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Comparison of kainate-induced seizures, cognitive impairment and hippocampal damage in male and female mice

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ABSTRACT

Kainate (KA) mouse model induced by intraperitoneal injection has been widely used for epilepsy and neurodegeneration studies. KA elicits sustained epileptic activity in mouse brain revealed by recurrent behavioral seizures, deteriorative neurodegeneration and various neurological deficits. However, to date, the vast majority of the studies used male mice only, and few studies on the comparison of brain injury between male and female mice in this model were reported. Epidemiological studies indicate that sex may affect the susceptibility to seizure response and neurodegeneration process. Therefore, this study focused on the effect of sex difference on KA-induced recurrent seizures and mortality, locomotor activity and cognitive impairment, and hippocampal neurodegeneration and reactive gliosis in mice. Our results showed that, compared to females, adult male mice exhibited worse performance in mortality rate, severity of epileptic seizures, and cognitive impairment indicated by novel object recognition task. Unexpectedly, post-KA male and female mice underwent similar decline and recovery of locomotor activity. KA-induced neurodegeneration in the whole hippocampus, particularly in CA1 and CA3 subregions, along with the deteriorative reactive gliosis in astrocytes and microglia, was more severe in males than that in females. These data provided the direct in vivo evidence that indicates the key role of sex difference in studies with KA mouse model, and this could be beneficial for optimizing the design of future studies.

1. Introduction

Kainate (KA) mouse model induced by intraperitoneal (i.p.) injection has been widely used in epilepsy and neurodegenerative studies. Most studies with KA mouse models used male mice only; few studies focused on the sex importance in brain injury outcomes. Epidemiological studies indicated that sex may affect the seizure response and neuronal injury process. Although the sex-dependent effects on seizure were observed in a number of rodent studies [21,33,45,47,55–57], few studies reported the role of sex on brain injury and resultant neurological deficits in KA mouse models through intrahippocampal or nasal administration [27,56,67]. Furthermore, as far as we know, no study reported the effect of sex difference on brain injury in KA mouse model through i.p. injection. In most recent years, sex has been considered as a key biological variable in both basic and clinical epilepsy studies [14].

KA is an analog of glutamate, and its neurotoxicity is 30-fold higher than glutamate [16,29,65]. KA acts on glutamate receptors and elicits sustained neuro-excitotoxic and epileptogenic effects in mouse hippocampus, revealed by recurrent behavioral seizures and progressive deterioration of neurons [49,60,66,68]. Such neuronal vulnerability occurs in limbic circuit, especially in the hippocampal CA1 and CA3 subregions, which is associated with the specific distribution of glutamate receptors (AMPARs/KARs) in hippocampus [59]. The synergistic interaction among glutamate excitotoxicity, oxidative stress and inflammation contributes to KA-caused seizures and neurodegeneration in vulnerable brain regions [38]. Increasing evidences indicate the reactive oxygen species (ROS) overproduction induced oxidative stress result from glutamate medicated excitotoxicity. These elements also lead to multiple functional deficits, such as cognitive impairment. Glial activation and proliferation is another important feature of brain damage.

This study aimed at the effect of sex difference on mouse brain damage following KA i.p. injection. Accordingly, the comparisons

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between KA-exposed males and females will be analyzed in several aspects: (1) Mortality rate, recurrent seizures and seizure severity; (2) Locomotor activity by open field test and cognitive impairment by novel object recognition task; (3) Neurodegeneration in hippocampal CA1 and CA3 subregions by Nissl staining; (4) Hippocampal reactive gliosis in astrocytes and microglia by immunostaining. These results will provide the direct in vivo evidence, indicating the role of sex difference in studies with this model.

2. Materials and methods

2.1. Animals

The age-matched male and female C57BL/6 mice (2–3 month old) were housed in an environment controlled for lighting (a 12 h light/dark cycle) and temperature, with food and water available ad libitum. All animal procedures conformed to the National Institute of Health guidelines and were approved by Institutional Animal Care and the relevant Institutional Ethical Review Committees.

2.2. Chemicals and antibodies

kainate (KA) (Sigma, St. Louis, MO, USA); Thionin acetate salt (for Nissl staining; Sigma, St. Louis, MO, USA); Mouse anti-GFAP antibody (GFAP; EMD Millipore, Billerica, Massachusetts, USA); Mouse monoclonal glutamine synthetase (GS; EMD Millipore, Billerica, Massachusetts, USA); Rabbit polyclonal ionized calcium-binding adapter protein 1 (Iba1; Wako Bioproducts, Richmond, VA, USA); 3,3-diaminobenzidine (DAB) substrate (Vector Laboratories; Lowellville, Ohio, USA).

2.3. Behavioral tests

2.3.1. KA-induced excitotoxicity and seizure scoring

KA administration through i.p. injection was employed to induce neurodegeneration [30,52,60,68]. Male and female mice received an i.p. injection of single dosage of KA (30 mg/kg) dissolved in sterilized saline. The behavioral performance of mouse was assessed by trained experimenter each 20 min for 2 h after KA injection. The behavioral seizures were scored 0–5 in accordance with the descriptions [3,64]: 0, normal behavior; 1, immobilization, “wet-dog shakes” occasionally; 2, head nodding, unilateral forelimb clonus, frequent “wet dog shaking”; 3, rearing, bilateral forelimb clonus; 4, generalized limbic seizures with falling and running; 5, continuous generalized seizures with tonic limbic extension, death.

In addition, the severity of seizures was calculated by integrating individual scores per mouse [64]. This data incorporates the seizure severity of mice that died within 2 h following KA injection. The maximum seizure severity = the maximum seizure score of a mouse during the experiment time. The average seizure severity of a mouse = Σ (all scores of the mouse)/experimental time.

2.3.2. Locomotor activity

The general spontaneous locomotor activity of mice was examined by the open field paradigm with automated video tracking software [28]. Mouse was placed in the apparatus (40 cm × 40 cm) and was allow exploring for 5 min. The total traveled distance was recorded.

2.3.3. Novel object recognition task

To evaluate the KA-induced cognitive impairment, novel object recognition task was performed using a square arena (45 cm × 45 cm × 35 cm) that was placed in a dimly lit room [25,37]. The test (over 3 days) included a habituation phase, a training phase, and a retention phase. For the habituation phase, mouse was moved into an empty arena and was allowed to explore for 5 min. For the training phase (24 h after habituation), mouse was exposed to the same arena with two identical objects for 15 min. For the retention phase (24 h after the training phase), one of the objects was replaced with a novel object, and the mouse was allowed to explore the novel and old objects for 5 min. The arena and the objects were cleaned with ethanol and air-dried between mice. All sessions were recorded using a video camera. A mouse was judged to be actively exploring an object when its nose was toward the object (< 2 cm). Discrimination Index (DI) = (time exploring the novel object – time exploring the old object)/total time exploring both objects x 100%.

2.4. Histology and immunohistochemistry

At 5 days after KA or saline injection, mice were transcardially perfused with PBS (pH 7.4) to flush the blood and followed with freshly prepared 4% PFA in 0.1 M sodium phosphate buffer (pH 7.4) [26]. The brains were dissected, postfixed, gradiently dehydrated with 20 and 30% sucrose in PBS. For each mouse, six sets of coronal brain sections (30-μm-thick) were sliced on Leica cryostat and preserved in PBS-buffered 50% glycerol at −20 °C until used for subsequent immunohistochemistry.

Brain sections were rinsed in PBS and quenched by incubating with 3% H_2O_2 (15 min, at room temperature), followed by three times of rinsing with PBS [26]. To avoid non-specific staining between primary antibody and the tissue, the sections were blocked by PBS containing 0.3% triton X-100 and 5% BSA for 1 h. The sections were then incubated with primary antibody overnight at 4 °C. The following primary antibodies were used: GFAP (1:3000); GS (1:3000); Iba1 (1:5000). Sections were washed on the second day, incubated with appropriate second antibody, and visualized with DAB substrate. Nissl staining was performed to evaluate neuronal degeneration as previously described [28]. Following Nissl assay, the pyramidal neurons were stained dark blue, the feature which was absence in dead neurons. Nissl-positive pyramidal cells in CA1 and CA3 hippocampal subregions were quantified. For the quantification analysis of Nissl, GFAP, or Iba1 staining, three consecutive sections (Bregma −1.70 ~ −2.06) were measured and generated a single value for each mouse [26]. The images were analyzed with ImageJ software (NIH).

2.5. Statistical analysis

Analyses were performed using GraphPad Prism 6.0 software. The number of animals for each experiment is specified in the figure or figure legend. Survival was assessed using Kaplan–Meier analysis [42], and the statistically significant difference between two groups was determined with the log-rank test. Data analysis of seizure scores was performed by using two-way analysis of variance (ANOVA) for repeated measurements. The comparison between the two groups was analyzed using unpaired Student's *t*-tests, while multigroup comparisons were carried out using two-way ANOVA followed by Bonferroni post hoc tests. Results are represented as mean ± SEM, and $p < 0.05$ was regarded as statistically significance.

3. Results

3.1. KA induced a slightly increased mortality rate and higher seizure response in the male mice than the females

We firstly examined whether sex may affect mortality rate and seizure susceptibility following KA injection. Based on our preliminary results (not show), we selected 30 mg/kg as an optimal dosage for this study. Adult male and female mice received a single dose of intraperitoneal injection of KA (30 mg/kg, Fig. 1A). The Kaplan–Meier survival curves revealed that KA induced slightly higher mortality rate over 5 days in male mice than that in female mice ($P > 0.05$; Fig. 1B). At 24 h, KA led to increased mortality in ~23.5% of males ($n = 17$), in contrast to 6.6% of females ($n = 15$). At 48 h, the mortality of males

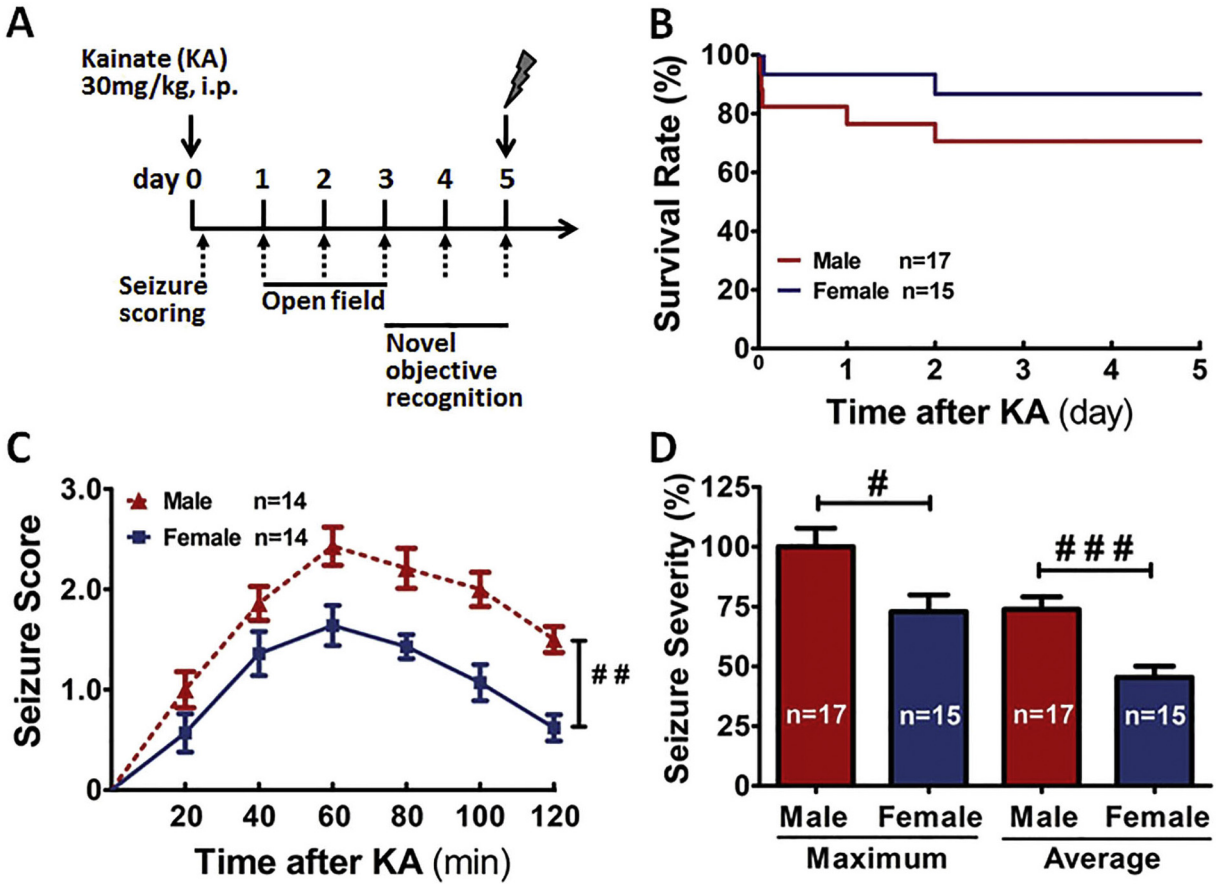

Fig. 1. Male mice exhibit relatively higher mortality rate and susceptibility to seizure response than female mice. (A) Scheme of the experimental design. (B) Kaplan–Meier survival curves showed that the male mice displayed the growth tendency of mortality rate compared to the female mice over 5 days ($P > 0.05$) after KA injection (30 mg/kg; i.p.). (C) Seizure activity in response to KA was scored for each 20-min interval. The male mice generalized significantly higher seizure activity ($\#\#P < 0.01$) within 120 min following KA administration. The mice that died during this time period were not included for this statistical analysis). (D) The maximum and average seizure score of each mouse within 120 min post-KA administration were analyzed. The males showed markedly higher seizure severity than females. $\#P < 0.05$; $\#\#P < 0.01$. Sample number per group is labeled on the figure. Data in (C) and (D) are presented as mean ± SEM. KA, kainate; i.p., intraperitoneal injection.

increased to 29.4% compared to 13.3% for females, emphasizing the important role of sex on this process.

The seizure responses to KA were assessed by trained experimenter blinded to group. During the 120 min following KA administration, either males or females displayed acute, yet transient seizures that (Fig. 1C) peaked at around 60 min and then decayed. Comparably, the male mice underwent significantly more severe seizures than that in females ($P < 0.01$). In addition, considering the mice that died during the 120 min were not included for this statistical analysis, seizure severity was also analyzed by integrating individual scores each mouse over the 120 min period. This calculation incorporated the seizure severity of mice that died within 120 min following KA administration and therefore can better reflect the overall seizure response. The male mice exhibited remarkable increase in either maximum ($P < 0.05$) or average ($P < 0.001$) seizure severity (Fig. 1D). Together, male mice exhibited dramatic higher susceptibility to KA-induced epileptic seizures.

3.2. KA-caused more severe cognitive impairment in the males than that in the females

To determine whether the sex affect the neurobehavioral outcomes after KA-induced seizures, we performed behavioral assessments in locomotor activity and cognitive function at the indicated times (Fig. 1A), including open field test (1–3d) and novel object recognition task (3-5d) tests. Quantitative analysis revealed that, compared to control saline-treated group, both post-KA groups underwent obvious decline in locomotor activity at 1d after KA administration and thereafter rapidly recovered; no significant difference was detected at any observed times between both post-KA groups (Fig. 2A). The locomotor activity was revealed by the total traveled distance of mouse within 5 min in the open field arena.

In the testing stage of novel object recognition task, all groups readily discriminated the novel object from the familiar one (Fig. 2C, left panel, $**P < 0.01$, $***P < 0.001$). As shown in Fig. 2C (middle panel), the analysis of the discrimination index pointed out that both post-KA groups displayed worse performance than control group ($*P < 0.05$, $**P < 0.01$), whereas such decline was more severe in post-KA male group than that in post-KA female group ($\#P < 0.05$). Interestingly, it was shown that the total exploring time on both objects was comparable among three groups ($P > 0.05$, Fig. 2C, right panel). In the control mice (saline; Fig. 2A–C), no significant difference was found in the tests of open field locomotor activity and novel object recognition between males and females. Therefore, the "control group" refers to the combination of males and females with saline injection. Together, the data indicated that the sex did affect the post-KA cognitive impairment in mice, but not the locomotor activity.

3.3. KA-induced comparably more exacerbated hippocampal neurodegeneration in the male mice

KA–induced seizures are often associated with complex

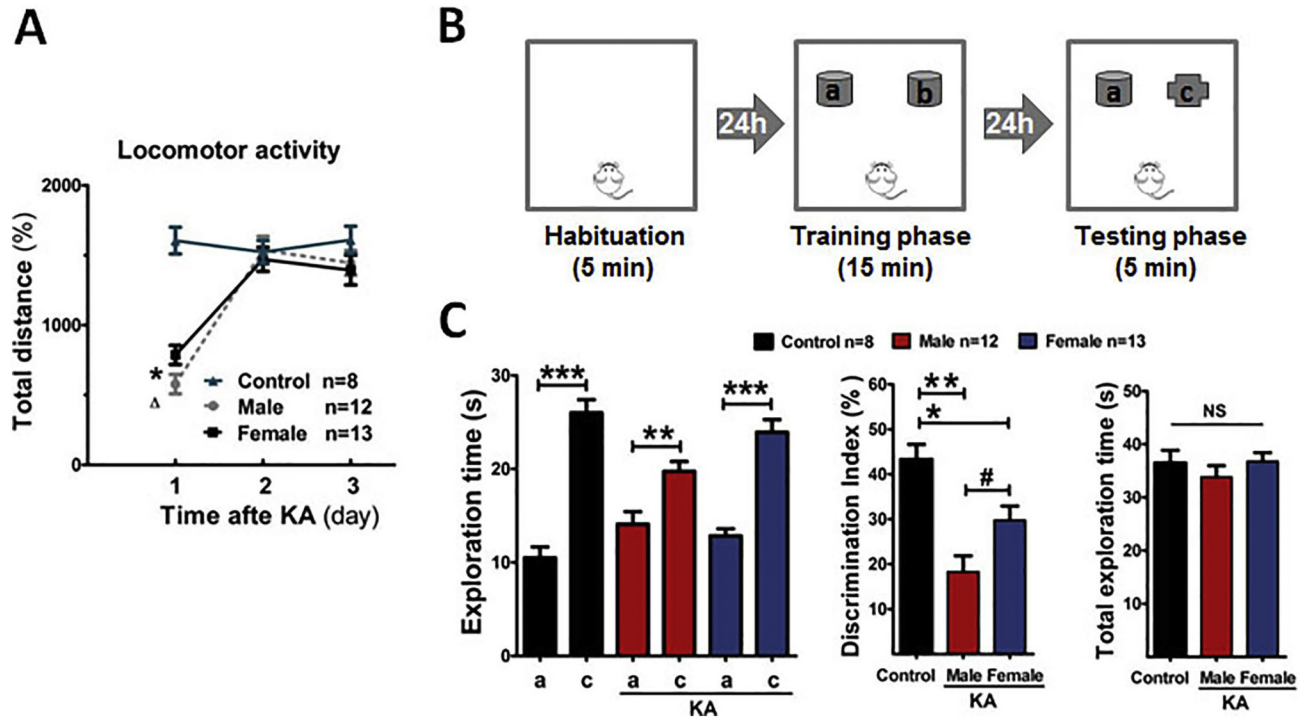

Fig. 2. KA-induced cognitive impairment is more severe in the male mice. (A) KA induced dramatic reduction of locomotor activity in both males and females at 1 day but not later time points after KA injection, revealed by the total traveled distance in 5 min in the open field paradigm (OP; *P < 0.05, female vs. control; ΔP < 0.05, males vs. control). No significant difference was detected between both post-KA groups at the indicated time points. (B) Overview of the novel object recognition (NOR) set-up. On the first day, the mice were allowed to habituate to the NOR arena in the absence of objects for 5 min. On the second and the third day, they were exposed to two same objects (a and b) for 15 min of training and underwent a 5 min testing in which a novel object (c) replaced one of the objects (b). (C) The mice in all groups showed comparable preference of exploration to the novel object (c) than the old object (c), indicated by the exploration time of each mice (**P < 0.01, ***P < 0.001, old vs. novel object). Both post-KA groups underwent marked decline in discrimination index (*P < 0.05, **P < 0.01), whereas the post-KA males showed more severe decline compared to post-KA females (#P < 0.05, post-KA male vs. post-KA female). In addition, there is no significant difference in the total exploration time among the three groups. In the control mice (saline; A–C), no significant difference was found in the tests of OP and NOV between males and females. Therefore, the "control group" refers to the combination of post-saline males and post-saline females. Sample number per group is labeled on the figure. Results are presented as the mean ± SEM. KA, kainate; NS, no significance; Total distance, the total traveled distance of mouse within 5 min in open field locomotor arena.

hippocampal pyramidal neuronal damage. To determine the effect of sex on KA-induced neurodegeneration, the hippocampal slides were examined by Nissl staining of nearby sections (Fig. 3). As expected, under the basal condition, no apparent abnormal hippocampal anatomy was observed in the males and the females. At 5 days after KA administration, the males but not the females displayed significant neuronal loss in whole hippocampus (including CA1-3 and dentate gyrus subregions, *P < 0.05). In contrast, when we examined the pyramidal neuronal damage in the hippocampal CA1 and CA3 subregions, the most susceptible regions following KA injection, both males and females displayed significant pyramidal neuronal loss (*P < 0.05, **P < 0.01, ***P < 0.001), whereas male mice exhibited comparably much deteriorative condition (#P < 0.05, ##p < 0.01). These morphological findings implied the critical role of sex on KA-induced hippocampal neurodegeneration.

3.4. KA-induced reactive gliosis in astrocytes and microglia was more severe in the male mice

Reactive gliosis, mainly in astrocytes and microglia, refers to the changes at molecular, gene expressional, morphological, and biochemical levels, is a notable characteristic of epilepsy in patients and most seizure animal models [43,62]. To assess the effect of sex on hippocampal reactive gliosis after KA injection, we examined the morphological changes in astrocytes and microglia by immunostaining of glial fibrillary acidic protein (GFAP), an astrocyte marker, and ionized calcium-binding adapter protein 1 (Iba1), a microglial marker (Figs. 4 and 5).

Under the normal condition, both astrocytes and microglia were regularly distributed in the whole hippocampus, and most displayed non-reactive status with small cell body and multiple slim branches. No apparent difference was observed between males and females. In contrast, KA exposure caused significant increase in reactive gliosis of astrocytes and microglia in the whole hippocampus, CA1 and CA3 subregions at 5 days after KA administration, revealed by the GFAP immunoreactive intensity and Iba1 immunoreactive area percentage (**P < 0.01, ***P < 0.001). Most glial cells displayed reactive status featured by hypertrophic cell bodies and thickened and retracted processes. Compared to the females, the males exhibited significant deteriorated condition (#P < 0.05, ##P < 0.01). Together, these findings supported that the sex play a key role in KA-induced reactive gliosis in astrocytes and microglia in the hippocampus of this mouse KA model.

4. Discussion

Systemic injection (i.p.; subcutaneous, s.c.; intravenously, i.v.) or local intracerebral injection of KA into a specific brain region of experimental animals can lead to reoccurred seizures and neurodegeneration [52]. Among systematic administration routes of KA, i.p. injection is frequently used for the induction of KA model. This study demonstrates the males exhibit worse performance than females in this mouse model of excitotoxic neurodegeneration. We showed that, in comparison with female mice, KA-exposed male mice displayed comparably higher mortality rate and sensibility to epileptic seizures. We

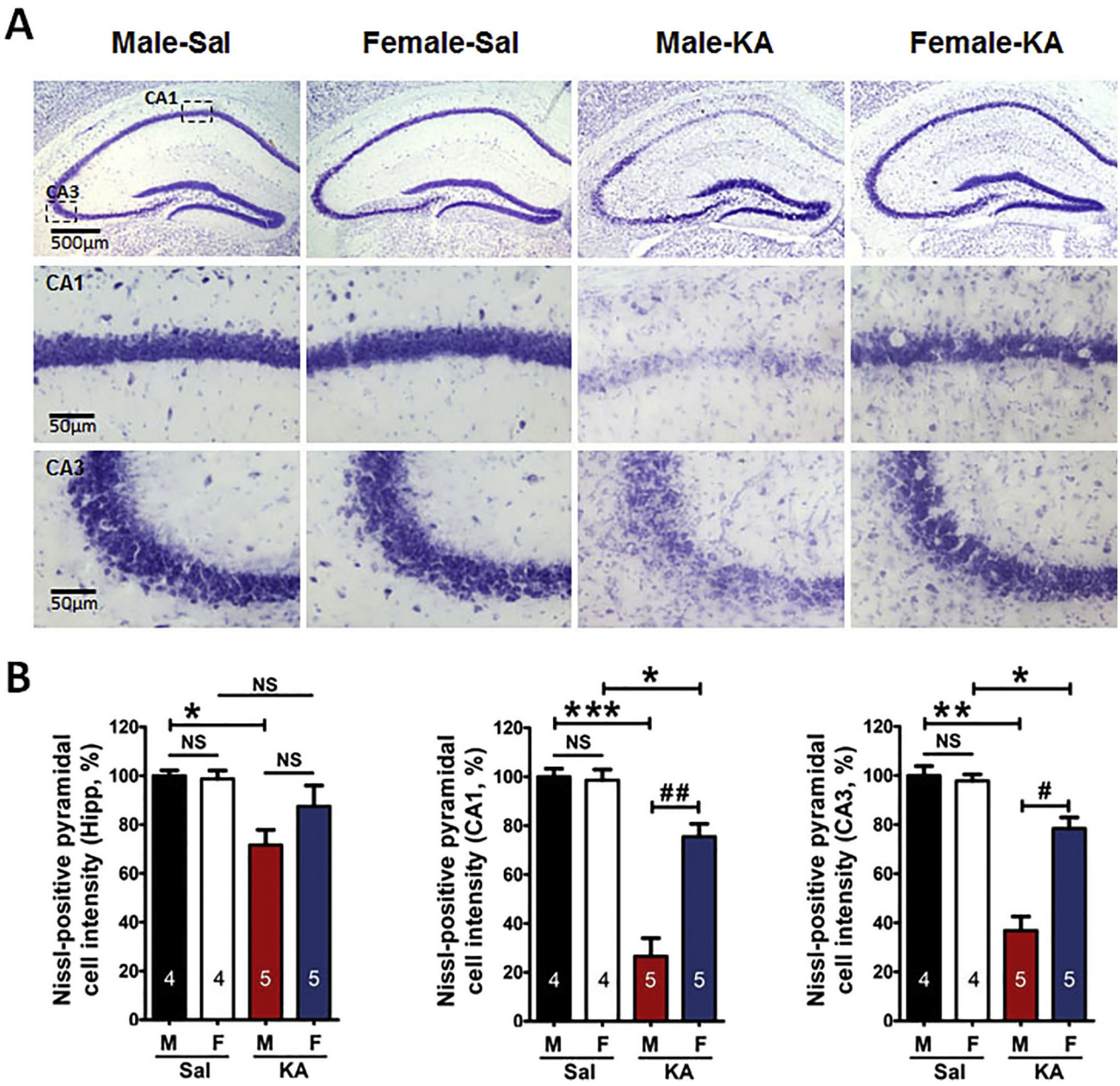

Fig. 3. KA causes greater hippocampal neuronal degeneration in the male mice. (A) Representative images depict the changes in Nissl-positive pyramidal neurons in the hippocampal region at 5 days after KA or saline injection. (B) Quantitative analysis revealed that KA caused the decline of neurons in the whole hippocampus, especially in the male mice ($*P < 0.05$, left panel). In CA1 and CA3 subregions of the hippocampus, KA caused significant increases of pyramidal neuronal loss, indicated by the intensity of neurons ($**P < 0.01$, $***P < 0.001$). In addition, the post-KA males exhibited significantly higher neuronal loss than that in females in both CA1 and CA3 subregions ($\#P < 0.05$, $\#\#P < 0.01$). Sample number per group is labeled on the figure. Data are presented as mean ± SEM. Sal, saline; KA, Kainate.

also showed that males have worse performance in KA-induced cognitive impairment than females, but not in the alternation of locomotor activity. Consistently with these behavioral declines, KA resulted in exacerbated hippocampal neurodegeneration and deteriorative reactive astrogliosis and microgliosis. Therefore, such behavioral and pathological comparison between male and female mice after KA lesion supports the critical role of sex difference on brain injury in our paradigms.

This work provides several lines of evidence in support of effect of sex difference on KA-induced brain injury. First, the finding that the male mice displayed higher mortality rate and seizure susceptibility was supported by multiply clinical and animal studies. Epilepsy is a serious neurological disorder characterized by spontaneous seizures. The recurrent epileptic seizures are the hallmark of clinical epilepsy condition, and the patients with epilepsy suffer from a 2–3 times increased mortality that varies with multiple risk factors [2,31,46,54]. Most studies suggest the slightly higher incidence of epilepsy in males, and sex difference is related to epilepsy type and treatment [9,41]. One report pointed out that woman with epilepsy may prone to enhanced seizure frequency at certain phases of their menstrual cycles [41]. In rodent studies, experimental seizures are usually evoked by drugs like KA, pentylenetetrazol or pilocarpine. KA induces status epilepticus (SE), a state of continuous seizures that sustain for hours before self-terminating, and severe neuronal loss in related brain regions where the KA receptors locate [47]. The SE appears to be closely related to the mortality, suggested by that all post-KA mice died within 2 days over 5 days experiment paradigm. In support of our finding, adults female rat in another study was shown to be resistant to another chemoconvulsant pilocarpine [47].

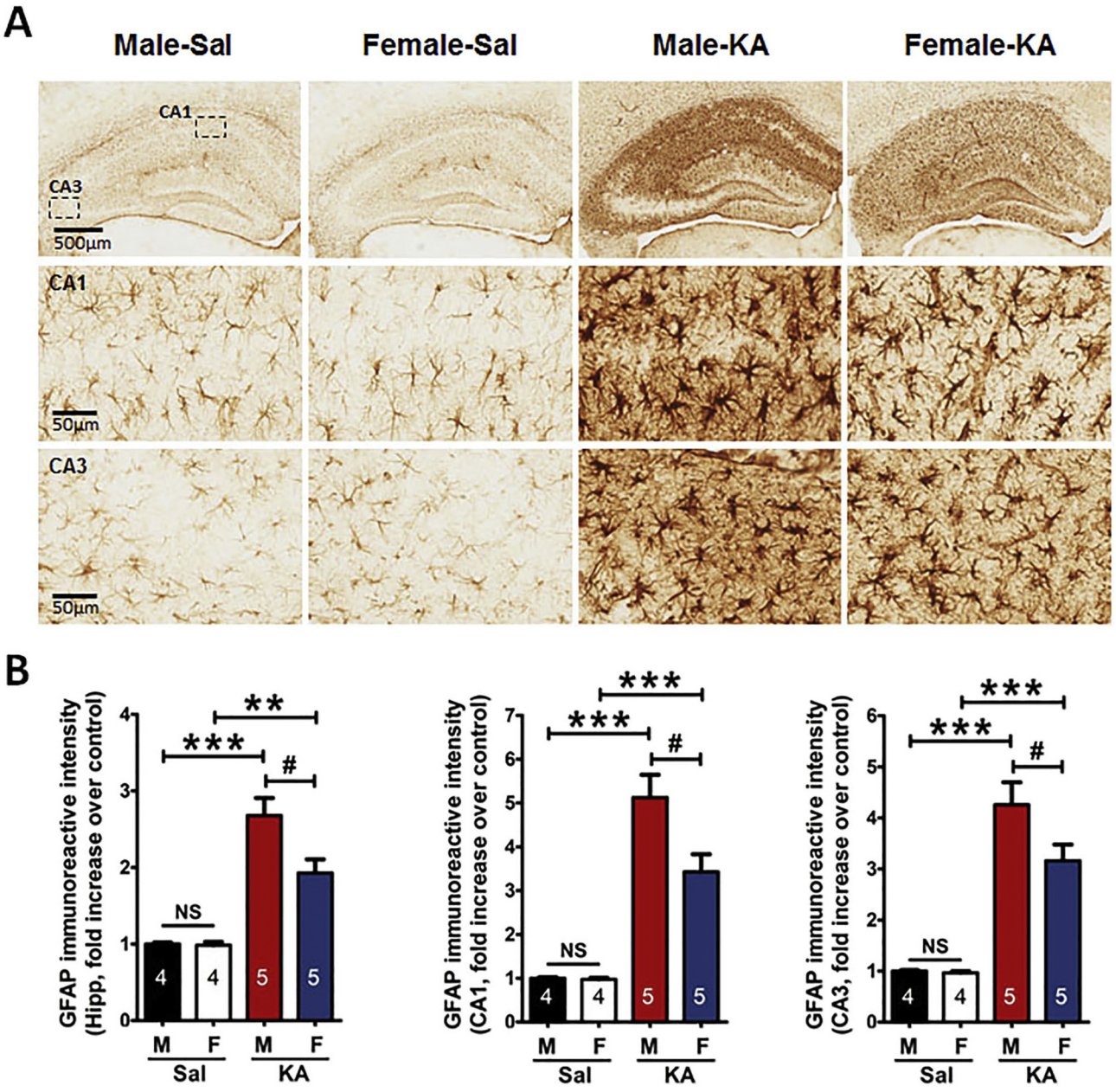

Fig. 4. The post-KA males exhibit a dramatic increase in hippocampal reactive astrogliosis. (A) Representative images depicting changes in GFAP-positive astrocytes at 5 days after KA or saline injection. (B) Quantitative analysis revealed that, compared to the saline-treated male or female mice, KA led to dramatic activation of astrocytes in the whole hippocampal, CA1 and CA3 subregions (**$P < 0.01$, ***$P < 0.001$), whereas this alternation was more severe in males than that in females (#$P < 0.05$). Sample number per group is labeled on the figure. Sal, saline; KA, kainate; GFAP, glial fibrillary acidic protein; CA1, CA3, the cornu ammonis subregions of the hippocampus.

Second, in addition to the afflicting by the constant stress of seizures, a crucial understudied clinical issue is that patients also often experience comorbid cognitive and psychiatric disorders [6,20,24]. SE and the hippocampal neurodegeneration are often associated with cognitive decline [1]. However, no study has reported the influence of sex-related difference on neurobehavioral cognitive alternation in systemic administration of KA rodent models. Multiple behavioral tasks are used to model the cognitive changes seen in the human disease, such as spatial memory tests (Morris water maze, radial arm water maze, Barnes maze), associative learning tasks (passive avoidance, fear conditioning), alternation tasks (Y-Maze, T-Maze), and recognition memory tasks (NOV) [61]. Herein, we utilized NOR task, which doesn't require external motivation, or exhaustive swim training in Morris water maze. The NOR was developed in the late 1980s based on the observation that access to novelty like an object or an environment can evoke approach behaviors in rodents [25]. Recognition memory refers to the capacity to recognize previously encountered events and is classified into long-term declarative memory. Not surprisingly, no significant difference was observed in locomotor activity between male and female mice at any indicated times. In order to detect the potential difference between groups, an optimized method of NOR task was applied [25,37,51]. Strong training stage of exploring both objects for 15 min in novel object recognition task conferred a long-term memory that last for at least 24 h [37], which provide an excellent discrimination window in testing phase. The important role of sex difference on cognitive deficits was supported by with many seizure, epilepsy, and cognitive rodent studies [12,19,35,39]. There are obvious sex differences in incidence and stress-related psychiatric disorders in humans. In

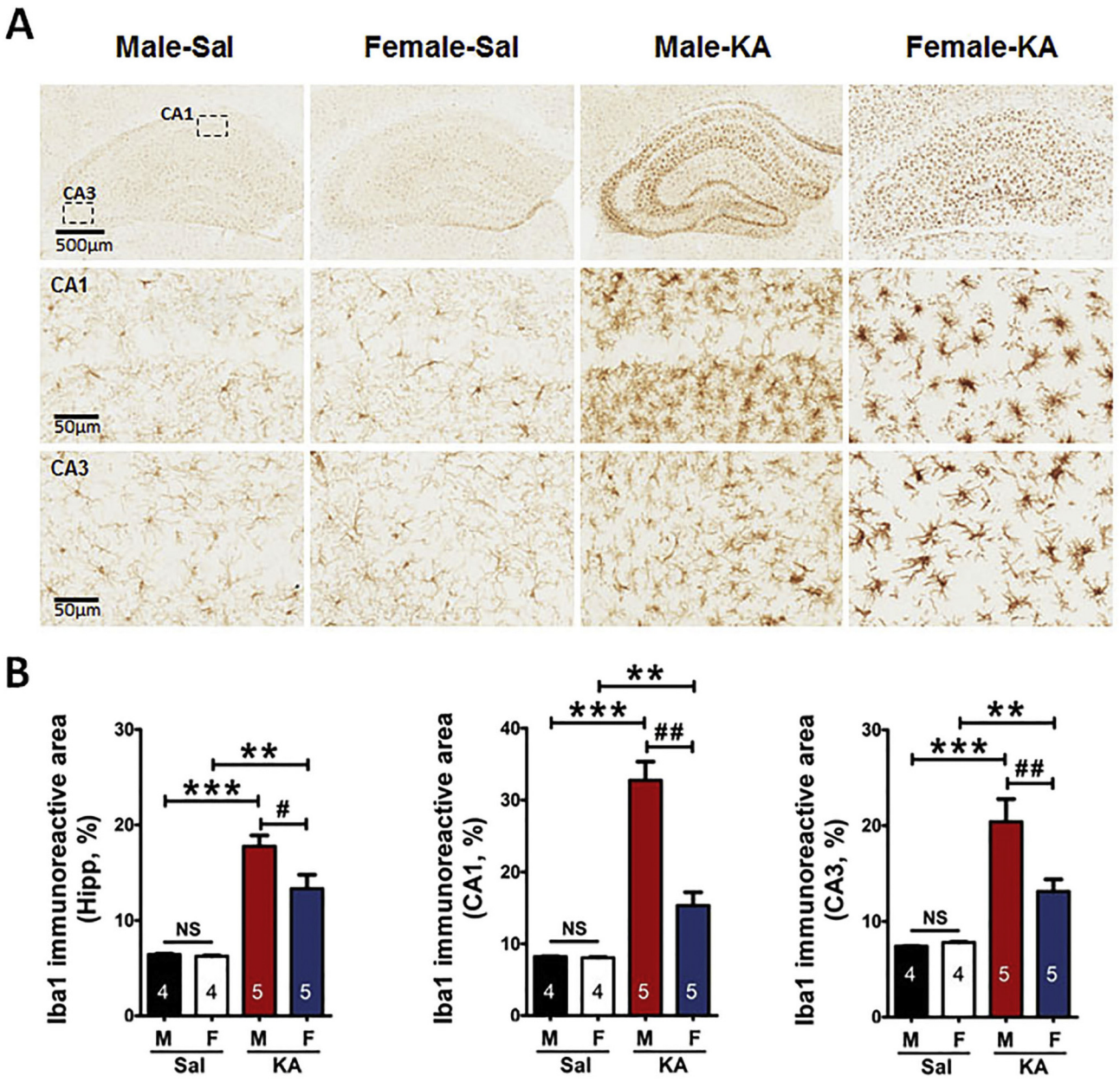

Fig. 5. The post-KA males display a marked increase in hippocampal reactive microgliosis. (A) Representative images depicting changes in Iba1-positive astrocytes at 5 days after KA or saline injection. (B) Quantitative analysis revealed that, compared to the saline-treated male or female mice, KA led to dramatic activation of microglia in the whole hippocampal, CA1 and CA3 subregions (**P < 0.01, ***P < 0.001), whereas this alternation was more severe in males than that in females (#P < 0.05). Sample number per group is labeled on the figure. Sal, saline; KA, kainate; Iba1, ionized calcium-binding adapter protein 1; CA1, CA3, the cornu ammonis subregions of the hippocampus.

contrast, rodent models are predominantly based on male animals. However, sex differences in epilepsy in laboratory animals are still unclear [47]. The strongest point concerning the female mice possibly related to their fluctuating sex hormones per phase and the estrous cycle [53]. Interestingly, one study reported that early life status epilepticus and stress cause more transient cognitive deficits in males but aggravate the consequences of subsequent SE in females [1].

Third, in human patients and animal models, recurrent seizures are accompanied by neuronal damage, which is associated with the mortality under epilepsy and other pathological conditions [8]. Indeed, in present study systemic injection of KA generated well-characterized reoccurred seizures and neurodegeneration in the whole hippocampus, especially CA1 and CA3 subregions, which was consistent with previous report [60]. The mechanisms involve glutamate receptors activation, consequent Ca^{2+}-mediated excitotoxicity, excessive ROS production, and reduced ATP production, ultimately leading to neuronal death [22,44]. It was reported that until 3–5 days following KA injection, the delayed neuronal cell death of CA1 pyramidal neurons becomes apparent [23]. The attenuated hippocampal neurodegeneration in female mice may be associated with the hormone levels, which have been reported by many reports [7,13,18,32,34,47].

Fourth, dysregulation of glial functions may elicit seizure or epileptogenesis, and epilepsy is characterized by astrocytic and microglial activation and proliferation [10]. In our study, following the acquired epilepsy, neuronal loss was observed accompanied with marked reactive gliosis in astrocytes and microglia. The KA-induced proliferative response of glial cells has been reported in 1982 [36], and exhibits steady increase during, at least, one day to one month [4,11]. Reactive gliosis, mainly in astrocytes and microglia, refers to the response to central nervous system (CNS) stressors, including structural and

metabolic changes that have been considered as a prominent feature of human epilepsy and most seizure animal models [40,43,50,62,63]. The reactive gliosis occurred in paralleled with the functional decline. Multiple mechanisms like water and potassium ion buffering, and regulating neurotransmission are involved [10,17,58]. Microglia has been shown to regulate neuronal activityes during epilepsy and seizure–induced neurodegeneration [48]. The sex difference in the reactive gliosis further highlighted its importance in KA-induced delayed neurodegeneration [5,15]. Interestingly, when compared to the slight decline of the neuronal injury in the post-KA female mice, both glial cell types displayed significant activation in the whole hippocampus, and CA1 and CA3 subregions, indicating the higher susceptibility of glial cells to the KA-induced injury. It needs to be pointed out that we only observed the neuronal loss, reactive gliosis in astrocytes and microglia at 5 days after KA injection. Further observations at later time points could be informative to discriminate the sex difference with this mouse model.

In summary, these evidence revealed the important role of sex differences in systematic administration of KA mouse model, which could be beneficial for optimizing the design of studies with this model.

Author contributions

L.L. conceived and designed the experiments; L.L. and F.L. performed the experiments and the data analyses. L.L. wrote the manuscript. Both authors discussed, revised, and approved the final manuscript.

Declaration of Competing Interest

The authors declare there is no conflict of interests.

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