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Original article

Investigating the potential of mirtazapine to induce drug-seeking behavior in free-choice drinking mouse model

Yusuf S. Althobaiti [a,b,1]

[a] Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia
[b] Addiction and Neuroscience Research Unit, Taif University, Taif, Saudi Arabia

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ABSTRACT

Addiction to various drugs and chemicals is a significant public health concern worldwide. Addiction to prescription medications has increased due to the psychoactive effects of these medications, their availability, low price, and the lack of legal consequences for abusers. One of such prescription medication is mirtazapine (MIRT). MIRT is an antidepressant that has recently been reported to be abused and could induce withdrawal symptoms in different case studies. No previous study has investigated its abuse potential in animal models of drug addiction. Here, we conducted a free-choice drinking paradigm to investigate voluntary drinking of MIRT at two different concentrations. Male BALB/c mice were given unlimited access to two water bottles for five days before being divided into three groups: the first group had free access to two water bottles. The second group (MIRT10) and the third group (MIRT20) was allowed unlimited choice to one bottle of water and one bottle of MIRT at concentrations of 0.03 and 0.06 mg/mL, respectively. The average daily MIRT intake in the MIRT20 group was significantly higher on all tested days than that in the MIRT10 group. Moreover, mice in the MIRT20 group preferred to self-administer MIRT over water, indicating that MIRT can induce drug-seeking behavior. To further investigate the addictive potential of MIRT and its possible deterioration of memory and recognition, as reported with several known drugs of abuse, animals underwent a novel object recognition test. Mice in the MIRT20 group demonstrated significant deterioration in memory and recognition, indicating its effects on different brain regions involved in recognition, similar to other known drugs of abuse. The forced swimming test and tail suspension test were used to test MIRT-induced withdrawal symptoms after forced abstinence. After eight days of abstinence, mice in the MIRT20 group demonstrated significant depression-like symptoms in both the TST and FST, manifested by a significant increase in immobility time. MIRT was shown to induce drug-seeking behavior, deteriorate recognition, and cause withdrawal symptoms. This might confirm that MIRT has the potential to induce drug dependence and further studies are warranted to explore the neurobiological basis of MIRT-induced drug-seeking behavior.

1. Introduction

Addiction to various drugs and chemicals is a significant public health concern worldwide. Drug dependence presents significant psychological, medical, and economic challenges (Ramey and Regier, 2019). Non-medical use of a psychotherapeutic drugs entails taking a substance that is not prescribed for the user or taking medicine solely for the experience or feelings it may elicit (Use et al., 2007; Hulme et al., 2018). Individuals with substance addiction disorders may experience drug cravings and seek previously abused drugs (Childress et al., 1999; O'Brien et al., 1998). Saudi Arabia has a high frequency of drug addiction and high rates of hepatitis, human immunodeficiency virus, criminality, and

Abbreviations: ANOVA, analysis of variance; CRF, corticotropin-releasing factor; 5-HT1, 5-serotonin 1 receptors; 5-HT2, 5-serotonin 2 receptors; 5-HT3, 5-serotonin 3 receptors; FST, forced swimming test; MIRT, mirtazapine; NORT, novel object recognition test; TST, tail suspension test.

E-mail address: Ys.althobaiti@tu.edu.sa
[1] Postal address: Department of Pharmacology and Toxicology, College of Pharmacy, Taif University, P.O. Box 11099, Taif 21944, Saudi Arabia.

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socioeconomic degradation (Bassiony, 2013). Notably, the abuse of prescription drugs for reasons other than its therapeutic effects has been growing in the past decade (Substance Abuse and Mental Health Services Administration, 2004; Compton and Volkow, 2006). This increase of drug abuse might be caused by increased availability, lower cost, and no significant legal consequences faced by the abusers.

Mirtazapine (MIRT) is a tetracyclic compound identified as a noradrenergic and selective serotonergic antidepressant used in humans. The pharmacological profile of MIRT is distinguished by its antagonistic effects on presynaptic α2 receptors, 5-serotonin 1 receptors (5-HT1), and 5-HT2 and 5-HT3 receptors (Jilani et al., 2021). MIRT antidepressant activity is most likely mediated by blocking presynaptic α2-adrenergic receptors (Graves et al., 2012). When MIRT was compared with amitriptyline in a previous study, more than 70 % of MIRT-treated individuals gained weight in the first four weeks of treatment (Goodnick et al., 1998). However, higher MIRT doses resulted in lower weight gain, indicating that higher doses of MIRT might work by different mechanisms (Kawachi, 1999).

MIRT has been on the market since 1996 as a specific serotonergic and noradrenergic antidepressant (Davis and Wilde, 1996). Notably, MIRT has been classified as a recreational substance whose sedative, hallucinogenic, and delirium-like effects make users rapidly develop tolerance to it (Davis and Wilde, 1996). Withdrawal symptoms were reported in some cases when MIRT was abruptly stopped. MIRT administered at 60 mg per day was abruptly discontinued in one patient after one month of treatment because it yielded no therapeutic benefit (Benazzi, 1998). The day after discontinuing MIRT, the patient experienced dizziness, nausea, anxiety, insomnia, and paresthesia despite taking clomipramine, nortriptyline, and alprazolam. However, the withdrawal symptoms disappeared when MIRT was restarted two days later.

Another case report (Klesmer et al., 2000) has described panic attacks caused by the discontinuation of MIRT. The patient used other drugs and had a history of substance abuse. When MIRT treatment was reintroduced, his panic attacks disappeared. Dizziness, nausea, insomnia, anxiety, and panic attacks are symptoms of abrupt MIRT withdrawal (Benazzi, 1998). MIRT-related fatal drug overdoses are uncommon and are usually caused by polypharmacy rather than by MIRT (Bremner et al., 1998). The signal patterns in the search analytics domain were similar to those found in the Food and Drug Administration Adverse Event Reporting System database domain, where signals of drug abuse–related events were detected for MIRT (Spachos et al., 2020). To the best of our knowledge, no previous study tested the ability of MIRT to induce drug-seeking behavior and its effects on memory and recognition as well as its withdrawal in mouse model of drug addiction.

The free-choice drinking model is a non-operational oral self-administration strategy and is most widely applied in alcohol addiction research (Planeta, 2013; Fuchs et al., 2019). The free-choice paradigm model has also been used to assess and validate the addictive features of nicotine in a mouse model (Bagdas et al., 2019). A study has reported that rats who were given a free choice between water and the opiate etonitazene became addicted to it (Heyne, 1996). Therefore, this report aimed to investigate the abuse potential of MIRT using a mouse model of drug dependence. This study also tested the possible effects of MIRT on recognition and withdrawal behavior.

2. Materials and methods

2.1. Animals

At the start of the study, twenty-two 8-weeks old male BALB/c mice weighing 20–30 g (King Fahd Medical Research Center, Jeddah, Saudi Arabia) were individually housed in conventional plastic cages with controlled temperature (21 °C) and humidity (30 %). A 12/12 h light–dark cycle was used and the animals were allowed seven days to get accustomed to their habitat before testing. The mice were provided with *ad libitum* food and water during the experiment. The Taif University's Research Ethics Committee approved the experimental methodologies of the animal study following the National Institutes of Health's Institutional Animal Care and Use Committee criteria (42–0112).

2.2. Drug

MIRT was a gift from Riyadh Pharma (Riyadh, Saudi Arabia).

2.3. Experimental protocol

The experimental design is summarized in Fig. 1. From day one to five, all animals had unlimited access to two bottles of water. On day 5, mice were randomly separated into three groups (n = 7–8/group). Group I: control group with free access to two bottles of water for eight days; Group II: MIRT10 group with free access to one bottle of water and one bottle containing a low concentration of MIRT for eight days; and Group III: MIRT20 group with free access to one bottle of water and one bottle containing a higher concentration of MIRT for eight days. On day 14, the mice underwent a forced abstinence period during which the MIRT bottles were exchanged for water bottles to induce possible withdrawal effects of MIRT.

2.4. Free choice drinking paradigm

The free-choice drinking model is a non-operational oral self-administration strategy and is most widely applied in alcohol and drug addiction research. In this test, two water bottles, one on the right and the other on the left, were added to the conventional wire cage tops. Mice drank freely from these containers, with fluid loss averaging<1 g/day. Bottles containing either tap water or MIRT solution were positioned on the left and right sides of each mouse's home cage. Two MIRT concentrations were prepared based on the total daily fluid consumption. The calculated concentrations were 0.03 and 0.06 mg/mL to yield individual mouse intake of approximately 10 mg/kg/day (MIRT10) and 20 mg/kg/day (MIRT20), respectively, if mice exclusively drank from the MIRT bottle. The low concentration of MIRT used in the MIRT10 group mimics the therapeutic dose, which is usually between 2.5 and 10 mg/kg/day (El-Tanbouly et al., 2017; Rogóż, 2013; Schreiber et al., 2002). The higher concentration of MIRT used in the MIRT20 group may yield doses exceeding the common therapeutic doses, which might affect other neuronal pathways that may be involved in addiction. The locations of the two bottles were switched every 24 h to avoid place preferences. The bottles were weighed to the nearest 0.1 g every 24 h, and the weight shift in each bottle was recorded. A "blank" was computed by deducting the weight loss due to leakage and evaporation from the change in bottle weight of four equal pairs of bottles placed on empty cages to estimate the weight of the fluids consumed.

2.5. Novel object recognition test

The novel object recognition test (NORT) was conducted as previously reported (Leger et al., 2013) with minor modifications. This experiment used glass bottles and an open square box (50 cm wide, 50 cm long, and 50 cm high). Each animal was given three minutes to investigate objects placed at two different locations during the familiarization trial. Subsequently, the animals were

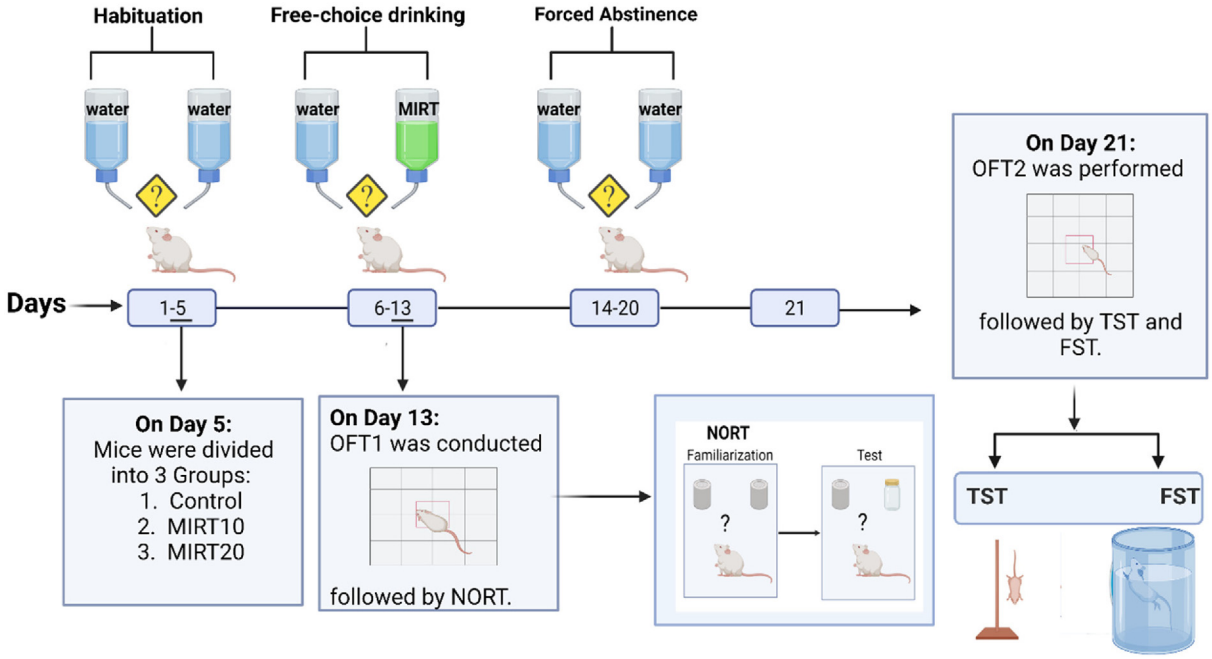

Fig. 1. Experimental design. Body weight and fluid intake were recorded daily.

returned to their original cages for a 10-minute resting period. One object was swapped out for another in the choice trial, and the animals were given three minutes to explore the new object. The ratio was calculated using the following formula: time spent exploring the object/total time exploring both objects × 100. The videos were analyzed using the ANY-maze system. NORT was employed at the end of the MIRT consumption day to investigate possible negative effects of the abuse of this drug on recognition and memory, as has been reported for several known drugs of abuse (Belcher et al., 2005; Gong et al., 2019; Ryabinin et al., 2002).

2.6. Forced swimming test

The forced swimming test (FST) was performed as reported previously (Can et al., 2012). This test was conducted on day 21, which was the last day of the forced abstinence period, in order to investigate if MIRT can induce withdrawal symptoms similarly to other drugs of abuse following a period of abstinence. In this test, each mouse was submerged in a clear cylindrical glass beaker containing water for six minutes. When a mouse floated erect and made only minor efforts to keep its head above the water, it was considered motionless. The swimming time criteria were significant actions of all four limbs, including jumping, struggling, thrashing, and climbing on the glass cylinder wall. During the final five minutes of the test, the duration of immobilization was assessed by two blinded observers. Each mouse was tested only once. After the test, the mice were dried with a towel and returned to their home cages.

2.7. Tail suspension test

The tail suspension test (TST) was performed as reported previously (Steru et al., 1985). Similarly to the forced swimming test, this test was conducted on the last day of the forced abstinence period, in order to investigate if MIRT can induce withdrawal symptoms. In this test, the agitation and immobility of mice were observed for six minutes while suspended by the tail. Two trained, blinded observers manually recorded the immobility length throughout the final five minutes of the test to avoid observer bias. Only when the mouse was motionless and passive was it considered immobile. The FST and TST were used to investigate MIRT-induced withdrawal symptoms after forced abstinence. These behavioral tests assess the despair aspect of depressive-like behavior, which is an indication of withdrawal symptoms.

2.8. Open field test

The open field test (OFT) was conducted to assess any effects of MIRT intake or its withdrawal on locomotion. This test was performed twice, once before the NORT and once before TST and FST. For this, the mice were placed in a rectangular box (70 × 35 cm with 50 cm high walls) and recorded for five minutes. The videos were then analyzed using the ANY-maze Video tracking system to calculate the distance travelled.

2.9. Statistical analysis

Two-way repeated-measures analysis of variance (ANOVA) (concentration × time), followed by the Bonferroni multiple comparisons test, was used to analyze the drinking and NORT data. One-way ANOVA followed by the Bonferroni multiple comparisons test was used to analyze the TST, FST, and OFT data. GraphPad Prism-9.3.1 was used to perform all statistical analyses in this study. Additionally, statistical significance was set at $P < 0.05$ and data was reported as mean ± S.E.M.

3. Results

3.1. Drinking assessment

3.1.1. Average daily MIRT intake

Two-way repeated-measures ANOVA showed a significant effect of both concentration [$F(1, 13) = 66.43$, $P < 0.0001$] and time [$F(7, 91) = 2.926$, $P = 0.0083$], whereas the concentration × time interaction was non-significant [$F(7, 91) = 2.035$, $P = 0.0590$]. The Bonferroni multiple comparisons test revealed that the MIRT20 group had a significantly higher average daily MIRT intake (mg/kg/day) on all tested days than the MIRT10 group (Fig. 2a).

3.1.2. Average daily water intake

A significant effect of both concentration [F (1, 13) = 23.73, P = 0.0003] and time [F (7, 91) = 5.298, P < 0.0001] was found, whereas the concentration × time interaction was non-significant [F (7, 91) = 0.3510, P = 0.9278]. The MIRT10 group had a significantly higher average daily water intake (g/kg/day) on days 1–4 and 6–7 than the MIRT20 group (Fig. 2b).

3.1.3. Drug preference

A significant effect of both concentration [F (1, 13) = 24.84, P = 0.0002] and time [F (7, 91) = 2.397, P = 0.0269] was revealed, whereas the concentration × time interaction was non-significant [F (7, 91) = 1.012, P = 0.4283]. A significant increase was found in daily MIRT preference in the MIRT20 group on all tested days compared to the MIRT10 group (Fig. 2c).

3.1.4. Body weight

A non-significant effect of concentration [F (1, 13) = 0.1689, P = 0.6878], time [F (7, 91) = 0.8828, P = 0.5234], and concentration × time interaction [F (7, 91) = 1.048, P = 0.4039] was found (Fig. 2d).

3.1.5. Total fluid intake

A non-significant effect of concentration [F (1, 13) = 0.2378, P = 0.6339], a significant effect of time [F (7, 91) = 9.008, P < 0.0001], and a non-significant concentration × time interaction [F (7, 91) = 1.189, P = 0.3171] was revealed (Fig. 3a).

3.1.6. Total MIRT intake

A significant effect of both concentration [F (1, 13) = 25.17, P = 0.0002] and time [F (7, 91) = 3.156, P = 0.0049] was found, whereas the concentration × time interaction was non-significant [F (7, 91) = 1.658, P = 0.1293]. The Bonferroni multiple comparisons test revealed a significant increase in average daily MIRT intake (mL) in the MIRT20 group on all tested days except day 2 compared to the MIRT10 group (Fig. 3b).

3.2. Effects on memory recognition

We performed NORT to assess memory recognition on the last day of MIRT administration. Two-way repeated-measures ANOVA showed a significant effect of both concentration [F (2, 19) = 58.65, P < 0.0001] and time [F (1, 19) = 41.45, P < 0.0001],

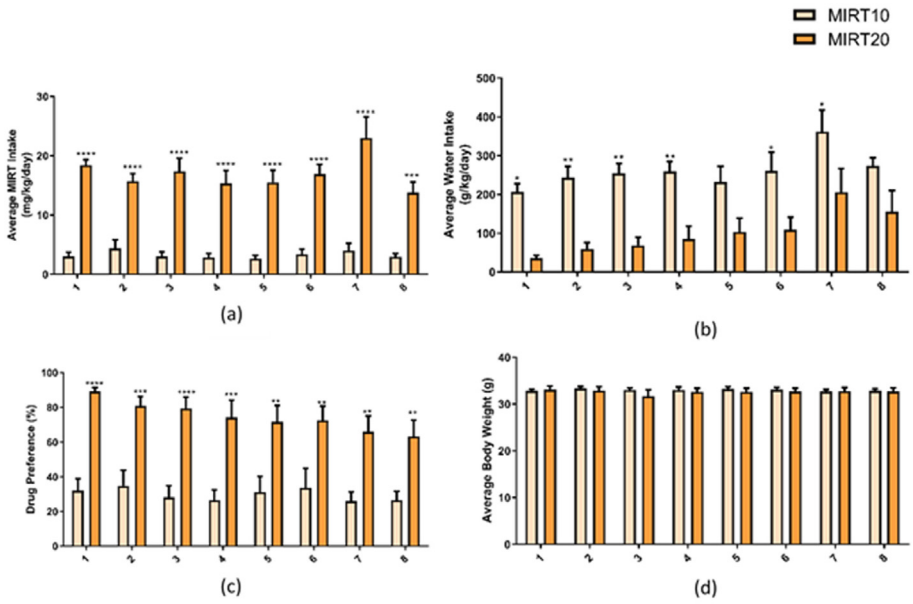

Fig. 2. (a) and (b) represent the average daily intake of MIRT and water, respectively. There were significant increases in average daily MIRT intake in the MIRT20 group compared to the MIRT10 group. Moreover, there were significant increases in the average daily water intake in the MIRT10 group compared to the MIRT20 group. (c) and (d) represent drug preference and body weight, respectively. Significant increases in daily MIRT preference in the MIRT20 group compared to the MIRT10 group were revealed. No significant changes in body weight were observed between the groups on any of the days tested. Values are presented as means ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001 and ****P < 0.0001.

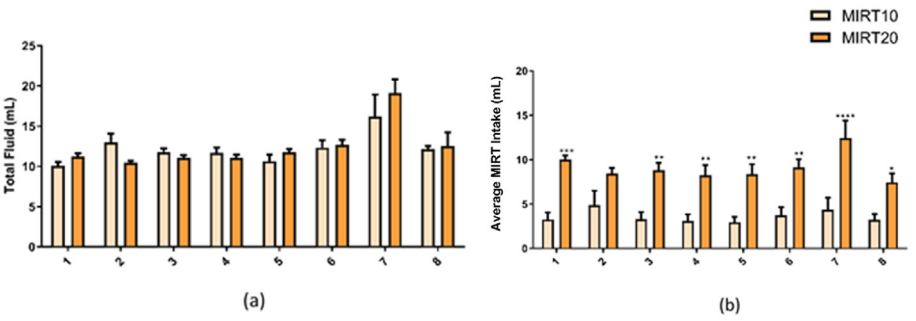

Fig. 3. (a) Total fluid intake (mL). No significant changes in the total fluid intake were observed between the groups on any of the tested days. (b) Total MIRT intake (mL). There were significant increases in average daily MIRT intake (mL) in the MIRT20 group compared to the MIRT10 group. Values are presented as means ± SEM. *P < 0.05, **P < 0.01, and ***P < 0.001.

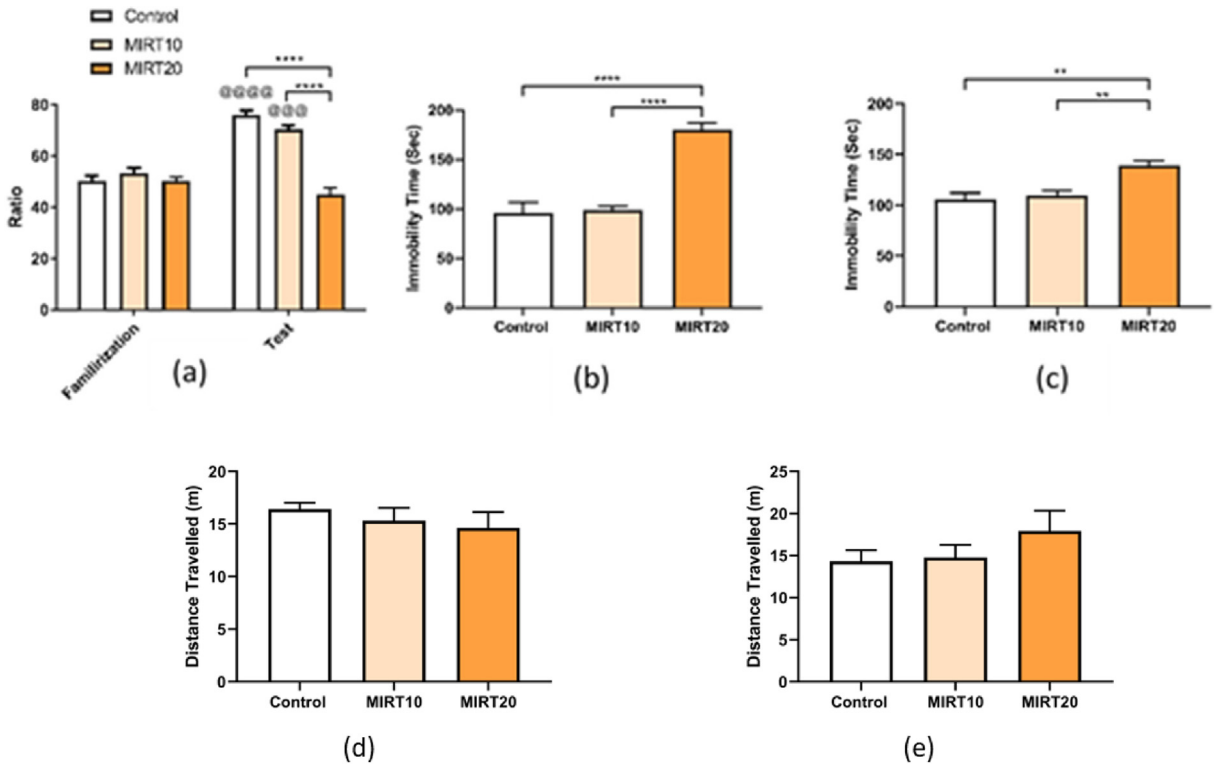

Fig. 4. (a) Effect of MIRT on NORT is presented. A significant increase was found in the time spent exploring the novel object during the test phase in the control and MIRT10 groups as compared to the familiarization phase and as compared to the MIRT20 group during the test phase.. No significant change was observed in the ratio of exploring the novel object during the test phase as compared to the familiarization phase in MIRT20 group. (b) and (c) represents FST and TST, respectively. A significant increase in the immobility time in the MIRT20 group as compared to the MIRT10 and control groups was found in both FST and TST. (d) and (e) represent OFT1 and OFT2, respectively. No significant changes were revealed between the control and treatment groups regarding the distance travelled in either OFT1 or OFT2. Values are expressed as mean ± SEM. $^{**}P < 0.01$ and $^{****}P < 0.0001$ ($^{@@@}P < 0.001$ and $^{@@@@}P < 0.0001$ as compared to the familiarization phase).

as well as a significant concentration × time interaction [$F(2, 19) = 23.64$, $P < 0.0001$]. The multiple comparisons test showed a significant increase in the time spent exploring the novel object during the test phase in the control and MIRT10 groups compared to the familiarization phase and the MIRT20 group during the test phase (Fig. 4a). There was no significant change in the ratio of exploring the novel object during the test session compared with the familiarization session in the MIRT20 group.

3.3. MIRT-induced withdrawal behavior assessment

We performed FST and TST to assess withdrawal behavior after forced abstinence from MIRT for eight days. In the FST, a significant main effect was found [$F = 43.03$, $P < 0.0001$]. The MIRT20 group had a significant increase in immobility time compared to the MIRT10 and control groups (Fig. 4b). In the TST, a significant main effect was revealed [$F = 13.41$, $P = 0.0015$]. The MIRT20 group had a significant increase in immobility time compared to the MIRT10 and control groups (Fig. 4c).

3.4. Effects on locomotion

The OFT was performed once before the NORT and once before TST and FST to assess any possible effects of MIRT intake and withdrawal on locomotor behavior, respectively. In OFT1 that was performed on the last day of free-choice drinking and before NORT, one-way ANOVA revealed a non-significant main effect [$F = 0.5568$, $P = 0.5821$] (Fig. 4d). Similarly, in OFT2 that was conducted on the last day of forced abstinence and before FST and TST, one-way ANOVA revealed a non-significant main effect [$F = 1.072$, $P = 0.3785$] (Fig. 4e).

4. Discussion

In this study, the ability of MIRT to induce free-choice drinking and motivate mice to drink MIRT instead of water was assessed. Mice in the MIRT10 group that had a lower concentration of MIRT in the drinking bottle did not prefer MIRT to water, with a maximum average daily intake of MIRT of approximately 4.4 mg/kg/-day. However, MIRT was able to induce drug-seeking behavior in mice at higher concentrations. Mice in the MIRT20 group preferred MIRT to water, with an average daily intake of MIRT reaching approximately 33 mg/kg/day. The daily intake of MIRT was higher than the therapeutic doses used for this drug, which are usually between 2.5 and 10 mg/kg/day (El-Tanbouly et al., 2017; Rogóż, 2013; Schreiber et al., 2002). Moreover, MIRT has been reported to depress locomotion at 10 and 20 mg/kg doses in mice, indicating that higher doses may affect other neuronal pathways (Yilmaz et al., 2007). Interestingly, in mice, MIRT injected intraperitoneally at a therapeutic dose of 10 mg/kg has been shown to be effective in improving behavioral parameters and normalizing protein expression level of cortical parvalbumin, immunoreactivity of parvalbuminergic cortical neurons, and perineuronal net thickness in a mice model of Rett syndrome (Gutiérrez et al., 2020, 2022). Moreover, MIRT was effective in alleviating psychosis and dyskinetic-like behaviors in a marmoset model of Parkinson's disease at a dose of 10 mg/kg (Hamadjida et al., 2017). Similarly, MIRT treatment using doses of 3 and 10 mg/kg has been documented to alleviate neuronal loss and behavioral deterioration, as well as to normalize serotonergic and histaminergic receptor gene expression in a mice model of myotonic dystrophy type 1 (Ramon-Duaso et al., 2020). This confirms that MIRT used at therapeutic doses might be beneficial for alleviating multiple

neurological disorders. However, consumption of this drug in higher doses and by healthy subjects might induce drug-seeking behavior as seen in this current study.

This increased self-administration of MIRT in mice is indicative of one of the most important criteria for drug dependence, as it results in a higher substance intake (Zou et al., 2017). The free-choice drinking paradigm is well-known in drug addiction research that was established for analyzing alcohol addiction in mice and rats (Griffin, 2014; Waller et al., 1982). Studies have revealed that nicotine can produce dependence in rats and mice, and that nicotine consumption is preferred in free-choice drinking to water (Aschhoff et al., 2000; Bagdas et al., 2019). Additionally, it has been revealed that in a free-choice drinking model of oxycodone and water, rats and mice preferred drinking oxycodone instead of water (Iyer et al., 2022; Zanni et al., 2020). Similarly, amphetamine self-administration has been successfully established in rat and mouse models of free-choice drinking (Heyne and Wolffgramm, 1998; Meliska et al., 1995). Hence, MIRT might also have abuse potential because it induced self-administration in the free-choice drinking paradigm in mice.

Furthermore, to investigate the addictive potential of MIRT and possible associated deterioration of memory and recognition, as has been reported for several known drugs of abuse (Belcher et al., 2005; Gong et al., 2019; Ryabinin et al., 2002), NORT was conducted on the last day of MIRT consumption. Mice in the MIRT20 group revealed a significant deterioration in memory and recognition, which is indicative of its effects on different brain regions involved in recognition, similar to other known drugs of abuse. This cannot be attributed to any effects of MIRT on locomotion as no significant changes were revealed between the control and treatment groups regarding the distance travelled in OFT1. Chronic ethanol intake is frequently associated with various cognitive problems, including long-term impairments in memory and learning (Hashemi Nosrat Abadi et al., 2013). A previous study revealed that novel object recognition was disrupted by paternal morphine exposure (Ellis et al., 2020). Moreover, morphine withdrawal in CRF_1- and CRF_2-deficient mice revealed that these receptors play a role in cognitive impairment assessed through NORT (Morisot and Contarino, 2016). Additionally, methamphetamine and cocaine dependence have been reported to induce several mental symptoms and cognitive deficits (Fole et al., 2015; Schwendt et al., 2012). Further studies are needed to assess the specific effects of MIRT on neuronal pathways in brain regions involved in recognition and memory, such as the hippocampus and perirhinal cortex (Antunes and Biala, 2012).

The FST and TST were used to investigate MIRT-induced withdrawal symptoms after forced abstinence. These behavioral tests can assess the despair aspect of depressive-like behavior, an indication of withdrawal symptoms. Due to the limitations of FST and its possible impact on animal behavior as reported previously (Carvalho et al., 2021; Reardon, 2019), FST was performed only once and at the end of the study to eliminate its possible confounding effects on the other behavioral paradigms. To eliminate any possible effects of MIRT withdrawal on locomotion that might affects the validity of FST and TST, OFT2 was performed on the last day of forced abstinence and before FST and TST. Following abstinence, no significant changes were revealed between the control and treatment groups regarding the distance travelled in OFT2. Mice in the MIRT20 group displayed a significant depressive-like effect in both the TST and FST after forced abstinence, manifested by significant increases in immobility time. These findings are in line with previous studies showing that MIRT can induce withdrawal symptoms. Sudden withdrawal of MIRT can cause depression, insomnia, anxiety, restlessness, diarrhea, vomiting, and rarely mania (De Boer, 1995). Anxiety, loss of appetite, nausea, tremor, and an eight-pound weight loss were reported in the case of a 53-year-old man who was prescribed MIRT for appetite stimulation and experienced these symptoms within 48 h after he discontinued his prescription (McGowan et al., 2021). Notably, withdrawal symptoms are very common after abrupt discontinuation of psychotropic medications (Brandt et al., 2020; Cosci and Chouinard, 2020). These drugs including MIRT are heterogeneous compounds with a high variability of receptor affinities. Receptor affinity is an important factor of efficacy and can influence side effects and withdrawal symptoms. Therefore, further research is needed to provide a safe discontinuation strategy for MIRT, considering its side effect, pharmacodynamics, and pharmacokinetic profiles.

Abusing MIRT at higher doses is unsafe and MIRT should be used as prescribed by healthcare practitioners. MIRT can cause substantial changes in neuronal function (such as tonic activation of 5-HT1A receptors) and gene expression (such as density of 5-HT2 and β1 adrenergic receptors) after repeated dosing. Ischemic stroke patient developed mania three days after starting MIRT (15 mg) (De León et al., 1999). In a global clinical trial, the rate of mania induction using MIRT was 0.25 % (Montgomery, 1995). Serotonin syndrome is a potentially fatal side effect of serotonergic medication. MIRT is a generally safe antidepressant with a reduced prevalence of adverse effects, however, when used in high doses or with other serotonergic drugs such as methadone and sertraline, it can cause serotonin syndrome (Martín-Lázaro, 2017). MIRT can also cause pancreatitis, a rare but significant side effect (Hussain and Burke, 2008). MIRT-induced pancreatitis has been reported in a 46-year-old African-American woman. The woman developed pancreatitis; therefore, MIRT was discontinued as the probable reason for her hypertriglyceridemia-induced pancreatitis (Bowers et al., 2019). The side effects of MIRT, including psychomotor restlessness and akathisia, are described as warnings, which affect<1 % of the populace (Koller, 2019).

5. Conclusions

The results of this investigation revealed for the first time that MIRT is able to induce drug-seeking behavior in mice that preferred to self-administer MIRT to water. These mice revealed a significant deterioration in memory and recognition, which is indicative of its effect on different brain regions involved in recognition, similar to other known drugs of abuse. Moreover, mice in the MIRT20 group displayed significant depression-like symptoms after forced abstinence. Healthcare practitioners should be aware of the drug's considerable potential for addiction and arrange prescriptions accordingly. MIRT was able to induce drug-seeking behavior, deteriorate recognition, and cause withdrawal symptoms. This might confirm that MIRT has the potential to induce drug dependence, and that its use should be controlled by legal authorities. Further studies are needed to investigate the neurobiological basis of MIRT-induced drug-seeking behavior.

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

Antunes, M., Biala, G.J.C.P., 2012. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn. Process. 13, 93–110.
Aschhoff, S. et al., 2000. Nicotine consumption of several mouse strains using a two bottle choice paradigm. J. Exp. Anim. Sci. 40, 171–177.
Bagdas, D. et al., 2019. Assessing nicotine dependence using an oral nicotine free-choice paradigm in mice. Neuropharmacology. 157, 107669.
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.
Benazzi, F., 1998. Mirtazapine withdrawal symptoms. Can. J. Psychiatry. 43, 525.
Bowers, R.D., Valanejad, S.M., Holombo, A.A., 2019. Mirtazapine-Induced Pancreatitis-A Case Report. J. Pharm. Pract. 32, 586–588.
Brandt, L. et al., 2020. Antipsychotic Withdrawal Symptoms: A Systematic Review and Meta-Analysis. Front Psychiatry. 11, 569912.
Bremner, J.D., Wingard, P., Walshe, T.A., 1998. Safety of mirtazapine in overdose. J. Clin. Psychiatry. 59, 233–235.
Can, A., Dao, D.T., Arad, M., Terrillion, C.E., Piantadosi, S.C., Gould, T.D., 2012. The mouse forced swim test. J. Visualized Exp. 59, 3638.
Carvalho, C. et al., 2021. Time to abolish the forced swim test in rats for depression research? Journal of Applied Animal Ethics Research 1 (aop), 1–9.
Childress, A.R. et al., 1999. Limbic activation during cue-induced cocaine craving. Am. J. Psychiatry. 156, 11–18.
Compton, W.M., Volkow, N.D., 2006. Major increases in opioid analgesic abuse in the United States: concerns and strategies. Drug Alcohol Depend. 81, 103–107.
Cosci, F., Chouinard, G., 2020. Acute and persistent withdrawal syndromes following discontinuation of psychotropic medications. Psychotherapy and psychosomatics 89 (5), 283–306.
Davis, R., Wilde, M.I., 1996. Mirtazapine: a review of its pharmacology and therapeutic potential in the management of major depression. CNS Drugs. 5, 389–402.
De Boer, T., 1995. The effects of mirtazapine on central noradrenergic and serotonergic neurotransmission. Int. Clin. Psychopharmacol. 10 (Suppl 4), 19–23.
De León, O.A., Furmaga, K.M., Kaltsounis, J., 1999. Mirtazapine-induced mania in a case of poststroke depression. J. Neuropsychiatry Clin. Neurosci. 11, 115–116.
Ellis, A.S. et al., 2020. Paternal morphine self-administration produces object recognition memory deficits in female, but not male offspring. Psychopharmacology. 237, 1209–1221.
El-Tanbouly, D.M., Wadie, W., Sayed, R.H., 2017. Modulation of TGF-β/Smad and ERK signaling pathways mediates the anti-fibrotic effect of mirtazapine in mice. Toxicol. Appl. Pharmacol. 329, 224–230.
Fole, A. 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. Learn. Mem. 123, 179–186.
Fuchs, R.A., Higginbotham, J.A., Hansen, E.J., 2019. Chapter 2 - Animal Models of Addiction. In: Torregrossa, M. (Ed.), Neural Mechanisms of Addiction. Academic Press, pp. 3–22.
Gong, D. et al., 2019. Differences in cocaine- and morphine-induced cognitive impairments and serum corticosterone between C57BL/6J and BALB/cJ mice. Pharmacol. Biochem. Behav. 182, 1–6.
Goodnick, P., Kremer, C., Wingard, P., 1998. Weight change during mirtazapine therapy. Prim. Psychiatry. 3, 103–108.
Graves, S.M. et al., 2012. Mirtazapine, and mirtazapine-like compounds as possible pharmacotherapy for substance abuse disorders: evidence from the bench and the bedside. Pharmacol. Ther. 136, 343–353.
Griffin III, W.C.J.A., 2014. Alcohol dependence and free-choice drinking in mice. Alcohol. 48, 287–293.
Gutiérrez, J.F. et al., 2020. Protective role of mirtazapine in adult female Mecp2+/− mice and patients with Rett syndrome. J. Neurodev. Disord. 12 (1), 1–20.
Hamadjida, A. et al., 2017. The effect of mirtazapine on dopaminergic psychosis and dyskinesia in the parkinsonian marmoset. Psychopharmacology 234, 905–911.
Hashemi Nosrat Abadi, T. et al., 2013. Effects of different exercise protocols on ethanol-induced spatial memory impairment in adult male rats. Alcohol. 47, 309–316.
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. Psychopharmacol. (Berl.) 140, 510–518.
Hulme, S., Bright, D., Nielsen, S., 2018. The source and diversion of pharmaceutical drugs for non-medical use: A systematic review and meta-analysis. Drug Alcohol Depend. 186, 242–256.
Hussain, A., Burke, J., 2008. Mirtazapine associated with recurrent pancreatitis — a case report. J. Psychopharmacol. 22, 336–337.
Iyer, V. et al., 2022. A limited access oral oxycodone paradigm produces physical dependence and mesocorticolimbic region-dependent increases in DeltaFosB expression without preference. Neuropharmacology. 205, 108925.
Kawachi, I., 1999. Physical and psychological consequences of weight gain. J. Clin. Psychiatry. 60 (Suppl 21), 5–9.
Klesmer, J., Sarcevic, A., Fomari, V., 2000. Panic attacks during discontinuation of mirtazepine. Can. J. Psychiatry. 45, 570–571.
Koller, K., 2019. Propranolol for mirtazapine-induced akathisia: single case report. Ment. Health Clin. 9, 61–63.
Leger, M. et al., 2013. Object recognition test in mice. Nat. Protoc. 8, 2531–2537.
Martín-Lázaro, J.F., 2017. A dangerous triad: sertraline, mirtazapine and methadone. Clin. Med. Rev. Case Rep. 4, 154–155.
McGowan, K.E., March, K.L., Finch, C.K., 2021. The hunger for mirtazapine: a discontinuation syndrome. J. Pain Palliat. Care Pharmacother. 35, 113–116.
Meliska, C.J. et al., 1995. Ethanol, nicotine, amphetamine, and aspartame consumption and preferences in C57BL/6 and DBA/2 mice. Pharmacol. Biochem. Behav. 50, 619–626.
Montgomery, S.A., 1995. Safety of mirtazapine: a review. Int. Clin. Psychopharmacol. 10 (Suppl 4), 37–45.
Morisot, N., Contarino, A., 2016. The CRF1 and the CRF2 receptor mediate recognition memory deficits and vulnerability induced by opiate withdrawal. Neuropharmacology. 105, 500–507.
O'Brien, C.P. et al., 1998. Conditioning factors in drug abuse: can they explain compulsion? J. Psychopharmacol. 12, 15–22.
Planeta, C.S., 2013. Animal models of alcohol and drug dependence. Braz. J. Psychiatry. 35. Suppl 2, S140–S146.
Ramey, T., Regier, P., 2019. Cognitive impairment in substance use disorders. CNS Spectrums 24 (1), 102–113.
Ramon-Duaso, C. et al., 2020. Protective effects of mirtazapine in mice lacking the Mbnl2 gene in forebrain glutamatergic neurons: Relevance for myotonic dystrophy 1. Neuropharmacology 170, 108030.
Reardon, S., 2019. Depression researchers rethink popular mouse swim tests. Nature 571 (7766), 456–458.
Rogóż, Z.J.P.R., 2013. Effect of combined treatment with mirtazapine and risperidone on the MK-801-induced changes in the object recognition test in mice. Pharmacol. Rep. 65, 1401–1406.
Ryabinin, A.E., Miller, M.N., Durrant, S., 2002. Effects of acute alcohol administration on object recognition learning in C57BL/6J mice. Pharmacol. Biochem. Behav. 71, 307–312.
Schreiber, S. et al., 2002. The antinociceptive effect of mirtazapine in mice is mediated through serotonergic, noradrenergic and opioid mechanisms. Brain Res. Bull. 58, 601–605.
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.
Spachos, D. et al., 2020. Combining big Data Search analytics and the FDA Adverse Event Reporting System database to detect a potential safety signal of mirtazapine abuse. Health Informatics J.26, 2265–2279.
Steru, L. et al., 1985. The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology. 85, 367–370.
Substance Abuse and Mental Health Services Administration, 2004. Overview of Findings from the 2003 National Survey on Drug Use and Health. Substance Abuse and Mental Health Services Administration, Office of Applied Studies, Rockville, MD.
Use, N.S.o.D., et al. Results from the national Survey on Drug Use and Health: national Findings, 2007. Department Of Health And Human Services, Substance Abuse and Mental Health.
Waller, M.B. et al., 1982. Induction of dependence on ethanol by free-choice drinking in alcohol-preferring rats. Pharmacol. Biochem. Behav. 16, 501–507.
Yilmaz, I. et al., 2007. Mirtazapine does not affect pentylenetetrazole- and maximal electroconvulsive shock-induced seizures in mice. Epilepsy Behav. 11, 1–5.
Zanni, G. 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.
Zou, Z. et al., 2017. Definition of substance and non-substance addiction. Adv. Exp. Med. Biol. 1010, 21–41.

Further Reading

Aboitiz, F., García, R., 2009. Merging of phonological and gestural circuits in early language evolution. Rev. Neurosci. 20, 71–84.
Bart, G. et al., 2004. Substantial attributable risk related to a functional mu-opioid receptor gene polymorphism in association with heroin addiction in central Sweden. Mol. Psychiatry. 9, 547–549.
Choi, D.-S., 2012. Addiction genetics: a harbinger of advanced research and new treatment for addiction. Addiction Genetics. 1, 1–2.
de Boer, T., 1996. The pharmacologic profile of mirtazapine. J. Clin. Psychiatry. 57. Suppl 4, 19–25.
Gutiérrez, J.F. et al., 2022. Mirtazapine treatment in a young female mouse model of Rett syndrome identifies time windows for the rescue of early phenotypes. Exp. Neurol. 353, 114056.
Jilani, T.N., et al., 2019. Mirtazapine, in: StatPearls. StatPearls Publishing, Treasure Island.
O'Connor, J.C. et al., 2009. Induction of IDO by bacille calmette-guerin is responsible for development of murine depressive-like behavior. J. Immunol. 182, 3202–3212.
Zacny, J. et al., 2003. College on Problems of Drug Dependence taskforce on prescription opioid non-medical use and abuse: position statement. Drug Alcohol Depend. 69, 215–232.