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Effects of Tramadol on Exploratory Behaviors, Anxiety and Memory in BALB/C Mice: 49-Day Oral Dosing Study

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Abstract

This study aimed to evaluate the behavioral effects of tramadol administered orally at doses of 20 mg/kg and 40 mg/kg for 49 days in BALB/c mice. Standardized behavioral tests, including the Open Field (OF), Elevated Cross Maze (ECM), Morris Water Maze (MWM), Y-maze, and Object Recognition Test (NOR), were used to measure motor activity, anxiety, and spatial and nonspatial memory. The results revealed that mice treated with the high dose of tramadol (40 mg/kg) exhibited significant reductions in motor activity, increased anxiety, and cognitive deficits in both spatial and nonspatial memory. In contrast, mice treated with 20 mg/kg tramadol exhibited increased exploratory activity during treatment, but these effects were transient. Control mice (treated with distilled water) showed no significant changes during the experiment. These results suggest that tramadol , although effective for its analgesic properties, may impair memory and increase anxiety when administered at high doses over a prolonged period. These findings highlight the importance of managing opioid side effects in a clinical and research setting.

Keywords: Tramadol, Motor Behavior, Anxiety, Memory, BALB/C Mice

Introduction

Opioids, and tramadol in particular , are frequently used for their ability to relieve pain. However, their impact on behavioral and cognitive functions remains an important area of research, given that these substances can cause adverse effects at the central nervous system (CNS) level (García-García et al., 2016; Ramsay & Bennett, 2019). Tramadol , an opioid receptor agonist and a serotonin-norepinephrine reuptake inhibitor, is commonly prescribed to treat moderate to severe pain (Ramsay & Bennett, 2019). Although its pharmacological profile is often considered less addictive than that of other opioids, studies suggest that prolonged exposure could lead to behavioral and cognitive alterations in animals, which deserves to be explored in experimental models (Bello & Jansen, 2018; Schmitt & Milde , 2017).

The effects of tramadol on exploratory behavior and anxiety have been assessed using several classical paradigms, such as the Open Field (OF) test, the Elevated Crosshair Maze (ECM), the Morris Water Maze (MWM), as well as memory tests such as the Object Recognition Test (OR) and the Y-maze (Ennaceur & Chazot , 2015; Franz, 2017). These tests are used to assess anxiety, exploratory behaviors, and spatial memory, respectively, providing clues about the effect of pharmacological treatment on cognitive and emotional functions (Routtenberg & Babb , 2014; Vann & Aggleton , 2018). Previous studies have shown that tramadol doses can induce anxiety-like behaviors in rodents and affect their spatial navigation abilities

(García-García et al., 2016), but data on the long-term effects of prolonged treatment, such as that administered over a 49-day period, remain limited.

The present work aims to study the effects of tramadol at doses of 20 mg/kg and 40 mg/kg on the behaviors of BALB/c mice. We used a series of behavioral tests to assess anxiety, memory, and motor exploration. This experimental protocol relies on the principles of behavioral pharmacology to provide information on how prolonged tramadol treatment could modify the behavioral responses of mice. The main hypothesis is that tramadol , depending on the dose and duration of treatment, could induce significant changes in the exploratory and cognitive behaviors of mice, thus leading to adverse effects.

The results obtained will allow us to better understand the neuropharmacological implications of tramadol , particularly in the context of prolonged treatment, and to determine whether this drug causes alterations similar to those observed in other opioids (Boulenger & Scatton , 2014). This information could have important implications for the clinical use of tramadol , particularly in terms of management of its side effects and prevention of potential abuse.

Methodology

Animals

The study was performed on BALB/c mice, provided by the Faculty of Health Sciences of Marien Ngouabi University (Brazzaville, Congo). A total of 30 adult male mice (Age: 8-10 weeks, average weight: 25-30 g) were used for this study. Mice were maintained under standard conditions (temperature: 22 ± 2°C, humidity: 50-60%, 12h/12h light/dark cycle) with ad libitum access to distilled water and standard food. Experiments were performed in accordance with the guidelines of local animal protection legislation and the Marien Ngouabi University statement , in accordance with the ethical principles of animal research (Council for International Organizations of Medical Sciences [CIOMS], 2016).

Treatment

The mice were divided into three groups of 10 animals each:

- **Group 1 (Control, ED)** : Mice received distilled water orally for 49 days.
- **Group 2 (TRA 20)** : Mice received 20 mg/kg tramadol hydrochloride orally for 49 days.
- **Group 3 (TRA 40)** : Mice received 40 mg/kg tramadol hydrochloride orally for 49 days.

Tramadol doses were chosen based on previous studies that showed significant behavioral effects at these concentrations (García-García et al., 2016; Schmitt & Milde , 2017). Tramadol was administered once daily at a dose of 0.1 mL per mouse. Treatment lasted 49 days, a period long enough to observe potential effects of the drug on the animals' exploratory and cognitive behaviors.

Behavioral tests

The behavioral tests used in this study are commonly used to assess pharmacological effects on anxiety, motor exploration, and memory in rodents. Each test was performed according to the standard protocols described below.

1. Open Field (OF)

The Open Field (OF) test is a widely used test to assess exploratory and anxiety behaviors in rodents (Ennaceur & Chazot , 2015). The device consisted of a 50 cm x 50 cm square box with opaque walls, covered with absorbent paper. The surface of the test area was divided into 25 equal squares of 10 cm^2 each. Mice were placed in the center of the field and filmed for 5 minutes to record the distance traveled,

the number of rightings (postural reflexes), and the number of entries into the central zone (10 cm side area in the center of the field). The distance traveled is an indicator of motor activity, while rightings and the number of entries into the central zone are indirect measures of anxiety (Ennaceur & Chazot , 2015).

2. Raised Crosshair Maze (RCM)

Elevated Crosshair Maze (ECM) is used to assess anxiety in rodents by measuring the preference for open or closed arms (Boulenger & Scatton , 2014). It consists of an apparatus with two open arms and two closed arms, arranged to form a raised "H". The mouse is placed in the center and its movements are recorded for 5 minutes. The number of entries into the open and closed arms, as well as the time spent in each arm, are used to assess the level of anxiety. Anxious mice tend to avoid the open arms and remain more in the closed arms (Ennaceur & Chazot , 2015).

3. Morris Water Maze (MWM)

The Morris Water Maze (MWM) is a classic test for assessing spatial memory in rodents (Franz, 2017). This test uses a 1.5 m diameter circular pool filled with room temperature water, in which a hidden platform is submerged approximately 1 cm below the water surface. Mice are trained to locate the platform based on visual cues located around the pool. Performance is measured by recording the time taken to find the platform (search latency), the number of trials required to find it, and the time spent in each quadrant of the pool.

4. Object Recognition Test (OR)

The object recognition test is used to assess non-spatial memory in rodents (Routtenberg & Babb , 2014). It consists of a task where mice are exposed to two identical objects in an open arena during an exploration phase. After a 24-hour period, one of the objects is replaced by a different object, and the time spent exploring each object is measured. Normal mice tend to spend more time with the new object, indicating recognition of the familiar object and intact memory.

5. Y-maze

The Y-maze is a test used to assess working memory and exploratory ability (Vann & Aggleton , 2018). It consists of a "Y"-shaped maze, with three equal arms, in which a mouse is placed in the center. Behaviors are recorded in terms of the number of arms visited and the percentage of the first correct choice, that is, the percentage of times the mouse explores an arm not visited first. This test is used to assess the cognitive flexibility and working memory of mice.

Statistical analysis

Statistical analysis of the data obtained from the different behavioral tests (Open Field, Elevated plus maze, Morris water maze, Y maze, Object recognition test) was performed to evaluate the effects of tramadol (TRA 20 mg/kg and TRA 40 mg/kg) on the motor and anxiety behaviors of mice. The analyses were performed using appropriate parametric tests to compare the different groups (control group with distilled water - ED, group treated with tramadol 20 mg/kg - TRA 20, and group treated with tramadol 40 mg/kg - TRA 40). The results are presented in terms of mean ± standard deviation (M ± SD) for each condition (before, during and after treatment), and the differences between groups were evaluated using ANOVA tests and post-hoc tests

Results and discussion

Results

1. Open Field

A. Distance traveled

The results show a significant difference during and after treatment between the TRA 20 group and the ED control group, as well as between the TRA 40 group and the control group ($p < 0.05$ for both comparisons). Specifically, during treatment, mice in the TRA 20 group traveled a significantly greater distance than those in the ED group, while those in the TRA 40 group traveled a significantly smaller distance. After treatment, these differences were maintained with p values < 0.01 for comparisons.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	567.5 ± 45.44	581.1 ± 41.15	568.3 ± 44.98
Group 2 (TRA 20)	562.7 ± 45.56 ^{ns}	786.4 ± 49.21*	338.9 ± 42.11**
Group 3 (TRA 40)	566.3 ± 44.96 ^{ns}	364.9 ± 39.86*	232.2 ± 39.61**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

B. Number of adjustments

Analysis of the number of rightings also revealed significant differences, particularly during and after treatment. Mice treated with 20 mg/kg tramadol (TRA 20) showed a significantly higher number of rightings than those in the ED control group during treatment ($p < 0.01$). In contrast, mice treated with 40 mg/kg tramadol (TRA 40) showed a marked decrease in the number of rightings, with significant differences compared with the ED control group at all time points ($p < 0.01$).

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	19.2 ± 1.69	21.0 ± 2.89	21.6 ± 2.63
Group 2 (TRA 20)	20.3 ± 2.16 ^{ns}	32.8 ± 3.22**	7.9 ± 1.20**
Group 3 (TRA 40)	20.2 ± 1.32 ^{ns}	10.8 ± 1.48**	5.6 ± 1.90**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

C. Number of entrances into the central area

The test revealed a significant reduction in entries into the central zone of mice treated with 20 mg/kg and 40 mg/kg tramadol during and after treatment ($p < 0.01$), suggesting an anxiogenic effect of tramadol at these doses.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	5.9 ± 0.99	6.2 ± 0.79	5.8 ± 1.03
Group 2 (TRA 20)	5.6 ± 0.84 ^{ns}	14.9 ± 2.23**	1.9 ± 0.88**
Group 3 (TRA 40)	5.9 ± 1.20 ^{ns}	2.1 ± 0.74**	1.4 ± 0.70**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

Elevated Cross Labyrinth (LCS)

A. Time spent in the open arm (TPBO)

The test results showed a significant increase in the time spent in the open arm for the TRA 20 group compared to the ED control group during treatment ($p < 0.01$). However, the TRA 40 group spent less

time in the open arm, with significant differences compared to the control group at all time points ($p < 0.01$). These results suggest that low-dose tramadol (20 mg/kg) has an anxiolytic effect, while the higher dose (40 mg/kg) appears to increase the anxiety of the mice

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	27.6 ± 2.37	26.3 ± 2.41	27.8 ± 2.85
Group 2 (TRA 20)	27.6 ± 2.84 ^{ns}	53.3 ± 2.98**	20.8 ± 2.30**
Group 3 (TRA 40)	27.2 ± 2.74 ^{ns}	18.6 ± 1.17**	17.2 ± 2.10**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

B. Number of entries into the open arm (NEBO)

A significant increase in the number of entries into the open arm was observed in the TRA 20 group during treatment ($p < 0.01$) compared with the ED control group. In contrast, TRA 40 mice showed a marked decrease in the number of entries into the open arms, with significant differences compared with the control group at all time points ($p < 0.01$).

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	8 ± 1.05	7.8 ± 1.03	8.6 ± 1.07
Group 2 (TRA 20)	7.4 ± 1.07 ^{ns}	18.8 ±3.79**	5.1 ± 0.88**
Group 3 (TRA 40)	7.8 ± 1.03 ^{ns}	10.8 ± 1.48**	4.3 ± 1.42**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

3. Morris Water Maze

A. Time spent next to the platform (TPCP)

Analyses revealed a significant reduction in time spent near the platform for both TRA 20 and TRA 40 groups during and after treatment ($p < 0.01$). In particular, mice treated with TRA 40 spent significantly less time near the platform, suggesting a possible deficit in spatial memory.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	18.8 ± 1.75	19.0 ± 1.56	20.0 ± 1.49
Group 2 (TRA 20)	19.7 ± 2.45 ^{ns}	12.9 ± 1.85**	11.7 ± 1.70**
Group 3 (TRA 40)	19.4 ± 1.58 ^{ns}	9.4 ± 1.58**	7.2 ± 1.81**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

B. Time to find the platform

The test showed that mice in the TRA 20 group took significantly less time to find the platform compared to mice in the TRA 40 group, which took longer, especially after treatment. The differences between groups are statistically significant ($p < 0.01$).

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	18.8 ± 1.75	19.0 ± 1.56	20.0 ± 1.49
Group 2 (TRA 20)	19.7 ± 2.45 ^{ns}	12.9 ± 1.85**	11.7 ± 1.70**
Group 3 (TRA 40)	19.4 ± 1.58 ^{ns}	9.4 ± 1.58**	7.2 ± 1.81**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

5. Y-maze

A. Percentage of first correct choice

The results showed that mice in the ED control group made 100% of the first choices correct before and after treatment, whereas the TRA 20 and TRA 40 groups showed significantly lower performance during and after treatment. The differences were significant ($p < 0.01$), suggesting that tramadol impairs recognition memory.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	100%	90%	100%
Group 2 (TRA 20)	100%	50%**	40%**
Group 3 (TRA 40)	100%	40%**	30%**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

B. Percentage of time spent in the new arm

A significant decrease in time spent in the new arm was observed in both TRA 20 and TRA 40 groups during and after treatment ($p < 0.01$), particularly in the TRA 40 group. This suggests a deficit in short-term memory in tramadol-treated mice , particularly at the highest dose.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	76.3 ± 6.80	76.9 ± 6.28	77.3 ± 12.51
Group 2 (TRA 20)	77.3 ± 6.21 ns	47.6 ± 9.00**	46.0 ± 7.45**
Group 3 (TRA 40)	78.7 ± 5.12 ns	38.8 ± 12.51**	33.6 ± 9.41**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

6. Object recognition test

A. Percentage of first correct choice

The test revealed a significant reduction in the percentage of correct first choices in the TRA 20 and TRA 40 groups during and after treatment ($p < 0.01$). Mice in the control group (ED) showed perfect performance before and after treatment, while the TRA 20 and TRA 40 groups showed a deterioration in performance, suggesting a negative effect of tramadol on recognition memory.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	80%	90%	100%
Group 2 (TRA 20)	100%	60%**	40%**
Group 3 (TRA 40)	90%	60%**	30%**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

B. Percentage of time spent next to the new object

Mice in the control group spent the majority of their time exploring the novel object, while those in the TRA 20 and TRA 40 groups spent significantly less time near the novel object after treatment ($p < 0.01$). This may reflect impairment of long- term memory caused by tramadol.

Band	Before treatment	During treatment	After treatment
Group 1 (ED)	79.3 ± 6.80	83.9 ± 6.28	85.3 ± 12.51

Group 2 (TRA 20)	80.3 ± 5.25	60.6 ± 9.00**	45.0 ± 5.45**
Group 3 (TRA 40)	82.7 ± 4.12	54.8 ± 13.71**	38.6 ± 9.52**

tramadol hydrochloride , ns: P >0.5 *: P <0.05; **: P <0.01.

Discussion

tramadol treatment at doses of 20 mg/kg and 40 mg/kg in BALB/c mice, using classical behavioral tests to assess anxiety, motor exploration, and memory. The results obtained show significant effects of tramadol on various behavioral parameters, suggesting that this drug could induce notable alterations in motor, anxiety-like, and cognitive behaviors of animals. These results are consistent with previous work on opioids and their impact on the central nervous system, but also provide new information on the effects of prolonged tramadol treatment in a context of moderate to high doses.

Impact of tramadol on anxiety and exploratory behavior

The Open Field (OF) test is commonly used to assess anxiety and motor activity in rodents. In this study, mice treated with 40 mg/kg tramadol (Group 3) showed a significant reduction in distance traveled compared to the control (ED) and 20 mg/kg (TRA 20) groups. This suggests that the high dose of tramadol may induce more inhibited behavior, which is often interpreted as a sign of increased anxiety or sedation (Ennaceur & Chazot , 2015). Indeed, several studies have shown that high doses of opioids can induce anxiety-like or social withdrawal behaviors in rodents, probably due to an alteration in the activity of brain circuits involved in the regulation of anxiety, such as the serotonin and norepinephrine system (García-García et al., 2016; Tzschentke , 2017).

In contrast, mice treated with 20 mg/kg tramadol (Group 2) showed a significant increase in exploratory activity during treatment compared with the control group, suggesting a moderate stimulation effect on motor behavior. This is consistent with other research suggesting that moderate doses of tramadol can induce mild analgesic and anxiolytic effects without causing excessive sedation (García-García et al., 2016). However, the increase in exploratory activity was not observed persistently after treatment ended, indicating that this effect may be transient and not reflect a lasting change in exploratory motivation.

Elevated Crosshair Maze (ECM) test, used to measure anxiety-like behaviors, corroborate these observations. Indeed, mice treated with the highest dose of tramadol (40 mg/kg) showed a tendency to avoid open arms, which is a typical behavior of anxious animals (Boulenger & Scatton , 2014). Lower doses of tramadol (20 mg/kg) did not induce such changes, which could suggest that the moderate anxiolytic effects observed in the Open Field test are mainly manifested at low doses and are abolished at higher doses.

Effects on memory and cognition

Spatial memory tests, such as the Morris Water Maze (MWM) and the Y-maze, have shown that tramadol , especially at the 40 mg/kg dose, induces significant cognitive deficits. In the MWM test, mice treated with 40 mg/kg took a longer time to locate the platform, and this latency was significantly increased compared with the control and 20 mg/kg groups. This result is consistent with previous studies that have reported that opioids, including tramadol , can impair spatial memory and learning performance (Schmitt & Milde , 2017; Tzschentke , 2017). Opioids have well-documented effects on the hippocampus, a brain region involved in spatial memory, and prolonged exposure to high doses of opioids can disrupt hippocampal function , leading to cognitive deficits (Bello & Jansen, 2018).

The Y-maze test also revealed changes in cognitive performance in tramadol- treated mice . Although all mice showed comparable initial exploration of the maze arms before treatment, mice in the TRA 40 group showed a significant reduction in the percentage of the first correct choice after 49 days of treatment. This result indicates an impairment in working memory and cognitive flexibility, which is consistent with studies that have demonstrated that chronic opioid exposure affects working memory processes (García-García et al., 2016; Schmitt & Milde , 2017).
tramadol interference with neurobiological mechanisms underlying memory and learning, particularly in brain regions involved in spatial and non-spatial cognition. Studies have shown that tramadol , as an opioid receptor agonist and serotonin reuptake inhibitor, can alter the functional integrity of neuronal networks involved in these processes (García-García et al., 2016).

Effects on non-spatial memory

The results of the object recognition test also confirm that tramadol , especially at the 40 mg/kg dose, causes a reduction in the ability of mice to recognize a novel object. This impairment of non-spatial memory is similar to that observed in previous studies using other opioids (Tzschentke , 2017). Tramadol may disrupt the formation of new memory traces by modulating key neurotransmitter systems such as dopamine and serotonin, which play a crucial role in memory and attention processes (Franz, 2017). The results also show a decrease in the percentage of time spent in proximity to the novel object, which could reflect a deficit in recognition memory and a tendency towards indifference, a phenomenon frequently observed after prolonged exposure to opioids.

Limitations of the study and future perspectives

Although our results provide important information on the effects of long-term tramadol on motor, anxiety-like, and cognitive behaviors in mice, some limitations should be considered. First, this study used fixed doses of tramadol without assessing more detailed dose-dependent effects. It would be relevant to test a wider range of doses to better understand the relationship between dose and the observed behavioral effects. Furthermore, long-term effects on brain structures, including the hippocampus and prefrontal cortex, were not studied in this research. Future studies could include histopathological analyses to determine neuronal changes associated with chronic tramadol exposure . Finally, the effects of combining tramadol with other medications, such as antidepressants or anxiolytics, could also be explored to assess pharmacological interactions and their impacts on cognition and anxiety.

Conclusion

In conclusion, this study highlights the effects of tramadol on motor, anxiety and cognitive behaviors in mice, especially after a prolonged treatment of 49 days. The results suggest that high doses of tramadol (40 mg/kg) can induce cognitive deficits, increase anxiety and reduce motor activity, while moderate doses (20 mg/kg) seem to have a more moderate effect on these parameters. These results highlight the importance of monitoring opioid side effects, especially during prolonged treatment, and suggest that lower doses may be preferable to limit the risks of behavioral and cognitive alterations. Future research on the neurobiological effects of tramadol and on interactions with other drugs will help to better understand its mechanisms of action and optimize its clinical use.

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