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Original article

Oral self-administration of pregabalin in a mouse model and the resulting drug addiction features

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ABSTRACT

Prescription drug abuse is an issue that is rapidly growing globally. Pregabalin, an anticonvulsant, analgesic, and anxiolytic medication, is effective in the management of multiple neurological disorders; however, there is increasing concern regarding its widespread illicit use. It has been previously reported in mice that pregabalin can induce conditioned place preference. In this current investigation, the potential of pregabalin to elicit free-choice drinking in a mouse model of drug addiction, and its effect on recognition and withdrawal behaviors after forced abstinence, were studied. Twenty-two male BALB/c mice were randomly divided into three groups (n = 7–8/group); control, pregabalin-30, and pregabalin-60. The study had three phases: habituation (days 1–5) with free water access, free-choice drinking (days 6–13) with pregabalin groups receiving one water and one pregabalin bottle, and forced abstinence (days 14–21) with free water access. On day 13, the first open field test was conducted, followed by the Novel Object Recognition Test. On day 21, the second open field test was performed, followed by the Tail Suspension Test and Forced Swimming Test. Pregabalin elicited voluntary drinking in the higher-dose group, concurrently causing a decline in recognition memory performance in the novel object recognition test. Moreover, pregabalin induced withdrawal behavior after a period of forced abstinence in the forced swimming and tail suspension tests. This is the first report to establish an animal model of free-choice pregabalin drinking that may be used for further molecular studies and targeted therapy for pregabalin addiction.

1. Introduction

Prescription drugs are increasingly abused and misused around the world. The non-medical use of prescription drugs is considered one of the most significant risk factors for compromised health on a global scale (Ibrahim et al., 2018). Notably, Saudi Arabia experiences a substantial prevalence of substance addiction, which is associated with several diseases, socioeconomic degradation, and crime (Bassiony, 2013). Individuals having substance use disorders might encounter intense drug cravings and actively pursue stimuli linked to the substances they have previously abused (O'Brien et al., 1998).

Gabapentinoids constitute a class of compounds derived from gamma-aminobutyric acid (GABA), an inhibitory neurotransmitter in the nervous system (Dooley et al., 2007, Calandre et al., 2016). Pregabalin, an alkylated analog of GABA, shares a structural relationship with gabapentin. Pregabalin binds to voltage-dependent calcium channels, thereby reducing excitatory neurotransmission (Montgomery et al., 2013). In addition, there has been a demonstrated effect of pregabalin in rats with regard to its GABA-mimicking properties (de Guglielmo et al., 2013). There are several conditions for which pregabalin is effective, such as diabetic neuropathy, generalized anxiety disorder, and partial epilepsy (Boschen, 2011, Feltner et al., 2011, Pexton et al., 2011).

However, there is increasing concern about illicit pregabalin use among young people in Saudi Arabia and globally (Aldemir et al., 2015, Halaby et al., 2015). Pregabalin addiction is unlikely to occur at therapeutic doses; however, a few instances of drug addiction have been reported following administration at higher doses (Loftus and Wright,

Abbreviations: ANOVA, analysis of variance; GABA, Gamma-aminobutyric acid; OFT, open field test; TST, tail suspension test; NORT, novel object recognition test; FST, forced swimming test.

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2014). Pregabalin addiction has been reported in a patient that had been taking the drug for seven years and developed a dose-dependent addiction (Sahu et al., 2021). The author of the present study previously reported that pregabalin can induce conditioned place preference when administered non-contingently through intraperitoneal injections of 60 mg/kg (Almalki et al., 2021; Althobaiti et al., 2019; Althobaiti et al., 2021). However, the animals were not allowed free access to the drug to investigate whether pregabalin would induce drug-seeking behavior similar to well-known drugs of abuse, such as alcohol, nicotine, and opiate etonitazene (Bagdas et al., 2019; Heyne, 1996; Planeta, 2013).

The aim of the current investigation is to evaluate the capability of pregabalin to elicit free-choice drinking in a drug addiction mouse model. Moreover, the effect of pregabalin free-choice drinking on recognition and pregabalin-induced withdrawal behavior after a period of forced abstinence were determined.

2. Materials and methods

2.1. Animals

Twenty-two male BALB/c mice, aged 8 weeks and weighing between 20 and 30 g, were procured from the King Fahd Medical Research Center in Jeddah, Saudi Arabia. The animals were individually accommodated in standard vivarium cages, subjected to a 12/12-hour light/dark cycle, maintained at a relative humidity of 25–35%, and housed at a temperature ranging from 22 to 25 °C. A habituation period of 7 days was allowed before the start of the experiments. The mice had unrestricted access to both food and water throughout the study. Approval for the animal study was granted by the Taif University's Research Ethics Committee (42–0112). The study was performed following to the criteria outlined by the Institutional Animal Care and Use Committee of the National Institutes of Health.

2.2. Drug

Pregabalin used in the study was provided as a donation by Jamjoom Pharmaceuticals located in Jeddah, Saudi Arabia. In the drug preparation process, pregabalin was directly added to water to achieve the desired concentrations, as pregabalin is freely soluble in water.

2.3. Experimental protocol

This study consisted of three phases. During the initial phase (days 1–5), known as the habituation phase, mice were allowed unrestricted access to water available in two bottles, positioned on either side of the wire cage top. On the fifth day, three groups of mice, with the same age, were randomly allocated for the experiments (n = 7–8/group); control, pregabalin-30, and pregabalin-60. The second phase (days 6–13) was the free-choice drinking phase. During this 8-day phase, group I (control group) had two bottles of water available at all times, whereas groups II (pregabalin-30) and III (pregabalin-60) were provided with open access to one bottle containing water and another one contains either a low or high concentration of pregabalin, respectively. The third phase (days 14–21) was the final phase in which all pregabalin-containing bottles were replaced with water bottles, and the animals underwent forced abstinence. The experimental design is depicted in Fig. 1.

2.4. Free choice drinking paradigm

Research on drug dependence has widely adopted this drinking paradigm to evaluate oral self-administration of alcohol and other substances. In this experiment, the total fluid intake per day during the habituation period was utilized to formulate two pregabalin solutions with varying concentrations. Considering the determined concentrations of 0.09 and 0.18 mg/mL, it was established that mice would be exposed to approximately 30 mg/kg/day (pregabalin-30) and 60 mg/kg/day (pregabalin-60) of pregabalin. These doses were determined in accordance with findings from our previously published studies, in which pregabalin was administered to mice at doses of 30 and 60 mg/kg, with the latter dose of 60 mg/kg demonstrating the ability to induce conditioned place preference (Althobaiti et al., 2019; Althobaiti et al., 2021).

Throughout the experiment, two water bottles were available to the control group. The pregabalin-30 and pregabalin-60 groups had unrestricted access to water bottle and pregabalin bottle, with concentrations of 0.09 and 0.18 mg/mL, respectively. One bottle was attached on the right side of the conventional wire cage top and the other to the left.

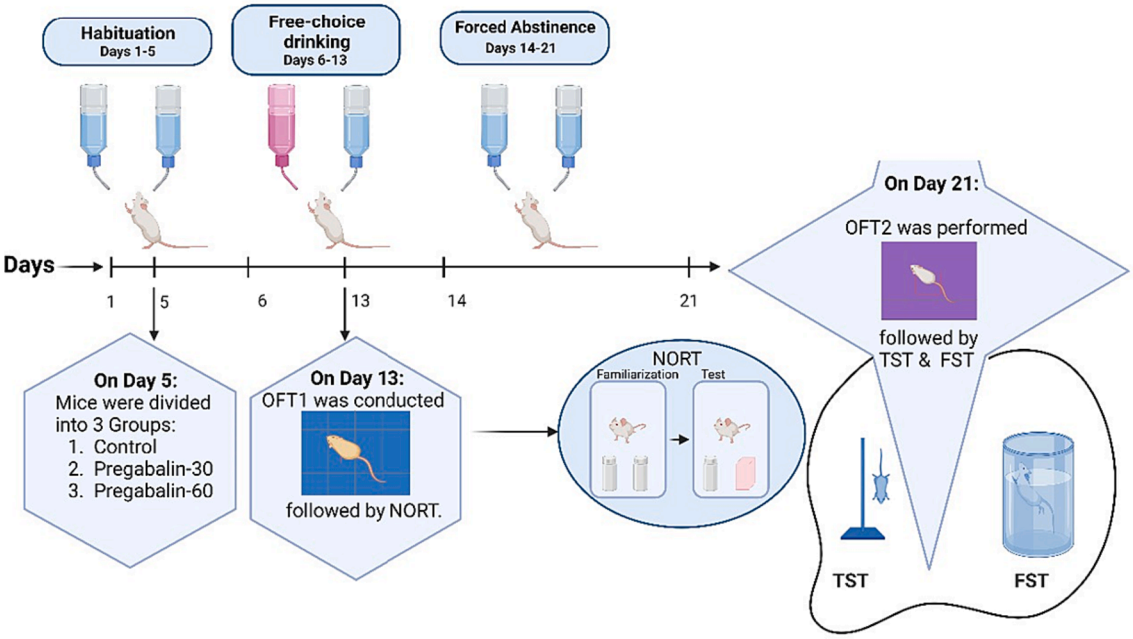

Fig. 1. Design of the pregabalin free-choice drinking and abstinence experiments. OFT1 and OFT2; the first and second open field test, respectively. NORT; novel object recognition test, TST; tail suspension test, and FST; forced swimming test.

Mice were allowed to drink freely from the containers.

Each bottle was rotated every 24 h to avoid a preference for one location over another. Throughout the study, the average fluid loss remained below 1 g per day. Weight changes of bottles were recorded every 24 h to the nearest 0.1 g. Four identical pairs of bottles were positioned on vacant cages, a blank value had been established by subtracting the weight loss attributed to evaporation or leakage from weight change of bottle. Drug preference (%) was determined by dividing the volume of pregabalin intake by the total fluid intake (pregabalin solution + water) and multiplying the result by 100 (Jaworski et al., 2005, Althobaiti, 2022).

2.5. Novel object recognition test (NORT)

The NORT was performed, during the light cycle, based on previously published procedure, with minor modifications (Leger et al., 2013). Before starting the NORT, the first open field test (OFT1) was conducted to examine any potential impact of pregabalin on locomotion that could influence the NORT results. The records movements of the mice for 5 min in a rectangular box, which measured 70 cm by 35 cm with walls 50 cm high, were obtained. The distance traveled, in meters, was computed by analyzing the videos through the ANY-maze video tracking system (Stoelting Co., USA). The NORT was conducted approximately 90 min after the completion of the OFT1 (de Almeida et al., 2020). During the familiarization trial, mice were allowed 3 min of exploring the two objects. Subsequent to this exploration, the mice were moved to their cages for a rest period of 10 min. In the test trial, a familiar object was removed and substituted with a new one (novel object), and the mice were allowed exploration of these objects for 3 min. The discrimination ratio was subsequently computed by dividing the time of exploring a particular object by total time of exploring the two objects and then multiplied by 100. The analysis of the videos was conducted using the ANY-maze system. This test was conducted to investigate potential adverse effects of pregabalin intake on recognition memory, as previously documented for different commonly abused substances (Ryabinin et al., 2002, Belcher et al., 2005, Gong et al., 2019).

2.6. Withdrawal behavior assessment

On the final day of the forced abstinence phase, the tail suspension test (TST) was executed following the established procedures as previously described (Can et al., 2012). Before TST and the forced swimming test (FST), the second open field test (OFT2) was performed, similar to the OFT1, to assess any potential impact of pregabalin withdrawal on locomotion that could influence the results of FST or TST.

During the TST, the immobility of the mice was observed for a duration of 6 min, while they were suspended by their tails. To mitigate observer bias, two trained and blinded examiners independently and manually tested the immobility time during the last 5 min of the TST. If mice were completely motionless, they were considered immobile. After completing TST, animals were returned to their home cages for a rest period of 2 h before starting FST, to allow more time for the animals to recover from possible stress (Dunn et al., 2005).

The FST was conducted following established procedures as previously reported (Porsolt, 2000). In brief, animals were immersed in water for a duration of 6 min in a transparent cylindrical glass beaker. When a mouse floats upright and tries to maintain its head above water with minimal effort, it is said to be motionless. As a criterion for swimming time, mice should jump, struggle, thrash, and climb on the glass cylinder wall. Two blinded observers evaluated the duration of immobilization during the last 5 min of the FST. The experiment was only conducted one time for each mouse. Following testing, the animals were dried using a towel and then moved back to their respective cages.

The FST and TST were employed, during the light cycle, to examine withdrawal symptoms induced by pregabalin following a period of forced abstinence. Depressive-like behavior can be assessed through these behavioral tests to determine if mice are experiencing withdrawal symptoms. Of note, no prior training was provided to the animals before conducting the TST and FST. Introducing training sessions for these tests could pose a risk to the validity of the results, as animals may develop learned responses that influence their behavior during the tests.

2.7. Statistical analysis

To analyze the drinking and NORT data, two-way repeated-measures Analysis of Variance (ANOVA) was performed, taking into account the factors of treatment and time, followed by a multiple comparison Bonferroni test. TST, FST, and OFT data were analyzed employing one-way ANOVA followed by Bonferroni multiple comparison tests. In the current study, all statistical analyses were carried out using GraphPad Prism 9.3.1. The data are presented as the mean ± standard error of the mean, with statistical significance set at $P < 0.05$.

3. Results

3.1. Free-choice drinking

3.1.1. Pregabalin intake and preference

The statistical analysis of the mean daily intake of pregabalin (mg/kg/day) demonstrated significant effects of treatment [$F(1, 13) = 238.1$, $P < 0.0001$], time [$F(7, 91) = 7.215$, $P < 0.0001$], and the interaction of treatment and time [$F(7, 91) = 10.59$, $P < 0.0001$]. Post-hoc examinations revealed a substantial elevation in mean daily pregabalin intake within the pregabalin-60 group across all examined days compared to the pregabalin-30 group (Fig. 2a). Similarly, the analysis of the average daily pregabalin intake (mL/day) exhibited significant effects of treatment [$F(1, 13) = 130.1$, $P < 0.0001$], time [$F(7, 91) = 5.221$, $P < 0.0001$], and the interaction of treatment and time [$F(7, 91) = 10.93$, $P < 0.0001$]. Consistently, the pregabalin-60 group manifested significantly higher pregabalin consumption compared to the pregabalin-30 group across all evaluated days (Fig. 2b). Lastly, the analysis unveiled a significant influence of treatment [$F(1, 13) = 131.2$, $P < 0.0001$], a non-significant effect of time [$F(7, 91) = 0.5137$, $P = 0.8221$], and a significant treatment × time interaction [$F(7, 91) = 2.426$, $P = 0.0252$] on pregabalin preference. The pregabalin-60 group exhibited significantly higher preference for pregabalin compared to the pregabalin-30 group on all testing days, as validated by the Bonferroni test (Fig. 2c).

3.1.2. Water intake and preference

Statistical analysis of the mean water intake (g/kg/day) showed a significant impact of treatment [$F(1, 13) = 41.83$, $P < 0.0001$], a non-significant effect of time [$F(7, 91) = 1.784$, $P = 0.0999$], and a significant effect of interaction [$F(7, 91) = 3.621$, $P = 0.0017$]. The post-hoc analysis indicated a statistically significant reduction in the average daily water intake observed in the pregabalin-60 group throughout all assessed days, as compared to the pregabalin-30 group (Fig. 3a). Similarly, statistical analysis of the mean water intake (mL/day) showed a statistically significant impact of treatment [$F(1, 13) = 42.01$, $P < 0.0001$], a non-significant impact of time [$F(7, 91) = 1.788$, $P = 0.0991$], and a significant effect of interaction [$F(7, 91) = 3.934$, $P = 0.0009$]. On all days tested, the pregabalin-60 group showed a significant reduction in mean daily water intake when compared with the pregabalin-30 group (Fig. 3b). Finally, the analysis showed a significant impact of treatment [$F(1, 13) = 131.2$, $P < 0.0001$], a non-significant impact of time [$F(7, 91) = 0.5137$, $P = 0.8221$], and a significant effect of interaction [$F(7, 91) = 2.426$, $P = 0.0252$] on water preference. According to multiple comparisons, the water preference of the pregabalin-60 group exhibited a statistically significant decrease compared to the pregabalin-30 group across all days of testing (Fig. 3c).

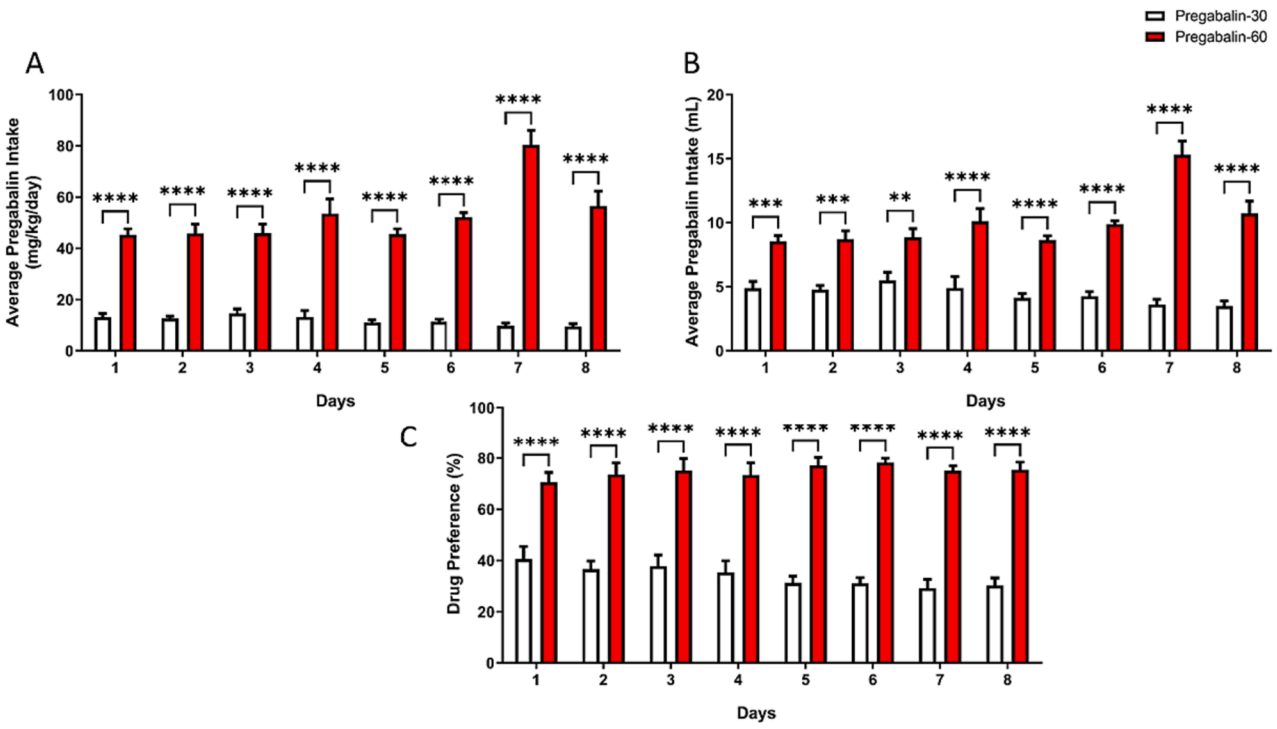

Fig. 2. (a), (b), and (c) illustrate the mean daily pregabalin intake (mg/kg/day) and (mL), and the drug preference, respectively. When compared with the pregabalin-30 group, significant increases in pregabalin intake (mg/kg/day), pregabalin intake (mL), and drug preference were observed in the pregabalin-60 group. Two-way repeated-measures ANOVA was performed followed by a multiple comparison Bonferroni test (n = 7–8/group). Values are expressed as mean ± standard error of the mean. **P < 0.01, ***P < 0.001, and ****P < 0.0001.

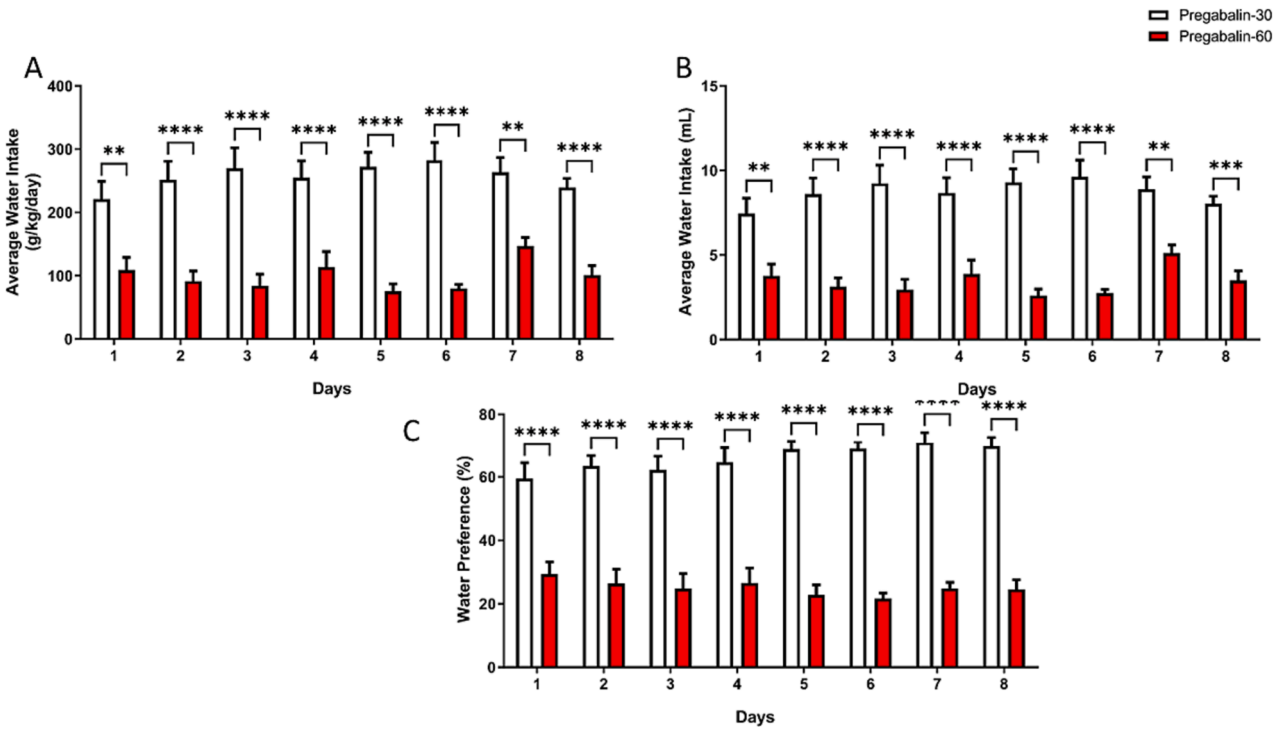

Fig. 3. (a), (b), and (c) illustrate the mean daily water intake (g/kg/day) and (mL) and water preference, respectively. Water intake (g/kg/day), water intake (mL), and water preference decreased significantly in the pregabalin-60 group when compared with the pregabalin-30 group. Two-way repeated-measures ANOVA was performed followed by a multiple comparison Bonferroni test (n = 7–8/group). Values are expressed as mean ± standard error of the mean. **P < 0.01, ***P < 0.001, and ****P < 0.0001.

3.1.3. Body weight and total fluid intake

The statistical analysis indicated a non-significant impact of treatment [F (2, 19) = 0.6332, P = 0.5417], a significant impact of time [F (7, 133) = 4.903, P < 0.0001], and a significant effect of interaction [F (14, 133) = 3.661, P < 0.0001] (Fig. 4a). The comparison of the body weights of the test groups on each day of testing failed to show any statistically significant differences among the groups. Regarding total daily fluid intake, relevant analysis showed a non-significant impact of treatment [F (2, 19) = 1.312, P = 0.2926] and a significant impact of both time [F (7, 133) = 8.322, P < 0.0001] and interaction [F (14, 133) = 8.744, P < 0.0001] (Fig. 4b). The pregabalin-60 group consumed significantly more fluid on day 7 than the control and pregabalin-30 groups, based on multiple comparisons. The remaining days did not lead to any significant differences between the groups.

3.2. Effects on recognition memory

On the final day of the voluntary pregabalin drinking experiment, OFT1 was performed followed by NORT to test the impact of pregabalin intake on recognition memory. For the OFT1, a significant main effect [F = 9.460, P = 0.0014] was observed (Fig. 5a). The distance traveled by mice in the pregabalin-30 and pregabalin-60 groups decreased significantly when compared with that of the mice in the control group. No significant changes in the distance traveled among the pregabalin-30 and pregabalin-60 group mice were detected.

Regarding the NORT, significant treatment [F (2, 19) = 23.61, P < 0.0001], time [F (1, 19) = 62.31, P < 0.0001] and interaction [F (2, 19) = 19.01, P < 0.0001] effects were observed (Fig. 5b). No significant difference between all groups in exploring objects was detected during the familiarization phase. The discrimination ratio of exploring the novel objects was significantly elevated in the control in comparison to pregabaline-30 and pregabalin-60 groups, during the test phase. However, the discrimination ratio of exploring the novel objects was significantly decreased in the pregabalin-60 group in comparison to the pregabaline-30 group, during the test phase.

3.3. Forced abstinence-induced withdrawal behavior

After the period of forced abstinence from pregabalin, OFT2 was performed, followed by TST and FST, to evaluate potential withdrawal behavior. For the OFT2, no significant main effect [F = 0.8433, P = 0.9198] (Fig. 6a) was found.

In the TST, a significant main effect was observed [F = 36.47, P < 0.0001]. The pregabalin-60 group demonstrated a statistically significant increase in immobility time when compared to both the control and pregabalin-30 (Fig. 6b). In the FST, the analysis indicated a significant main effect [F = 12.74, P = 0.0018]. Multiple comparisons showed a significantly elevated immobility time in the pregabalin-60 when compared with those in control and pregabalin-30 (Fig. 6c).

4. Discussion

In this current investigation, pregabalin induced free-choice drinking in the high-dose pregabalin-60 group. This is consistent with our previously published reports, where the drug induced conditioned place preference when administered non-contingently through intraperitoneal injections at a high dose of 60 mg/kg (Almalki et al., 2021; Althobaiti et al., 2019; Althobaiti et al., 2021). In this current investigation, for the first time, it is demonstrated that pregabalin can be voluntarily orally consumed by mice, and is preferred over water when the concentration of the solution is higher, at dosage of 60 mg/kg. Moreover, we demonstrate, for the first time, that pregabalin addiction can deteriorate recognition memory in an animal model, similar to well-known drugs of abuse. The results also showed that pregabalin can induce withdrawal behavior in mice after a period of forced abstinence.

Notably, the low concentration pregabalin solution, which yielded a dosage of approximately 30 mg/kg, did not result in observable drug-seeking behavior as evidenced by the outcomes within the free-choice drinking paradigm. This finding aligns with prior investigations and aligns with the observations reported by the authors of the current study, where the dosage of 30 mg/kg did not elicit place preference in animal models (Althobaiti et al., 2019; Andrews et al., 2001; Ruttenl et al., 2011; Althobaiti et al., 2021). Nevertheless, when the concentration of pregabalin solution was increased to 60 mg/kg, free-choice drinking and drug preference were induced. This might be due to its rewarding effects at higher doses, as shown in previous reports of pregabalin-induced euphoric effects as side effects in participants (Lang et al., 2006, Chua et al., 2012, Chew et al., 2014).

It is possible that pregabalin induces drug-seeking behavior as a result of its effects on glutamatergic receptors. This system has been extensively documented to have a crucial role in the manifestation of drug-seeking behavior across various substances of abuse (Kalivas et al., 2003, Gipson et al., 2013, Sari et al., 2013). Interestingly, we have previously documented that the administration of ceftriaxone, acknowledged for its role in up-regulating glutamate transporter type-1, effectively mitigates the development of pregabalin-induced place preference. This observation suggests the potential involvement of glutamatergic mechanisms in the initiation of drug-seeking behavior induced by pregabalin (Althobaiti et al., 2019). This aligns with previous findings where ceftriaxone demonstrated efficacy in preventing drug-seeking behavior associated with various abused substances, including heroin, cocaine, nicotine, ethanol, and methamphetamine (Sari et al., 2009, Knackstedt et al., 2010, Abulseoud et al., 2012, Alajaji et al., 2013, Qrunfleh et al., 2013, He et al., 2014).

Research on substance addiction has traditionally employed the free-choice drinking model as a prominent methodological approach. This

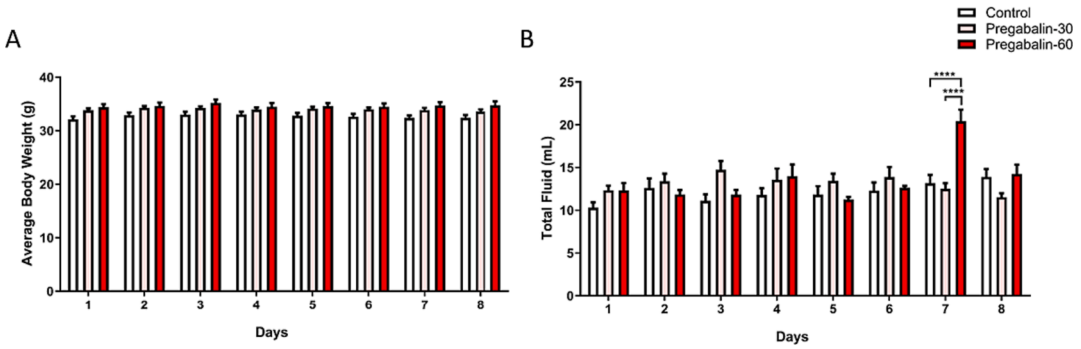

Fig. 4. (a) Body weight (g). No statistically significant changes in body weight were observed across all tested days and groups. (b) Total fluid intake (mL). Only the pregabalin-60 group experienced significant increases in total fluid intake on day 7 when compared with the pregabalin-30 group. Two-way repeated-measures ANOVA was performed followed by a multiple comparison Bonferroni test (n = 7–8/group). Values are reported as mean ± standard error of the mean. ***P < 0.001.

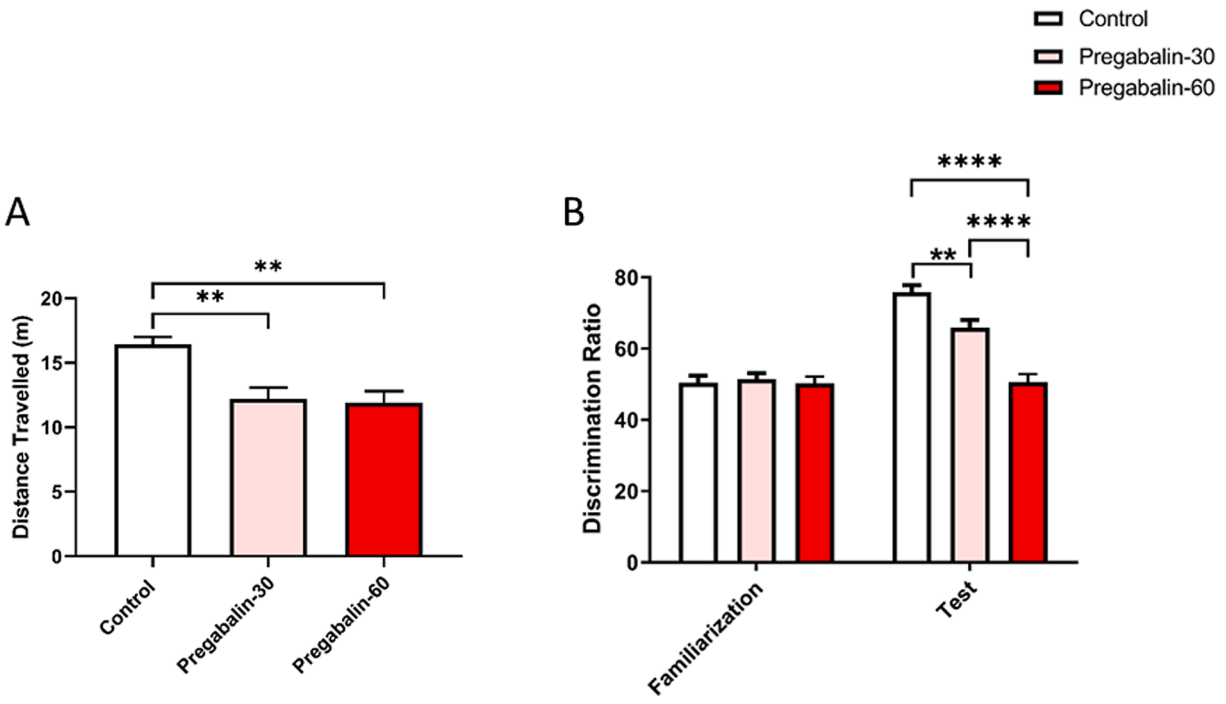

Fig. 5. (a) and (b) show OFT1 and NORT, respectively. A significant reduction in the distance traveled was observed in both the pregabalin-30 and pregabalin-60 groups in comparison to the control group. No statistically significant differences in the distance traveled were observed between the pregabalin-30 and pregabalin-60 groups. When compared with the familiarization session, the exploration of the novel object was significantly higher in the control and pregabalin-30 groups during the test phase. However, in the pregabalin-60, a nonsignificant difference between the test and the familiarization phase was found. Relative to both the pregabalin-30 and pregabalin-60 groups and during the test session, a significant increase in the the novel object exploring was revealed in the control group. One-way ANOVA and two-way repeated-measures ANOVA was performed followed by a multiple comparison Bonferroni test to analyze OFT1 and NORT, respectively (n = 7–8/group). Values are expressed as mean ± standard error of the mean. **P < 0.01 and ****P < 0.0001 ($^{@@@}$P < 0.001 and $^{@@@@}$P < 0.0001 compared to the familiarization phase).

model was utilized as a method to explore the voluntary oral intake of various substances, such as alcohol, oxycodone, nicotine, and amphetamine (Heyne and Wolffgramm 1998; Bagdas et al., 2019; Zanni et al., 2020; Montanari et al., 2021). To the best of our understanding, this investigation is the first to establish free-choice oral consumption of pregabalin in an animal model. The findings could lay a foundation for further research into the neurobiological basis of pregabalin addiction, as well as targeted therapy for pregabalin dependence.

A previous study investigated whether pregabalin would deteriorate recognition memory, as is the case with several substances (Belcher et al., 2005, Gong et al., 2019, Li et al., 2019). To rule out potential influences of pregabalin intake on locomotor activity, which may affect animal performance in the NORT, the OFT1 was performed before NORT. A substantial decline in locomotor activity was observed in both pregabalin treated groups, when compared with that in the control. Notably, no significant changes were detected in locomotor activity between the pregabalin-30 and pregabalin-60 groups. In the NORT, significant deteriorations in recognition memory were observed in the pregabalin-60 group than in the pregabalin-30 and control groups. A significant deterioration in recognition memory was also observed in the pregabalin-30 group compared to the control group. Given the absence of a significant difference in locomotion between the pregabalin-30 and pregabalin-60 groups, it is improbable that the pronounced impairment in recognition memory observed in the pregabalin-60 group can be attributed to the effects of pregabalin on locomotion. Despite variances in locomotor activity among pregabalin groups and control group, the NORT results remain robust because there is no significant changes between all groups in exploring objects during the familiarization phase. This observed locomotor variations did not hinder the curiosity of mice to explore the objects during the familiarization phase. The induction of drug-seeking behavior by pregabalin, along with the concomitant impairment in recognition memory, aligns with phenomena documented in established substances of abuse, resembling characteristics notably observed in alcohol consumption (Abadi et al., 2013), morphine (Morisot and Contarino 2016, Ellis et al., 2020), methamphetamine, and cocaine (Schwendt et al., 2012, Fole et al., 2015). Additional studies are necessary to explore the neurobiological changes involved in pregabalin-induced decline in recognition memory. Withdrawal symptoms, such as depressive-like behavior, were documented for different substances of abuse and psychotropic medications due to abstinence (Cryan et al., 2003, Mannucci et al., 2006, Rauf et al., 2014, Kim et al., 2017, Brandt et al., 2020, Ghavimi et al., 2021). In the present study, forced pregabalin abstinence caused withdrawal symptoms, as indicated by the FST and TST results. In the pregabalin-60 group, immobility time was significantly longer than in the control and pregabalin-30 groups, suggesting withdrawal-induced depressive-like behavior. Due to the acknowledged limitations of FST and its potential impact on animal behavior, as reported in previously (Carvalho et al., 2021), FST was conducted as a single event at the study's conclusion to minimize any confounding effects on other behavioral paradigms. According to the OFT2, which was conducted before the TST and FST, the increased time of immobility in the pregabalin-60 mice was not due to withdrawal effects on locomotion.

5. Conclusion

The findings of this research show that pregabalin induced free-choice drinking in the higher dose group and deteriorated memory and recognition in the NORT, similar to well-known drugs of abuse. Moreover, pregabalin induced withdrawal behavior after forced abstinence period in the TST and FST. This is the first report to establish an animal model of free-choice pregabalin drinking that may be used for

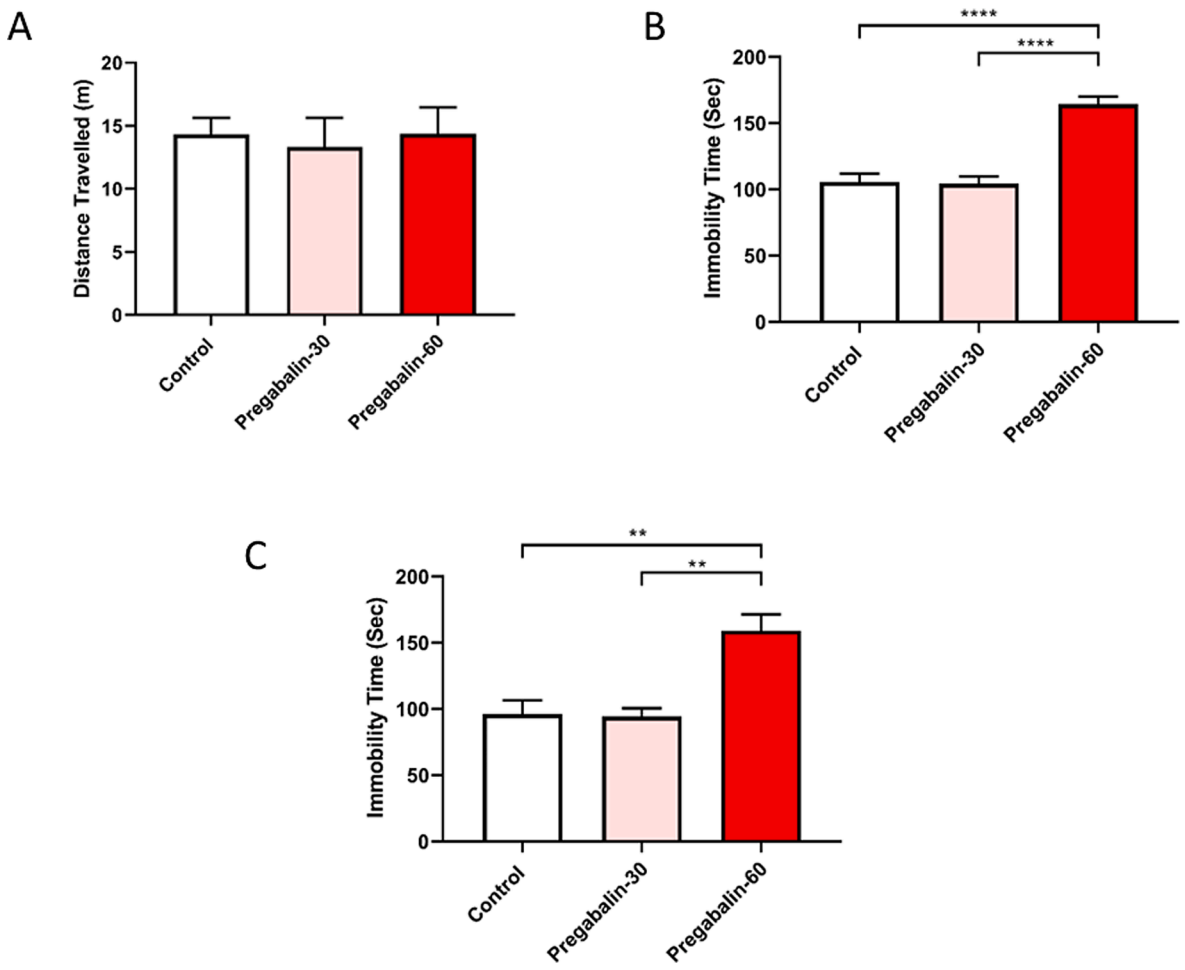

Fig. 6. (a), (b), and (c) represent OFT2, TST, and FST, respectively. In the OFT2, no changes in the distance traveled are observed in any of the tested groups. In the TST and FST, the pregabalin-60 group demonstrated a statistically significant elevation in the immobility time when compared to both the control and pregabalin-30 groups. One-way ANOVA was performed followed by a multiple comparison Bonferroni test. Values are reported as the mean ± standard error of the mean. **P < 0.01 and ****P < 0.0001.

developing targeted therapies for pregabalin addiction.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Abadi, T.H.N., Vaghef, L., Babri, S., et al., 2013. Effects of different exercise protocols on ethanol-induced spatial memory impairment in adult male rats. Alcohol 47, 309–316.

Abulseoud, O.A., Miller, J.D., Wu, J., et al., 2012. Ceftriaxone upregulates the glutamate transporter in medial prefrontal cortex and blocks reinstatement of methamphetamine seeking in a condition place preference paradigm. Brain Res. 1456, 14–21.

Alajaji, M., Bowers, M., Knackstedt, L., et al., 2013. Effects of the beta-lactam antibiotic ceftriaxone on nicotine withdrawal and nicotine-induced reinstatement of preference in mice. Psychopharmacology (Berl) 228, 419–426.

Aldemir, E., Altintoprak, A.E., Coskunol, H., 2015. Pregabalin dependence: a case report. Turk Psikiyatri Derg. 26, 217–220.

Almalki, A.H., Alsaab, H.O., Alsanie, W.F., et al., 2021. Potential Benefits of N-Acetylcysteine in Preventing Pregabalin-Induced Seeking-Like Behavior. Healthcare, MDPI.

Althobaiti, Y.S., 2022. Investigating the potential of mirtazapine to induce drug-seeking behavior in free-choice drinking mouse model. Saudi Pharm. J. 30, 1809–1815. 10.1016/j.jsps.2022.10.010.

Althobaiti, Y.S., Almalki, A., Alsaab, H., et al., 2019. Pregabalin: potential for addiction and a possible glutamatergic mechanism. Sci. Rep. 9, 1–8.

Althobaiti, Y.S., Almutairi, F.M., Alshehri, F.S. et al., 2021. Involvement of the dopaminergic system in the reward-related behavior of pregabalin. 11, 1-9.

Andrews, N., Loomis, S., Blake, R., et al., 2001. Effect of gabapentin-like compounds on development and maintenance of morphine-induced conditioned place preference. Psychopharmacology (Berl) 157, 381–387.

Bagdas, D., Diester, C.M., Riley, J., et al., 2019. Assessing nicotine dependence using an oral nicotine free-choice paradigm in mice. Neuropharmacology 157, 107669.

Bassiony, M., 2013. Substance use disorders in Saudi Arabia. J. Subst. Abus. 18, 450–466.

Belcher, A.M., O'Dell, S.J., Marshall, J.F.J.N., 2005. Impaired object recognition memory following methamphetamine, but not p-chloroamphetamine-or d-amphetamine-induced neurotoxicity. Neuropsychopharmacology 30, 2026–2034.

Boschen, M.J., 2011. A meta-analysis of the efficacy of pregabalin in the treatment of generalized anxiety disorder. Can. J. Psychiatry 56, 558–566.

Brandt, L., Bschor, T., Henssler, J., et al., 2020. Antipsychotic withdrawal symptoms: a systematic review and meta-analysis. Front. Psychiatry 11, 569912.

Calandre, E.P., Rico-Villademoros, F., Slim, M., 2016. Alpha2delta ligands, gabapentin, pregabalin and mirogabalin: a review of their clinical pharmacology and therapeutic use. Expert Rev. Neurother. 16, 1263–1277.
Can, A., Dao, D.T., Terrillion, C.E. et al., 2012. The tail suspension test. e3769.
Carvalho, C., Herrmann, K., Marques, T.A. et al., 2021. Time to abolish the forced swim test in rats for depression research? 4, 170-178.
Chew, M.L., Plotka, A., Alvey, C.W., et al., 2014. Pharmacokinetics of pregabalin controlled-release in healthy volunteers: effect of food in five single-dose, randomized, clinical pharmacology studies. Clin. Drug Invest. 34, 617–626.
Chua, Y., Ng, K., Sharma, A., et al., 2012. Randomised clinical trial: pregabalin attenuates the development of acid-induced oesophageal hypersensitivity in healthy volunteers–a placebo-controlled study. Aliment. Pharmacol. Ther. 35, 319–326.
Cryan, J.F., Hoyer, D., Markou, A., 2003. Withdrawal from chronic amphetamine induces depressive-like behavioral effects in rodents. Biol. Psychiatry 54, 49–58.
de Almeida, R.F., Pocharski, C.B., Rodrigues, A.L.S., et al., 2020. Guanosine fast onset antidepressant-like effects in the olfactory bulbectomy mice model. Sci. Rep. 10, 8429. https://doi.org/10.1038/s41598-020-65300-w.
de Guglielmo, G., Cippitelli, A., Somaini, L., et al., 2013. Pregabalin reduces cocaine self-administration and relapse to cocaine seeking in the rat. Addict. Biol. 18, 644–653.
Dooley, D.J., Taylor, C.P., Donevan, S., et al., 2007. Ca2+ channel α2δ ligands: novel modulators of neurotransmission. Trends Pharmacol. Sci. 28, 75–82.
Dunn, A.J., Swiergiel, A.H., 2005. Effects of interleukin-1 and endotoxin in the forced swim and tail suspension tests in mice. Pharmacol. Biochem. Behavior 81, 688–693.
Ellis, A.S., Toussaint, A.B., Knouse, M.C., et al., 2020. Paternal morphine self-administration produces object recognition memory deficits in female, but not male offspring. Psychopharmacology 237, 1209–1221.
Feltner, D.E., Liu-Dumaw, M., Schweizer, E., et al., 2011. Efficacy of pregabalin in generalized social anxiety disorder: results of a double-blind, placebo-controlled, fixed-dose study. Int. Clin. Psychopharmacol. 26, 213–220.
Fole, A., Martin, M., Morales, L., et al., 2015. Effects of chronic cocaine treatment during adolescence in Lewis and Fischer-344 rats: novel location recognition impairment and changes in synaptic plasticity in adulthood. Neurobiol. Learning Memory 123, 179–186.
Ghavimi, H., Bayani Ershadi, A.S., Dastvar, S. et al., 2021. The effects of minocycline in improving of methamphetamine withdrawal syndrome in male mice. 1-9.
Gipson, C.D., Reissner, K.J., Kupchik, Y.M., et al., 2013. Reinstatement of nicotine seeking is mediated by glutamatergic plasticity. Proc. Natl. Acad. Sci. USA 110, 9124–9129. https://doi.org/10.1073/pnas.1220591110.
Gong, D., Zhao, H., Liang, Y. et al., 2019. Differences in cocaine-and morphine-induced cognitive impairments and serum corticosterone between C57BL/6J and BALB/cJ mice. 182, 1-6.
Halaby, A., Kassm, S., Naja, W.J., 2015. Pregabalin dependence: a case report. Curr. Drug Saf. 10, 184–186.
He, Z., Chen, Y., Dong, H., et al., 2014. Inhibition of vesicular glutamate transporters contributes to attenuate methamphetamine-induced conditioned place preference in rats. Behav. Brain Res. 267, 1–5.
Heyne, A., 1996. The development of opiate addiction in the rat. Pharmacol. Biochem. Behav 53, 11–25.
Heyne, A., Wolffgramm, J.J.P. 1998. The development of addiction to d-amphetamine in an animal model: same principles as for alcohol and opiate. 140, 510-518.
Ibrahim, Y., Hussain, S.M., Alnasser, S., et al., 2018. Patterns and sociodemographic characteristics of substance abuse in Al Qassim, Saudi Arabia: a retrospective study at a psychiatric rehabilitation center. Ann. Saudi Med. 38, 319–325.
Jaworski, J., Francis, D., Brommer, C., et al., 2005. Effects of early maternal separation on ethanol intake, GABA receptors and metabolizing enzymes in adult rats. 181, 8-15.
Kalivas, P.W., McFarland, K., Bowers, S., et al., 2003. Glutamate transmission and addiction to cocaine. Ann. N. Y. Acad. Sci. 1003, 169–175.
Kim, H.-J., Park, S.-D., Lee, R.M. et al., 2017. Gintonin attenuates depressive-like behaviors associated with alcohol withdrawal in mice. 215, 23-29.
Knackstedt, L.A., Melendez, R.I., Kalivas, P.W., 2010. Ceftriaxone restores glutamate homeostasis and prevents relapse to cocaine seeking. Biol. Psychiatry 67, 81–84.
Lang, N., Sueske, E., Hasan, A., et al., 2006. Pregabalin Exerts Oppositional Effects on Different Inhibitory Circuits in Human Motor Cortex: A Double-blind, Placebo-controlled Transcranial Magnetic Stimulation Study. Epilepsia 47, 813–819.
Leger, M., Quiedeville, A., Bouet, V. et al., 2013. Object recognition test in mice. 8, 2531-2537.
Li, Q., Xu, F., Zhang, Q. et al., 2019. Effect of α-asarone on ethanol-induced learning and memory impairment in mice and its underlying mechanism. 238, 116898.
Loftus, H., Wright, A., 2014. Potential misuse of pregabalin and gabapentin. BMJ 348.
Mannucci, C., Tedesco, M., Bellomo, M., et al., 2006. Long-term effects of nicotine on the forced swimming test in mice: an experimental model for the study of depression caused by smoke. 49, 481-486.
Montanari, C., Secci, M.E., Driskell, A. et al., 2021. Chronic nicotine increases alcohol self-administration in adult male Wistar rats. 238, 201-213.
Montgomery, S., Emir, B., Haswell, H., et al., 2013. Long-term treatment of anxiety disorders with pregabalin: a 1 year open-label study of safety and tolerability. Curr. Med. Res. Opin. 29, 1223–1230.
Morisot, N., Contarino, A.J.N. 2016. The CRF1 and the CRF2 receptor mediate recognition memory deficits and vulnerability induced by opiate withdrawal. 105, 500-507.
O'Brien, C.P., Childress, A.R., Ehrman, R., et al., 1998. Conditioning factors in drug abuse: can they explain compulsion? J. Psychopharmacol. 12, 15–22.
Pexton, T., Moeller-Bertram, T., Schilling, J.M., et al., 2011. Targeting voltage-gated calcium channels for the treatment of neuropathic pain: a review of drug development. Expert Opin. Invest. Drugs 20, 1277–1284.
Planeta, C.S., 2013. Animal models of alcohol and drug dependence. Brazilian J. Psychiatry. 35, S140–S146.
Porsolt, R.D., 2000. Animal models of depression: utility for transgenic research. Rev. Neurosci. 11 (1), 53–58.
Qrunfleh, A.M., Alazizi, A., Sari, Y., 2013. Ceftriaxone, a beta-lactam antibiotic, attenuates relapse-like ethanol-drinking behavior in alcohol-preferring rats. J. Psychopharmacol. 0269881113482529.
Rauf, K., Subhan, F., Abbas, M., et al., 2014. Inhibitory effect of bacopasides on spontaneous morphine withdrawal induced depression in mice. Phytother. Res. 28, 937–939.
Ruttenl, K., Vry, J., Robens, A., et al., 2011. Dissociation of rewarding, anti-aversive and anti-nociceptive effects of different classes of anti-nociceptives in the rat. Eur. J. Pain 15, 299–305.
Ryabinin, A.E., Miller, M.N., Durrant, S.J.P.B., et al., 2002. Effects of acute alcohol administration on object recognition learning in C57BL/6J mice. Pharmacol. Biochem. Behavior 71, 307–312.
Sahu, S., Kumar, S., Chaudhury, S., et al., 2021. A case of pregabalin addiction. Industr. Psychiatry J. 30, 352-352.
Sari, Y., Smith, K.D., Ali, P.K., et al., 2009. Upregulation of GLT1 attenuates cue-induced reinstatement of cocaine-seeking behavior in rats. J. Neurosci. 29, 9239–9243.
Sari, Y., Sreemantula, S.N., Lee, M.R., et al., 2013. Ceftriaxone treatment affects the levels of GLT1 and ENT1 as well as ethanol intake in alcohol-preferring rats. J. Mol. Neurosci. : MN. 51, 779–787. https://doi.org/10.1007/s12031-013-0064-y.
Schwendt, M., Reichel, C.M., See, R.E., 2012. Extinction-dependent alterations in corticostriatal mGluR2/3 and mGluR7 receptors following chronic methamphetamine self-administration in rats. PLoS One 7, e34299.
Zanni, G., DeSalle, M.J., Deutsch, H.M., et al., 2020. Female and male rats readily consume and prefer oxycodone to water in a chronic, continuous access, two-bottle oral voluntary paradigm. Neuropharmacology 167, 107978.