiety, as well as horizontal and vertical locomotor activity. Decreased locomotion (Tamada et al. 2010) and rearing (Sestakova et al. 2013) are suggested to reflect increased emotionality to the novel environment. In the present study, there were no significant differences in open field data between the control animals and animals with seizure scores of 1–2 or 3–5. The absence of motor activity and anxiety-like behavioral changes in the seizing animals was similar to the observations obtained in pilocarpine-induced single seizures in mice in the open field test performed 9-12 weeks after the initial seizures (Müller et al. 2009a). Contrasting and somewhat conflicting data are also present in the literature for both brief and prolonged seizures. Gröticke et al. (2008) demonstrated that locomotor, exploratory, and anxiety-related behavior measurements in the open field, hole-board, elevated-plus maze, light-dark box, and novel object exploration tests performed starting 5–11 weeks after *status epilepticus* did not differ between the control and intrahippocampal kainate-injected mice. Müller et al. (2009b) reported that mice with pilocarpine-induced *status epilepticus* exhibited significant increases in anxiety-related behavior in the open field, i.e., less time spent in the aversive center and less rearing, and reduced locomotor activity in the Irwin observation test. Cardoso et al. (2009) examined behavioral alterations of repeated brief seizures evoked by electroconvulsive shock and compared them with those resulting from prolonged *status epilepticus* induced by pilocarpine in rats. Elevated levels of anxiety as indicated by decreased overall locomotor activity, defecation and urination scores in the open field, and time spent in the enclosed arms of the elevated

Table III. Open field and novel object recognition test measurements following convulsions in fasted mice after atropine treatment and food intake

Groups (n)	Motor Activity		Grooming (sec)		Recognition index (%)
	Square crossed	Rearing	Latency	Duration	
Control (9)	36.7±3.4	42.2±6.6	53.2±17.4	17.4±7.5	0.7±0.03
Seizure score 1–2 (9)	34.1±4.6	45.2±4.2	82.3±24.5	7.4±3.2	0.5±0.13
Seizure score 3–5 (14)	37.6±3.3	38.7±5.3	48.4±20.1	7.9±2.8	0.7±0.04

Number of square crossed, number of rearing, latency to grooming and total time spent in grooming were assessed in the open field 4 days, and recognition index was determined in the novel object recognition test 7 days after convulsions induced by atropine treatment and food intake in 24-h fasted mice; (n): number of animals.

plus-maze were detected approximately 10 weeks following both seizures, and were more potent in the electroconvulsive shock model. Akula et al. (2007) found hyperlocomotor activity and anxiogenic response in mice 24 h after the termination of 9-days kindling with pentylenetetrazole.

Decreased latency and increased duration in grooming behavior are often consistent with higher stress responses in novel environments, and therefore are generally presented as fear- or anxiogenic-like behaviors in rodents (Escorihuela et al. 1999, Enginar et al. 2008). However, according to the existing literature, grooming is rarely used to assess seizure-induced behavioral alterations. Similar to the locomotor activity measurements mentioned above, both measurements of grooming behavior did not differ among seizing and control animals in the open field in the present study. Portugal-Santana et al. (2004) also reported that grooming, indicative of motor performance, was normal in the post-ictal period in rats with electroshock seizures. In contrast, Bernard et al. (2015) found reduced grooming in the presence of an unfamiliar rat in the novel environment, which supported altered social anxiety following a single episode of kainic acid-induced early-life seizures in rats.

Cognitive deficits have been identified as marked sequelae of prolonged continuous seizures in patients with epilepsy (Holmes 2004). Accordingly, various learning and memory tests showed seizure-induced memory impairments in animals (Szyndler et al. 2006, Akula et al. 2007, Müller et al. 2009a, 2009b, Cardoso et al. 2011). The novel object recognition test was used to assess memory performance in the present study. This test is based on the spontaneous tendency of rats and mice to spend more time exploring a novel object than a familiar one. Increased exploration of the novel object was interpreted as successful retention of memory for the familiar object (Bertaina-Anglade et al. 2006). The present results showed that mice with seizure score 3–5, as well as, 1–2 displayed no differences from the control group by the recognition index calculated for each animal using the time spent exploring the familiar or novel object (Arque et al. 2008, Zhang et al. 2012). This suggests that seizures in the fasted animals did not induce impairment in the encoding (sample presentation), storage (interval) or retrieval (choice phase) of the recently learned information (Winters et al. 2006). Similar to the present data, the measurement of behavioral alterations starting 9–12 weeks after the initial seizures demonstrated that there was no memory impairment in the Morris water maze test in mice in which pilocarpine induced single seizures (Müller et al. 2009a). Rats subjected to a single pentylenetetrazole-induced seizure also displayed no difference from the control group in the novel object recognition test performed on day 10 (Aniol et al. 2013). Nevertheless, due to the appearance of impairments of short-term visual and social memory on day 70, the authors emphasized that the cognitive deficits did not reach statistical significance earlier. In contrast to brief seizures, several studies demonstrated learning and memory impairments following severe seizures in the novel object recognition (Rojas et al. 2016), elevated plus-maze (Akula et al. 2007) or Morris water maze (Gröticke et al. 2008, Müller et al. 2009a, 2009b, Brewster et al. 2013; Schultz et al. 2014) tests. These findings indicate that the severity and/or duration of seizures in fasted animals might not be sufficient to induce memory impairments. However, timing of the tests after the initial seizure (less than 1 week *vs.* several weeks) may have also been a factor in the lack of behavioral alterations in the present study.

Using Fluoro-Jade C, a sensitive and specific fluorescent marker of degenerating neurons (Schmued et al. 1997), histological analysis in the hippocampal sections was performed to determine neuronal damage. To establish a correlation between the behavioral measurements and the histological findings, animals were killed 7 days following convulsions. Gröticke et al. (2007) showed damage in the hippocampus in the CA1 and CA3 pyramidal cell layers and the hilus of the dentate gyrus 1 week following pilocarpine-induced *status epilepticus* in mice. In contrast to this finding, neurons in the hippocampal sections of the seizing animals in the present study had a similar staining pattern as control sections (Fig. 1). Accordingly, the percentage of the Fluoro-Jade C positive neurons in the granular layer of the dorsal dentate gyrus of the hippocampus did not differ between the control animals and animals with seizure scores 1–2 or 3–5. This finding seems consistent with the absence of memory impairment in the novel object recognition task, which is suggested to be sensitive to hippocampal damage (Arque et al. 2008), and is in accordance with the findings of some earlier studies. Pilocarpine-induced short single seizures (Müller et al. 2009a) and hyperthermia-induced tonic-clonic seizures lasting for 30 sec to 6 min (Jiang et al. 1999) also showed no observable neurodegeneration in the hippocampus. Depending on the total number of seizures or the interval between each seizure, brief generalized seizures elicited by electroshock are capable of producing moderate loss of neurons in the hippocampal formation (Cardoso et al. 2011). However, more severe seizures cause massive death of neurons in different regions of the hippocampus (Cavazos et al. 1994, Jiang et al. 1999, Brandt et al. 2003, Cardoso et al. 2011, Schultz et al. 2014).

An overview of the results mentioned above indicates that the common features of brain damage re-

ported in almost all studies are the association with the spontaneous recurrent seizures, development after a latency period, and correlation with the seizure intensity. Seizure-induced behavioral changes, memory disruption and spontaneous recurrent seizures are suggested to be related to the cellular and molecular alterations implicated in epileptogenesis. The present results seem consistent with each other because this study revealed no evidence of neuropathologic, behavioral, cognitive or epileptogenic consequences following convulsions.

CONCLUSIONS

Convulsions in fasted animals that develop after atropine treatment and food intake do not induce spontaneous recurrent seizures, motor activity and emotional changes, or hippocampal neuronal death. These results provide supporting evidence for reports that single brief seizures in animals (Müller et al. 2009a) and patients with epilepsy (Rocha et al. 2007) are not associated with behavioral alterations, cognitive deficits or hippocampal damage.

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