Ashdin Publishing
Journal of Drug and Alcohol Research
Vol. 13 (2024), Article ID 236300, 08 pages
DOI: 10.4303/JDAR/236300

Research Article

Anxiety-like Behavior Increases after Recent, but not Prenatal, Cannabis Exposure

Francisca Bertin[1,2*], Gonzalo Miguez[1], Mario A. Laborda[1], Vanetza Quezada-Scholz[1], Felipe Alfaro[3], Viviana Sáez[1], Matías González[1], Angélica Buendía[1]

[1]*Departamento de Psicología, Universidad de Chile, Santiago, Chile*
[2]*Facultad de Medicina Veterinaria y Agronomía, Universidad de Las Américas, Santiago, Chile*
[3]*Departamento de Ciencias Sociales y Humanidades, Universidad de Aysén, Coyhaique, Chile*

[*]*Address Correspondence to Francisca Bertin, E-mail: francisca.bertin@uchile.cl*

Received: 01 May 2024; Manuscript No: JDAR-24-137282; **Editor assigned:** 03 May 2024; PreQC No: JDAR-24-137282 (PQ); **Reviewed:** 17 May 2024; QC No: JDAR-24-137282; **Revised:** 22 May 2024; Manuscript No: JDAR-24-137282 (R); **Published:** 29 May 2024; **DOI:** 10.4303/JDAR/236300

Abstract

Cannabis consumption is globally prevalent, and its gestation use has increased despite the unclear psychological effects, such as anxiety and risk behaviors.

Objective: To determine the effects of prenatal and recent cannabis exposure on anxiety-like behaviors.

Methodology: Sprague-Dawley rats (both sexes) were used, with (P+) or without (P+) a prenatal exposure to cannabis. Post-weaning, litters were subdivided into groups with (R+) or without (R-) recent cannabis exposure before testing. Anxiety-like behavior was assessed in an elevated plus maze by quantifying entries and time spent in each zone. Results: The R+ condition increased entries in the center of the maze and tended to increase entries in the closed arms.

Discussion: We found that R+ has at least partially anxiogenic effects by increasing risk assessment behaviors in a novel environment. Considering cannabis high consumption, future research should explore the long-term effects of both exposure conditions on anxiety and other psychological aspects.

Keywords: Cannabis; Prenatal exposure delayed effects; Anxiety; Risk-taking; Animal models

Introduction

Cannabis is widely recognized as one of the most commonly used drugs, with increasing consumption observed during pregnancy. This tendency persists despite the lack of consensus regarding its impact on mental health [1–4]. This stresses significance as THC (Δ9-tetrahydrocannabinol, the primary psychoactive compound in cannabis), influences the endocannabinoid system, which regulates various psychological processes, including anxiety [5]. Given these uncertainties and the global prevalence of consumption, it is pertinent to investigate cannabis's effects on anxiety across 3 dimensions:

1. Prenatal exposure to cannabis (P), considering its increased use during pregnancy, potentially exposing the foetuses to the drug [6,7].
2. Recent exposure to cannabis (R), level of study on which the cannabis literature tends to be focused; and
3. the potential interactive effects of both P and R conditions, an area underexplored despite escalating cannabis consumption among the general populace and pregnant women.

Anxiety is a complex psychological phenomenon that primes an organism to respond to threats, potential harm, or uncertainty through a range of behavioral, psychological, and physiological reactions [8,9]. Anxiety also impacts other processes such as risk behavior and decision-making, suggesting a direct relationship between the level of anxiety and these processes [10–13]. Although anxiety inherently serves an adaptive purpose, prolonged periods of heightened anxiety or exaggerated responses can significantly impact mental well-being [14]. The endocannabinoid system plays a crucial role in emotional processing in stress and anxiety, as well as influencing their associated behavioral response [5,15]. This has been assessed through various experimental approaches. For example, Cannabinoid Receptor Type 1 (CB1) knock-out mice evidenced an increase in anxiety and risk assessment behavior (known as the behavior of acquiring information in a potentially risky environment) in an anxiety task (elevated plus maze, described below), compared to the heterozygous and wild type [16]. However, scientific evidence regarding the relationship between cannabis and anxiety presents conflicting findings. While some researchers advocate for cannabis as a therapeutic option for managing anxiety disorders, others contend that the drug may exacerbate anxiety symptoms [17,18].

Various methodological approaches are employed to assess anxiety in both humans and animals. In human studies, anxiety is predominantly evaluated through subjective reports (e.g., STAI) [19]. Conversely, animal studies often rely on physiological measures or the observation of anxiety-like behaviors [14]. Animal models of anxiety complement human studies, providing the advantage of allowing the study of several aspects of anxiety or anxiety like-behaviors under controlled conditions, contributing significantly to research in diverse fields such as psychology and pharmacology [20,21]. One widely utilized behavioral test in rodent models is the elevated plus maze. This apparatus comprises a raised maze shaped like a cross, with 2 enclosed arms, 2 open arms, and a central area [22]. Leveraging on rodents' natural aversion to brightly lit and exposed areas, along with the height of the maze, the open arms induce an anxiogenic response in the animals, allowing the assessment of innate anxiety-like behavior provoked by this novel and anxiety-inducing environment [22,23]. Additionally, this test allows for the assessment of other anxiety-related behaviors or reactions such as risk assessment and their influence on decision-making, which are measured by their activity in the center of the maze and the conflict in approaching towards or avoiding the open arms [24–26]. Parameters assessed include the percentage of time spent and number of entries into each zone (center, open arms, and closed arms), where decreased values in the open arms are indicative of higher anxiety levels [27]. Thus, this test is frequently employed to investigate the potential anxiolytic or anxiogenic effects of pharmacological agents [27,28].

Regarding the consequences of prenatal exposure to cannabis on anxiety, human studies report that the offspring of mothers who consumed cannabis during pregnancy often exhibit a higher frequency of anxiety; with the severity of these effects seemingly contingent upon the dosage and timing of cannabis consumption [29,30]. However, these studies often rely on longitudinal reports, where results have been inconsistent, therefore a consensus on the prenatal effects of this drug has not been reached [31–33]. Moreover, interpreting the effects of prenatal cannabis in human studies is often complicated by the co-occurring drug consumption during pregnancy, the gestational period during which consumption, and underlying health inequities and socio-economic disparities [29,34,35]. Animal models allow the study of prenatal exposure to drugs on anxiety in an ethical manner, enabling control over environmental and nutritional variables for both the offspring and mothers, as well as providing consistent drug composition, dose, and administration route [36,37]. Like humans, animal models have suggested an increase in anxiety-like behaviors following prenatal cannabis exposure. Nonetheless, conflicting findings persist, likely due to methodological variations such as task type and route of drug administration [23,38,39]. These antecedents show that are contradictions regarding the consequences of prenatal cannabis on anxiety and more studies are required to comprehend these effects.

On the other hand, recent exposure to cannabis has shown to have a bimodal effect (anxiolytic/anxiogenic) in both humans and animals, depending on the dosage and timing of consumption [40,41]. However, the meta-analysis by Sharpe, et al. has proposed that clinical studies in humans show that THC consumption reports a common anxiogenic effect [18]. In contrast, Fokos and Panagis observed in rats that acute administration of THC at any dose has anxiolytic effects; however, in stressed animals an anxiolytic effect was observed at high dose and anxiogenic at low dose [42]. These discrepancies reveal the importance of further research into the effects of cannabis exposure, particularly in determining these effects in subjects with fetal exposure to the drug.

This study aims to investigate the effects of prenatal and recent exposure to cannabis on anxiety-like behavior (including risk assessment) using the elevated plus maze. Offspring of pregnant rats exposed to either a cannabis solution (P+ condition) or vehicle (P-condition) were utilized. After weaning, each litter was divided into 2 groups to facilitate subsequent assessment of recent exposure condition. Prior to testing, all experimental subjects were exposed to either cannabis (R+ condition) or vehicle (R-condition), resulting in the formation of 4 experimental groups (P-/R-, P+/R-, P-/R+, P+/R+). We hypothesize that the P+ condition will increase anxiety-like behavior in the elevated plus maze (indicated by increased time and entries to the closed arms), while the R+ condition will reduce it (resulting in more time and entries to the open arms), and the simultaneous presence of both P+R+ conditions will produce a medium (i.e., additive) effect, similar to the control condition.

Methods

Subjects

A total of 71 Sprague Dawley rats of both sexes were used across 5 experimental replications, each sourced from different mother, fathers and dams. These animals were descendants of parental pairs obtained from the animal facility at the Faculty of Chemical and Pharmaceutical Sciences, University of Chile. Upon arrival at the laboratory, the animals were kept on a 12/12 light/dark cycle in the animal facility at the Experimental Psychology Laboratory: Prof. Ronald Betancourt Mainhard, Faculty of Social Sciences, University of Chile. To synchronize gestations, the parental pairs were kept together for 5 nights to mate, after which the females were housed in pairs and exposed daily to vaporized cannabis or vehicle until parturition (approximately 21 days of vaporization). After birth, all litters were culled to 8 pups per mother, consisting of 4 females and 4 males. From weaning (PND21), all experimental animals (pups) were housed in same-sex pairs. To habituate the animals to handling, they were manipulated for 1 minute, 3 times a week, both experimental and parental animals. All experimental subjects underwent the anxiety test on postnatal day 28. It is important to note that the experimental subjects were naive to both the experiment and the treatment (R+). Details of these methods were pre-registered (https://doi.org/10.17605/OSF.IO/MKSP8).

Study design

This study used a 2 × 2 factorial experimental design, with prenatal and recent cannabis exposure conditions as between-subjects factors. Anxious-like behavior was measured using an elevated plus maze by recording the number of entries and the percentage of time spent in each zone (open arms, closed arms, and center).

Materials

Cannabis: The cannabis extract was obtained from a confidential local laboratory in Chile (name under a non-disclosure agreement). This extract contained 40 mg/ml THC, diluted in ethanol. For both prenatal and recent exposure procedures, a total volume of 250 μl (10 mg THC) per session was vaporized.

Vaporization apparatus: Two Volcano Classic vaporizers (Storz and Bickel GmbH) were used for the vaporized administration of the cannabis solution or vehicle, respectively.

Elevated plus maze: A custom-made maze was used, with acrylic walls and floor, supported on an 80 cm high wooden stand. All walls and floor of the maze were black. The arms of the maze were 40 cm long and 9 cm wide, and the closed arms also had 20 cm high walls, which prevented the subjects from being exposed to the outside. To prevent the subject from falling from the open arms, a small 0.5 cm plastic barrier was placed. A Razer Kiyo camera was used for behavioral recordings, which also provided illumination via an LED ring light.

Procedures

Cannabis exposure: Prenatal and recent exposure to cannabis were conducted using the same method validated by Manwell, et al. (2014) who observed similar THC blood concentrations using this method and drug dose compared to 1.5 mg of THC administered intraperitoneally [43]. This pulmonary administration also replicates the most common administration route used by cannabis consumers. In each session, a 10 mg THC loaded pad was used, following the protocol of Nelong, et al. (2019) and Hamidullah, et al. (2021), who observed several effects on behavior and brain activity [44,45]. Specifically, the day before each session, a pad was loaded with 250 μl of cannabis extract (containing 10 mg of THC) or vehicle (95% ethanol), allowing the ethanol to evaporate overnight from both solutions. On the day of the session, the pads were placed in the Volcano vaporizer, which was then heated to 226°C (level 9). Once reached this temperature, the solutions were vaporized, and the vapor was captured in 8 L balloons. After the balloon was filled, the vapor was immediately released into a sealed plastic box containing the subjects, where they were exposed to vapor for 5 minutes.

Anxiety task: The anxiety test was conducted in a single session, without prior training, in a dark room illuminated by the camera's LED light ring. The test began by placing the subject in the center of the maze, facing an open arm. All behaviors were recorded using a camera positioned perpendicular to the center of the maze for 5 minutes [46]. From these recordings, the percentage of time spent and the number of entries into each zone (open arms, closed arms, center of the maze) were measured.

Data analysis

Coding: Data processing and behavioral coding was performed using Ethovision XT 16 software, which automatically quantifies the permanence time and number of entries to each zone of the maze (open arms, closed arms and center). From these data, the percentage of time spent in each zone (time in a zone/total time) was quantified.

Statistical analysis: The data were analyzed using factorial ANOVAs in Jamovi and Statistica 12. The effects of Prenatal (P) and Recent (R) cannabis exposure conditions on anxiety-like behavior were evaluated using the elevated plus maze, with measurements including the percentage of time spent and the number of entries in each zone. A significance level of α=.05 was employed for all analyses, with effect sizes reported using η^2p. Mean Square Error (MSE) was reported for variables showing main or marginal effects, and post hoc power analyses were conducted for marginal effects to assess their statistical significance.

Ethical aspects: All experimental procedures were approved by the Institutional Animal Care and Use Committee of the University of Chile (21451-FCS-UCH).

Results

Anxiety-like behavior in the elevated plus maze was assessed by quantifying the number of entries and the percentage of time spent in each zone of the maze (open arms, closed arms, and center) in subjects approximately 28 days of age. Regarding the number of entries, it was determined that the R+ condition generates an increase in the number of entries to the center of the maze, $F(1,67)=9.6$, $p=.0028$, MSE=236.2, $\eta^2p=0.12$ (Figure 1). No differences in this variable were observed for the P+ condition ($F=0.01$, $p=0.9$, $\eta^2p<0.001$), nor was there an interaction between the P and R conditions ($F=0.7$, $p=.393$, $\eta^2p=0.011$).

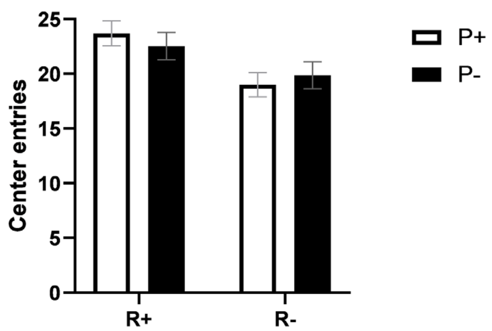

Figure 1: Recent cannabis exposure increases the number

of entries into the center of the maze: The number of entries through the center of the maze was compared between the P+ and R+ conditions. The R+ condition had a main effect of increasing this variable, whereas the P+ condition or the simultaneous presence of both conditions had no difference. White bars represent the P+ condition (with prenatal cannabis exposure), while black bars represent the P- condition (without prenatal cannabis exposure). Both bars are ordered under their respective R condition, where R+ represent with a recent exposure to cannabis and R- without a recent exposure. Error bars represent the standard error. Abbreviations: P+: With prenatal cannabis exposure; P-: Without prenatal cannabis exposure; R+: With recent cannabis exposure; R-: Without recent cannabis exposure

Regarding entries into the closed arms, a marginal increase was observed for the R+ condition, F (1,67)=3.1, p=.079, MSE=55.7, η^2p=0.045 (Figure 2). A power analysis detected a power of .43. No differences were observed for the P+ condition (F=0.8, p=.325, η^2p=0.013), and no interaction between variables was evident (F=0.5, p=.479, η^2p<0.001).

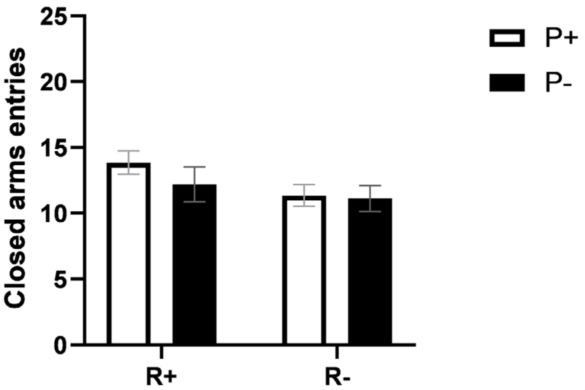

Figure 2: Recent cannabis exposure marginally increases the number of entries in the closed arms of the maze. The number of entries per closed maze arm was compared between the P+ and R+ conditions. The R+ condition had a marginally increasing effect on this variable, while the P+ condition or the simultaneous presence of both conditions had no difference. White bars represent the P+ condition (with prenatal cannabis exposure), while black bars represent the P- condition (without prenatal cannabis exposure). Both bars are ordered under their respective R condition, where R+ represent with a recent exposure to cannabis and R- without a recent exposure. Error bars represent the standard error.

Finally, the number of entries into the open arms was evaluated, and no significant effects were observed for either the R+ or P+ conditions, nor was there any interaction between the variables (all p>.232; graph not shown).

As for the variable percentage of time spent in each arm, no significant effects were observed for either the R+ or P+ conditions, nor was there any interaction between the variables in any of the maze zones (all p>.139; graphs not shown).

Discussion

Our findings show that the R+ condition increased entries to the center and tended to increase entries to the closed arms of the maze, independent of the prenatal exposure condition. Interestingly, the R+ effects were only observed for the number of entries, but not for the percentage of time spent in each zone of the maze. This suggests that these subjects increased their exploration of the less exposed zones of the maze but maintained similar times spent in each zone. These findings are interesting but do not support our hypothesis, particularly due to the absence of an effect in the P+ condition. Additionally, while effects were detected for the R+ condition, they differed from the original hypothesis. These results will be discussed further below. Finally, as proposed, no differences were observed between the control group (P-/R-) and those subjects with both exposure modalities simultaneously (P+/R+), behaving both in a similar manner.

To understand these results, it is important to consider the characteristics of the task used. The elevated plus maze measures a subject's emotional behavior, such as anxiety, through exploratory performance, where avoidance of the open arms is interpreted as anxiety-like behavior. Additionally, these changes in activity patterns within the maze reflect risk assessment behaviors in a novel situation, behaviors that have shown a high sensitivity to anxiolytic/anxiogenic drugs [23,47-49]. In this apparatus, both the closed arms and the center of the maze can be considered 'protected' areas compared to the open arms [50–52]. Thus, behaviors evaluated in the center of the maze are usually related to novelty seeking and risk assessment, with this sector acting as a 'decision point' where the subject faces the conflict of whether to approach or avoid exploring the open arms, processes which reflects elements of anxiety [26,53,54]. Considering this, and that anxious responses include apprehension and modifications in decision-making under conditions of uncertainty, it is suggested that the increased entries into the center zone in the R+ condition are associated with greater conflict in avoiding exposed zones compared to the other exposure conditions [55,56].

The increase in entries into the center in the R+ condition was observed alongside a tendency to increase entries into the closed arms of the maze. This close arm entry behavior reflects greater exploration of these protected areas, which are usually considered less anxiogenic than the open arms due to their lack of exposure to the outside [47]. Taken together, these results support the proposition that the R+ condition modifies the pattern of motor behavior in the maze, reflecting a conflict in avoiding the exposed area while increasing the transit of subjects through the safer or less risky areas of the maze.

Interestingly, despite the observed differences in the number of entries for the R+ condition, no differences were observed in the percentage of time spent in each arm for any conditions or their interactions. This indicates that the effects of R+ are mainly on the pattern of motor activity, rather than on the time in each zone. These findings suggest that the R+ condition generate partial effects on anxiety, increasing transit through the safer or less risky zones of the maze, where the conflict about entering the unprotected zone seems to decrease during the test, as no differences were observed in the variables measured in the open arms. Different results could be detected by analyzing chronological differences in these variables during specific time segments (e.g., minutes) of the test, allowing a deeper exploration of risk avoidance and risk assessment behavior. Such an analysis would increase sensitivity to detect changes in behavioral states, as used by Casarrubea, et al. (2015) [57]. Additionally, future studies should complement this analysis with other behavioral variables of anxiety and risk assessment, such as the number of stretch attempts and head dips [58].

In reference to the absence of changes in the P+ condition, it's interesting to note that although human and animal studies have suggested greater anxiety in the offspring of consuming mothers, the lack of observable effects in our study could be attributed to specific aspects of the task employed. Weimar, et al. (2020) discuss that while this task is regularly used to assess anxiety, its results also allow for the assessment of other aspects of this variable, such as differences in risk assessment behaviors [23]. These behaviors may differ from the anxiety domain present in human offspring of consumptive mothers. Another aspect that may differ from an increased anxiety is the age when the subjects of this study were tested.

For this study, the age of the subjects (average 28 days) could be a potential factor influencing the discrepancy between the proposed hypothesis and the obtained results. Anxiety is differentially expressed at different stages of postnatal development, so the sensitivity to detect these behaviors using the elevated plus maze could be affected by age [59]. Previous research using this same task has shown differences in the expression of these behaviors between adolescent and adult rats, which varied according to the experimental manipulation, suggesting that these results could be due to differences in neurodevelopmental stages and anxiety regulation [60]. In reference to anxiety and P+, Weimar, et al. (2020) using the elevated plus maze, determined that P+ generates an increase in anxiety-like behavior that is observable when the offspring are adults (73 days old) but not when they are juveniles (29 days old) [23]. Their results are consistent with the lack of effects in the P+ condition observed in this study and suggest that the determination of the consequences of P+ on anxiety is sensitive to the period in which they are evaluated.

A limitation of this study was not acknowledging anxiety as a multifaceted construct, and that our assessment covered only the behavioral dimension of anxiety. Future studies could address this limitation by complementing these measures with physiological variables such as heart rate. Additionally, it's important to note that while the effects of the drug were not observed in all behavioral measures of anxiety, the lack of effects does not necessarily indicate their absence. Another potential limitation of this study is the inability to measure plasma THC concentrations, however, the administration protocol and drug dose employed were selected based on vast literature supported by behavioral and physiological evidence of its efficiency [44,45,61].

Conclusion

In conclusion, the R+ condition, but not the P+ condition, generates a partial effect on anxiety by increasing the pattern of motor activity in the elevated plus maze. This pattern change is associated with avoidance and conflict in entering the unprotected zones of the maze, without impacting the time spent in all zones, suggesting that recent cannabis exposure has partial anxiogenic effects that may alter risk assessment and other anxiety related processes in a novel environment. Given the rising cannabis use among the general population and pregnant women, coupled with the significant impact of anxiety on mental health, it is crucial to continue studying the effects of cannabis use on anxiety.

Funding and Acknowledgment

This experiment was funded by the National Agency for Research and Development (ANID-Chile) through Fondecyt grant #3210212 awarded to F.B. G.M. was supported by Fondecyt project #1220797. M.A.L. was supported by Fondecyt grant #1191619. F.A. was supported by Fondecyt grant #3200226. V.Q-S was supported by Fondecyt grant #11170143. Also, we thank Felipe I. Varas, Jonathan Badilla, and Kasely Esteban, who are currently involved in the coding associated with this project.

Conflict of Interest

Authors declare no conflict of interest.

References

1. K. Cohen, A. Weizman, A. Weinstein, Positive and negative effects of cannabis and cannabinoids on health, Clin Pharmacol Ther, 105(2019):1139-1147.

2. D. Hammond, S. Goodman, E. Wadsworth, V. Rynard, C. Boudreau, et al. Evaluating the impacts of cannabis legalization: The International cannabis policy study, Int J Drug Policy, 77(2020):102698.

3. L. Korn, D.L. Haynie, J.W. Luk, B.G. Simons-Morton, Prospective associations between cannabis use and negative and positive health and social measures among emerging adults, Int J Drug Policy, 58(2018):55-63.

4. S. Zarei, Y. Salimi, E. Repo, N. Daglioglu, Z. Safaei, et al. A global systematic review and meta-analysis on illicit drug consumption rate through wastewater-based epidemiology, Environ Sci Pollut Res, 27(2020):36037–51.

5. R. Maldonado, D. Cabañero, E. Martín-García, The endocannabinoid system in modulating fear, anxiety, and stress, Dialogues Clin Neurosci, 22(2020):229–39.

6. Y.L. Hurd, Cannabis and the developing brain challenge

risk perception, J Clin Invest, 130(2020):3947-3949.

7. S.J. Weisbeck, K.S. Bright, C.S. Ginn, J.M. Smith, K.A. Hayden, et al. Perceptions about cannabis use during pregnancy: A rapid best-framework qualitative synthesis, Can J Public Health, 12(2020):49-59.
8. M. La-Vu, B.C. Tobias, P.J. Schuette, A. Adhikari, et al. To approach or avoid: An introductory overview of the study of anxiety using rodent assays. Front Behav Neurosci, 14(2020):566016.
9. T. Steimer, The biology of fear-and anxiety-related behaviors, Dialogues Clin Neurosci, 4(2002):231.
10. S.J. Bishop, C. Gagne, Anxiety, depression, and decision making: A computational perspective, Annu Rev Neurosci, 41(2018):371–388.
11. K. Haegler, R. Zernecke, A.M. Kleemann, J. Albrecht, O. Pollatos, et al. No fear no risk! Human risk behavior is affected by chemosensory anxiety signals, Neuropsychologia, 48(2010):3901–3908.
12. M.A. Soleimani, S. Pahlevan Sharif, N. Bahrami, A. Yaghoobzadeh, K.A. Allen, et al. The relationship between anxiety, depression and risk behaviors in adolescents, Int J Adolesc Med Health, 31(2019):20160148.
13. M.T. Tull, A.C. Barbano, K.M. Scamaldo, J.R. Richmond, K.A. Edmonds, et al. The prospective influence of COVID-19 affective risk assessments and intolerance of uncertainty on later dimensions of health anxiety, J Anxiety Disord, 75(2020):102290.
14. G.N. Petrie, A.S. Nastase, R.J. Aukema, M.N. Hill, Endocannabinoids, cannabinoids and the regulation of anxiety, Neuropharmacology, 195(2021):108626.
15. R.M. Cysneiros, F.T. Ribeiro, The endocannabinoid system: Signaling and social motivation, Neurobiol Physiol Endocannabinoid Syst, (2023):469–478.
16. D. Soriano, A. Brusco, L. Caltana, Further evidence of anxiety-and depression-like behavior for total genetic ablation of cannabinoid receptor type 1, Behav Brain Res, 400(2021):113007.
17. N.R.P.W. Hutten, T.R. Arkell, F. Vinckenbosch, J. Schepers, R.C. Kevin, et al. Cannabis containing equivalent concentrations of Delta-9-Tetrahydrocannabinol (THC) and Cannabidiol (CBD) induces less state anxiety than THC-dominant cannabis, Psychopharmacology (Berl), 239(2020):3731–3741.
18. L. Sharpe, J. Sinclair, A. Kramer, M. de Manincor, J. Sarri, Cannabis, a cause for anxiety? A critical appraisal of the anxiogenic and anxiolytic properties, J Transl Med, 18(2020):1–21.
19. T.P.S. Oei, L. Evans, G.M. Crook, Utility and validity of the STAI with anxiety disorder patients, Br J Clin Psychol, 29(1990):429–432.
20. A. Gasparyan, F. Navarrete, J. Manzanares, Cannabidiol and sertraline regulate behavioral and brain gene expression alterations in an animal model of PTSD, Front Pharmacol, 12(2021):694510.
21. A.P.M. Cruz, V. Castro-Gomes, J. Landeira-Fernandez, An animal model of trait anxiety: Carioca high freezing rats as a model of generalized anxiety disorder, Personal Neurosci, 7(2024):e6.
22. D. Sarkar, A review of behavioral tests to evaluate different types of anxiety and anti-anxiety effects, Clin Psychopharmacol Neurosci, 18(2020):341.
23. H.V. Weimar, H.R. Wright, C.R. Warrick, A.M. Brown, J.M. Lugo, et al. Long-term effects of maternal cannabis vapor exposure on emotional reactivity, social behavior, and behavioral flexibility in offspring, Neuropharmacology, 179(2020):108288.
24. R. Cui, H. Ruan, L. Liu, X. Li, Involvement of noradrenergic and serotonergic systems in risk-based decisions between options of equivalent expected value in rats, Neurobiol Learn Mem, 175(2020):107310.
25. T.A. Green, S.J. Baracz, N.A. Everett, K.J. Robinson, J.L. Cornish, Differential effects of GABAA receptor activation in the prelimbic and orbitofrontal cortices on anxiety, Psychopharmacology (Berl), 237(2020):3237–3247.
26. E.P. Zorrilla, G.R. Valdez, J. Nozulak, G.F. Koob, A. Markou, Effects of antalarmin, a CRF type 1 receptor antagonist, on anxiety-like behavior and motor activation in the rat, Brain Res, 952(2002):188–199.
27. G. Guillén-Ruiz, J. Cueto-Escobedo, F. Hernández-López, L.E. Rivera-Aburto, E.V. Herrera-Huerta, et al. Estrous cycle modulates the anxiogenic effects of caffeine in the elevated plus maze and light/dark box in female rats, Behav Brain Res, 413(2021):113469.
28. K. Zhang, J. Lu, L. Yao, Involvement of the dopamine D1 receptor system in the anxiolytic effect of cedrol in the elevated plus maze and light–dark box tests, J Pharmacol Sci, 142(2020):26–33.
29. K.S. Grant, E. Conover, C.D. Chambers, Update on the developmental consequences of cannabis use during pregnancy and lactation, Birth Defects Res, 112(2020):1126–1138.
30. G. Rompala, Y. Nomura, Y.L. Hurd, Maternal cannabis use is associated with suppression of immune gene networks in placenta and increased anxiety phenotypes in offspring, Proc Natl Acad Sci USA, 118(2021):e2106115118.
31. N.M. De Genna, J.A. Willford, G.A. Richardson, Long-term effects of prenatal cannabis exposure: Pathways to adolescent and adult outcomes, Pharmacol Biochem Behav, 214(2022):173358.
32. S.E. Paul, A.S. Hatoum, J.D. Fine, E.C. Johnson, I. Hansen, et al. Associations between prenatal cannabis exposure and childhood outcomes: Results from the

ABCD study, JAMA Psychiatry, 78(2021):64.

33. J. Tirado-Muñoz, A.B. Lopez-Rodriguez, F. Fonseca, M. Farré, M. Torrens, et al. Effects of cannabis exposure in the prenatal and adolescent periods: Preclinical and clinical studies in both sexes, Front Neuroendocrinol, 57(2020):100841.

34. H. El. Marroun, H. Tiemeier, V.W.V. Jaddoe, A. Hofman, J.P. Mackenbach, et al. Demographic, emotional and social determinants of cannabis use in early pregnancy: The generation R study, Drug Alcohol Depend, 98(2008):218–226.

35. K.A. Richardson, A.K. Hester, G.L. McLemore, Prenatal cannabis exposure-the "first hit" to the endocannabinoid system, Neurotoxicol Teratol, 58(2016):5–14.

36. A.F. Olyaei, L.R. Campbell, V.H.J. Roberts, J.O. Lo, Animal models evaluating the impact of prenatal exposure to tobacco and marijuana, Clin Obstet Gynecol, 65(2022):334-346.

37. T.M. Sandini, T.J. Onofrychuk, A.J. Roebuck, S.A. Hammond, D. Udenze, et al. Cognition and behavior repeated exposure to high-THC cannabis smoke during gestation alters sex ratio, behavior, and amygdala gene expression of sprague dawley rat offspring, eNeuro, 10(2023):1-16.

38. R. Frau, V. Miczán, F. Traccis, S. Aroni, C.I. Pongor, et al. Prenatal THC exposure produces a hyperdopaminergic phenotype rescued by pregnenolone, Nat Neurosci, 22(2019):1975-1985.

39. R.J. Newsom, S.J. Kelly, Perinatal delta-9-tetrahydrocannabinol exposure disrupts social and open field behavior in adult male rats, Neurotoxicol Teratol, 30(2008):213-219.

40. S. Bhattacharyya, J.A. Crippa, R. Martin-Santos, T. Winton-Brown, P. Fusar-Poli, Imaging the neural effects of cannabinoids: Current status and future opportunities for psychopharmacology, Curr Pharm Des, 15(2009):2603-2614.

41. L.P. Iglesias, L. Bedeschi, D.C. Aguiar, L. Asth, F.A. Moreira, Effects of Δ9-THC and type-1 cannabinoid receptor agonists in the elevated plus maze test of anxiety: A systematic review and meta-analysis, Cannabis Cannabinoid Res, 8(2023):24–33.

42. S. Fokos, G. Panagis, Effects of δ9-tetrahydrocannabinol on reward and anxiety in rats exposed to chronic unpredictable stress, J Psychopharmacol, 24(2010):767-777.

43. L.A. Manwell, A. Charchoglyan, D. Brewer, B.A. Matthews, H. Heipel, et al. A vapourized Δ9-Tetrahydrocannabinol (Δ9-THC) delivery system part I: Development and validation of a pulmonary cannabinoid route of exposure for experimental pharmacology studies in rodents, J Pharmacol Toxicol Methods, 70(2014):120–127.

44. T.F. Nelong, B.W. Jenkins, M.L. Perreault, J.Y. Khokhar, Extended attenuation of corticostriatal power and coherence after acute exposure to vapourized Δ9-Tetrahydrocannabinol in rats, Can J Addict, 10(2019):60-66.

45. S. Hamidullah, C.D. Lutelmowski, S.D. Creighton, K.R. Luciani, J.A. Frie, et al. Effects of vapourized THC and voluntary alcohol drinking during adolescence on cognition, reward, and anxiety-like behaviours in rats, Prog Neuro-Psychopharmacology Biol Psychiatry, 106(2021):110141.

46. S. Cloutier, R.C. Newberry, Use of a conditioning technique to reduce stress associated with repeated intra-peritoneal injections in laboratory rats, Appl Anim Behav Sci, 112(2008):158-173.

47. N.B. Bertagna, P.G.C. Dos Santos, R.M. Queiroz, G.J.D. Fernandes, F.C. Cruz, et al. Involvement of the ventral, but not dorsal, hippocampus in anxiety-like behaviors in mice exposed to the elevated plus maze: Participation of CRF1 receptor and PKA pathway, Pharmacol Reports, 73(2021):57–72.

48. A.J. Schrader, R.M. Taylor, E.G. Lowery-Gionta, N.L.T. Moore, Repeated elevated plus maze trials as a measure for tracking within-subjects behavioral performance in rats (Rattus norvegicus), PLoS One, 13(2018):e0207804.

49. A.P. Carobrez, L.J. Bertoglio, Ethological and temporal analyses of anxiety-like behavior: The elevated plus-maze model 20 years on, Neurosci Biobehav Rev, 29(2005):1193–205.

50. E.F. Espejo, Structure of the mouse behaviour on the elevated plus-maze test of anxiety, Behav Brain Res, 86(1997):105–12.

51. A. Holmes, S. Parmigiani, P.F. Ferrari, P. Palanza, R.J. Rodgers, Behavioral profile of wild mice in the elevated plus-maze test for anxiety, Physiol Behav, 71(2000):509–516.

52. A.A. Walf, C.A. Frye, The use of the elevated plus maze as an assay of anxiety-related behavior in rodents, Nat Protoc, 2(2007):322–8.

53. H. Shoji, T. Miyakawa, Effects of test experience, closed-arm wall color, and illumination level on behavior and plasma corticosterone response in an elevated plus maze in male C57BL/6J mice: A challenge against conventional interpretation of the test, Mol Brain, 14(2021):1–12.

54. P.M. Wall, C. Messier, Methodological and conceptual issues in the use of the elevated plus-maze as a psychological measurement instrument of animal anxiety-like behavior, Neurosci Biobehav Rev, 25(2001):275–286.

55. S. Glasgow, G. Imbriano, J. Jin, X. Zhang, A. Mohanty, Threat and uncertainty in the face of perceptual decision-making in anxiety, J Psychopathol Clin Sci,

131(2022):265–277.

56. K. Jaśko, A. Czernatowicz-Kukuczka, M. Kossowska, A.Z. Czarna, Individual differences in response to uncertainty and decision making: The role of behavioral inhibition system and need for closure, Motiv Emot, 39(2015):541–52.

57. M. Casarrubea, F. Faulisi, F. Sorbera, G. Crescimanno, The effects of different basal levels of anxiety on the behavioral shift analyzed in the central platform of the elevated plus maze, Behav Brain Res, 281(2015):55–61.

58. D.B.S. Penna, Í. Raony, D. Jardim-Messeder, A. dos Santos-Rodrigues, P. Pandolfo, Adenosine receptors participate in anabolic-androgenic steroid-induced changes on risk assessment/anxiety-like behaviors in male and female rats, Physiol Behav, 261(2023):114071.

59. S.H. Albani, M.M. Andrawis, R.J.H. Abella, J.T. Fulghum, N. Vafamand et al. Behavior in the elevated plus maze is differentially affected by testing conditions in rats under and over three weeks of age, Front Behav Neurosci, 9(2015):98316.

60. T.L. Doremus, E.I. Varlinskaya, L.P. Spear, Age-related differences in elevated plus maze behavior between adolescent and adult rats, Ann N Y Acad Sci, 1021(2004):427–430.

61. C.F. Moore, J.W. Stiltner, C.M. Davis, E.M. Weerts, Translational models of cannabinoid vapor exposure in laboratory animals, Behav Pharmacol, 33(2022):63-89.