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Anxiety Mouse Model Constructed by Single Intraperitoneal Injection of m-Chlorophenpiperazine

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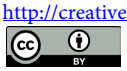

Abstract

Anxiety disorder is a common mental disorder. It is necessary to establish a rapid, stable and specific anxiety model to provide a theoretical basis for further research on the pathogenesis of anxiety and drug development. A single intraperitoneal injection of m-chlorophenylpipera-zine (mCPP) (1, 2, 4 mg/kg) was given to male ICR mice to establish an anxiety model, and the effects of mCPP on anxiety behavior, pain, athletic ability, passive avoidance response ability and depressive behavior of male ICR mice were evaluated. A single intraperitoneal injection of mCPP shortened the time in open arms and decreased the percentage of time in open arms of mice in the elevated plus-maze test. mCPP also shortened center zone distance and reduced the number of entries to the central zone in the open field test. Moreover, mCPP reduced head-dip counts and increased the head-dip latency of mice in the hole-board test. After being administrated with a single intraperitoneal injection of mCPP for 24h, the mice showed no significant difference in the entry into the light side and the percentage of time in the light side of the light-dark box test. A single intraperitoneal injection of mCPP had no effects on tail flick latency, rotating time, number of errors and the step-down latency, the immobility time of mice in the tail-flick test, rotarod test, step-down test and TST respectively. In conclusion, we established a rapid and stable anxiety mouse model by single intraperitoneal injection of mCPP.

Keywords

Anxiety Model, mCPP, Behavior

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1. Introduction

Anxiety disorders seriously affect the physical, psychological and social functions of patients, and increase the total burden of disease in the world. The prevalence of anxiety is estimated to be 18% among adults, with a lifetime prevalence of more than 28% [1] [2] [3]. The World Health Organization ranks anxiety disorders as the sixth largest contributor to global disability. Existing drugs for the treatment of anxiety have a variety of adverse reactions, such as dependence and withdrawal. Therefore, there are no ideal therapeutic drugs in the clinic at present. It is an important research direction to find new drug targets and explore drugs with higher safety. It is necessary to establish a rapid, stable and specific anxiety model to provide tools for drug research and development.

Anxiety animal models mainly include stress-induced models and chemical drug-induced models. Models induced by stress mainly include exposure to predators [4], maternal deprivation [5], social defeat [6], restraint stress [7], chronic unpredictable mild stress (CUMS) [8], empty bottle stress [9], foot shock [10], etc. This kind of model has some shortcomings, such as poor specificity, time-consuming, requirement on laboratory conditions and poor reproducibility. Models induced by chemicals are short in time and easy to operate. Drugs that can cause anxious behavior include corticosterone [11], caffeine [12], m-chlorophenylpipera-zine (mCPP) [13], etc. Caffeine has side effects that can affect the central nervous system [14]. Although corticosterone is widely used, it can cause anxiety and depression, and the specificity of this model is poor. In addition, the anxiety animal models also include gene knockout models NCS-1 gene knockout, and PRNP gene knockout induce anxiety behavior in mice [15] [16]. At present, there are few studies on gene knockout models, and further studies are needed.

mCPP is a commonly used chemical drug to establish an anxiety model [17]-[23]. mCPP-induced anxiety is an excellent animal model with a short time, simple operation and high success rate. However, there is no research to evaluate whether the model induced by mCPP only causes anxiety in mice without other behaviors.

In order to investigate whether mCPP only causes anxiety behavior, but does not affect pain, athletic ability, ability of learning and memory, and depressive behavior, after a single intraperitoneal injection of mCPP, we employed the elevated plus-maze (EPM), light-dark box (LDB), open field box (OFT) and hole-board test (HBT) to observe the degree of anxiety in mice. And the pain, athletic ability, passive avoidance response ability, and depression behavior of mice were measured by the tail-flick test, rotarod test, step-down test and tail suspension test (TST) respectively. Based on the above experiments, we found m-CPP model is a specific anxiety model.

2. Materials and Instruments

2.1. Animal

30 SPF grade 6-week-old male ICR mice, a total of 30 purchased from Beijing

Weitong Lihua Company, license number: SCXK (Jing) 2016-0006 and raised in the Experimental Center of Shandong University of Traditional Chinese Medicine. During the experiment, there were 6 animals in each cage, the temperature was maintained at 22˚C ± 2˚C, the humidity was maintained at 50% - 60%, the black and white cycle was 12 hours, and the food was free. Before the experiment, all mice were acclimatized to the experimental environment for 6 days. All animal experiments were reviewed and approved by the ethics committee of Shandong University of Traditional Chinese Medicine.

2.2. Drugs

mCPP (sigma, 125180-5G) was dissolved in saline to make 0.4 mg/mL. Take an appropriate amount of solution and dilute it to the required concentration.

2.3. Apparatus

Elevated plus-maze experiment system, Light-dark box experiment system, Open field experiment system, Super Maze analysis system, SuperTST analysis system (Shanghai Xin Ruan Information Technology Co., Ltd.), RotaRod (ugo basile, 47650), Tail Flick (Chengdu Taimeng Technology Co., Ltd, SW-200), Step-down recorder (Jinan Yiyan Technology Development Co., Ltd, YLS-3TB), Tail suspension box (Xmaze, XR-XX203).

3. Experimental Method

3.1. Animal Screening

After adaptive feeding for a week, 6-week-old male ICR mice were observed for the general conditions of all animals, and the autonomous activity experiment was conducted. According to the results of the autonomous activity experiment and the body weight of the mice, 24 mice were selected for the experiment.

3.2. Experimental Grouping and Administration

Twenty-four 6-week male ICR mice were randomly divided into four groups by body weight and total motion distance detected by autonomic mobility, namely the control group (C), the mCPP low-dose group (L, 1 mg/kg), the mCPP medium-dose group (M, 2 mg/kg), and the mCPP high-dose group (H, 4 mg/kg), with 6 in each group. A single intraperitoneal injection of mCPP was administered with a volume of 0.1 mL/10g. The control group was given 0.9% sodium chloride injection, and a behavioral test was performed 30 minutes after a single intraperitoneal injection. The specific animal grouping and dosage are shown in the following table.

3.3. Experiment Process

After adaptive rearing for one week, mice were screened for autonomous activity. Except for the LDB, all behavioral experiments were performed 30 minutes after injected with mCPP. The specific experimental procedure was conducted as follows (**Figure 1**).

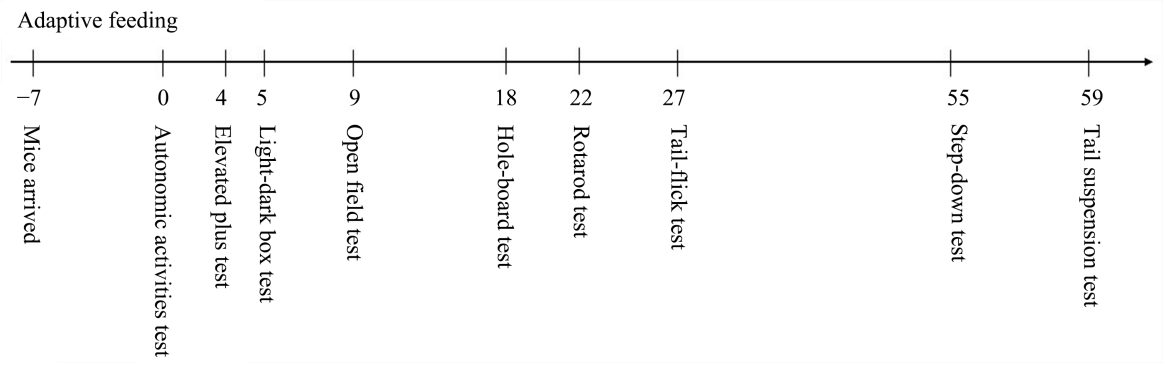

Figure 1. Experimental procedure of a mouse model of anxiety induced by a single intraperitoneal injection of mCPP.

4. Behavioral Evaluation

4.1. Effects of Single Intraperitoneal Injection of mCPP on Anxiety Behavior in Mice

4.1.1. Elevated Plus-Maze

EPM was conducted 30 min after a single intraperitoneal injection of mCPP. In this study, the anxiety state of animals was measured by using the conflicted behavior of exploring the new environment and the fear of hanging open arms. SuperMaze software was used to collect and analyze the behavior of mice. The mice were placed in the center of the central platform with their heads facing the open arms, and the following indicators within 5 minutes after release: 1) Number of open arms entries (OE); 2) Time in open arms (OT); 3) Number of close arms entries (CE); 4) Time in close arms (CT). Based on the above indexes, the following results can be obtained: 1) Entries to open arms/total arms (%): OE/(OE + CE) × 100%; 2) Time in open arms/total arms (%): OT/(OT + CT) × 100%. Animals with lower anxiety levels were more likely to explore the open arm, so in the EPM, the higher the percentage of open arm entry and open arm time, the less anxious the animal was at present.

4.1.2. Light-Dark Box

This experiment was carried out 24h after single intraperitoneal injection of mCPP before the EPM. Use SuperMaze software to collect and analyze the behavior of mice. Mice were put in the light box, pulled out the insert in the middle of the light and dark box. We recorded behavioral indicators of the mouse within 5 minutes. At the end of each test, the device was wiped with 75% alcohol. Observation indexes were: 1) The number of entrances in the light box; 2) Time spent in light box (%): Time spent in light box/total area × 100%. When the mouse is less anxious, the time and frequency of exploring the bright box will be longer. On the contrary, when the mouse was more anxious, it tended to move in the dark box.

4.1.3. Open Field Box

OFT was conducted 30 min after single intraperitoneal injection of mCPP. We used SuperMaze software to collect and analyze the behavior of mice. The open

field box (50 cm × 50 cm × 50 cm) was divided into 9 grids on average. Mice were placed in the center grid of the box, and motion state of the mice was tracked and recorded for 5min under normal light. Before the detection of the next mice, the chamber was cleaned with 75% ethanol to completely remove the odor left by the previous mice. The main indicators collected in the experiment are total distance (m), center zone distance (dm), number of entries to central zone. The less the mice enter the central area, the shorter the residence time in the central area, and the shorter the distance to the central area, the more anxiety the mice have. The fewer times the mice entered the central area and the fewer the distances in the central area, the more anxious mice were.

4.1.4. Hole-Board Test

HBT was conducted 30 min after single intraperitoneal injection of mCPP. The instrument is assembled by putting a plastic plate with 16 holes in the box (50 cm × 50 cm × 50 cm). The HBT was based on the nature of mice to explore caves, using novelty and fear to control the animal's behavior under new environment, and avoiding to reflect the effects of these two factors. The movement of the mice was recorded for five minutes, and the chamber. Observe the head-dip counts and head-dip latency of mice. The decrease of head-dip counts and the increase of head-dip latency indicated anxious behavior in mice.

4.2. Effects of Single Intraperitoneal Injection of mCPP on the Athletic Ability of Mice

Rotarod tests require animals to maintain balance and continuous movement on a rotating roller. Mice with dyskinesias or poor coordination will fall down faster. The fall time of the mice is statistically analyzed to evaluate the exercise athletic ability of the mice. The shorter the mice persist on the rotarod, the worse the coordination ability of their exercises. The day before the experiment, mice were placed on the rotarod for regular rotarod training. The mice were adapted to the rotarod for 30 seconds. After the mice were stable, the rod rotator was started, and the rotation speed was set at 10 r/min.

The rotarod test was conducted 30 min after a single intraperitoneal injection of mCPP. In the formal experiment, we placed the mice that had undergone conventional rotarod training on the rotarod with a constant speed of 25 r/min, recorded time the mouse stayed on the rotarod, dropped or grasped the rotarod and followed the rotarod to rotate three times all regarded as the end of the experiment. The rotation time was set to 300 seconds to ensure that the difference in the exercise balance ability between the mice was displayed without causing fatigue damage to the mice. Each mouse performed 3 rotarod tests with an interval of 30 minutes each time, the final experimental result was the average of three experiments.

4.3. Effects of Single Intraperitoneal Injection of mCPP on the Pain Sensation of Mice

Tail-flick test was conducted 30 min after a single intraperitoneal injection of

mCPP. The time of tail-flick response in mice was regarded as the indicator of pain response. The light intensity of the mice tail light pain tester to 50%, and the detection time is 10 s. We put the mice in the mouse holder, placed the mouse fixed tube seat on the top surface of the instrument, and adjusted its position so that the tail tip of the mice on the tail-tip positioning line, exposed the tail for light irradiation. The exposure site was fixed at the middle and lower 1/3 of the tail of each mouse. After the mice were quiet, the pain test was carried out, and the light bulb was lit. When the rat tail swings, the photoelectric switch automatically turns off the light and stops timing. The minimum unit of timing was 0.1 s. The incubation period from the beginning of the irradiation to the appearance of the tail-flick reaction was recorded as the pain threshold. In order to protect the tails of mice from damage, those who did not flick their tails for more than 10 s were recorded as 10 s, and the latency of tail flicks in each group was statistically processed.

4.4. Effects of Single Intraperitoneal Injection of mCPP on Passive Avoidance Response Ability of Mice

Training period: After 30 minutes of intraperitoneal injection, mice (one in each group in order, and 2 mice were detected at the same time) were placed in the jumping platform experimental device to acclimate for 3 min, and all mice were placed on the safety platform before power-on, and then the power supply (voltage 60 - 80 V, current 0.8 - 1.5 mA) was switched on. The mice would flee to the safety platform after receiving the electric shock. It was a wrong reaction for the mouse to jump from the safety platform and touch the copper grid with its two forefeet. The incubation period of jumping off the platform for the first time within 5 min and the number of shocks from jumping off the platform were recorded, which were used as the learning performance of mice.

Test period: The test was conducted at 8:30 the next day. The mice were placed on a safe platform, with a voltage of 60 - 80 V and a current of 0.8 - 1.5 mA. The experiment lasted for 5 min (no need to adapt). The incubation period of the mice in each group jumping off the platform for the first time within 5 minutes and the number of electric shocks received by jumping off the platform was recorded as the memory performance of the mice.

4.5. Effects of Single Intraperitoneal Injection of mCPP on Depression Behavior in Mice

TST was conducted 30 min after single intraperitoneal injection of mCPP. Super TST software was used to collect and analyze the behavior of the mice. The tail of each mouse was attached to the hook in the suspension box with medical tape, and the changes of the movement state of the mice were collected within 5 min. The first 1 min was the adaptation period, and the state of the mice was analyzed after 4 min. The main indexes collected in the experiment are: immobility time and struggle time. Immobility time was main parameter, and the increase of immobility time indicated that the mice had depression-like behavior.

4.6. Statistical Analysis

All data were expressed as Mean ± S.D and analyzed by employing the GraphPad 8.0 software. Data between the two groups were compared using Student's t-test. Comparisons of data from multiple groups against one group were analyzed using a one-way analysis of variance (ANOVA) followed by Dunnett's post hoc test. The statistical analysis used $P < 0.05$ as a basis for the level of significance.

5. Results

5.1. Effects of Single Intraperitoneal Injection of mCPP on Anxiety Behavior in Mice

5.1.1. Effects of Single Intraperitoneal Injection of mCPP on the Behavior of Mice in Elevated Plus-Maze

After mice were treated with a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 30 min, the EPM was conducted to detect the anxiety of mice. The results showed (Figure 2) that the time in open arms was significantly shorter ($P < 0.05$) (Figure 2(B)) and the percentage of time in open arms was significantly lower ($P < 0.05$) (Figure 2(D)) in mice treated with high-dose mCPP (4 mg/kg) than control mice.

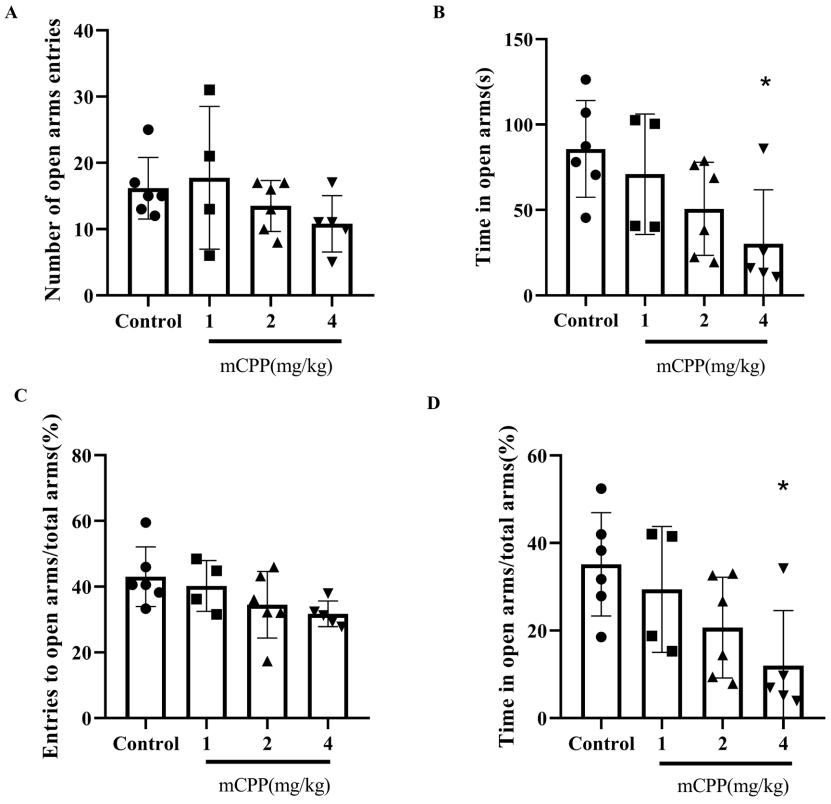

Figure 2. Effects of single intraperitoneal injection of mCPP on the behavior of mice in elevated plus-maze test. Number of open arms entries (A), Time in open arms (s) (B), Entries to open arms/total arms (%) (C), Time in open arms/total arms (%) (D); Mean ± S.D., n = 4 - 6, $^*P < 0.05$, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

5.1.2. Effects of Single Intraperitoneal Injection of mCPP on the Behavior of Mice in Open Field Box

After the mice were intraperitoneally injected with mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) once for 30 min, the OFT was performed to test the anxiety of mice. The results showed (Figure 3) that the center zone distance was significantly shorter at dose 2 mg/kg ($P < 0.05$) and 4 mg/kg ($P < 0.01$) (Figure 3(B)), the number of entries to central zone was significantly lower at dose 2 mg/kg ($P < 0.05$) and 4 mg/kg ($P < 0.01$) (Figure 3(C)) in mice treated with mCPP than that of control mice.

5.1.3. Effects of Single Intraperitoneal Injection of mCPP on the Behavior of Mice in Hole-Board Test

After the mice were given a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 30 min, the HBT was used to observe the anxiety of mice. Comparing with the control group, the significant reduction of the head-dip counts was observed in the mice treated with 1 mg/kg ($P < 0.05$), 2 mg/kg ($P < 0.05$) and 4 mg/kg ($P < 0.01$) m-CPP (Figure 4(A)). The treatment with 2 mg/kg and 4 mg/kg m-CPP significantly increased head-dip latency of the mice ($P < 0.05$) compared to the control group (Figure 4(B)).

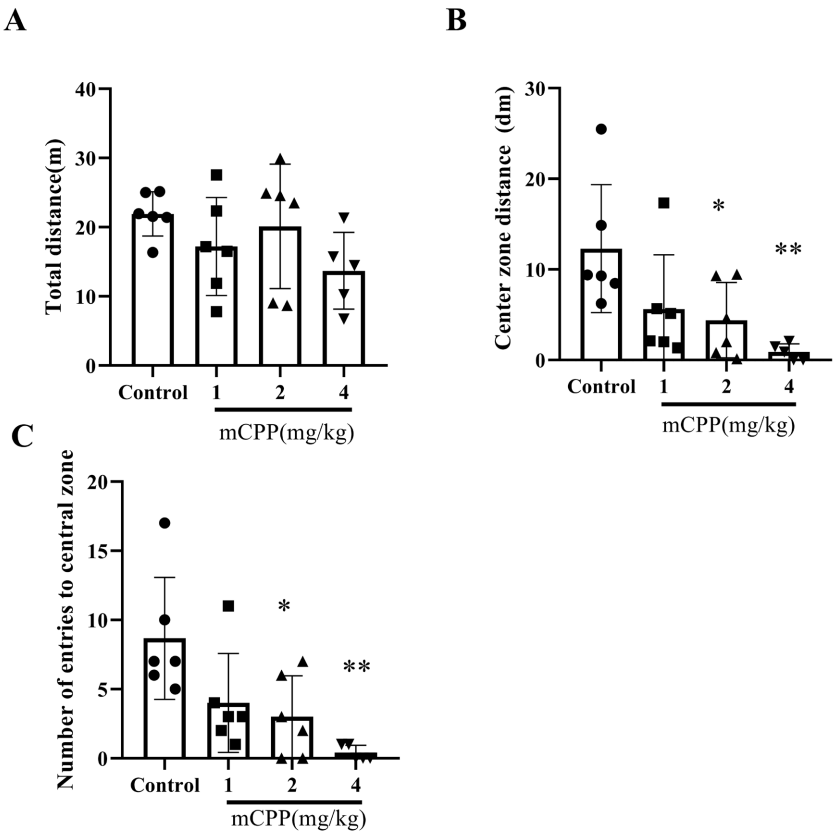

Figure 3. Effects of single intraperitoneal injection of mCPP on the behavior of mice in open field test. Total distance (A), Center zone distance (dm) (B), Number of entries to central zone (C); Mean ± S.D., n = 5 - 6, $^{*}P < 0.05$, $^{**}P < 0.01$ *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

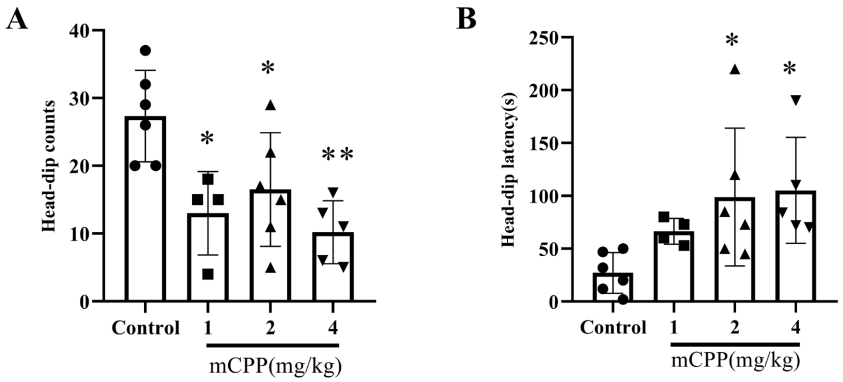

Figure 4. Effects of single intraperitoneal injection of mCPP on the behavior of mice in hole-board test. Head-dip counts (A), Head-dip latency (B); Mean ± S.D., n = 4 - 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

5.1.4. Effects of Single Intraperitoneal Injection of mCPP on the Behavior of Mice in Light-Dark Box

After the mice were administrated with a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 24 h, the LDB was carried out to evaluate the anxiety of mice. The results showed that there was no significant difference for the entry into the light side and the percentage of time in light side between groups treated with mCPP and control group (**Figure 5**).

5.2. Effects of Single Intraperitoneal Injection of mCPP on the Athletic Ability of Mice in Rotarod Test

After the mice were intraperitoneally injected with mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) once for 30 min, the rotarod test was used to estimate the athletic ability of mice. The results showed that the injected with m-CPP (1 mg/kg, 2 mg/kg, 4 mg/kg) did not affect the rotating time of the mice compared to the control group (**Figure 6**).

5.3. Effects of Single Intraperitoneal Injection of mCPP on Pain Sensation of Mice in Tail-Flick Test

After the mice were treated with single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 30 min, the tail-flick test was used to measure the pain sensation of mice. The results showed that there was no significant difference for the tail flick latency between groups treated with mCPP and control group (**Figure 7**).

5.4. Effects of Single Intraperitoneal Injection of mCPP on Passive Avoidance Response Ability of Mice in Step-Down Test

After the mice were administrated with a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 24 h, the step-down test was conducted to appraise the passive avoidance response ability of mice. The results showed that there was no significant difference for the number of errors and the step-down latency between groups treated with mCPP and control group (**Figure 8**).

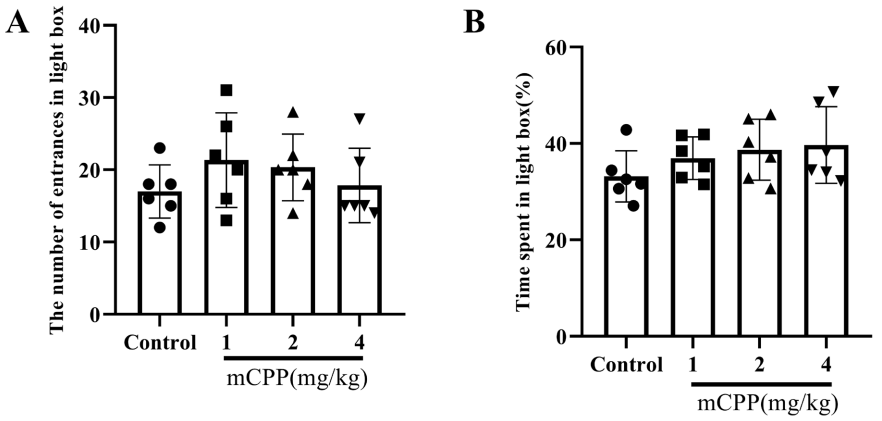

Figure 5. Effects of single intraperitoneal injection of mCPP on the behavior of mice in light-dark box test. The number of entrances in light box (A), Time spent in light box (%) (B); Mean ± S.D., n = 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

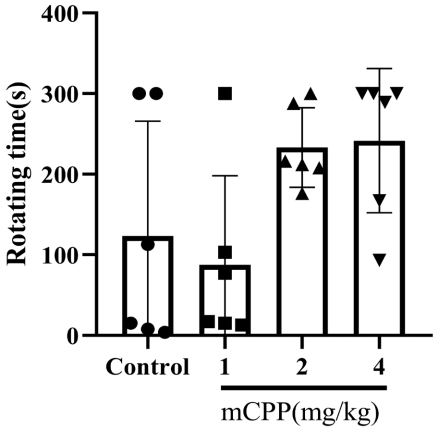

Figure 6. Effects of single intraperitoneal injection of mCPP on the behavior of mice in rotarod test. Mean ± S.D., n = 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

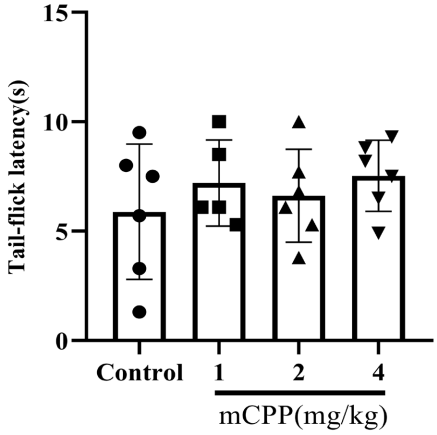

Figure 7. Effects of single intraperitoneal injection of mCPP on the behavior of mice in tail-flick test. Mean ± S.D., n = 5 - 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

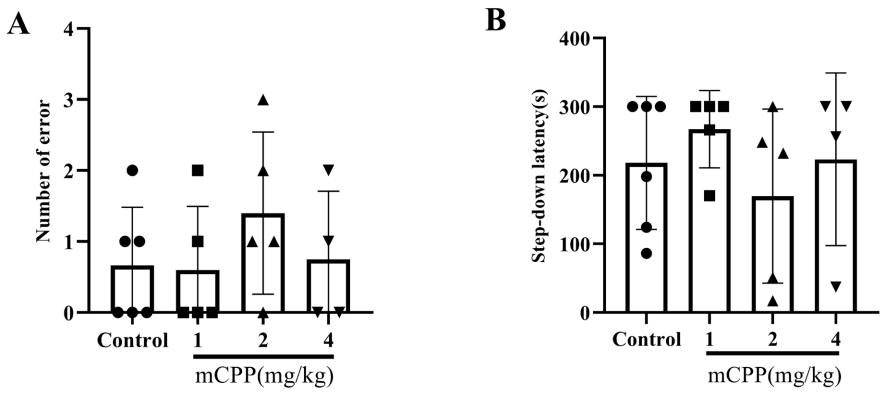

Figure 8. Effects of single intraperitoneal injection of mCPP on the behavior of mice in step-down test. Number of errors (A), Step-down latency (B); Mean ± S.D., n = 4 - 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphad Prism8.0.1 software.

5.5. Effects of Single Intraperitoneal Injection of mCPP on Depression Behavior of Mice in Tail Suspension Test

After the mice were given single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) for 30 min, the TST was used to observe the depression behavior of mice. The results showed that there was no significant difference for the immobility time and struggling time between groups treated with mCPP and control group (**Figure 9**).

6. Discussion

Since the pathogenesis of anxiety disorders is not well understood, it has been short of ideal anxiety animal models. This study showed that a single intraperitoneal injection of mCPP could induce anxiety behaviors with no effects on athletic ability, depression, pain and the ability of learning and memory. Therefore, it is a rapid, stable and specific anxiety model.

mCPP has been widely used with some success as a model of anxiety. The following studies all used EPM to evaluate the mCPP-induced ICR anxiety mouse model. Nan Zhang used a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) to treat male ICR mice and found a single intraperitoneal injection of mCPP 2 mg/kg caused a significant decrease in time in open arms/total arms (%) in mice. A single intraperitoneal injection of mCPP 4 mg/kg caused a significant decrease in time in open arms and time in open arms/total arms (%) of the mice [13]. Cui XY used a single intraperitoneal injection of mCPP (0.7 mg/kg) to model anxiety in male ICR mice, and it was found that time in open arms and time in open arms/total arms (%) were significantly reduced [19]. Mehmet Kurt used a single intraperitoneal injection of mCPP (2.5 mg/kg) to model anxiety in male ICR mice and found that time in open arms and time in open arms/total arms (%) were significantly reduced [18]. Takayoshi M used a single intraperitoneal injection of mCPP (2.5 mg/kg) to model anxiety in male ICR mice, and it was found that time in open arms/total arms (%) were

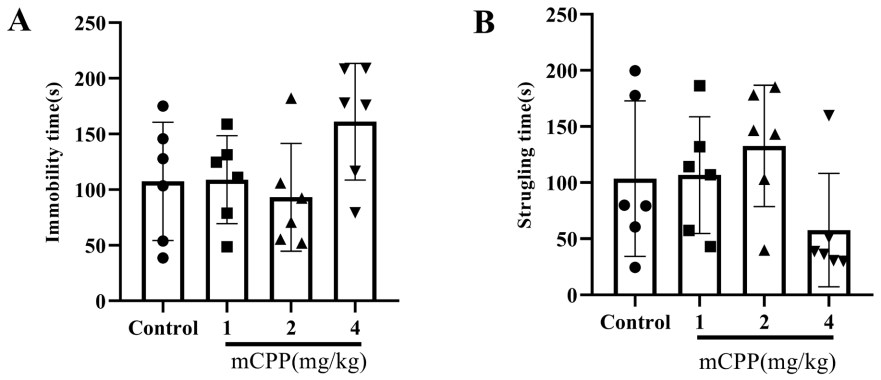

Figure 9. Effects of single intraperitoneal injection of mCPP on the behavior of mice in tail suspension test. Immobility time (A) Struggling time (B) Mean ± S.D., n = 6, *vs* Control, one-way ANOVA analysis followed by Dunnett's post hoc test, Graphpad Prism8.0.1 software.

significantly reduced [17]. The above studies suggest that a single intraperitoneal injection of mCPP (0.7 or 2 or 2.5 or 4 mg/kg) mCPP can cause anxiety in mice. In our study, male ICR mice given a single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) showed anxiety which was consistent with the results in the literature.

Compared with previous studies, in addition to using EPM, it was the first time we also used LDB, OFT, and HBT to evaluate the effects of a single intraperitoneal injection of mCPP on anxiety behavior in ICR mice. In the LDB, there was no significant change in the number of entrances in a light box and time spent in a light box, indicating that anxiety behavior disappeared 24 hours after a single intraperitoneal injection of mCPP in mice. This suggested it had a time-dependent effect for a single intraperitoneal injection of mCPP to induce anxiety. These results indicated that it is a rapid and stable anxiety model.

At present, there are no reports on the effects of mCPP on pain, athletic ability, passive avoidance response ability and depressive behavior in mice. In this study, we found mCPP has no effect on tail flick latency, rotating time, number of errors and the step-down latency, and the immobility time of mice in tail-flick test, rotarod test, step-down test and TST respectively. These results indicated that a single intraperitoneal injection of mCPP had no effect on pain, athletic capacity, ability to learn and memory and depressive behavior in mice, meaning this is a specific anxiety model.

mCPP is one of the first 5-HT receptor agonists used in psychiatry to examine the 5-HT receptor system [24], it is a metabolite of trazodone that crosses the blood-brain barrier [25]. m-CPP can bind to a variety of receptors, such as 5-HT1A, 5-HT1B, 5-HT2A, 5-HT2B, 5-HT2C, 5-HT3, histamine H1 and adrenergic α2A subtype receptors [26] [27] [28]. mCPP can aggravate clinical anxiety symptoms, including acute anxiety, panic disorder and agoraphobia [29]. A number of studies have shown that mCPP also induces anxiety responses in animal models [12] [18]. The anxiety-causing effect of mCPP is thought to be

mediated by the agonistic effect of 5-HT2C receptors [30] [32], and 5-HT2C receptor antagonists show anxiolytic effects in animal models [32]. This might be the mechanism of a single intraperitoneal injection of mCPP inducing rapid, stable and specific anxiety model.

At present, the anxiety mouse model induced by mCPP has been employed to study the effect of anti-anxiety drugs. For example, odor exposure of ylang-ylang essential oil reversed the anxiety based on this model [21]. Alianda found that intra-amygdaloid injection of mCPP increased anxiety-like behaviour in the mouse EPM, an effect that was completely blocked by local infusion of SDZ SER-082 (a preferential 5-HT2C receptor antagonist) [20]. Darin J Knapp found that CVT-10216 had no anti-anxiety effect in the mCPP model, but had anti-anxiety effect in repeated alcohol-withdrawal-induced anxiety and restraint stress-induced anxiety [33]. Pretreatment with 5-HT3 receptor antagonist N-cyclohexyl-3-methoxyquinoxalin-2-carboxamide (QCM-13) was unable to reverse anxiogenic effect of mCPP, but potentiated anxiolytic effect of buspirone [22]. Takayoshi Mamiya gave the soybean powder-added food pellets (soybean pellets) to investigate anti-anxious effects of soybean in male mice, they could not observe the m-CPP-induced anxiety-like behavior in mice fed soybean pellets in this test, and the results indicated that soybean pellets may attenuate anxiety-like behavior in mice [17].

In addition, the mCPP model has been used to study the anti-anxiety effects of non-pharmacological interventions. James H Fox found that exercise may help to reduce anxiety by down-regulating postsynaptic 5HT 2B/2C receptors using this model [34]. The mCPP model is also used to evaluate the relationship between anxiety and other functions. Hiroyuki Takamatsu studied the relationship between anxiety and brain function by PET measurement. They found that anxiety influences conscious brain function. Furthermore, the study suggests that the prevention of anxiety is important when measuring conscious brain function in monkeys [35]. As a recognized drug that can cause anxiety, mCPP is also used to evaluate the effectiveness of anxiety behavioral testing methods. Nicholas Jones used unstable elevated exposed plus maze (UEEPM) to detect the behavior of rats after taking mCPP, a drug known to cause anxiety, to study the predictive validity of UEEPM and its sensitivity to anxiogenic agents [36].

7. Conclusion

A single intraperitoneal injection of mCPP (1 mg/kg, 2 mg/kg, 4 mg/kg) can cause anxiety in mice, and has no effect on athletic ability, depression, pain, and passive avoidance response ability. A rapid, stable and specific anxiety mouse model can be constructed using a single intraperitoneal injection of mCPP.

Author Contributions

Xiaorui Cheng designed the study and modified the manuscript. Tianyuan Ye and Maijia Li carried out the specific studies. Tianyuan Ye and Maijia Li con-

tributed to writing articles and modifying the manuscript.

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Disclosure Statement

The authors report there are no competing interests to declare.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Calhoon, G.G. and Tye, K.M. (2015) Resolving the Neural Circuits of Anxiety. *Nature Neuroscience*, **18**, 1394-1404. https://doi.org/10.1038/nn.4101

[2] Kessler, R.C., Chiu, W.T., Dernier, O., Merikangas, K.R. and Walters, E.E. (2005) Prevalence, Severity, and Comorbidity of 12-Month DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, **62**, 617-627. https://doi.org/10.1001/archpsyc.62.6.617

[3] Kessler, R.C., Berglund, P., Demler, O., Jin, R., Merikangas, K.R. and Walters, E.E. (2005) Lifetime Prevalence and Age-of-Onset Distributions of DSM-IV Disorders in the National Comorbidity Survey Replication. *Archives of General Psychiatry*, **62**, 593-602. https://doi.org/10.1001/archpsyc.62.6.593

[4] Nasca, C., Orlando, R., Marchiafava, M., Boldrini, P., Battaglia, G., Scaccianoce, S., Matrisciano, F., Pittaluga, A. and Nicoletti, F. (2013) Exposure to Predator Odor and Resulting Anxiety Enhances the Expression of the $\alpha_2\delta$ Subunit of Voltage-Sensitive Calcium Channels in the Amygdala. *Journal of Neurochemistry*, **125**, 649-656. https://doi.org/10.1111/j.1471-4159.2012.07895.x

[5] Shin, S.Y., Baek, N.J., Han, S.H. and Min, S.S. (2019) Chronic Administration of Ketamine Ameliorates the Anxiety- and Aggressive-Like Behavior in Adolescent Mice Induced by Neonatal Maternal Separation. *The Korean Journal of Physiology & Pharmacology*, **23**, 81-87. https://doi.org/10.4196/kjpp.2019.23.1.81

[6] Stein, D.J., Vasconcelos, M.F., Albrechet-Souza, L., Cereser, K.M.M. and De Almeida, R.M.M. (2017) Microglial Over-Activation by Social Defeat Stress Contributes to Anxiety- and Depressive-Like Behaviors. *Frontiers in Behavioral Neuroscience*, **11**, Article 207. https://doi.org/10.3389/fnbeh.2017.00207

[7] Assad, N., Luz, W.L., Santos-Silva, M., Carvalho, T., Moraes, S., Picanco-Diniz, D.L.W., Bahia, C.P., Oliveira Batista, E.J., Da Conceicao Passos, A., Oliveira, K., *et al*. (2020) Acute Restraint Stress Evokes Anxiety-Like Behavior Mediated by Telencephalic Inactivation and GabAergic Dysfunction in Zebrafish Brains. *Scientific Reports*, **10**, Article No. 5551. https://doi.org/10.1038/s41598-020-62077-w

[8] Sun, X.L., Zhang, T.Z., Zhao, Y., Cai, E.B., Zhua, H.Y. and Liu, S.L. (2020) Panaxynol Attenuates CUMS-Induced Anxiety and Depressive-Like Behaviors via Regulating Neurotransmitters, Synapses and the HPA Axis in Mice. *Food & Function*, **11**, 1235-1244. https://doi.org/10.1039/C9FO03104A

[9] Wang, Y., Li, P., Zhang, L., Fu, J., Di, T., Li, N., Meng, Y., Guo, J. and Zhao, J. (2020) Stress Aggravates and Prolongs Imiquimod-Induced Psoriasis-Like Epidermal Hyperplasis and IL-1*β*/IL-23p40 Production. *Journal of Leukocyte Biology*, **10**, 267-281. https://doi.org/10.1002/JLB.3MA0320-363RR

[10] Zhang, Z.S., Qiu, Z.K., He, J.L., Liu, X., Chen, J.S. and Wang, Y.L. (2017) Resveratrol Ameliorated the Behavioral Deficits in a Mouse Model of Post-Traumatic Stress Disorder. *Pharmacology Biochemistry and Behavior*, **161**, 68-76. https://doi.org/10.1016/j.pbb.2017.09.004

[11] Yang, N., Ren, Z., Zheng, J., Feng, L., Li, D., Gao, K., Zhang, L., Liu, Y. and Zuo, P. (2016) 5-(4-Hydroxy-3-Dimethoxybenzylidene)-Rhodanine (RD-1)-Improved Mitochondrial Function Prevents Anxiety- and Depressive-Like States Induced by Chronic Corticosterone Injections in Mice. *Neuropharmacology*, **105**, 587-593. https://doi.org/10.1016/j.neuropharm.2016.02.031

[12] Mahdi, S., Almosawi, S., Baksh, H., Qareeballa, A., Alsaleh, B., Falamarzi, F., Alrabaani, M., Alkalbani, A. and Kamal, A. (2019) Effect of Chronic Administration and Withdrawal of Caffeine on Motor Function, Cognitive Functions, Anxiety, and the Social Behavior of BLC57 Mice. *International Journal of Health Sciences*, **13**, 10-16.

[13] Zhang, N., Zhang, L., Feng, L. and Yao, L. (2018) Cananga Odorata Essential Oil Reverses the Anxiety Induced by 1-(3-Chlorophenyl) Piperazine through Regulating the MAPK Pathway and Serotonin System in Mice. *Journal of Ethnopharmacology*, **219**, 23-30. https://doi.org/10.1016/j.jep.2018.03.013

[14] Nehlig, A., Daval, J.L. and Debry, G. (1992) Caffeine and the Central Nervous System: Mechanisms of Action, Biochemical, Metabolic and Psychostimulant Effects. *Brain Research Reviews*, **17**, 139-170. https://doi.org/10.1016/0165-0173(92)90012-B

[15] De Rezende, V.B., Rosa, D.V., Comim, C.M., Magno, L.A., Rodrigues, A.L., Vidigal, P., Jeromin, A., Quevedo, J. and Romano-Silva, M.A. (2014) NCS-1 Deficiency Causes Anxiety and Depressive-Like Behavior with Impaired Non-Aversive Memory in Mice. *Physiology & Behavior*, **130**, 91-98. https://doi.org/10.1016/j.physbeh.2014.03.005

[16] Onodera, T., Sakudo, A., Tsubone, H. and Itohara, S. (2014) Review of Studies That Have Used Knockout Mice to Assess Normal Function of Prion Protein under Immunological or Pathophysiological Stress. *Microbiology and Immunology*, **58**, 361-374. https://doi.org/10.1111/1348-0421.12162

[17] Mamiya, T., Asanuma, T., Kawai, Y., Hasegawa, Y., Nishimura, A., Kumazawa, T. and Ukai, M. (2006) Effects of Soybean Food Pellets on M-CPP-Induced Anxiety Model of Mice. *Biological and Pharmaceutical Bulletin*, **29**, 1498-1500. https://doi.org/10.1248/bpb.29.1498

[18] Kurt, M., Bilge, S.S., Kukula, O., Celik, S. and Kesim, Y. (2003) Anxiolytic-Like Profile of Propofol, A General Anesthetic, in the Plus-Maze Test in Mice. *Polish Journal of Pharmacology*, **55**, 973-977.

[19] Cui, X.Y., Zhao, X., Chu, Q.P., Chen, B.Q. and Zhang, Y.H. (2007) Influence of Diltiazem on the Behavior of Zolpidem-Treated Mice in the Elevated-Plus Maze Test. *Journal of Neural Transmission*, **114**, 155-160. https://doi.org/10.1007/s00702-006-0535-1

[20] Cornelio, A.M. and Nunes-De-Souza, R.L. (2007) Anxiogenic-Like Effects of MCPP Microinfusions into the Amygdala (but Not Dorsal or Ventral Hippocampus) in Mice Exposed to Elevated Plus-Maze. *Behavioural Brain Research*, **178**, 82-89.

https://doi.org/10.1016/j.bbr.2006.12.003

[21] Yonezawa, A., Yoshizumi, M., Ebiko, M., Ise, S., Watanabe, C., Mizoguchi, H., *et al*. (2008) Ejaculatory Response Induced by a 5-HT2 Receptor Agonist m-CPP in Rats: Differential Roles of 5-HT2 Receptor Subtypes. *Elsevier Pharmacology Biochemistry and Behavior*, **88**, 367-373. https://doi.org/10.1016/j.pbb.2007.09.009

[22] Gupta, D., Radhakrishnan, M., Thangaraj, D. and Kurhe, Y. (2015) Pharmacological Evaluation of Novel 5-HT3 Receptor Antagonist, QCM-13 (N-Cyclohexyl-3-Methoxyquinoxalin-2-Carboxamide) as Anti-Anxiety Agent in Behavioral Test Battery. *Journal of Pharmacy and Bioallied Sciences*, **7**, 103-108. https://doi.org/10.4103/0975-7406.154429

[23] Benjamin, D., Lal, H. and Meyerson, L.R. (1990) The Effects of 5-HT1B Characterizing Agents in the Mouse Elevated Plus-Maze. *Life Sciences*, **47**, 195-203. https://doi.org/10.1016/0024-3205(90)90320-Q

[24] Kahn, R.S. and Wetzler, S. (1991) M-Chlorophenylpiperazine as a Probe of Serotonin Function. *Biological Psychiatry*, **30**, 1139-1166. https://doi.org/10.1016/0006-3223(91)90184-N

[25] Rurak, A. and Melzacka, M. (1983) Effect of Dosage and Route of Administration of Trazodone on Cerebral Concentration of 1-M-Chlorophenylpiperazine in Rats. Kinetics of Trazodone Biotransformation in Rats. *Polish Journal of Pharmacology*, **35**, 241-247.

[26] Kreiss, D.S. and De Deurwaerdere, P. (2017) Purposeless Oral Activity Induced by Meta-Chlorophenylpiperazine (M-CPP): Undefined Tic-Like Behaviors? *Journal of Neuroscience Methods*, **292**, 30-36. https://doi.org/10.1016/j.jneumeth.2017.05.007

[27] Tucci, M.C., Dvorkin-Gheva, A., Johnson, E., Wong, M. and Szechtman, H. (2015) 5-HT2A/C Receptors Do Not Mediate the Attenuation of Compulsive Checking by MCPP in the Quinpirole Sensitization Rat Model of Obsessive-Compulsive Disorder (OCD). *Behavioural Brain Research*, **279**, 211-217. https://doi.org/10.1016/j.bbr.2014.11.017

[28] Khaliq, S., Haider, S., Saleem, S., Memon, Z. and Haleem, D.J. (2012) Influence of Serotonergic 5-HT2C Receptor Antagonist Mesulergine in the Reversal of Memory Deficits Induced by MCPP. *Journal of College of Physicians and Surgeons Pakistan*, **22**, 75-79.

[29] Charney, D.S., Woods, S.W., Goodman, W.K. and Heninger, G.R. (1987) Serotonin Function in Anxiety. II. Effects of the Serotonin Agonist MCPP in Panic Disorder Patients and Healthy Subjects. *Psychopharmacology*, **92**, 14-24. https://doi.org/10.1007/BF00215473

[30] Wood, M.D. (2003) Therapeutic Potential of 5-HT2C Receptor Antagonists in the Treatment of Anxiety Disorders. *Current Drug Targets—CNS & Neurological Disorders*, **2**, 383-387. https://doi.org/10.2174/1568007033482698

[31] Campbell, B.M. and Merchant, K.M. (2003) Serotonin 2C Receptors within the Basolateral Amygdala Induce Acute Fear-Like Responses in an Open-Field Environment. *Brain Research*, **993**, 1-9. https://doi.org/10.1016/S0006-8993(03)03384-5

[32] Fiorella, D., Helsley, S., Rabin, R.A. and Winter, J.C. (1995) 5-HT2C Receptor-Mediated Phosphoinositide Turnover and the Stimulus Effects of M-Chlorophenylpiperazine. *Psychopharmacology*, **122**, 237-243. https://doi.org/10.1007/BF02246545

[33] Overstreet, D.H., Knapp, D.J., Breese, G.R. and Diamond, I. (2009) A Selective ALDH-2 Inhibitor Reduces Anxiety in Rats. *Pharmacology Biochemistry and Behavior*, **94**, 255-261. https://doi.org/10.1016/j.pbb.2009.09.004

[34] Fox, J.H., Hammack, S.E. and Falls, W.A. (2008) Exercise Is Associated with Reduction in the Anxiogenic Effect of MCPP on Acoustic Startle. *Behavioral Neuroscience*, **122**, 943-948. https://doi.org/10.1037/0735-7044.122.4.943

[35] Takamatsu, H., Noda, A., Murakami, Y., Tatsumi, M., Ichise, R. and Nishimura, S. (2003) A PET Study after Treatment with an Anxiety-Provoking Agent, M-Chlorophenyl-Piperazine, in Conscious Rhesus Monkeys. *Journal of Nuclear Medicine*, **44**, 1516-1521.

[36] Jones, N., Duxon, M.S. and King, S.M. (2002) Ethopharmacological Analysis of the Unstable Elevated Exposed plus Maze, A Novel Model of Extreme Anxiety: Predictive Validity and Sensitivity to Anxiogenic Agents. *Psychopharmacology*, **161**, 314-323. https://doi.org/10.1007/s00213-002-1029-y