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Chronic pregabalin treatment reduced anxiety, and acute pregabalin treatment increased depression-like behaviors in rats

Hasan Çalışkan[1*], Fırat Akat[2], Ali Doğan Dursun[3] and Nezahet Zaloğlu[2]

Abstract

Background Pregabalin is an antiepileptic drug that binds to the alpha-2/delta unit at presynaptic voltage-dependent calcium channels. We aimed to investigate the effect of acute and chronic pregabalin administration on anxiety and depression-like behaviors.

Methods Fifty-six male Wistar albino rats were divided into seven groups: control, vehicle, and five different dose groups (5, 10, 30, 60, and 100 mg/kg). Pregabalin was administered for two weeks. Depression-like behaviors were evaluated by Forced swimming test. Anxiety-like behavior (ALB) was evaluated by Open field test (OFT), Elevated Plus Maze (EPM), and light-dark box. Subjects underwent the forced swimming test (FST) after the first dose, while the open field test (OFT), elevated plus maze (EPM), and light-dark box (LDB) were performed after two weeks of treatment. Further sucrose preference test was conducted to evaluate anhedonia until the end of the experiment.

Results In the forced swimming test, depression-like behaviors increased after acute single-dose administration of 10, 30, 60, 100 mg/kg pregabalin. According to OFT results, chronic 100 mg/kg pregabalin showed anxiolytic effects by decreasing grooming, and freezing behaviors. In addition, 100 mg/kg chronic pregabalin administration significantly increased the time spent in the central region, the number of entries to the center, and the unsupported rearing number without causing any change in locomotor activity. According to EPM results, both chronic 60 and 100 mg/kg pregabalin treatments showed anxiolytic effects by increasing open arm time and head dipping behavior. In addition, 60 and 100 mg/kg chronic pregabalin administration significantly decreased stretch attend posture. All pregabalin administrations between 5 and 100 mg/kg displayed anxiolytic effects in the LDB. Sucrose preference was above 65% for the duration of all experiments and subjects did not show anhedonia.

Conclusion Acute pregabalin treatment triggered depression-like behaviors. Anhedonia, which may be associated with depression, was not observed during chronic treatment. Moreover, chronic treatment with pregabalin revealed potent anxiolytic effects in different behavior patterns and doses for all tests of unconditional anxiety. In particular, 100 mg/kg chronic pregabalin administration decreased anxiety-like behaviors in all experiment setups. Although the anxiolytic effect was demonstrated in chronic treatment, acute treatment of pregabalin induced depression-like behaviors, and thus in clinical practice should be done with caution, especially in patients with anxiety-depression comorbidity.

*Correspondence:
Hasan Çalışkan
hasan.caliskan@balikesir.edu.tr

Full list of author information is available at the end of the article

Keywords Pregabalin, Anxiety-like behaviors, Depression-like behaviors

Introduction

Anxiety disorders are one of the most common types of psychiatric illness [23]. According to the World Health Organization, worldwide anxiety prevalence is estimated at 3.6%, with approximately 264 million people suffering from anxiety disorders [38]. Further anxiety disorders put a severe load on [53]. Arias et al. reported that the economic value associated with mental disorders is estimated at about 5 trillion United States dollars [1]. Anxiety disorders can be accompanied by depression [49], and this comorbidity is approximately 45.7% [27].

Psychiatry and neurology intersect at many points, and researchers have addressed neuropsychiatry as an important field of medical science [47]. In terms of the neuropsychiatric approach, such an intersection is clear between anxiety and epilepsy. There is a common point and similarity between anxiety disorders and epilepsy in different aspects such as neuroanatomy, neurotransmitters, treatment, animal models, and epilepsy-anxiety comorbidity. Several evidences of this are listed: (1) Certain brain regions, such as amygdala and hippocampus, play important roles in the neurobiology of both epilepsy and anxiety [21]. (2) GABA is an important mediator responsible for both anxiety and epilepsy pathophysiology [34, 50]. (3) Benzodiazepines have positive allosteric modulators on the (GABA)-A receptor and increase in inhibition effect—due to this effect, benzodiazepines are used for anxiety and epilepsy [19]. (4) pentylenetetrazole (PTZ), a GABA receptor antagonist, is used to create a chemically-induced seizure animal model. A 60–100 mg PTZ injection triggered different epilepsy seizure stages on the Racini scale (Epilepsy evaluation scale) [14], and a low PTZ dose (20 mg/kg) was used to generate animal models of anxiety and increase anxiety-like behaviors [10]. (5) Patients who are diagnosed with epilepsy have 2 to 5 times the risk of developing a psychiatric disorder [32]; anxiety disorder comorbidity was reported at a higher rate than in the general population [6, 37]. Wiglusz et al. reported that the prevalence of anxiety disorder was 16.7% in epilepsy patients and could affect quality of life [52].

Pregabalin is an antiepileptic drug that binds to the α2δ unit at presynaptic voltage-dependent calcium channels and thus results in the inhibition of excitatory neurotransmission [45]. The calcium channel α2δ type 1 (α2δ-1) subunit was found to be the major binding protein for pregabalin in the neocortex, hippocampus, amygdala, and spinal cord in an autoradiographic mouse study [5] and the calcium channel α2δ type 2 (α2δ-2) subunit was the major binding protein in the cerebellum [48].

In terms of the interaction between pregabalin and the α2δ-1 and α2δ-2 subunits, other central nervous system drug classes do not have this characteristic [29]. Pregabalin is used for the treatment of anxiety, diabetic neuropathic pain, fibromyalgia, and epilepsy [17]. Some clinical studies showed that pregabalin triggers depression and suicidal ideation [20, 28].

Behavioral tests are useful for understanding neuropsychiatric disease treatment and mechanisms [40]. The effect of pregabalin, an antiepileptic agent, on behavioral patterns related to anxiety and depression was examined in detail from a neuropsychiatric point of view in this research. For this purpose, we investigated pregabalin's effects on depression-like behaviors, anti-depressant-like behaviors, and anhedonia. Our future goals include investigating different anxiety-related behaviors, such as self-grooming parameters, aggression behaviors, social behaviors, and classical unconditioned anxiety test parameters, such as high altitude, high light, open area, and new environment-induced anxiety-like behaviors.

Method

Animals

56 Wistar Albino rats (male, aged 10–12 weeks at the start of the experiment, 200–300 g, $n=8$) were purchased from the Laboratory Animals Center of Ankara University. At the end of a 1-month adaptation period, drug treatment was initiated when the subjects were 12 weeks old. Animals were housed under standard laboratory conditions (12 h light/dark cycle; temperature = 22 ± 2 °C; humidity = 50 ± 5%). Subjects had access to ad libitum food pellets and tap water. All experiments were carried out as approved by the Ankara University Experimental Animals Ethics Committee, with the approval reference number 2015-14-163.

Drug treatment and experiment groups

We used Pregabalin (Lyrica, which was generated from Pfizer). Pregabalin dissolved in saline + Tween solution. Every day we prepared a fresh 100 ml solution. Preparation of 1% v/v Tween-20 in saline: 100 ml saline contains 1 ml Tween 20). The solutions were homogenized by vortexing at 20 °C. Pregabalin was added into 1% Tween 20 saline solution. With the addition of pregabalin, a suspension was formed. The suspension was vortexed and immediately injected into the subjects. Tween provided both solubility and stabilization of the suspension. As far as tween for drug delivery is concerned, various advantages have been reported [26]. Despite several advantages, Tween may affect behaviors [9]. Therefore, in the present study, a vehicle group (Tween + saline) was

formed in addition to the control (saline) group. Rats were divided into seven groups: control (received saline 1 ml/kg, i.p), vehicle (received: 1% Tween 20+saline, 1 ml/kg, i.p), and five different dose of pregabalin (5, 10, 30, 60, and 100 mg/kg was dissolved in saline+1% Tween, 1 ml/kg i.p) for acute and chronic treatment. In the acute phase, rats received the first dose of pregabalin three hours before the forced swimming test, and in the chronic phase, rats daily received fresh pregabalin solution for two weeks. Then, anxiety behaviors assessment was evaluated following treatments. 50 mg/kg Sodium Thiopental was injected intraperitoneally into the subjects. Exsanguination from the heart was performed under deep anesthesia and afterward, sacrification procedure was conducted (After the exsanguination from the heart, the heart was removed from the body. Afterward, other organs were removed).

Behavioral testing

Behavioral testing was conducted in the Banu Ocakçıoğlu Learning and Behavior Laboratory. Two hours before the experiment started, animals were placed in a testing room for acclimatization. The tests (open field test [OFT], elevated plus maze [EPM], and forced swimming test [FST]) were conducted in the morning (09:00–14:00) in a sufficiently lighted (110 lx, warm light), silent room in all cases. The light-dark box test (LDB) was carried out during a night training session (20:00–24:00) in a lighted (850 lx, intense light), silent room. Digital cameras recorded the vertical and horizontal movements of the animals. The researcher waited outside of the room during the experiment. The test apparatus was cleaned with 65% ethanol after each test to prevent an effect of odors left by another animal. Additionally, the water in the swimming tank was changed for each FST. Pauses of at least two seconds were classified as "freezing" and analyzed as a distinct parameter. The OFT always preceded the EPM test, and there was no break between these tests. All behavioral tests were performed in a temperature-controlled room (25 °C).

Forced swimming test

The FST analyzed depression-like behavior [39]. The FST protocol was performed according to Detke and Lucki, who modified the procedure [13]. The FST apparatus consisted of a 50-cm high and 25-cm diameter rectangular container made of glass. The water height was adjusted to 40 cm and a temperature of 25 °C. Rats were forced to swim for 15 min in the pretest, and after 24 h, the rats were forced to swim for 5 min in the test. Climbing (vertical movement), swimming (horizontal movement), floating (immobility), head twitch, latency (first immobility behavior time), total mobility, and fecal boli numbers were recorded by the camera.

Sucrose preference test (SPT)

Anhedonia was assessed by using the SPT. Animals were offered 1% sucrose solution or tap water placed on the right or left side of the home cage. To prevent the possible effect of side preference or neophobia, the positions of the bottles were switched after 12 h, using a balanced method. At the end of the test, the volume of liquid consumed was recorded and used to measure the sucrose preference index. Sucrose preference was calculated with the formula [sucrose solution consumed volume/total consumed volume × 100), [4].

Open field test

The OFT is frequently used to assess general locomotor activity and anxiety-like behavior in rodents [7, 8]. We performed the test in a hypethral box, which was made of 100×100×40 cm whiteboard. The floor was divided into 25 equal squares. The squares adjacent to the edges of the box are defined as the peripheral region, and the squares in the middle of the box are defined as the central region. The subject was initially placed in the center of the open field and allowed to explore freely for 5 min. At the end of the test, the subject was returned home to the cage. All test sessions were recorded via camera and analyzed by two different researchers. The total distance traveled (four paws placed into a new square; horizontal locomotor activity), the number of rearings (with both front paws raised from the floor and both front paws touching the wall; vertical locomotor activity), time in the center zone, central zone entrance, unsupporting rearing behavior (without touching the wall rearing behavior; sensitive exploratory behavior), total grooming time, nonsequential grooming, freezing time, and fecal boli number were evaluated.

Elevated plus maze

An EPM test was used to investigate anxiety-related behaviors [7, 8]. The EPM consisted of two opposing arms (50×10 cm) without side walls, two perpendicular closed arms (50×40×10 cm), and a central zone common to all arms (10×10 cm). The EPM was 70 cm above the ground. Each rat was placed in the central area of the EPM with the head facing an open arm. At the end of the test, the subject was returned to the cage. All test sessions were recorded via camera and analyzed by two different researchers. Open arm time, head dipping behavior (occurring in the open arm), stretch-attend posture number, anxiety index, and fecal boli number were evaluated.

Light-dark box test (LDB)

The LDB test is widely used to assess anxiety-like behavior in rodents. The test was carried out as described in our previous study [12]. The test was carried out under standardized conditions on a single day (20:00–24:00).

The wooden box (40 cm in width and 110 cm in length) consisted of two equal rectangular compartments: a light zone (850 lx illumination intensity) and a dark zone. A wall with an aperture (7.5×7.5 cm) was placed on the floor between the light and dark zones. At the beginning of the test, the subject was placed in the center of the light chamber, facing away from the entrance, and allowed to freely explore both chambers for 5 min. End of the test, the subject was returned to the cage. All behaviors were recorded via camera and analyzed by two different researchers. Light zone time, light-dark entrance numbers, and fecal boli numbers were evaluated.

Statistical analysis

We performed One-way ANOVA for every parameter separately to compare four groups between each other. Afterward, the Tukey test was used as a post-Hoc test to determine the origin of the variation. Data were presented as Mean±Standard Error of mean in column graphs. $p<0.05$ value was accepted as statistically significant.

Result

Forced swimming test

The acute administration effects of pregabalin were examined in the forced swim test.

We observed climbing, swimming, floating (immobility time), latency time, head shaking, and fecal boli numbers in the FST, shown in Fig. 1.

Climbing time ($F_{(6,48)}=10$) dramatically decreased in all pregabalin groups, excluding the 5 mg/kg group ($p<0.0001$). There was no significant difference between the groups in swimming time ($F_{(6,48)}=1.2$), which is another active behavior ($p>0.05$). Furthermore, head shaking ($F_{(6,49)}=0.73$) and fecal boli number ($F_{(6,49)}=0.68$) did not differ among groups ($p>0.05$).

Floating time ($F_{(6,48)}=7.6$), defined as a depression-like behavior, increased in all pregabalin dose groups except for the 5 mg/kg group ($p<0.0001$). Latency time ($F_{(6,48)}=7.29$) to reach the first immobility period was dramatically reduced in all pregabalin groups versus control ($p<0.0001$). 10, 30, 60 and 100 mg/kg pregabalin induced depression-like behaviors.

Sucrose perference test

The SPT results are shown in Fig. 2. For both the adaption and experiment time, the sucrose preference percentage increased to higher than 65% in all groups and did not differ among groups ($p>0.05$).

Open field test

The chronic administration effects of pregabalin were examined in the open field test.

Both locomotor activity and anxiety-like behaviors were analyzed using the OFT. Locomotor activity parameters are shown in Fig. 3. According to statistical analysis, total distance traveled ($F_{(6,49)}=4.52$) activity was significantly higher in the 60 mg/kg group compared

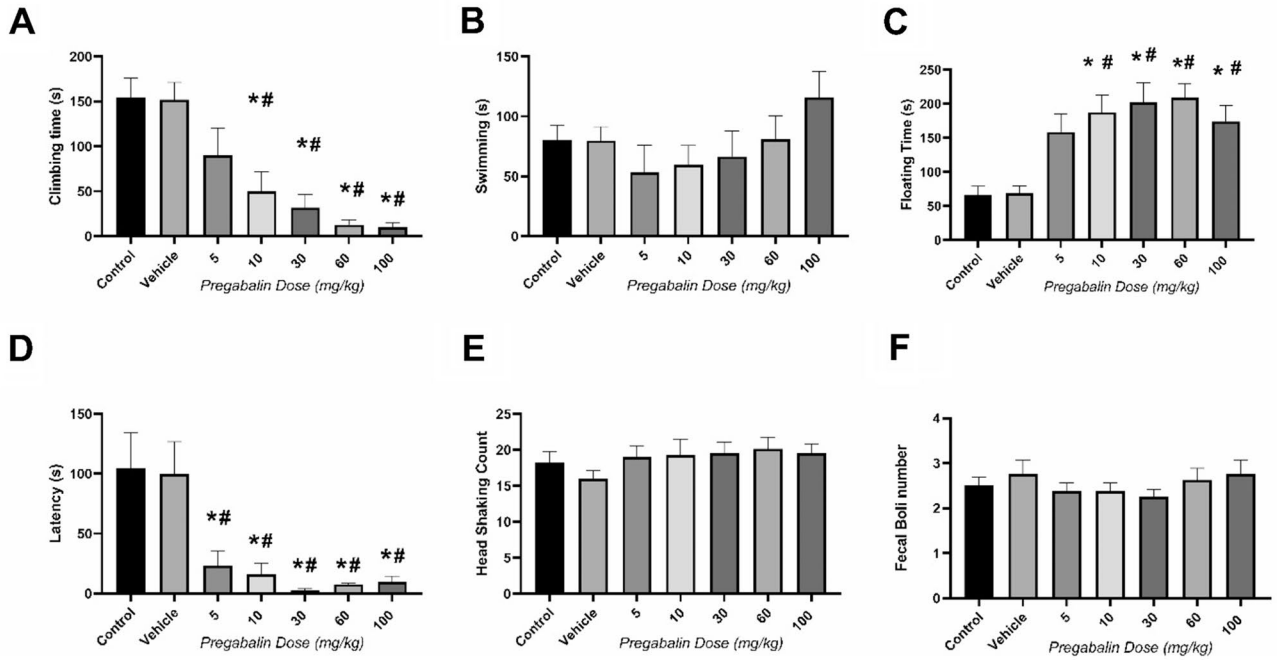

Fig. 1 Effect of acute pregabalin on forced swimming test-associated behavior patterns. (**A**) climbing (**B**) swimming (**C**) floating (**D**) latency (**E**) head shaking (**F**) fecal boli. Figure 1 shows depression-like behaviors examined in the acute period 3 h after the first dose of pregabalin (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

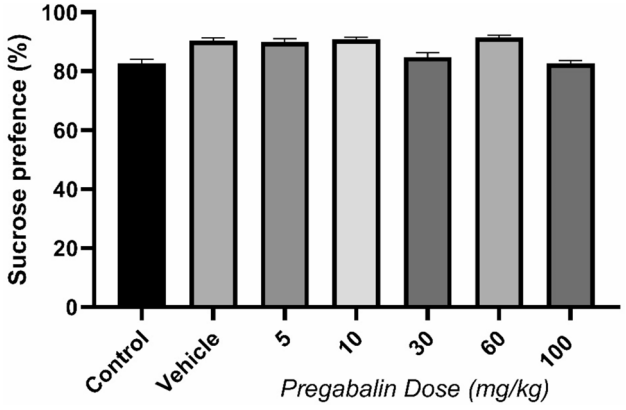

Fig. 2 Effect of pregabalin on sucrose preference. Figure 2 shows the anhedonic behavior examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests. (There is no significant difference between the experimental groups and sucrose preference is above 65%)

to the control group ($p<0.01$). Total rearing numbers ($F_{(6,49)}=0.39$) were not significant in all groups ($p>0.05$).

We observed central zone time, central entrance number, and unsupported rearing number in the OFT, which are shown in Fig. 4. Central zone time ($F_{(6,49)}=2.74$) did not differ among groups ($p>0.05$), but central zone entrance numbers ($F_{(6,49)}=13.40$) were higher in both the 60 and 100 mg/kg group ($p<0.0001$). Furthermore, unsupported rearing numbers ($F_{(6,49)}=13.96$) were higher only in the 100 mg/kg group vs. the control group ($p<0.0001$).

We also analyzed extra behavioral parameters in the OFT to separate anxiety-like behavior (ALB) and locomotion deficits (Fig. 5). Stress-related total grooming time, incorrect transition time (out-of-sequence grooming), grooming latency, and freezing times were analyzed.

Total grooming time ($F_{(6,49)}=13.65$), incorrect transition time ($F_{(6,42)}=9.89$), and freezing time ($F_{(6,49)}=42.72$) were significantly reduced in the 100 mg/kg group vs. control ($p<0.0001$). Furthermore, grooming latency ($F_{(6,42)}=28.76$) was delayed in the 100 mg/kg group

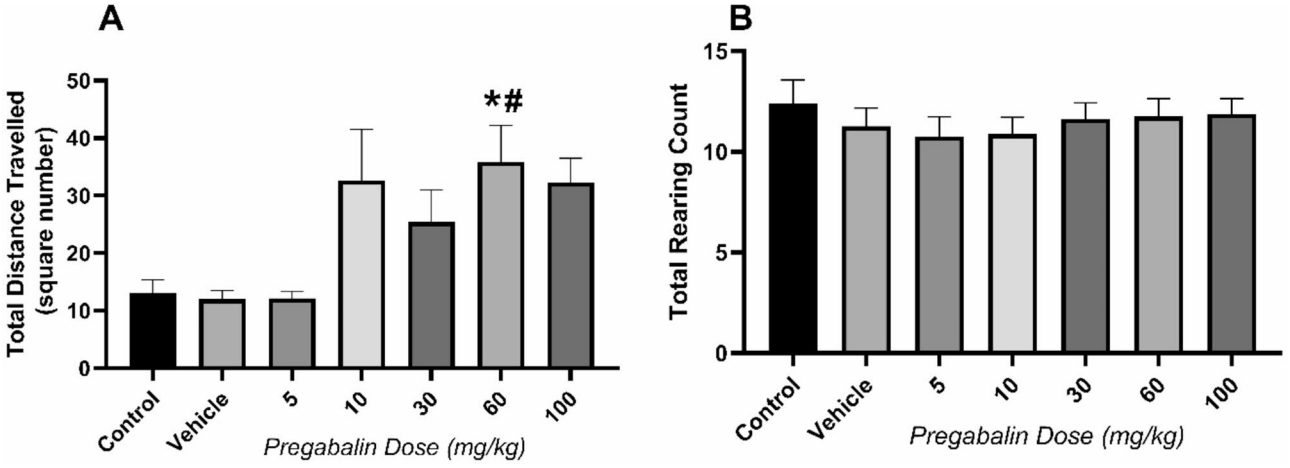

Fig. 3 Effect of pregabalin on open field test associated locomotor activity (**A**) Total Distance Travelled (**B**) Total Rearing Number (*: 0.05 vs. control, #: 0.05 vs. vehicle). Figure 3 shows the locomotor activity examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

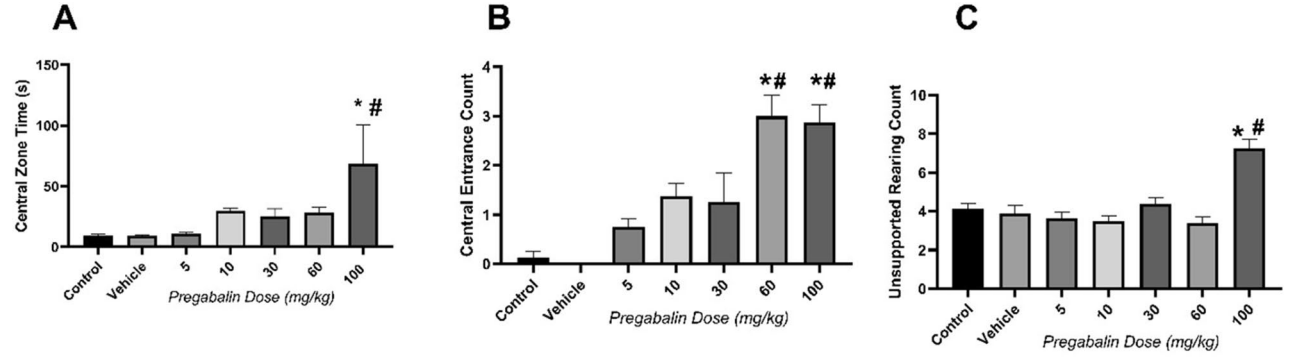

Fig. 4 Effect of pregabalin on open field test associated anxiety behavior patterns (**A**) Central Zone (**B**) Central Entrance (**C**) Unsupported Rearing (*: 0.05 vs. control, #: 0.05 vs. vehicle). Figure 4 shows anxiety-like behaviors examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

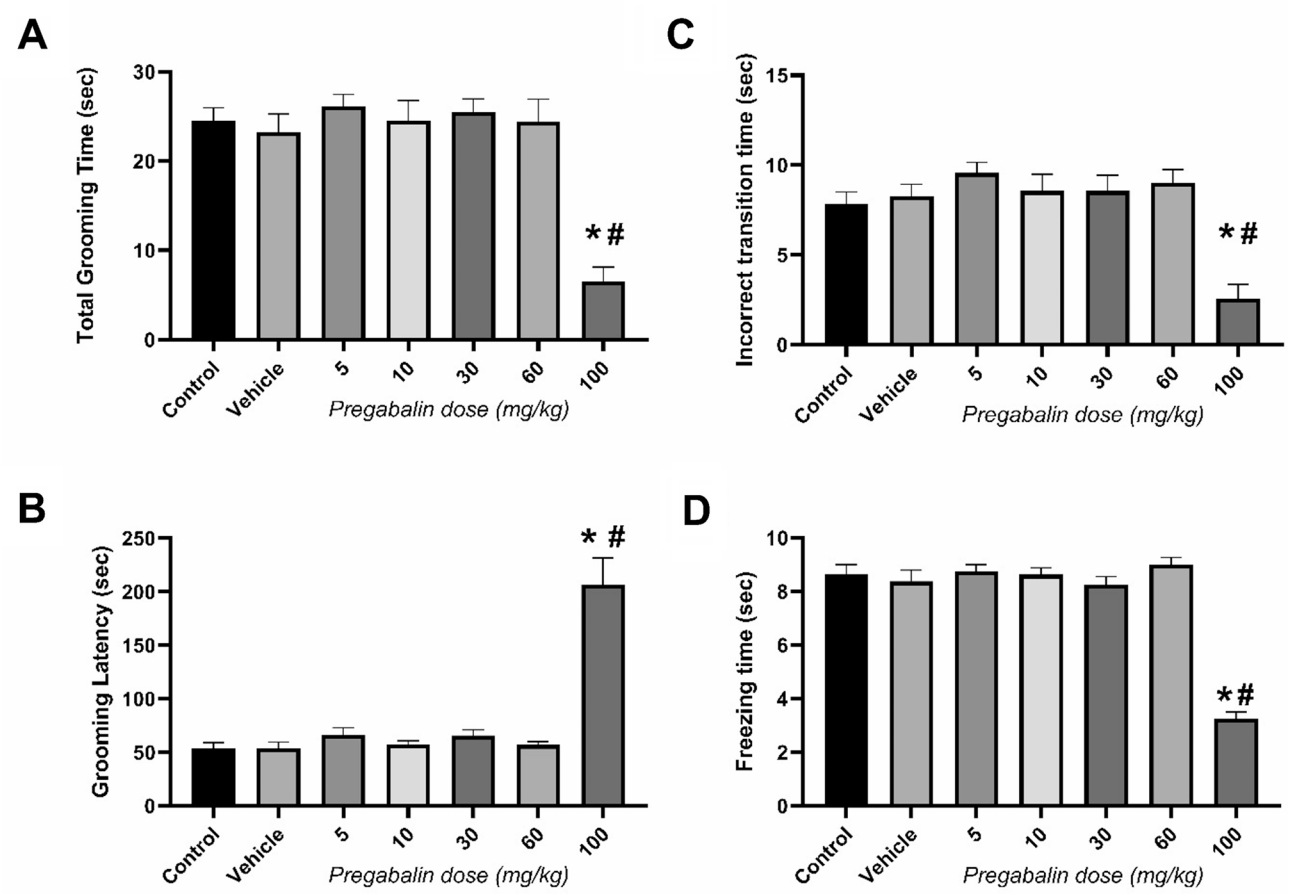

Fig. 5 Effect of pregabalin on open field test associated anxiety behavior patterns (**A**) Total Grooming (**B**) Incorrect transition time (**C**) Grooming Latency (**D**) Freezing. Figure 5 shows anxiety-like behaviors examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

($p<0.0001$). To summarise open field result, 100 mg/kg pregabalin displayed anxiolytic effects.

Elevated plus maze

The chronic administration effects of pregabalin were examined in the elevated plus maze.

We observed open arm time, open arm entries, head dipping behavior, and stretch-attend posture in EPM, as shown in Fig. 6.

According to the EPM results, open arm time ($F_{(6,49)}=9.27$) increased for both the 60 and 100 mg/kg groups ($p<0.0001$) but for open arm entrances ($F_{(6,49)}=3.50$) only in the 100 mg/kg group ($p<0.05$).

The anxiolytic and exploratory behavior of head dipping ($F_{(6,49)}=11.02$) increased in the 60 and 100 mg/kg versus control ($p<0.0001$). Stress-related posture behavior, the stretch attend posture measure, decreased ($F_{(6,49)}=16.93$) in the 60 and 100 mg/kg group ($p<0.0001$).

To summarise elevated plus maze, both 60 mg/kg and 100 mg/kg pregabalin displayed anxiolytic effects.

Light dark box test

The chronic administration effects of pregabalin were examined in the light-dark box test.

We observed light zone time in the LDB test, shown in Fig. 7. After 14 doses of pregabalin were administered, light zone time ($F_{(6,49)}=10.60$) was significantly higher in all pregabalin groups (5–100 mg/kg) versus control ($p<0.0001$). To summarise the light-dark box test, 5, 10, 30, 60 and 100 mg/kg pregabalin displayed anxiolytic effects.

Discussion

In the present study, it was observed that depression-like behaviors increased in naive rats after acute pregabalin administration. The study also examined climbing behavior related to the noradrenergic system and swimming behavior related to the serotonergic system [11, 13]. Climbing behavior was severely affected and decreased after acute pregabalin administration. Consistent with the reduction of noradrenergic system-related behavior in our study, pregabalin has been shown to reduce noradrenaline secretion [42]. Furthermore, due to the

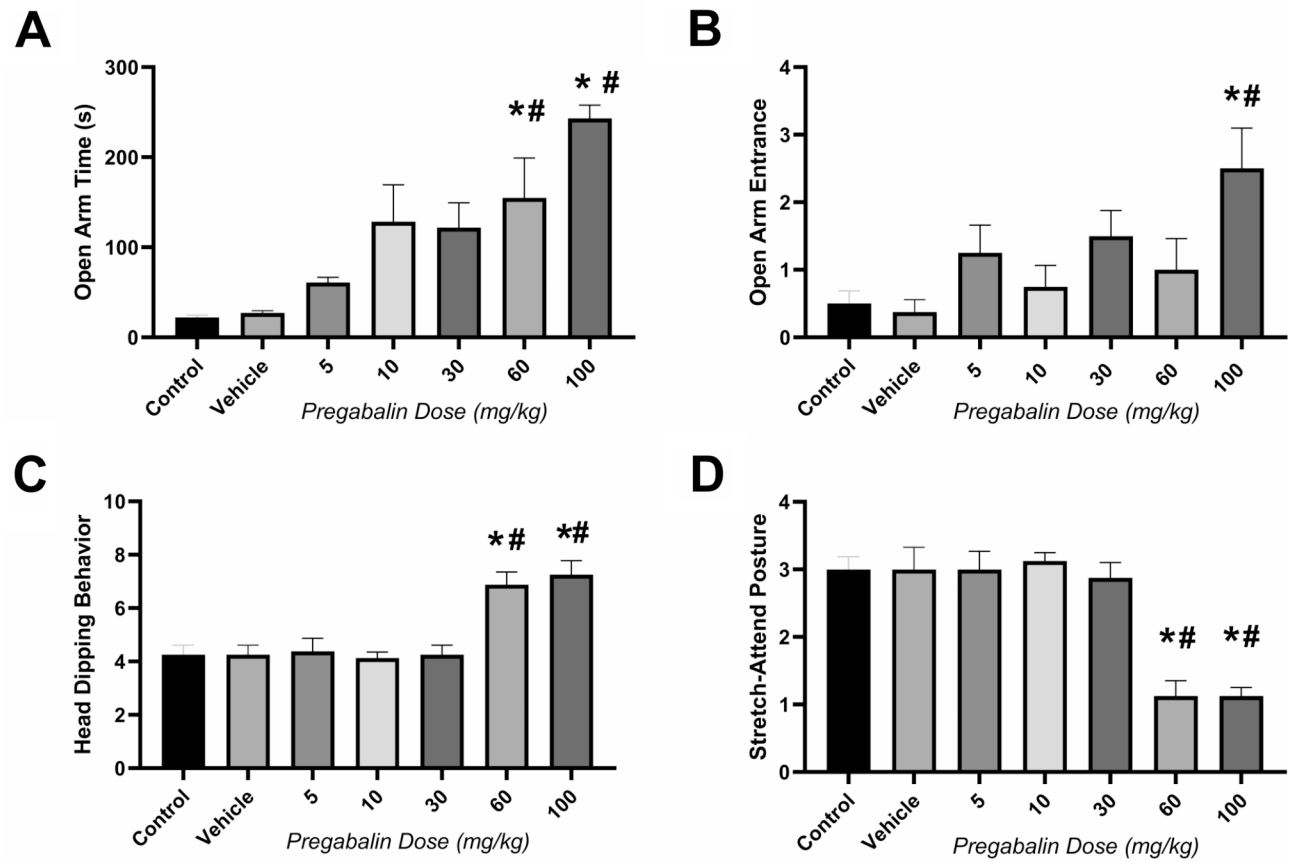

Fig. 6 Effect of pregabalin on elevated plus maze associated anxiety behavior patterns (**A**) Open Arm (**B**) Open Arm Entrance (**C**) Head Dipping (**D**) Strech-attend posture. Figure 6 shows anxiety-like behaviors examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

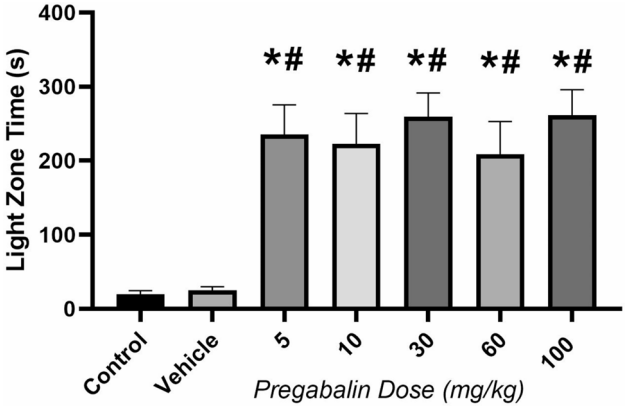

Fig. 7 Effect of pregabalin on light dark box test associated anxiety behavior pattern. Figure 7 shows anxiety-like behaviors examined in the chronic period after two weeks of pregabalin treatment (i.p, 5, 10, 30, 60, 100 mg/kg). One-way ANOVA and Tukey were used as statistical tests (*: 0.05 vs. control, #: 0.05 vs. vehicle)

decrease in the duration of the climbing behavior (vertical movements), floating (total immobility) increased as a depression-like behavior (DLB). In this study, swimming behavior (horizontal movements) associated with the serotonergic system did not change after pregabalin administration. Parallel to this data, head-shaking behavior, regarded as serotonergic systems-associated behavior, was unchanged after the pregabalin protocol. Defecation (fecal boli number) is an autonomic nervous system-related factor, which increased dose-dependently slightly but not significantly. Valdivieso et al. also reported no difference in fecal boli [51].

The FST was defined first by Porsolt in 1977. In the first FST, depressive behavior was examined as total immobility time (floating) and mobility time, which indicates hope for survival [39]. Although it is the most commonly utilized test, the FST has some limitations. Some authors emphasize that a major weakness of the FST is that it includes short-term stress applied to naive animals, while human depression has a long-lasting behavioral pathology [22]. In the FST, antidepressant/depressant activity is usually evaluated after treatment with 1–3 doses. We observed that depression-like behaviors increased after a single dose of pregabalin administration. In parallel with our data, depression and suicide attempts caused by pregabalin have been reported in clinical articles [20, 28].

The sucrose preference test importantly models the lack of pleasure seen in depression. According to our sucrose preference test result, pregabalin did not distort pleasure-taking behavior for any experimental group, and adult naive rats exhibited a high consumption of sucrose solution. In other animal studies investigating anhedonic behavior, after prenatal exposure to pregabalin, there was no change in pleasure behavior [31, 43].

The present study found that pregabalin dose-dependently reduced ALBs as measured by several different aspects. Similar results were reported in both conditional and unconditional anxiety tests [3, 15, 16, 30, 35].

Among the parameters examined in the OFT, the increase in the time spent in the center zone and the increase in the number of entries into this zone are indicators of decreased anxiety. The central zone of the OFT is a risk for animals and triggers thigmotaxis behavior (escaping to the border of the open field) [44]. In the present study, the time spent in the central zone increased slightly in the 100 mg/kg group, while the number of entries into the central zone increased in the 60 and 100 mg/kg groups. This data, which has not previously been examined in other studies, showed that chronic pregabalin treatment had a positive effect on both central zone time and central zone entrance.

In the open field test, the anxiolytic effect with chronic treatment was significantly observed in the 100 mg/kg group. Subjects spent more time in the center area of the test which is the risk zone. The behavior of wandering in the bounded area described as thigmotaxis and fear of open space decreased significantly. In addition, the frequency of entry to the center zone increased.

Another anxiolytic behavior examined in the OFT is rearing. This behavior, in which the subjects stand on two legs in the open field, is divided into two categories: unsupported rearing and supported rearing. Unsupported rearing (without touching the walls in the OFT) is sensitive to stress and anxiety [46]. According to the data, pregabalin improved unsupported rearing dose-dependently (60, 100 mg/kg groups). Total rearing is more sensitive to vertical locomotor activity. Zimcikova et al. and Meymandi et al. showed total rearing numbers as vertical locomotor activity in the OFT (2017; 2020). Chronic administration of pregabalin did not change the total rearing behavior profile [54]. Prenatal administration of pregabalin decreased vertical activity [31, 43]). Our results showed no significant changes in the supported rearing and total rearing numbers.

We analyzed additional behavior patterns as self-grooming in the OFT. Self-grooming behavior has complexity and organization, including highly stereotyped patterns [24]. Self-grooming consists of 4 major stages, 1: Bilateral paw strokes made near the nose (paw and nose grooming stage), 2: series of unilateral strokes (each made by one paw) from whiskers to below the eye (face grooming), stage 3: bilateral strokes backward and upwards made by both paws simultaneously (head grooming), stage 4: body licking (body grooming). Stressors result in a disorganized grooming pattern, increasing the frequency and duration of grooming [7]. Anxiolytic treatments decrease rodent self-grooming activity and normalize its sequential organization [25, 36]. We assessed total grooming time and incorrect transition (unsequential time) parameters. Pregabalin ameliorated incorrect transition time and total grooming time.

Zimcikova et al. reported similar results in grooming patterns, but they reported that they could not distinguish between stress-induced grooming and self-grooming [54]. Firstly, our observation showed that 100 mg/kg pregabalin decreases stress-induced grooming behavior. Chronic pregabalin treatment also reduced freezing known as anxiogenic behavior.

Summarising the findings of 100 mg/kg pregabalin, stress-induced grooming and freezing behaviors were significantly reduced and subjects spent more time in open area. The strong anxiolytic effects of chronic pregabalin administration were demonstrated in different behavioral patterns.

High-induced and new environment areas trigger off anxiety-like behavior. The elevated plus maze, which is the model used in this respect, is the most preferred experimental apparatus. Acute or chronic treatment of pregabalin has shown diverse effects on anxiety-like behavior in previous studies [2, 16, 18, 41, 55]. This study focused on risky zone-related behavior parameters such as open arm time, and open arm entrance. In addition to the duration and frequency of the behaviors in the risky area, head dipping, which is a new environment exploratory behavior that increases with the decrease of anxiety, and the stretched-attend posture, whose amount increases with stress, were examined in the presented study. Both head dipping behavior and in the risk zone spent duration and entrance number increased whereas stretch attend- posture number decreased.

To further evaluate anxiety-like behavior, we applied the light-dark test with high-light exposure. Unlike the results in other anxiety tests, the anxiolytic effect was observed in all groups except for the control and vehicle groups Navarrete et al. reported that pregabalin was a potent anxiolytic against light-induced anxiety in the LDB, but in the same study, pregabalin did not show an anxiolytic effect in the hole board test [33].

Pregabalin did not induce severe aggressive behavior such as clinch attack, upright posture, or bites of the ear, tail, testis, throat, and waist. Autonomic nervous system-related a sign of defecation (fecal boli number) increased dose-dependently slightly but not significantly in FST After a single dose. This slight increase was not

demonstrated in anxiety tests such as OFT, EPM, or LDB after chronic pregabalin administration.

Conclusion

In summary, after administration of a single dose of pregabalin, depression-like behaviors increased, and climbing behavior was significantly negatively suppressed. During chronic treatment, anhedonia that might be associated with depression was not observed. Furthermore, chronic treatment of pregabalin resulted in potent anxiolytic effects in all unconditional anxiety tests measured by different aspects. Chronic Administration of 100 mg/kg pregabalin showed anxiolytic effects in all three anxiety tests. Interestingly, all pregabalin administrations between 5 and 100 mg/kg reduced anxiety-like behaviors in the light-dark box test. According to the elevated plus maze test's result, 60 and 100 mg/kg pregabalin produced anxiolytic effects. Although an anxiolytic effect was shown in the present study, extrapolations should be made with caution, especially in patients with anxiety-depression comorbidities in the clinic. Patients should be informed of and monitored for a possible deterioration in mood. The consequences of modulating voltage-gated calcium channels in the presynaptic region and their underlying mechanisms require further physiological and pharmacological studies.

Abbreviations

OFT	Open field test
EPM	Elevated plus maze
LDB	Light dark box
ALB	Anxiety-like behaviors
DLB	Depression-like behaviors
SPT	Sucrose preference test
FST	Forced swimming test

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Author contributions

H.Ç. and N.Z. designed and supervised Project N.Z.H.Ç and F.A. performed the experiments.All authors analyzed the data. All authors wrote the manuscript. All authors read and approved the final manuscript.

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Data availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval

All experiments were carried out as approved by the Ankara University Experimental Animals Ethics Committee, with the approval reference number 2015-14-163.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

Author details

[1]Department of Physiology, School of Medicine, Balıkesir University, Balıkesir, Turkey
[2]Department of Physiology, Faculty of Medicine, Ankara University, Ankara, Turkey
[3]Department of Physiology, Faculty of Medicine, Atılım University, Ankara, Turkey

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