



## Effect of PTZ-induced acute seizures on anxiety-like behaviors in open field test in male mice



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### ABSTRACT

**Background:** Seizures induce transient changes in behavior, including changes in anxiety-related exploration. In the present study the effect of acute pentylenetetrazol (PTZ)-induced seizures on activity and exploration in open-field test was investigated.

**Methods:** Eight adult male C57BL/6 mice (weighing 20-25 g) were subjected to the open-field test before and 24 hours after intraperitoneal administration of PTZ (60 mg/kg) to induce an acute seizure model. Behavioral parameters, including time spent in the center zone (center time), number of crossings, and distance traveled in the center zone, were evaluated.

**Results:** PTZ injection significantly reduced center time ( $P < 0.05$ ) and had a large effect on reducing the number of crossings ( $d = 1.16$ ) and center distance moved ( $d = 0.8$ ) parameters. These data suggested a meaningful alterations in exploratory behavior following PTZ injection.

**Conclusion:** These findings indicated that acute PTZ-induced seizures are associated with changes in anxiety-like behavior, particularly reflected by reduced exploration of the central area of the open-field arena.

**Keywords:** Seizure, Open field, Exploration, Pentylenetetrazol, Anxiety.

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### Introduction

A seizure is a transient occurrence of signs and symptoms resulting from excessive, abnormal, and hypersynchronous activity of a population of neurons in the brain. Epilepsy is a chronic neurological brain disorder characterized by recurrent and unprovoked seizures and is associated with a wide range of neurological, cognitive, psychological, and social consequences (1). Each brain region contains local excitatory and inhibitory circuits and also receives excitatory and inhibitory inputs from other brain areas. Any factor that disrupts the balance between excitatory and inhibitory circuits in favor of excitation can lead to the induction of epileptic activity. Seizures occur when the normal balance between glutamatergic (excitatory) and GABAergic (inhibitory) systems, which under physiological conditions regulate neuronal firing and prevent abnormal spontaneous discharges, is disturbed (2). One of the proposed mechanisms underlying the initiation and propagation of seizures is an increase in the synchrony of neuronal discharges. Despite the high prevalence

of epilepsy, a definitive cure is still unavailable in many cases (3).

Approximately 70 million people worldwide—nearly 1% of the global population—are affected by epilepsy (4), and about one-third of patients are resistant to antiepileptic drug therapy. In drug-resistant epilepsy, surgical intervention and resection of the epileptogenic focus may be considered in a limited number of cases (5). However, when seizures originate from multiple or widespread regions of the brain, surgical treatment is not feasible (5). Moreover, epilepsy surgery is associated with irreversible and potentially severe consequences (6). In addition, antiepileptic drugs can exert various adverse effects on the brain and other organs. Therefore, alternative therapeutic strategies are required for the effective control or treatment of patients with epilepsy (7).

A previous study demonstrated that rats subjected to a temporal lobe epilepsy model exhibited reduced exploratory behavior in the open field test compared with control animals (8).

In this study, we used an acute high-dose

administration of pentylenetetrazol (PTZ) to induce a model of generalized tonic-clonic seizures. PTZ is a GABA<sub>A</sub> receptor antagonist that binds to the picrotoxin site on the GABA<sub>A</sub> receptor complex and blocks the receptor extracellularly (9).

Hall (1934) originally described the open field test for the study of emotionality in rats. The procedure consists of subjecting an animal, usually a rodent, to an unknown environment from which escape is prevented by surrounding walls (10). Open field test is widely used to study anxiety-like behavior in rodents (11). Stressful experiences are linked to anxiety disorders in humans. Similar effects are observed in rodent models, where anxiety is often measured in classic conflict tests such as the open-field test. Spontaneous rearing behavior, in which rodents stand on their hind legs to explore, can also be observed in this test yet is often ignored (12). Animals display an increased percent of entries in the central area could be interpreted as anxiolytic-like behavior. In such a situation, rodents spontaneously prefer the periphery of the apparatus to activity in the central parts of the open field. Indeed, mice and rats walk close to the walls, a behavior called thigmotaxis. Increase of time spent in the central part as well as of the ratio central/total locomotion or decrease of the latency to enter the central part are indications of anxiolysis (13).

An important mechanism underlying the development of seizure is the disruption of the balance between excitatory and inhibitory neural inputs (14). Alterations in excitatory and inhibitory inputs, changes in extracellular ion concentrations such as potassium, and modifications in the opening and closing kinetics of voltage-gated ion channels are among the proposed mechanisms involved in the initiation and propagation of seizures (15).

This study was performed to indicate whether PTZ injection has significant effect on exploratory behavior of animals in the open field test. Despite the widespread use of the pentylenetetrazol (PTZ)-induced seizure model in preclinical epilepsy research, the non-convulsive behavioral consequences of acute seizures, particularly anxiety-related alterations, remain insufficiently characterized. Previous studies have largely focused on gross locomotor outcomes, making it difficult to dissociate

anxiety-related behavioral changes from generalized activity alterations. Moreover, within-subject assessments directly comparing behavioral performance before and after acute PTZ administration are scarce. Therefore, the present study was designed to address this gap by systematically evaluating anxiety-related behavioral parameters in the open field test 24 hours following acute PTZ-induced seizures using a within-subject experimental design.

## Materials and methods

### Animals

Eight adult male C57BL/6 mice (weighing 20–25 g) were used in this study. All animals were included in a single experimental group (n = 8) and were assessed at two time points (pre-PTZ and post-PTZ) using a within-subject design. All animals were housed under standard laboratory conditions (12:12 h light/dark cycle, temperature  $22 \pm 2^{\circ}\text{C}$ , and free access to food and water). Mice were allowed to acclimate to the laboratory environment for at least one week prior to the beginning of behavioral testing. Only male mice were included to avoid potential hormonal variations associated with the estrous cycle. All experimental procedures were performed in accordance with Zanjan University of Medical Sciences guidelines for the care and use of laboratory animals (Ethical approval code: IR.ZUMS.AEC.1403.007).

### Open-field testing

All open-field testing took place inside sound insulated, ventilated multiconditioning chambers. The open-field arena (45 cm (l)  $\times$  45 cm (w)  $\times$  40 cm (h)) consisted of Plexiglas walls and floor. Prior to testing each animal, the entire open-field arena was cleaned using ethyl alcohol. Animals were removed from their home cage by the tail and placed directly into the center of the open field. Recording/tracking was initiated immediately after placing the animal in the center of the open field arena. Behavioral tracking and analysis were performed using EthoVision XT (Noldus Information Technology, Netherlands). The Open Field test was conducted twice for each animal:

1. Baseline assessment (Pre-PTZ; before pentylenetetrazol injection), and
2. Post-PTZ assessment (24 hours after PTZ administration).

Each mouse was gently placed in the center of the arena, and video recording was initiated immediately. The floor of the arena was digitally segmented into predefined zones (center and periphery) using EthoVision XT. All behavioral parameters were automatically quantified by the software.

Behavioral analysis focused on three standard indices of anxiety-related behavior: a) Center time: total duration spent in the central zone; reduced center time typically reflects increased anxiety; b) Center distance moved: reduction of distance moved by the animal in central area of the open field arena is considered an index of thigmotaxis and elevated anxiety; c) Number of Crossings: total number of grid-line crossings, used as a combined measure of locomotor activity and exploratory behavior.

#### **PTZ-induced acute seizure model**

For acute seizure, pentylenetetrazol (PTZ; Sigma-Aldrich, USA) was administrated intraperitoneally (i.p.) at the dose of 60 mg/kg to animals. By performing the test both before and after PTZ administration, within-subject comparisons were used to determine whether PTZ induced changes in locomotor or anxiety-related parameters.

#### **Statistical analysis**

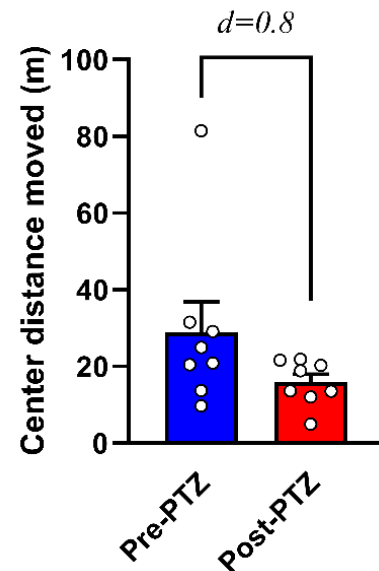
Data are presented as mean  $\pm$  standard error of the mean (SEM). Normality of data distribution was assessed using the Shapiro–Wilk test. For within-subject comparisons between pre-PTZ and post-PTZ conditions, paired t-tests were applied to normally distributed variables, while non-normally distributed data were analyzed using the Wilcoxon signed-rank test. Effect sizes were calculated to estimate the magnitude of observed effects. Statistical analyses were performed using GraphPad Prism software, and a p value  $< 0.05$  was considered statistically significant.

#### **Results**

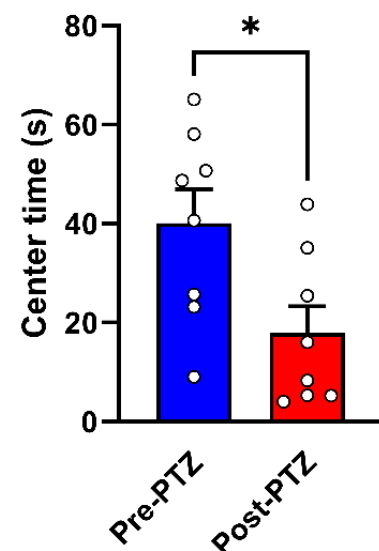
Open field parameters were evaluated to assess changes in exploratory behavior before and after PTZ administration. Comparison of baseline and post-PTZ measurements demonstrated a consistent significant reduction in all analyzed parameters following PTZ injection.

A significant decrease was observed in the distance traveled within the center zone following PTZ administration. Although the reduction in center distance moved did not reach statistical

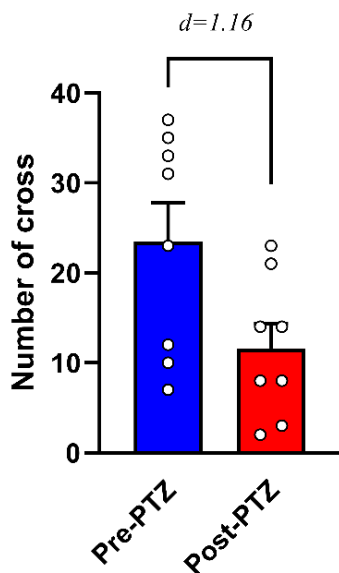
significance, effect size analysis revealed a large effect ( $d = 0.8$ ) (Fig.1). The time spent in the center zone of the arena showed statistically significant reduction ( $p < 0.05$ ) after PTZ administration compared with pre-PTZ values (Fig.2). Also the number of crossings did not reach statistical significance; however, effect size analysis showed a very large effect ( $d = 1.6$ ) (Fig.3).



**Figure 1.** Comparison of the distance traveled within the center zone of the open field arena before and after PTZ administration. Data are expressed as mean  $\pm$  SEM. statistical analysis was performed using the non-parametric Wilcoxon signed-rank test. The calculated effect size ( $d = 0.8$ ) suggests a large effect.



**Figure 2.** Comparison of time spent in the center zone of the open field arena before and after PTZ administration. Data are presented as mean  $\pm$  SEM. Statistical analysis was performed using a paired t-test. A significant reduction in center time was observed following PTZ injection ( $p < 0.05$ ). \*  $p < 0.05$



**Figure 3.** Comparison of the number of crossings in the open field arena before and after PTZ administration. Data are shown as mean  $\pm$  SEM. Statistical analysis was conducted using a paired t-test. A large effect size was observed ( $d = 1.16$ ).

## Discussion

Data of the present study showed that PTZ injection has significant effect on behavioral parameters in open-field test. The parameters analyzed—center time, number of crossings, and center distance moved—are commonly used to reflect an animal's willingness to explore the central area of a novel environment, which is considered more anxiogenic than peripheral zones (13, 16) and it is in line with our findings. These parameters are well-established behavioral indices of anxiety in rodents. Reduced exploration of the central area reflects increased avoidance of exposed and anxiogenic environments, which is a hallmark of anxiety-like behavior in the open-field test (17). Our results demonstrated a reduction in time spent in the center of the open field, number of crossings and center distance moved following PTZ administration that suggests PTZ probably increases anxiety state and reduces the exploratory behavior of the animals. Consistent with our observations, previous studies have shown that seizure-experienced animals exhibit increased anxiety-like behavior in the elevated plus maze test (18).

Anxiety has been associated with an increased glutamate-to-GABA ratio in the brain. Since seizure activity is known to shift this balance toward excitation (17), it is plausible that one of

the mechanisms through which seizures enhance anxiety-like behavior involves alterations in the excitatory–inhibitory neurotransmitter balance.

Stress is a precipitating factor for anxiety-related disorders, which are among the leading forms of psychiatric illness and impairment in the modern world. Rodent-based behavioral tests and models are widely used to understand the mechanisms by which stress triggers anxiety-related behaviors and to identify new treatments for anxiety-related disorders (19). Anxiety and fear produce similar behavioral responses, including increased vigilance, freezing and/or hypoactivity, elevated heart rate, and suppressed food consumption (20).

Stressful experiences have a powerful impact on brain function and can lead to both short and long-term behavioral alterations (21). Observing stress-related anxiety in rodents typically relies on species-specific behaviors such as increased risk assessment, the reduction of exploration, seeking shelter, escape, burying or defecation (22). One of the most frequently used behavioral tests in rodents is the open-field test (OFT), a conflict test based on the opposing drives to explore new environments and fearful avoidance of bright/exposed areas. The OFT was originally developed by Calvin Hall in the 1930s to measure defecation as an index of timidity in mice (10). Nowadays, automated tracking allows the use of time spent in the center of the arena as a readout for anxiety, which has made the OFT the most widely used test for anxiety (16). The results suggest that PTZ exposure may induce short-term behavioral adaptations related to emotional or stress-related processes. Assessment of such behavioral changes provides important contextual insight for interpreting outcomes in experimental models of epilepsy.

## Conclusion

The present findings demonstrate that acute PTZ administration is associated with measurable alterations in open field behavior, characterized by reduced exploration of the central area of the arena. These results suggest that PTZ-induced seizures are accompanied by short-term behavioral changes related to emotional or stress-related processes. Overall, the open field data provide important contextual information for the interpretation of seizure-related outcomes and underscore the relevance of assessing behavioral

state alongside electrophysiological and cognitive measures in experimental epilepsy models.

### Acknowledgment

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### Conflict of interests

The authors declare no conflicts of interest.

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