Video Article

Pentylenetetrazole-Induced Kindling Mouse Model

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Abstract

Pentylenetetrazole (PTZ) is a GABA-A receptor antagonist. An intraperitoneal injection of PTZ into an animal induces an acute, severe seizure at a high dose, whereas sequential injections of a subconvulsive dose have been used for the development of chemical kindling, an epilepsy model. A single low-dose injection of PTZ induces a mild seizure without convulsion. However, repetitive low-dose injections of PTZ decrease the threshold to evoke a convulsive seizure. Finally, continuous low-dose administration of PTZ induces a severe tonic-clonic seizure. This method is simple and widely applicable to investigate the pathophysiology of epilepsy, which is defined as a chronic disease that involves repetitive seizures. This chemical kindling protocol causes repetitive seizures in animals. With this method, vulnerability to PTZ-mediated seizures or the degree of aggravation of epileptic seizures was estimated. These advantages have led to the use of this method for screening anti-epileptic drugs and epilepsy-related genes. In addition, this method has been used to investigate neuronal damage after epileptic seizures because the histological changes observed in the brains of epileptic patients also appear in the brains of chemical-kindled animals. Thus, this protocol is useful for conveniently producing animal models of epilepsy.

Video Link

The video component of this article can be found at https://www.jove.com/video/56573/

Introduction

Epilepsy is a chronic neurological disorder that is characterized by recurrent seizures and affects approximately 1% of people. The underlying mechanisms of epileptogenesis and seizure generation in epilepsy patients cannot be fully clarified in clinical studies. Therefore, an appropriate animal model is required for the study of epilepsy[1].

A variety of animal models of epilepsy have been used to investigate the physiology of epilepsy and to identify anti-epileptic drugs[2,3]. Among these models, pharmacological seizure induction is a common method used to generate an animal model for the investigation of the pathology of epilepsy[4]. This method is inexpensive and simple. Electrode-mediated kindling is also a commonly used method, but the costs of this procedure are higher, and the method requires surgical and electrical skills to induce repetitive seizures[5].

Pharmacological induction is also advantageous because the timing and number of seizures are easily controlled. Genetic mouse models that exhibit spontaneous seizures are also used in the study of epilepsy. However, predicting when and how often the seizures arise in these genetic models may be impossible[6]. A monitoring system is required to observe the epileptic behavior of genetically modified mice[6].

Kainic acid, pilocarpine and pentylenetetrazole (PTZ) are widely used as seizure-inducing drugs[7]. Kainic acid is an agonist for glutamate receptors, and pilocarpine activates cholinergic receptors. PTZ is a gamma aminobutyric acid (GABA)-A receptor antagonist[8]. PTZ suppresses the function of inhibitory synapses, leading to increased neuronal activity. This regulation causes generalized seizures in animals[9]. A single injection of kainic acid and pilocarpine can induce acute seizures, especially status epilepticus (SE)[10,11] and kainic acid- or pilocarpine-mediated SE promotes chronic spontaneous and recurrent seizures[12,13]. Electroencephalographic (EEG) recordings and behavior analysis have indicated that spontaneous recurrent seizures are observed a month after a single injection[12,13]. A single injection of a convulsive dose of PTZ also induces acute seizure. However, chronic spontaneous seizures after a single injection of PTZ are difficult to promote. Chronic administration of PTZ is required to induce repetitive seizures[14]. In either method, the generation of repetitive seizures is able to induce a pathology more similar to that of human epilepsy than the generation of acute seizures. In the case of PTZ, each injection evokes a seizure, and seizure severity becomes more severe in a stepwise manner with each injection. Finally, a single low-dose PTZ injection induces a severe tonic-clonic seizure. In this phase, each injection evokes severe seizures. In addition, the seizure latency and duration also change over the course of the injections. The latency to tonic seizure often becomes shorter in the latter phase of kindling[15]. Furthermore, seizure aggravation is accompanied by a prolonged seizure duration[16]. Investigating the molecular mechanism regulating the seizure severity, latency, and duration is useful for screening anti-epileptic drugs[17,18,19].

Seizures are commonly induced by a single systemic administration of PTZ, and the recovery is very fast, within 30 min[4,5]. Thus, the number of seizures is more controllable in the PTZ-kindling model. However, EEG monitoring has indicated that generalized spikes may be seen up to 12 h

after PTZ-mediated seizure[20]. Therefore, animals should preferably remain under observation for 24 h after the myoclonic or tonic seizure[21] for more precise analysis of the kindling mechanisms.

The administration of anti-epileptic drugs, such as ethosuximide, valproate, phenobarbital, vigabatrin, and retigabine[3], before or after PTZ injection mitigates the aggravation of the seizure severity[3,22,23]. Similarly, knockout mice that lack genes involved in seizure exacerbation, such as matrix metalloproteinase-9[24], FGF-22[25] and neuritin[26], have been shown to exhibit reduced seizure severity after multiple PTZ injections. In addition, observing histopathological alterations after epileptic seizures is possible with this method. In patients with temporal lobe epilepsy, there are typical histological changes in the brain, such as mossy fiber sprouting[27,28], abnormal granule neuron migration[29], astrogliosis[30], neuronal cell death in the hippocampus[31,32], and hippocampal sclerosis[33]. Similar changes are observed in epileptic model animals. Among the available methods, PTZ-mediated chemical kindling is a good, reproducible and inexpensive method to produce an animal model of epilepsy. In a pilocarpine-mediated SE model, seizure control is difficult and many mice die or fail to develop SE[34]. In contrast, mortality and seizure severity are more controllable in the PTZ model. Additionally, PTZ is less expensive than kainic acid, and skills in mouse brain surgery are not required for drug administration.

Protocol

All experimental procedures were approved by the Animal Care and Use Committee of the Tokyo Metropolitan Institute of Medical Science. Postnatal 8 - 16-week-old mice are recommended. Any inbred strain is acceptable for the experiment. C57BL/6 mice are more resistant to PTZ, whereas BALB/c and Swiss albino mice are more sensitive to PTZ. C57BL/6 were used in this study. Vulnerability to PTZ also depends on the age of the mouse. Compared to younger mice, older mice are more refractory to PTZ[35]. The number of animals used for this method can vary, but at least 6 - 10 animals are required for each condition.

1. Preparation of PTZ

1. Dissolve 2 mg/mL PTZ in sterile 0.9% (w/v) NaCl. Prepare PTZ on the day of use.

2. Injection of PTZ

1. Perform all experiments between 9:00 a.m. and 12:00 p.m. (noon).
2. Measure the animal's body weight.
3. Place the animal in an observation chamber for habituation.
4. During the habituation period (3 min), calculate the volume of PTZ solution for the injection based on the body weight of the animal and the previously determined injection dose.
   NOTE: For example, when 35 mg/kg PTZ is used, 0.875 mg of PTZ is required for a mouse weighing 25 g (35 mg/kg x 0.025 kg = 0.875 mg). Therefore, 437.5 µL of a 2 mg/mL PTZ solution should be injected (0.875 mg / 2 mg/mL = 0.4375 mL). The injection dose of PTZ depends on the mouse genotype and strain. For wild-type C57BL/6 mice, a dose of 30-35 mg PTZ/kg body weight is recommended for the first trial. Sensitivity to PTZ depends on the mouse strain used[36,37]. The injection dose varies according to the purpose of the experiment.
5. Inject PTZ intraperitoneally with a 1-mL syringe attached to a 27-gauge needle into the left or right quadrant of the abdomen of the animal. Avoid injecting at the midline.
   1. Avoid repeated injections at the same position.

6. Observe animal behaviors for 30 min after PTZ administration (**Figure 1A**), and classify and score the abnormal behavior as shown below. If possible, keep the animals under observation for 24 h, or at least an additional 6-10 h post-injection, especially once the seizure severity score reaches 3 or above.
   1. Note any mild seizures or behavioral changes in the animals beyond the 30-min observation period. This prolonged observation period may address a particularly important and complete effect of a subconvulsive dose of PTZ in the generation of chronic unprovoked seizures in epilepsy.
   2. In addition, measure the seizure duration of each observed seizure as changes in seizure duration relate to the seizure severity. Moreover, the latency to the first seizure after the PTZ injection is another important measure to collect. Monitoring the seizure frequency, duration, and latency is critical for any post-seizure molecular studies.

7. Inject PTZ every other day (**Figure 1A**). The number of injections depends on the objective of the experiment. Representative examples are as follows.
   1. To generate completely kindled animals, once an animal experiences a seizure with a score of 5 (tonic-clonic seizure), finish the injection within additional three administrations. In this case, increase the injection dose if the seizure score does not increase for three consecutive administrations. Each animal can receive a different number and dose of PTZ injections.
   2. Fix the number and dose of PTZ injection in every condition to evaluate the vulnerability to PTZ, including in assessments of anti-epilepsy drugs and studies of the phenotype of genetically modified mice. Give all animals the same number of PTZ injections; a total of 8 to 12 injections is recommended. If an animal dies, the seizure score should be denoted as 6.
   3. Determine the injection dose necessary to maintain a high seizure score (4 or 5) for 10 or more injections to study the histopathological changes that occur epileptic seizures. In this case, the total injection number should range from 25 to 30. Decrease the injection dose if the seizure score reaches 5. If the seizure score decreases to 3 or lower, increase the injection dose.

3. Seizure Score

1. Observe the animal behaviors and record the scores.
   1. Classify and score the epileptic behaviors as follows[38,39] (**Figure 1B**):

0: normal behavior, no abnormality.
1: immobilization, lying on belly.
2: head nodding, facial, forelimb, or hindlimb myoclonus.
3: continuous whole-body myoclonus, myoclonic jerks, tail held up stiffly.
4: rearing, tonic seizure, falling down on its side.
5: tonic-clonic seizure, falling down on its back, wild rushing and jumping.
6: death.

2. Change the behavioral criteria based on the Racine score[40], depending on the experimental conditions.

4. Post-Seizure Analysis

1. Immunohistopathological analysis
   1. Perfusion fixation
      1. Preparation
         1. Dissolve 4% (w/v) paraformaldehyde (PFA) in 0.1 M phosphate buffer (pH 7.4). Heat the solution to approximately 70 °C with the addition of a drop of 1 M NaOH (approximately 1 µL of 1 M NaOH for 50 mL PFA solution).
         2. Prepare ice-cold 4% PFA and phosphate-buffered saline (PBS).
         3. Set up a peristaltic pump, with a tube and needle. Run approximately 20 mL of water through the tube to clear out residue. Then, place the open end of the tube in ice-cold PBS.

      2. Transcardial perfusion and preparation of the fixed brain
         1. Deeply anesthetize the mouse with 50% isoflurane/50% ethanol in a small jar.
         2. Keep the mouse in a supine position by pinning the tips of their forelimbs and hindlimbs.
         3. Cut the ventral midline of the abdomen and expose the diaphragm.
         4. Cut the diaphragm along the costal margin. Then, cut both lateral sides of the ribs and body. Pinch the xiphoid process by the locking forceps and evert the rib cage. Maintain the position of the xiphoid process by pinning it with a needle or pinching it with forceps. Expose the heart.
         5. Insert the needle into the left ventricle and make a cut in the right atrium using scissors. Be aware not to push the needle too far into the heart, as it can pierce an interior wall.
         6. Begin a steady flow of ice-cold PBS through the needle to wash out the blood (approximately 15 - 20 mL/min).
         7. When the blood has been cleared from the body, move the tube to a 4% paraformaldehyde solution (approximately 60 mL) without the addition of bubbles. Then, stop the perfusion.
         8. Cut the rear neck and spine and peel off the head skin and the dorsal part of the skull with dissecting scissors.
         9. Cut the premaxillary bone between the eye orbits and completely remove the dorsal portion of the skull.
         10. Create a gap between the brain and jugal bone from the posterior side and cut the trigeminal nerves and optic chiasm underneath the brain. Carefully remove the brain and place it in a vial containing 4% PFA. Post-fix the brain at 4°C for 12-16 h.

   2. Brain slice preparation
      1. Transfer the fixed brain to 20% sucrose/PBS until the brain sinks into the solution to allow for cryoprotection.
      2. Cut the fixed brain based on the required slice orientation and required part of the brain.
      3. Set the cryomicrotome. Set the blade in position and maintain the microtome stage at a temperature under -20 °C.
      4. Place the pre-cut brain on the stage and coat the brain with 20% sucrose/PBS. The sucrose/PBS solution will gradually freeze, covering the brain on the stage, until the brain is completely embedded in frozen 20% sucrose/PBS.
      5. Slice the brain (30 µm thickness) by sliding the blade through the tissue. Transfer the slices into PBS and keep them at 4 °C.

   3. Fluorescent immunohistochemistry
      1. Wash the necessary slices in 0.1% Triton X-100/PBS (PBST) for 10 min, 3 times at room temperature (RT).
      2. Block the slices by incubating them in PBST with 2% goat serum or 5% bovine serum albumin (BSA) for 1 - 2 h at RT.
      3. Incubate the slices in an antibody solution with the suitable concentration of primary antibody in PBST with 2% goat serum or 5% BSA for 1 to 7 overnights at 4 °C.
      4. Wash the slices in PBST for 10 min, 3 times at RT.
      5. Incubate the slices in a secondary antibody solution with the suitable concentration of secondary antibody in PBST for 1 - 2 h at RT, light-protected.
      6. Mount the slices onto glass slides and cover with mounting medium and cover glass.
      7. Observe the slices with fluorescence microscopy.

2. Three-chamber test
   1. Preparation
      1. Prepare at least 2 2 - 6-month-old 129/Sv mice of the same sex as the subject mice to be the "Stranger mouse." Habituate all mice in the cage before the analysis. For each training session, place the mouse in the cage and leave the mouse for 15 min.
      2. Before each habituation phase with the subject mouse, clean the entire apparatus and both cages by wiping the surface with 70% ethanol.
      3. Place the empty cages in the two side chambers of the 3-chamber apparatus and open the doors between the chambers.
      4. To habituate the subject mouse, place a subject mouse in the center chamber and allow the mouse to freely move throughout the apparatus until the mouse has investigated both cages, plus an additional 5 min. Subject animals that may be fearful of the cage

do not investigate the cage for 20 min and should not be used for this analysis. Such animals avoid the cage and rarely come close to the cage.

   5. After the animal moves into the center chamber, close the doors and let the mouse move freely in the center chamber for 5 min. During this period, put one of the 129/Sv mice in a cage to habituate to the cage.

2. Sociality analysis
   1. Carry out this analysis immediately after the habituation period of the subject mouse.
   2. Place the cage containing a 129/Sv mouse, termed the "Stranger 1 cage", in one of the side chambers and place the empty cage, termed the "Object cage", in the other side chamber.
   3. Open the door and monitor the behavior of the subject mouse.
   4. Allow the subject mouse to move freely for 10 min and record the following behavioral parameters. If the subject mouse is afraid of the mouse in the Stranger 1 cage, as indicated by the mouse avoiding the Stranger 1 cage and remaining in the corner of one of the chambers, the animal should not be used for the analysis.
      a) Time spent in each chamber, the Stranger 1 chamber, the Object chamber, and center chamber.
      b) Time spent investigating each cage, the Stranger 1 cage and the Object cage. (The mouse is classified as investigating the cage if the mouse is sniffing or toughing the mouse or cage)
      c) The number of entrances into each chamber (optional)
      d) Total distance travelled (optional)
   5. After the mouse moves into the center chamber, close the doors.

3. Social novelty analysis
   1. Carry out this analysis immediately after the sociality analysis.
   2. Wipe both cages with 70% ethanol.
   3. Place another 129/Sv mouse in one of the cages, termed the "Stranger 2 cage" and place the Stranger 2 cage in one of the chambers. Place the Stranger 1 mouse in the other cage, termed the "Stranger 1 cage", and place the Stranger 1 cage in the other chamber.
   4. Open the door and monitor the behavior of the subject mouse.
   5. Allow the subject mouse to move freely for 10 min, and measure the same behavioral parameters described for the sociality analysis.
      NOTE: The Stranger 1 and Stranger 2 mouse should be chosen randomly. The Stranger 1 chamber and Object chamber as well as the Stranger 1 chamber and Stranger 2 chamber should be randomly determined.

3. Contextual fear discrimination
   1. Conditioning
      1. Before each conditioning trial, clean the experimental apparatus by wiping the surface with 70% ethanol.
      2. Place a mouse in a conditioning apparatus with specific conditions (apparatus shape, wall color, floor material, odor, lighting, and background noise volume should be pre-determined). For example, the conditioning apparatus used here was a square apparatus with clear plexiglass walls, a metal grid floor, an ethanol odor, 100 lux brightness, and 65-dB background white noise.
      3. Condition the mouse by foot-shock with pseudo-random timing of 0.1-mA electrical shocks for 2 s x 3 times over the course of 5 min.
      4. Return the mouse to the home cage after conditioning.

   2. Memory assessment
      1. Before each assessment, clean the experimental apparatus by wiping the surface with 70% ethanol.
      2. The next day following conditioning, place the mouse into the same apparatus as shock condition and measure the freezing time over the course of 5 min.
      3. Later the same day, place the mouse in a novel apparatus and measure the freezing time during a 5-min assessment. A triangular apparatus with white plexiglass walls, plate floor, no odor, 30 lux brightness, and 70-dB white noise was used here.
      4. Compare the freezing time between the same condition and novel condition. The mice with a normal memory ability will freeze more in the same condition than in the novel condition. Freezing is defined immobility of the animal for more than 2 s.

Representative Results

Repetitive injection of PTZ induces an increase in seizure severity. Six C57BL/6 mice were treated with PTZ, and another 6 mice were treated with saline as a control group. The PTZ dose was 35 mg/kg, and 10 injections were administered. The seizure score gradually increased with PTZ injections, whereas no seizures or abnormal behaviors were evoked by saline injections (**Figure 2**). ANOVA followed by Bonferroni test showed a significant difference between the PTZ-treated group and the saline-treated group.

Repetitive seizure promotes aberrant axonal branch formation (mossy fiber sprouting) and abnormal migration of granule cells in the hippocampus. Mice were treated with PTZ for 25 injections (dose was adjusted between 24 mg/kg and 35 mg/kg to maintain severe seizure in mice without inducing death caused by a severe seizure). The mouse brains were fixed 3 weeks after the last injection. Control brains were fixed before PTZ injections. Brain slices were immunostained with anti-synaptoporin (x 500) and anti-ZnT3 (x 500) antibodies to observe the mossy fiber sprouting (**Figure 3A**) and with the anti-doublecortin antibody (x 200) to observe the abnormal migration of granule cells (**Figure 3A**). Mossy fiber sprouting in the granule cell layer was observed in PTZ-treated slices (**Figure 3A**). Newborn granule cells, which are immunoreactive for doublecortin, were observed within the hilus in the PTZ-treated slices (**Figure 3A**). The granule cell layer and hilus shown in **Figure 3A** is illustrated in **Figure 3B**.

Repetitive seizures also impair normal behavior of mice. Twelve C57BL/6 mice were treated with PTZ (35 mg/kg, 10 injections), and another 12 mice were treated with saline as a control group. Two weeks after the last injection, the mice were analyzed in a 3-chamber test (**Figure 4A**) and contextual fear discrimination test (**Figure 5A**). PTZ-treated mice showed normal sociality (**Figure 4B**). Mice spent more time in the Stranger 1 chamber than in the Object chamber (saline: p = 0.003, PTZ: p = 0.027) and investigated the Stranger 1 cage more than they investigated the Object cage (saline: p = 0.009, PTZ: p = 0.004). However, PTZ-treated mice showed abnormal social novelty (**Figure 4C**) indicative of impaired social memory. Control mice spent more time in the Stranger 2 chamber than in the Stranger 1 chamber and investigated the Stranger 2 cage more than they investigated the Stranger 1 cage, whereas the kindled mice did not show any significant difference in time spent in the chambers or time spent investigating the cages (Time in chamber: saline: p = 0.006, PTZ: p = 0.126. Time investigating: saline: p = 0.002, PTZ: p = 0.426). PTZ-treated mice also showed impaired memory in the contextual fear test (**Figure 5B**). Control mice showed a longer freezing time in the shock condition than in the novel condition, whereas PTZ-treated mice did not show any significant difference in freezing time (saline: p < 0.001, PTZ: p = 0.060). Unpaired t-tests were carried out for statistical analyses.
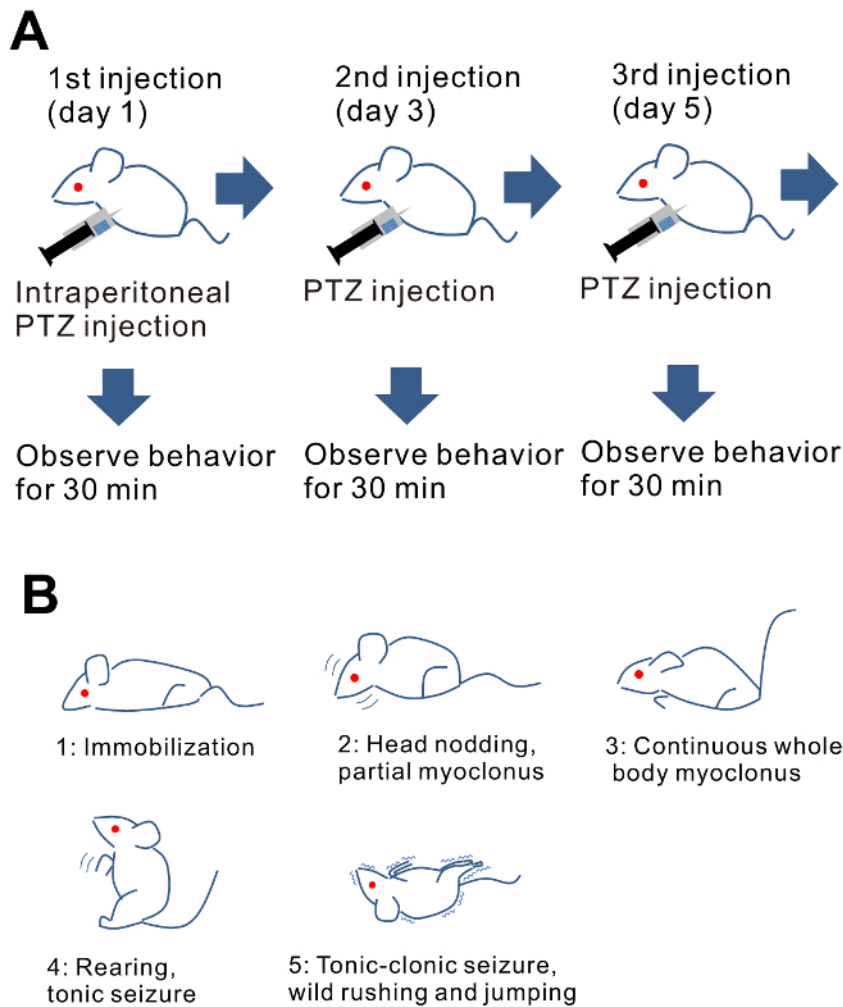

Figure 1: Brief description of the protocol. (**A**) Schematic illustration of PTZ-mediated kindling. (**B**) Illustrations of representative animal behaviors for respective seizure scores. Please click here to view a larger version of this figure.
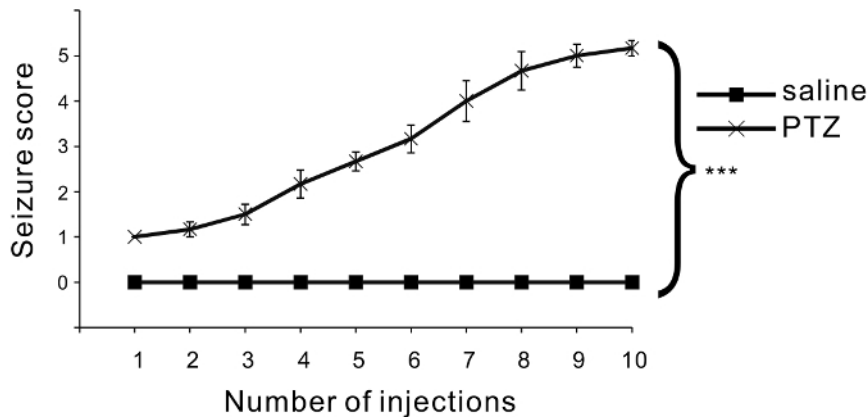
Figure 2: Assessment of convulsive behavior. The mean seizure scores are indicated in the graph. Six mice were used in each condition, and one series of injections was carried out. After each injection, the seizure scores were monitored and scored. Compared to saline injections, PTZ injections significantly increased seizure severity ($p < 0.001$: repeated-measures ANOVA). Each seizure score is shown as the mean ± SEM. Please click here to view a larger version of this figure.
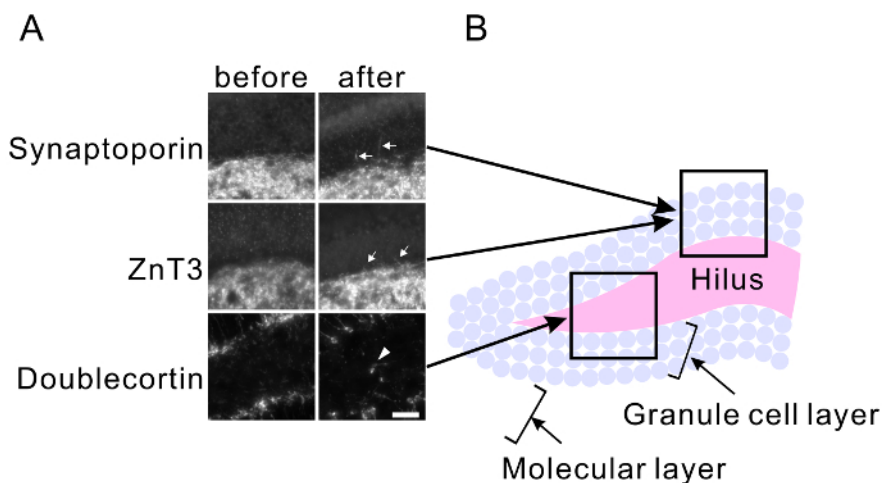

Figure 3: PTZ-mediated mossy fiber sprouting and abnormal migration of granule cells. (A) Maximum projected immunohistochemical images of the hilus, granular layer, and molecular layer of hippocampal slices of the mice before kindling (left) and after kindling (right). Anti-synaptoporin (top) and anti-ZnT3 antibodies (middle) were used to visualize the axons of granule neurons (mossy fibers). The anti-doublecortin antibody was used to visualize newborn granule neurons (bottom). PTZ-mediated repetitive seizures induce mossy fiber sprouting (arrows) and abnormal migration of granule cells into the hilus (arrowheads). Scale bar, 50 μm. The approximate position of each image is indicated in **(B).** Please click here to view a larger version of this figure.
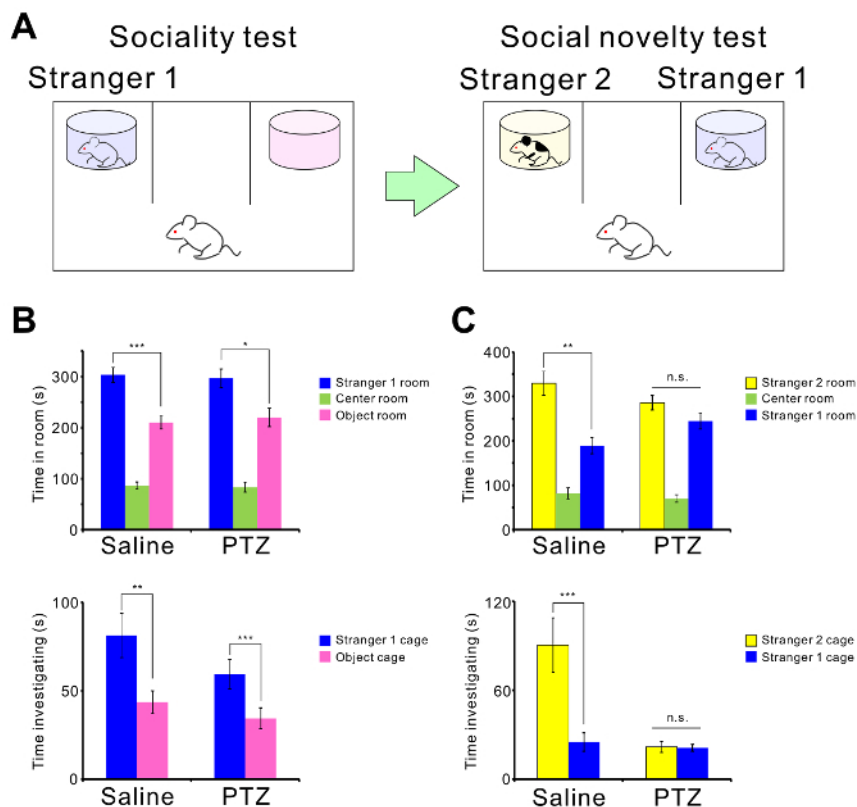
Figure 4: PTZ-mediated abnormal social behavior. (A) Schematic illustration of the 3-chamber test. **(B)** The mean amount of time spent in each chamber and the mean amount of time spent investigating each cage in the sociality test are shown. **(C)** The mean amount of time spent in each chamber and the mean amount of time spent investigating each cage in the social novelty test are shown. There were twelve mice in both the PTZ- and saline-treated groups. PTZ-mediated seizures induced abnormal social behavior (***p < 0.001, n.s. = not significant). All times are shown as the mean ± SEM. Please click here to view a larger version of this figure.
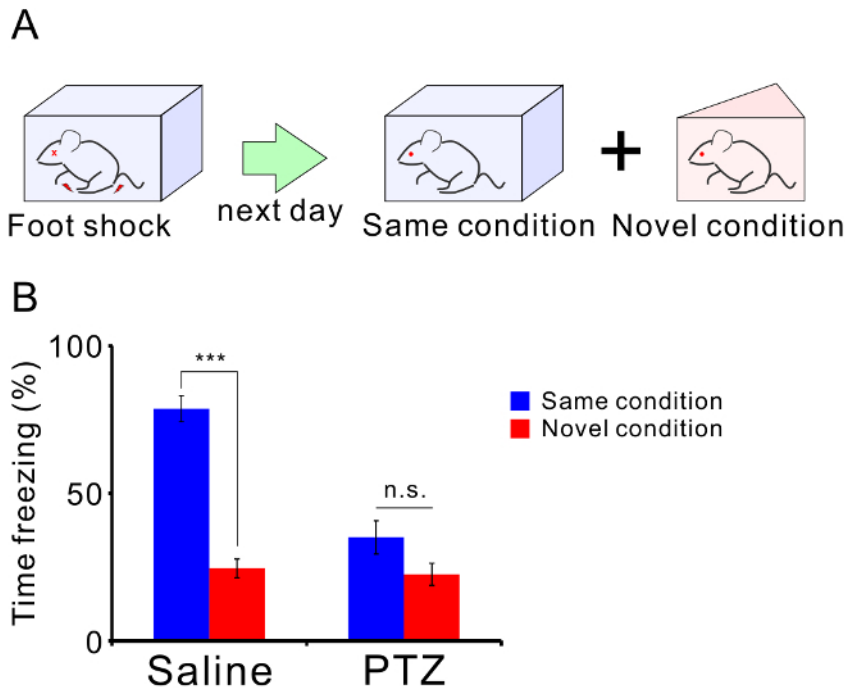
Figure 5: PTZ-mediated memory impairment. (A) Schematic illustration of the contextual fear discrimination test. **(B)** The mean percentage of freezing times in each condition are shown. There were twelve mice in both the PTZ- and saline-treated groups. Graph shows the mean ± SEM. Please click here to view a larger version of this figure.

Discussion

Here, we present a widely accessible protocol for the establishment of a pharmacological animal model of epilepsy. PTZ-mediated chemical kindling has a long history and is a commonly accepted model for the study of the histopathology and cellular pathology of epilepsy[41]. The chemical kindling model of epilepsy has been reviewed previously by Suzdak and Jansen, 1995[42]. Pharmacological seizure induction, especially with PTZ, is an easy and simple method for evoking severe seizures. Changes in the injection dose correlate with seizure severity. Thus, identifying the appropriate dose over several trials by changing the PTZ dose and examining the resulting behavior is very easy.

Many researchers have attempted to create knockout or knock-in mice as epilepsy models and have succeeded in producing mice that exhibit spontaneous seizures[43,44]. However, pharmacological seizure induction is still considered a good model for epilepsy. Another common method for seizure induction other than gene modification involves implanting electrodes into an animal's brain and inducing electroshock-mediated seizures. This method is costly, difficult, and requires surgical skill to implant the electrode at the precise locus in the brain[5].

Chemical induction of convulsions enables the rapid investigation of epileptogenesis and anti-epileptic drug screening at low cost[2,4]. The frequency and severity of spontaneous seizures and SE vary depending on the drug used. Kainic acid and pilocarpine are mainly used to provoke SE and subsequent chronic spontaneous or recurrent seizures[45,46,47]. On the other hand, PTZ is used both to promote SE when given in a high-dose and to develop chemically kindled animals when given in a subconvulsive dose[48,49]. In addition, the mechanisms of convulsive drugs that induce seizures are well known. Thus, blocking the mechanism necessary to induce seizure may help to identify anti-epileptic drugs. On the other hand, genetic models are required to investigate the mechanisms that evoke epileptic seizures[50]. After the mechanisms by which seizures are induced are elucidated, the screening of anti-epileptic drugs may be started.

In the representative results shown here, animal behavior was observed for 30 min after PTZ administration. However, as mentioned in the protocol section, animal behavior is preferably observed for 24 h or at least 6-10 h after PTZ injection, especially once the seizure score reaches 3 or higher. Although an animal monitoring system may be required to observe the animal for the whole day, a prolonged observation is critical for obtaining a deep understanding of epilepsy. In addition, the seizure duration and seizure latency are useful measures to collect. These measurements are important when relating changes in seizure duration and latency to time and seizure severity.

The post-seizure histopathology of chemically kindled animals has been investigated, especially in the hippocampus. Repetitive seizures or SE induce mossy fiber sprouting[27,28], abnormal migration of newborn granule neurons[25,29], astrogliosis in the hippocampus[30], and apoptosis of pyramidal neurons[31,32]. These histological changes in the brain disrupt neurological functions in epileptic patients. For example, the association of epilepsy with autism spectrum disorders (ASD) and intellectual disability (ID) is well recognized[51,52,53]. Whether epileptic seizures induce ASD and ID or ASD and ID induce epileptic symptoms is still a complicated question. Recent studies have shown that PTZ-mediated seizures induce ASD-like social-cognitive impairments[54] and ID-like learning deficits [55,56,57]. These findings indicate that the epilepsy-mediated histopathology elicits the neuronal dysfunction and psychiatric disorders.

Histological changes promoted by epilepsy take time to develop after seizure induction. In this regard, pharmacological seizure induction is advantageous because researchers can control the timing, number and severity of seizures in animals. Including chemical kindling, animal

models of epileptogenesis will continue to promote the investigation of both the mechanism of epilepsy induction and related neurophysiological disorders.

Disclosures

The authors declare no conflicts of interest.

Acknowledgements

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