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Effects of Chronic Prenatal Alcohol Exposure on Nociceptive Responses to Mechanical and Thermal Stimuli in Rats

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Abstract: The present study sought to investigate the effects of chronic prenatal alcohol exposure (PAE) on nociceptive responses to mechanical and thermal stimuli in rats. The Von Frey and Hot Plate tests were employed to assess the nociceptive responses of 10 control rats and 7 experimental rats whose mothers had been administered ethanol from day 5 to day 20 of gestation. In healthy animals, a decrease in pain sensitivity was observed between days 28 and 70, which was not observed in the experimental group. The findings also indicated that rats with PAE exhibited diminished sensitivity to nociceptive stimuli during the early postnatal period, as evidenced by a higher threshold response to mechanical stimuli at day 28 than in the control group. However, those observations did not apply to thermal stimuli. It appears that this may be a result of distinctiveness in neural pain pathways for particular stimuli at the receptor or ion channel level, while a disruption in the equilibrium between the sympathetic and parasympathetic nervous systems may be a contributing factor. The results of this study highlight a critical aspect of the harmful systemic effects of alcohol, while also underscoring the need for further research to elucidate the underlying mechanisms, including the role of the hypothalamic-pituitary-adrenal axis and the serotonergic system in modulating pain responses in individuals prenatally exposed to alcohol.

Keywords: fetal alcohol spectrum disorders, prenatal alcohol exposure, pain perception, Von Frey test, Hot Plate test.

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Introduction

Since the recognition of alcohol as a teratogen in 1973, a significant amount of research has been dedicated to investigating the long-lasting consequences of prenatal alcohol exposure (PAE). This research consistently proves that consuming alcoholic beverages during pregnancy can have a considerable impact on the development of the fetus [1, 2]. Certain newborns prenatally exposed to alcohol demonstrate microcephaly, low body mass or below-average stature [3]. Moreover, patients with a diagnosis of Fetal Alcohol Spectrum Disorder (FASD) often present some atypical facial characteristics such as a flattened nasal bridge, a smooth ridge between the nose and upper lip, a thin upper lip, an additional crease in the outer ears, an upturned nose, and a curled pinky finger [4]. Other issues comprise visual or auditory impairments, difficulties with sleep and feeding during infancy, as well as complications affecting the heart, kidneys, or skeletal system [5]. Finally, severe neurodevelopmental problems of children prenatally exposed to alcohol may be observed such as impaired cognitive functioning with learning deficits, challenges in following directions, problems with understanding the implications of actions, limited memory capacity, hyperactive behavior, insufficient concentration, defective reasoning ability or delays in speech and language development [6]. In addition to these well-documented alterations in cognitive skills, research from preclinical and human newborn studies has provided strong evidence linking PAE to changes in attention, failure to regulate arousal, and the response to stress [7]. As a consequence, these deficiencies frequently result in challenges with regard to academic performance and the formation of peer relationships. This is due to difficulties in adhering to social norms and regulations, as well as delays in communication [5]. FASD is one of the most common preventable diseases globally. The impact of this condition on a global scale is significant, resulting in considerable social and economic burdens. This problem affects individuals from diverse socioeconomic and ethnic backgrounds and those with the diagnosis require ongoing medical treatment and assistance with social and occupational needs throughout their lives [8, 9].

Some of the deficits seen in individuals with FASD may be caused by the effect of PAE on the autonomic nervous system. The consumption of alcohol has a significant impact on the development of the sympathetic nervous system in several organs especially in the cardiac muscle, where sympathetic neurons take part in response and adaptation to such stressors as pain or anxiety. Due to the developmental changes in the influence of parasympathetic and sympathetic nervous system on the heart activity in the early neonatal period, stress stimuli results in a deceleration of heart rate. After two weeks from delivery, the sympathetic nervous system becomes more active, which results in an increased heartbeat in response to the introduction of new stimuli [10]. In PAE individuals, a blunted heart rate response to stress stimuli has been observed in infants during the early neonatal period [7]. In contrast, older infants have been reported to exhibit an increased heart rate response to stress [11].

The impact of PAE on the stress response mechanism is crucial and affects the hypothalamic-pituitary-adrenal (HPA) axis. Research has shown that PAE leads to hyperresponsiveness of the HPA axis, which results in increased cortisol levels after stress exposure in both humans and animals [12]. However, Oberlander *et al.* have reported that in early neonatal life, there can be observed hyporesponsiveness of the HPA axis with decreased cortisol levels, similar to cardiac autonomic reactivity [7]. It is important to consider the differences in HPA activity between sexes and the timing of alcohol exposure, as they may impact the outcomes [12].

Another crucial element is the involvement of a neurotransmitter, serotonin (5-hydroxytryptamine, 5-HT), in the regulation of a multitude of physiological and behavioral functions, including mood, emotion, sleep, and appetite [12]. Moreover, 5-HT plays a vital role in modulating the HPA axis thus a disruption in the interaction between the HPA axis and 5-HT systems may result in altered stress reactivity. Preclinical research has demonstrated that PAE can influence the formation of the 5-HT system in fetal life and weanling rats. It is noteworthy that during the early stages of fetal development, 5-HT also plays a pivotal role in modulating pain responses in the developing brain [7].

The objective of the presented study was to investigate the abnormalities in nociception among rat offspring prenatally exposed to alcohol, including an analysis of sex-associated abnormalities.

Materials and Methods

Ethical approval

The study was conducted in accordance with ethical, regulatory, and scientific principles, under the consent and supervision of the local Animal Welfare Committee of Jagiellonian University (protocol number 705/2022).

Animals

A total of nine Wistar rats, consisting of six females and three males, with an average body weight of 160 g and 200 g respectively, were utilized for the purpose of reproduction. Animals from the Jagiellonian University Medical College Animal House were housed under regulated conditions upon arrival. These settings included a 12-hour light and 12-hour dark cycle, a temperature of 22 ± 2°C, and a humidity of 55 ± 10%. Transparent cages were arranged to facilitate visual, auditory, and olfactory communication. Every cage was furnished with suitable bedding materials and environment enrichment. Unlimited amounts of tap water and regular dry chow were provided (containing 25% protein, 8% fat, 67% carbs, and 2.86 kcal/g of metabolizable energy). (Labofeed B, Kcynia, Poland).

Model protocol

The PAE procedure was initially developed by Thomas *et al.* and subsequently modified [13]. After an initial adaptation period, male rats were placed in cages with female rats for mating over a 48-hour period. Males were used for breeding purposes only, at a ratio of one male to two females. The rats were then removed from the shared cages once pregnancy was confirmed. After the insemination process, one female rat was found to be infertile. The remaining five female rats were weighed and then divided into the following groups:

1. The control group (n = 2) received an isocaloric glucose solution with a concentration of 40% (w/v) through oral gavage. This was supplied daily from the 5th to the 20th prenatal day.
2. The PAE group (n = 3) received ethanol with a dosage of 3 g/kg body weight through oral gavage. This was administered daily from the 5th to the 20th gestational day, with varying concentrations. From gestational day 5 to 17, a solution containing 28.5% ethanol by volume was administered. On the 18th, 19th, and 20th day, the ethanol dose was gradually reduced to 75%, 50%, and 25% of the maximum dose, respectively, in order to prevent withdrawal symptoms in the offspring after birth.

In addition, female rats were administered 8 g/kg b.w. of thiamine intramuscularly on the 8th, 11th, 15th, and 18th day of pregnancy to prevent thiamine shortage following alcohol administration [14].

The control group of rats gave birth to a total of 10 pups (5 females and 5 males) following a 22-day gestation period. In contrast, the PAE group delivered a total of seven offspring, comprising 3 females and 4 males. The offspring remained in the presence of their mothers for the duration of the nursing phase, specifically 21 days following birth. Subsequently, the subjects were separated from their mothers and organized into clusters consisting of four to five members, with the intention of reducing the psychological strain caused by solitude. The mothers were then humanely euthanized after the process of weaning their offspring.

Pain sensitivity testing

The von Frey test

One of the tests to assess response to pain in studied animals was the Von Frey test, which measures hind paw threshold responses to light mechanical stimuli. The electronic Von Frey test was performed using a Dynamic Plantar Aesthesiometer (Dynamic Plantar Aesthesiometer 37450; Ugo Basile, Italy). The device comprises a movable touch-stimulator, a microprocessor, a large testing surface (perforated metal platform) and a modular animal enclosure offering 6 spaces. Before performing the test rats were habituated to the testing environment for 30 min for two consecutive days. Hind paw assessment occurred on postnatal days 28 and 70.

To assess rat hind paw mechanical threshold, rats were first acclimatized to the plastic box with a metal wire mesh table for 15 min. The cessation of exploratory and grooming behavior was determined by the researcher and represented the end of the habituation period. The mechanical stimulus was delivered to the plantar surface of the hind paw from below the floor of the test chamber by an automated testing device operated by the researcher. A steel rod of diameter 0.5 mm was pushed against the hind paw with an ascending force of 0 to 50 g over a period of 50 s at a rate of 1 g/s. When the animal withdrew its hind paw, the mechanical stimulus automatically stopped, and the force at which the animal withdrew its paw was recorded to the nearest 0.1 g. Animals were subjected to 4–6 consecutive trials with at least 3–5 min intervals between the trials and the results were then averaged.

The Hot Plate test

The hot plate test was conducted using a hot plate that was fitted with a transparent plexiglass shell and a corresponding cover (Hot/cold plate 3510; Ugo Basile, Italy). Prior to each measurement, a period of acclimatization lasting roughly 15–20 minutes was observed. Every animal was individually placed in a distinct enclosure to adapt to the unfamiliar surroundings. Similar to the Von Frey test, the experimenter assessed the end of the habituation period based on the cessation of exploration and grooming behaviors. The animals were individually positioned on a hot plate that had been preheated to a temperature of 50°C. Starting from this moment, the duration until the specific nociceptive response, which involves the licking of the hind paw (sometimes accompanied by vocalization), was recorded using a stopwatch. The outcome is a measure of delay, expressed in seconds. Upon witnessing the aforementioned reaction, prompt action was taken to extract the animal off the heated surface in order to avert any potential harm to its skin. Subsequently, the animal was returned to a distinct enclosure. After each rat, the hot plate surface was cleaned using a 20% ethanol solution.

Statistical analysis

The results were reported as the mean value with the standard error (SE) or standard deviation (SD). A two-way repeated measures analysis of variance (ANOVA) was conducted, with ethanol exposure and sex as independent variables, followed by a Bonferroni post hoc test. The level of statistical significance was set at $p < 0.05$. The statistical analysis was performed using the Statistica software (version 13, TIBCO Software).

Results

General Characteristics

No notable discrepancies were observed in the mean body weight of the animals tested between the control and PAE groups. At the time of the initial assessment utilizing pain behavior tests, the mean weight for the control group was 125.63 ± 8.4 g, while the PAE group exhibited a mean weight of 123.46 ± 9.6 g. In the control group, males demonstrated a statistically significant slight weight advantage over females, a trend that was less pronounced in the PAE group. At the time of the second measurement, the difference in weight between males and females was statistically significant in both groups (Fig. 1).

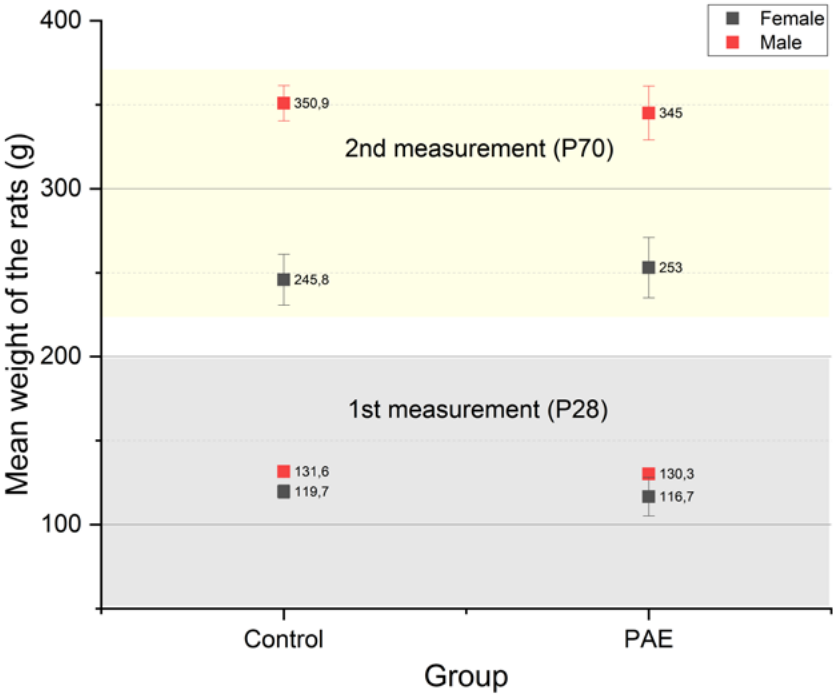

Fig. 1. The mean body weight of the control and PAE groups by sex is presented for the days of the Von Frey tests (gray box — first series of tests, yellow box — second series of tests). Data is presented as mean ± standard error (SE). P-postnatal days.

Pain behavior assessment

In both assessments of the hind paw mechanical threshold using the Von Frey test, the mean force applied was higher in the FASD group than in the control group (Table 1). However, in the second assessment conducted after a six-week interval, the applied force exhibited an increase in the control group. Thus, the statistically significant difference between the FASD and control groups was only observed in the initial measurement ($p < 0.001$). Furthermore, it was demonstrated that, in the control group, the applied pressure force was greater in the second measurement than in the first ($p = 0.38$). With regard to the Hot Plate test, the mean latency to a nociceptive response was longer in the FASD group than in the control group, although this difference was not statistically significant ($p = 0.148$).

Table 1. The results of pain behavior tests, which included two measurements using the von Frey test on days 28 and 70 postpartum and one measurement using the hot plate test, are presented as the mean ± SD.

	Control group	PAE group	P Value
Von Frey Test 1st measurement (P28)	31.25 ± 3.71 g	43.4 ± 4.16 g	<0.001
Von Frey Test 2nd measurement (P70)	37.65 ± 6.71 g	42.31 ± 6.03 g	0.1297909
Hot Plate Test	44.06 ± 19.72 s	56.71 ± 15.39 s	0.148006

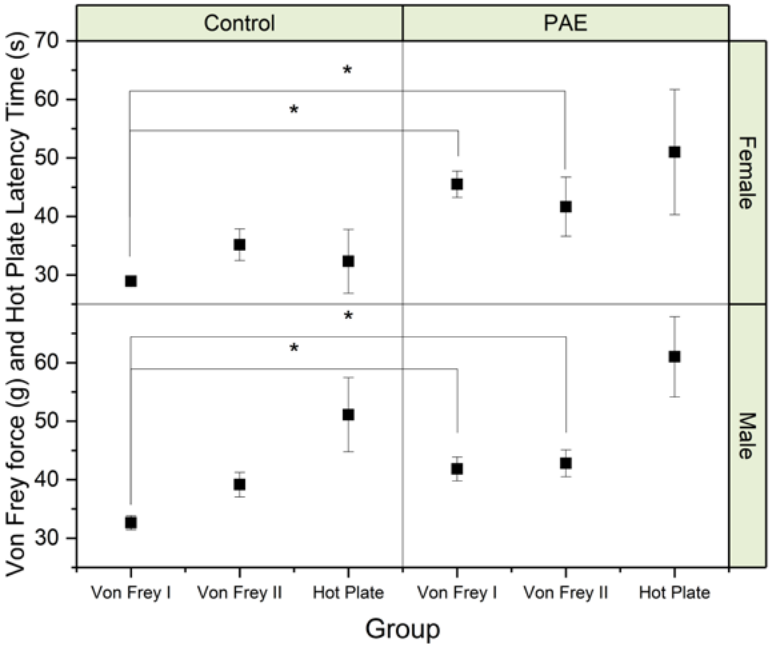

Fig. 2. The results of two Von Frey test measurements and one Hot Plate test measurement divided into control and PAE groups according to sex. Data are expressed as mean ± standard error (SE).

A post hoc analysis revealed statistically significant differences in the results of the initial assessment with the Von Frey test between the study groups, contingent on whether or not the subjects had been exposed to alcohol and on their sex (Fig. 2). The most notable discrepancies were identified between the control and PAE groups within the same sex. In particular, the primary measurement obtained via the von Frey test in the control group exhibited lower values in comparison to both the initial and subsequent measurements obtained in the PAE group. For groups of the opposite sex the differences were less pronounced but still statistically significant. There were no significant differences between the sexes within the same experimental groups (control males vs control females and experimental males vs. experimental females). No statistically significant differences were observed in the Hot Plate test, regardless of sex or study group.

Discussion

This study investigated the impact of PAE on pain like behaviors in rodents. The behavioral response of the animals to mechanical and thermal stimuli was evaluated through the administration of Von Frey and Hot plate tests, respectively. It was revealed that the offspring of female rats administered with ethanol daily from the 5th to the 20th gestational day presents decreased sensitivity to a nociceptive stimulus at an early stage of postnatal development. The study demonstrated that a higher filament pressure is necessary to elicit a hind paw withdrawal response in PAE subjects, compared to the control group. However, this was only the case when the measurement was taken at 28 postnatal days, not 70. Interestingly, most previous reports on this topic have tended to indicate no changes in basic sensory thresholds or even an increased sensitivity and decreased latency to pain in PAE animals [15–17]. One possible explanation for these discrepancies is the different methodology of the experiment. In contrast to the studies designed by Fish *et al.* and Bergenson *et al.*, which examined the effects of acute, binge-like alcohol consumption during the early stages of neural development, our model investigated chronic alcohol ingestion. Moreover, our study's protocol incorporated the use of tests with both mechanical and thermal stimuli to quantify nociception, whereas studies, such as that by Lugo and colleagues, often included a single stimulus type, namely heat (e.g. the tail-flick or hot plate test) [16, 17]. From this perspective, it is noteworthy that in our study, statistically significant alterations in the measurement of nociception were observed only for the mechanical stimulus and not for heat.

In examining the results of our experiment, it is essential to consider the fundamental physiological mechanisms underlying processing of pain. PAE has been demonstrated to impact a number of those pathways that are involved in the processes of pain conduction and modulation, including structural and functional disruption of the HPA axis and the autonomic nervous system. These factors play a significant role in this phenomenon, particularly in light of the bidirectional interaction between the HPA axis and the sympathetic nervous system. In 1997, it was found by Kelly *et al.* that PAE enhances parasympathetic activity and has the potential to decrease heart rate in preweanling rats [18]. The observed increased latency in pain sensitivity could reflect such parasympathetic dominance, however, further research is needed to confirm this association as the direct impact of the parasympathetic system on pain perception is not well established. Nevertheless, the sympathetic nervous system typically decreases latency times in pain behavioral tests like the von Frey test by increasing pain sensitivity and promoting a heightened state of physiological arousal.

Another noteworthy observation is that the PAE group exhibited comparable mean withdrawal forces in both von Frey test measurements, whereas in the control group, these forces increased

in the second measurement. This may suggest that nociceptive impairment due to PAE remains consistent over time, without clear signs of improvement or deterioration [19, 20]. The maturation of control rats changes their perception of pain, allowing them to tolerate higher levels of pain stimuli. This is particularly the case given the six-week interval between measurements, which is less likely to result in noticeable habituation effects. This may be attributed to elevated stress levels during the first exposure to the stimulus, which may result in stress-induced hyperalgesia [21]. The phenomenon was not observed in PAE rats, possibly due to dysfunction of the HPA axis and changes in how they process stressful stimuli. One hypothesis is that PAE can impair the function of the pituitary gland, resulting in lower levels of ACTH and consequently reduced corticosterone production. This impairment results in the disruption of normal stress responses and pain processing. Nevertheless, due to the multiplicity of mechanisms implicated in the perception of painful stimuli and the extensive range of systemic consequences associated with the deleterious effects of alcohol, it is not possible to ascertain whether this particular pathophysiological pathway is a principal contributor to the observed discrepancies.

It is important to note that the experience of pain is influenced by a multitude of factors, including biological sex. It has been observed that males and females who were prenatally exposed to alcohol exhibit disparate behavioral responses [10]. However, the present study did not identify any differences in pain sensitivity between males and females in either the PAE group or the control group. Similarly, Lugo *et al.* reported no variations in pain sensitivity between ethanol-exposed and control groups. They also examined the testosterone levels in ethanol-exposed rats and found that while reduced testosterone levels did not affect pain sensitivity, they did lead to reduced aggressive behaviors [17]. Just as testosterone affects male rats, ovarian hormones may also play a role in pain perception in females. Pupikina *et al.* investigated differences in behavior and tactile sensitivity between male and female littermates in adulthood. The study revealed that the tactile threshold in females, specifically during the diestrus phase, was lower than that in males. However, the direct influence of hormonal changes on this phenomenon remains inconclusive [22]. In regard to the heat stimulus, Fish *et al.* identified a notable effect of sex on the time required for a mouse to lift or lick one of its hind paws from a heated surface. Female mice exhibited longer response latencies compared to their male counterparts. However, PAE did not exert a significant influence on latency time [23]. In conclusion, the inconsistent results may be attributed to various factors, including the limited sample size and the age of the rats on the day of the test. When comparing males and females, it is essential to consider the estrous cycle in females, as Pupikina *et al.* did in their research. Additionally, it has been established that females have higher tactile sensitivity, which suggests that similar differences in pain perception may occur after PAE [22].

Conclusions

The study demonstrated that the offspring of mothers who had been chronically exposed to alcohol during pregnancy exhibited an elevated pain threshold to a nociceptive mechanical stimulus at 28 days postpartum in a rat model. Moreover, in healthy animals, a decrease in pain sensitivity was observed between days 28 and 70, which was not found in the experimental group. However, these regularities did not apply to thermal stimuli. Despite the shared neural pathways for mechanical and thermal pain, the differences in receptors and activated ion channels may result in variations in the perception of these two types of stimuli. The multifaceted impact of PAE on the organism's

state, coupled with the vast array of potential damage points and mechanisms affecting the pathways responsible for pain perception, impedes a precise delineation of the underlying pathophysiology. Nevertheless, a disruption in the equilibrium between the sympathetic and parasympathetic nervous systems may be a contributing factor. To confirm the findings and further explore the proposed explanations, more research is needed.

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Author contributions

Conceptualization, M.J., P.S., K.G.; methodology, M.J., P.S., A.M., A.B.-C.; validation and formal analysis, M.J., P.S.; writing and editing, M.K., P.S., K.S., M.K.-Ł., K.G. All authors have read and agreed to the published version of the manuscript.

Conflict of interest

None declared.

References

1. *Jones K.L., Smith D.W.*: Recognition of the fetal alcohol syndrome in early infancy. The Lancet. 1973 Nov; 302 (7836): 999–1001. doi: 10.1016/S0140-6736(73)91092-1.
2. *Mattson S.N., Bernes G.A., Doyle L.R.*: Fetal Alcohol Spectrum Disorders: A Review of the Neurobehavioral Deficits Associated With Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2019 Jun; 43 (6): 1046–1062. doi: 10.1111/acer.14040.
3. *Murawski N.J., Moore E.M., Thomas J.D., Riley E.P.*: Advances in Diagnosis and Treatment of Fetal Alcohol Spectrum Disorders: From Animal Models to Human Studies. Alcohol Res. 2015; 37 (1): 97–108. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/26259091.
4. *Wattendorf D.J., Muenke M.*: Fetal alcohol spectrum disorders. Am Fam Physician. 2005 Jul; 72 (2): 279–282, 285. [Online]. Available: http://www.ncbi.nlm.nih.gov/pubmed/16050451.
5. *Wilhoit L.F., Scott D.A., Simecka B.A.*: Fetal Alcohol Spectrum Disorders: Characteristics, Complications, and Treatment. Community Ment Health J. 2017 Aug; 53 (6): 711–718. doi: 10.1007/s10597-017-0104-0.
6. *Kable J.A., et al.*: Neurobehavioral Disorder Associated with Prenatal Alcohol Exposure (ND-PAE): Proposed DSM-5 Diagnosis. Child Psychiatry Hum Dev. 2016 Apr; 47 (2): 335–346. doi: 10.1007/s10578-015-0566-7.
7. *Oberlander T.F., Jacobson S.W., Weinberg J., Grunau R.E., Molteno C.D., Jacobson J.L.*: Prenatal Alcohol Exposure Alters Biobehavioral Reactivity to Pain in Newborns. Alcohol Clin Exp Res. 2010 Apr; 34 (4): 681–692. doi: 10.1111/j.1530-0277.2009.01137.x.
8. *Popova S., Lange S., Probst C., Gmel G., Rehm J.*: Estimation of national, regional, and global prevalence of alcohol use during pregnancy and fetal alcohol syndrome: a systematic review and meta-analysis. Lancet Glob Health. 2017 Mar; 5 (3): e290–e299. doi: 10.1016/S2214-109X(17)30021-9.
9. *Popova S., et al.*: Fetal alcohol spectrum disorders. Nat Rev Dis Primers. 2023 Feb.; 9 (1): 11. doi: 10.1038/s41572-023-00420-x.

10. *Jurczyk M., Dyląg K.A., Skowron K., Gil K.*: Prenatal alcohol exposure and autonomic nervous system dysfunction: A review article. Folia Med Cracov. 2019; 59 (3): 15–21. doi: 10.24425/fmc.2019.131132.
11. *Haley D.W., Handmaker N.S., Lowe J.*: Infant Stress Reactivity and Prenatal Alcohol Exposure. Alcohol Clin Exp Res. 2006 Dec; 30 (12): 2055–2064. doi: 10.1111/j.1530-0277.2006.00251.x.
12. *Weinberg J., Sliwowska J.H., Lan N., Hellemans K.G.C.*: Prenatal Alcohol Exposure: Foetal Programming, the Hypothalamic-Pituitary-Adrenal Axis and Sex Differences in Outcome. J Neuroendocrinol. 2008 Apr; 20 (4): 470–488. doi: 10.1111/j.1365-2826.2008.01669.x.
13. *Thomas J.D., Abou E.J., Dominguez H.D.*: Prenatal choline supplementation mitigates the adverse effects of prenatal alcohol exposure on development in rats. Neurotoxicol Teratol. 2009 Sep; 31 (5): 303–311. doi: 10.1016/J.NTT.2009.07.002.
14. *Wang R., et al.*: Prenatal ethanol exposure impairs sensory processing and habituation to visual stimuli, effects normalized by enrichment of postnatal environmental. Alcohol Clin Exp Res. 2022 May; 46 (5): 891–906. doi: 10.1111/ACER.14818.
15. *Fish E.W., et al.*: Acute alcohol exposure during neurulation: Behavioral and brain structural consequences in adolescent C57BL/6J mice. Behav Brain Res. 2016 Sep; 311: 70–80. doi: 10.1016/j.bbr.2016.05.004.
16. *Bergeson S.E., et al.*: Binge Ethanol Consumption Increases Inflammatory Pain Responses and Mechanical and Cold Sensitivity: Tigecycline Treatment Efficacy Shows Sex Differences. Alcohol Clin Exp Res. 2016 Dec; 40 (12): 2506–2515. doi: 10.1111/acer.13252.
17. *Lugo J.N., Marino M.D., Gass J.T., Wilson M.A., Kelly S.J.*: Ethanol exposure during development reduces resident aggression and testosterone in rats. Physiol Behav. 2006 Feb; 87 (2): 330–337. doi: 10.1016/j.physbeh.2005.10.005.
18. *Kelly S.J., Richards J.E.*: Development of Heart Inter-beat Interval Variability in Preweanling Rats: Effects of Exposure to Alcohol and Hypoxia. Physiol Behav. 1997 Feb; 61 (2): 231–241. doi: 10.1016/S0031-9384(96)00365-4.
19. *Chung D.D., Pinson M.R., Bhenderu L.S., Lai M.S., Patel R.A., Miranda R.C.*: Toxic and Teratogenic Effects of Prenatal Alcohol Exposure on Fetal Development, Adolescence, and Adulthood. Int J Mol Sci. 2021 Aug; 22 (16): 8785. doi: 10.3390/ijms22168785.
20. *Abel E.B.R.*: Long-term behavioral effects of prenatal alcohol exposure in rats. Neurotoxicol Teratol. 1994 Oct; 16 (5): 467–470. doi: 10.1016/0892-0362(94)90124-4.
21. *Jennings E.M., Okine B.N., Roche M., Finn D.P.*: Stress-induced hyperalgesia. Prog Neurobiol. 2014 Oct; 121: 1–18. doi: 10.1016/j.pneurobio.2014.06.003.
22. *Pupikina M., Sitnikova E.*: Sex Differences in Behavior and Learning Abilities in Adult Rats. Life. 2023 Feb; 13 (2): 547. doi: 10.3390/life13020547.
23. *Fish E.W., et al.*: The enduring impact of neurulation stage alcohol exposure: A combined behavioral and structural neuroimaging study in adult male and female C57BL/6J mice. Behav Brain Res. 2018 Feb; 338: 173–184. doi: 10.1016/j.bbr.2017.10.020.