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Effect of acetaminophen use on GABA, NMDA, and synaptic plasticity-related genes in the immature mouse brain: A preliminary study

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Abstract

Background/aim: Acetaminophen is frequently used as an analgesic during pregnancy. The purpose of the present study was to evaluate the effects of acetaminophen administered to pregnant mice on the fetal brain, attention, memory, and learning functions in the postnatal period, and genetic mechanisms in these mice.

Materials and methods: The study was designed with two different groups. The first group consisted of pregnant mice that were injected with acetaminophen, while the second group was comprised of pregnant mice that were injected with saline. 1st, 2nd, and 3rd days of pregnancy, one of the mice was injected subcutaneously with 100 mg/kg acetaminophen, and the other mouse was injected subcutaneously with 0.9% saline. On the 21st day after birth, five female and five male mice were randomly selected for the experimental and control groups. Behavioral tests were performed on mice at 2 months of age. In addition, changes in the transcript levels of 93 genes were evaluated by Real-Time PCR in the hippocampus.

Results: The control group showed more interest in the new object than the acetaminophen group (p=0.002). In the marble burying test, greater burying activity was observed in the control group than in the acetaminophen group (p=0.0345). No significant difference was observed between the control and acetaminophen groups in the social interaction and tail suspension tests. *GABRG3*, *GRM3*, *PICK1*, *CEBPB*, and *EGR4* mRNA expression levels increased in the acetaminophen group (0.0317, 0.0159, 0.0069, 0.0457, 0.015, p value respectively).

Conclusions: Prenatal acetaminophen exposure affected both behavioral tests and transcript levels. Therefore, the potential effects of prenatal acetaminophen exposure should be carefully investigated.

Keywords: Acetaminophen; synaptic plasticity; GABA; NMDA; immature brain

Introduction

Acetaminophen has been used in patients of all ages for its analgesic and antipyretic effects since its introduction in the mid-1950s [1]. Acetaminophen is recommended as the first choice for pain and fever at therapeutic doses for a short time in every period during pregnancy [2].

Acetaminophen can cross the placenta, and acetaminophen and its metabolites are found in the urine of newborn babies when used during pregnancy immediately before birth [3]. Long-term acetaminophen treatment may disrupt the integrity of the blood-brain barrier [4]. Acetaminophen, which crosses the blood-brain barrier, is metabolized to N-acetyl-p-benzoquinone imine by cytochrome P450 2E1(CYP2E1) [5]. In the brain, the CYP2E1 enzyme is concentrated in the frontal cortex and hippocampus, which plays an important role in learning and memory [6, 7].

Although the use of acetaminophen in pregnant women is considered harmless, some recent studies have shown that some cognitive effects of acetaminophen are overlooked in the fetus and are associated with a risk of fetal malformations, low birth weight, preterm delivery, and miscarriage [8, 9].

A study conducted with Norwegian mothers and children showed that children with prenatal exposure to acetaminophen had worse gross motor function and communication

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skills [10]. Long-term therapeutic doses of acetaminophen alter the balance of amino acid neurotransmitters in the rat brain at significant levels, which may lead to cognitive and behavioral impairment [11].

In the 2023 study, mice were exposed to acetaminophen at different doses and during different periods of pregnancy. It has been shown that dose, repeated exposure, and gestation period are determinants of hippocampal damage [12].

The formation of the neural network in the central nervous system is contingent upon the growth of axons, which reach their postsynaptic targets, and the formation of synapses. Synapse formation is most common during the first few years of life. There are approximately 15 thousand synapses in each cortical nerve cell during the period when synapses are most common, which corresponds to the formation of 1.8 million synapses every second during the first two months of pregnancy and the first two years after birth [13].

The present study aimed to evaluate the effects of acetaminophen administered to pregnant mice on the fetal brain, attention, memory, and learning functions in the postnatal period, and related genetically possible mechanisms in these mice. For this purpose, the changes in the transcript levels of 93 genes that play important roles in GABAergic receptors (GABAA, GABAB, GABAC), glutamate receptors (AMPA, Kainate, NMDA, metabotropic receptors), and synaptic plasticity were evaluated.

Materials-Methods

The study was conducted using Balb/c mice, with the approval of the Animal Experiments Local Ethics Committee (decision number 17/121). The mice were maintained in a facility under controlled conditions, with a light cycle from 06:00 to 18:00, a temperature of 22°C, and a humidity level of 55%. All animal models were maintained in the same animal house and on the same time scale, with well-controlled food, water, temperature, light, and care conditions. The Principles of Laboratory Animal Care (European rules) cared for and treated the animals. All tests were performed between 10:00 and 16:00 in isolated rooms.

Animal Design

The study was designed with two different groups (Figure 1). The first group consisted of pregnant mice that were injected with acetaminophen, while the second group was comprised of pregnant mice that were injected with saline. Two female mice and one male mouse were mated to obtain pregnant mice. The following morning, the plaque was monitored and a mating control was performed. Acetaminophen (100 mg/kg) was administered subcutaneously to two pregnant mice on days 1, 2, and 3 of their gestational period. The control group was administered 0.9% saline via subcutaneous injection on days 1, 2, and 3 of their respective pregnancies. Twenty-one days after birth, the offspring were separated from their mothers. After that, 5 female and 5 male newborn mice were randomly selected for the acetaminophen-injected and control groups, respectively. Behavioral experiments were conducted on the offspring that reached adulthood.

Behavior Tests

Behavioral experiments began when the acetaminophen and control mice were 2 months old. Before the commencement of the experiment, the mice were transported to the designated experimental room and allowed to acclimate to their surroundings for a period of 15 to 30 minutes.

a.Novel Object Recognition Test

The apparatus employed for the novel object recognition test comprised a square-shaped box measuring 50 cm in each dimension, with a transparent upper surface and surrounded by lines and a wall. The central area was illuminated at a light intensity of 300 lx. In this experiment, two identical objects, each no larger than a mouse, were employed. On the initial day of the experiment, the mice were placed in the center of the apparatus and observed for five minutes using an EthoVision system (Noldus Information Technology, Wageningen, the Netherlands). On the second day of the experiment, one of the objects was replaced with a new object, and the data were collected anew. The time spent by the mouse on novel and familiar objects, the number of visits made to each object, and the discrimination index were quantified [14].

b.Social Interaction Test

The test is performed in a rectangular, three-compartment box. Each room is 19 × 45 cm in size and comprises a system of divided walls and a central compartment constructed from transparent Plexiglas, which allows for unobstructed access to each room. Before the commencement of the experiment, the mice were placed in the room where the experiment was conducted for 30 minutes, during which time they were permitted to become familiar with the experimental setup environment. Subsequently, one of the mice was placed in a chamber for observation. While the other compartment was left unoccupied, the original experimental mouse was positioned in the middle compartment and recorded with a camera system for five minutes. The data were generated using the EthoVision system (Noldus Information Technology, Wageningen, the Netherlands), by the parameters of interest to the mouse in the compartment. Subsequently, a statistical analysis was conducted, and the results were compared with those of the control group [14].

c.Tail Suspension Test

The Tail Suspension Test is a mouse behavioral paradigm for assessing acquired helplessness and behavioral despair, or "depression-like" behavior [14].

The experimental design was established in a manner that enabled the simultaneous testing of three mice, thus allowing for the investigation of the effects of the treatment on each animal individually. To prevent visual contact between the

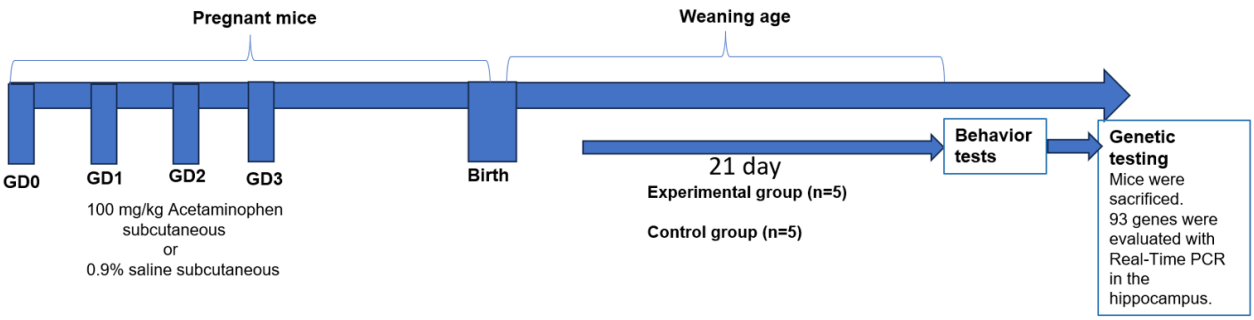

Figure 1. Study Design. -GD: Gestation date

mice, 25 cm thick, visually opaque plates were constructed from cardboard and placed between them at a distance of 25 cm. Given that the mice were white, a black background was employed. Twelve-centimetre-long tapes were cut and the mouse tails were affixed to the experimental setup by adhering to the tip of the tail without causing any damage. The mice were filmed for six minutes using a video camera (Samsung, South Korea). The data was subsequently analyzed using a computerized system, and the immobility time of the mice was calculated to be six minutes [14].

d.Marble Burying Test

The marble burial test is a commonly employed methodology for the assessment of neophobia, anxiety, obsessive-compulsive behaviors, and repetitive behaviors in rodents[15-20]. Performance in the ball burial test is associated with general digging behavior. The fundamental structure that is assessed in this experiment, which is concerned with the measurement of social curiosity and fear of novel objects, remains uncertain. This behavior is likely to be a typical defensive burial observed in rodents [21]. It appears that the mice are unable to discern the balls with clarity, suggesting that the utilization of more conspicuous colored balls may enhance the burial behavior [22,23]. The precise region of the neuronal system to which this behavior is related remains unclear. Nevertheless, the hippocampus and septum are believed to be significantly impacted regions, as lesions in these areas have been shown to diminish digging behavior [14].

The corncobs employed for the maintenance of the mice were situated within an unoccupied cage at a height of 5 cm. Twenty marbles were placed, four marbles in each row. The experimental mouse was released from one corner of the cage. Following a 30-minute observation period, the mouse was removed from the cage and the total number of buried marbles under the corn cob was recorded.

Molecular Analysis

Once the behavioral experiments had been concluded, the mice were sacrificed by cervical dislocation, and the hippocampal tissues were placed in PureZOL (Biorad, Germany, Cat No: 7326890) for molecular assays to be conducted. After analysis of behavioral tests, two female and three male mice were chosen for Q-PCR analysis, and their hippocampal RNA was isolated.

Total RNA Isolation

In order to ascertain the levels of gene expression, RNA was isolated from hippocampal tissues using Purezol. Each sample was added to Purezol and homogenized with the aid of 1 ml injectors. A volume of 180 µL of chloroform was added to each homogenized tissue sample and vortexed for 15 seconds. Subsequently, the samples were incubated for a period of five minutes at room temperature, after which they were subjected to centrifugation at 12,000 g for 20 minutes at 4°C. Subsequently, the upper phase was transferred to new reaction tubes and a 1:1 ratio of isopropanol was added to the upper phase, which was then subjected to centrifugation at 12,000 g at 4 °C for 10 minutes. Subsequently, the supernatant was removed, and 1 ml of 75% ethanol was added to the pellet. The pellet was then removed by inverting the tube and centrifuging at 7,500 g at 4 °C for five minutes. Subsequently, the supernatant was removed, and the residual alcohol within the pellet was extracted using a 10 µL pipette. The pellet was then left to dry. Upon completion of the drying process, the pellet was resuspended with the addition of 30 µL of nuclease-free water. The resulting RNAs were then placed on ice and maintained at 4 °C for a period of 10 to 15 minutes. Subsequently, the RNA concentrations were quantified utilizing a NanoDrop device (Shimadzu, Japan) [24].

cDNA Synthesis and Q-PCR Analysis

cDNAs were obtained using the EvoScript cDNA Synthesis Kit (Roche, Germany; Cat No: 07912374001). The Custom Panel (Roche, Germany, Cat No: 05532949001) was used to determine the expression levels of 93 different genes and three reference genes (Table 1). Compatible with probe assays, a

Table 1. Custom Panel Plate Design and Genes

	1	2	3	4	5	6	7	8	9	10	11	12
A	*GABRA1*	*GABRA2*	*GABRA4*	*GABRA5*	*GABRA6*	*GABRB1*	*GABRB3*	*GABRD*	*GABRE*	*GABRG1*	*GABRG2*	*GABRG3*
B	*GABRQ*	*GABBR1*	*GABBR2*	*GABRR1*	*GABRR2*	*GRIA1*	*GRIA2*	*GRIA3*	*GRIK1*	*GRIK2*	*GRIK4*	*GRIK5*
C	*GRIN1*	*GRIN2A*	*GRIN2B*	*GRIN2C*	*GRM1*	*GRM3*	*GRM4*	*GRM5*	*GRM6*	*GRM7*	*GRM8*	*ADCY1*
D	*ADCY8*	*BDNF*	*CAMK2A*	*CAMK2G*	*CDH2*	*CNR1*	*GNL1*	*GRIN2D*	*MAPK1*	*MMP9*	*NTF5*	*NTRK2*
E	*PLCG1*	*PPP1CA*	*PPP1CC*	*PPP3CA*	*PRKCA*	*PRKCC*	*RAB3A*	*YWHAQ*	*GRIA4*	*GRIP1*	*IGF1*	*NOS1*
F	*NGFR*	*PICK1*	*PLAT*	*PPP1R14A*	*PPP2CA*	*PRKG1*	*ARC*	*CEBPB*	*CEBPD*	*CREB1*	*CREM*	*EGR1*
G	*EGR2*	*EGR3*	*EGR4*	*FOS*	*HOMER1*	*JUN*	*JUNB*	*KLF10*	*NFKB1*	*NFKBIB*	*NGF*	*NPTX2*
H	*NR4A1*	*NTF3*	*PCDH8*	*PIM1*	*RELA*	*RGS2*	*RHEB*	*SRF*	*TNF*	*ACTB**	*GAPDH**	*HPRT1**

* Reference gene

probe master (Roche, Germany, Cat No: 05 532 914 001) was used to study the panels. The cTs obtained were normalized to three reference genes using the 2-ΔΔ Ct method [14, 24].

Statistical Analysis

Following the acquisition of the behavioral experiments and gene expression results, a comparison was made between the groups, with the appropriate parameters selected for this purpose. The compliance of the data with a normal distribution was evaluated through the use of histograms, Q-Q graphs, and the Shapiro-Wilk test. Subsequently, the data were analyzed using Student's t-test and Mann Whitney-U tests, as appropriate, following the determination of whether the data exhibited a normal distribution. The data were analyzed using the statistical software packages SPSS (version 22, IBM, USA) and Prism (version 8, GraphPad, USA). The threshold for statistical significance was set at a p-value of less than 0.05.

Results

Behavioral Tests

a.Novel Object Test

In the control group, mice showed more interest in novel objects than in old objects (p=0.0066). When the male and female mice were evaluated separately, the mice showed more interest in novel objects than in old objects in both genders in the Control Group (p=0.04; p=0.02). The mice showed a similar interest in novel and old objects in the acetaminophen group (p>0.05).

According to the findings, the acetaminophen group had a significantly lower tendency toward innovation and discovery than the control group (p=0.002). In particular, this tendency was lower in females than in males (Figure 2a).

When the groups were compared, the control group showed more interest in the novel object (p=0.002). When male and female mice in the control and acetaminophen groups were evaluated separately, greater interest was observed in the novel object in both sexes in the control group (p=0.008; p=0.01). When total movement and velocity were compared, there was no difference between the groups (p>0.05). The results of the Novel Object Test are shown in Figures 2a and 2b.

b.Social Interaction Test

The mean time (± SEM) in the empty and stranger-side chambers (± SEM) is shown in Figure 3. Mice in the control and experimental groups spent more time on the stranger side than on the empty side, showing a preference for social intimacy (p<0.001).

c.Tail Suspension Test

No statistically significant differences were identified between the control and experimental groups in the tail suspension test (p > 0.05). Figure 4 illustrates the immobility time observed in the tail suspension test.

d.Marble Burying Test

More burying activities were observed in the control group than in the acetaminophen group (p=0.0345). When evaluated in terms of sex, it was found that burying activity was similar in both male and female mice in both groups (p>0.05). The results of the marble-burying test are shown in Figure 5.

The Evaluation of GABAergic Receptors (GABAA, GABAB, GABAC), Glutamate Receptors (AMPA, Kainate, NMDA, metabotropic receptors), and Synaptic Plasticity

In our panel study, which included 93 genes that investigated the effects of acetaminophen on GABAergic receptors, glutamate receptors, and synaptic plasticity at the molecular level, only five gene transcript levels were found to be significantly different between the acetaminophen and control groups (p<0.05).

When transcript levels were compared according to fold change, *GABRG3*, *GRM3*, *PICK1*, *CEBPB*, and *EGR4* transcript levels were increased in the acetaminophen group (p =0.0317, p =0.0159, p =0.0069, p =0.0457, and p =0.015, respectively (Figure 6). The effects of acetaminophen on the gene transcript levels in mice are shown in Table 2.

Table 2. Effect of acetaminophen on gene expression in mice

Gene symbol	Acetaminophen Average 2^Delta Ct	Control Average 2^Delta Ct	p-value
GABRG3	200,796780875997	47,1314693700739	**0,0317**
GRM3	14,0513690285586	3,45958673160061	**0,0159**
PICK1	10,160580658233	2,22986424309863	**0,0069**
CEBPB	12,1928049272542	2,58209087644705	**0,0457**
EGR4	32,2634534790576	2,51046337656831	**0,015**

The median value of five gene expressions between acetaminophen and control groups. Statistically significant p values are shown in bold.

Discussion

The expression of genes in neurons is subject to change and is influenced by neuronal activity in the brain [25]. Acetaminophen is widely employed as an analgesic and antipyretic medication in both adults and children [1]. Recently, it has become common for pregnant women to use acetaminophen [2]. Despite evidence suggesting that acetaminophen may not be as safe as previously thought, little is known about the extent to which it affects cognition and behavior and the underlying

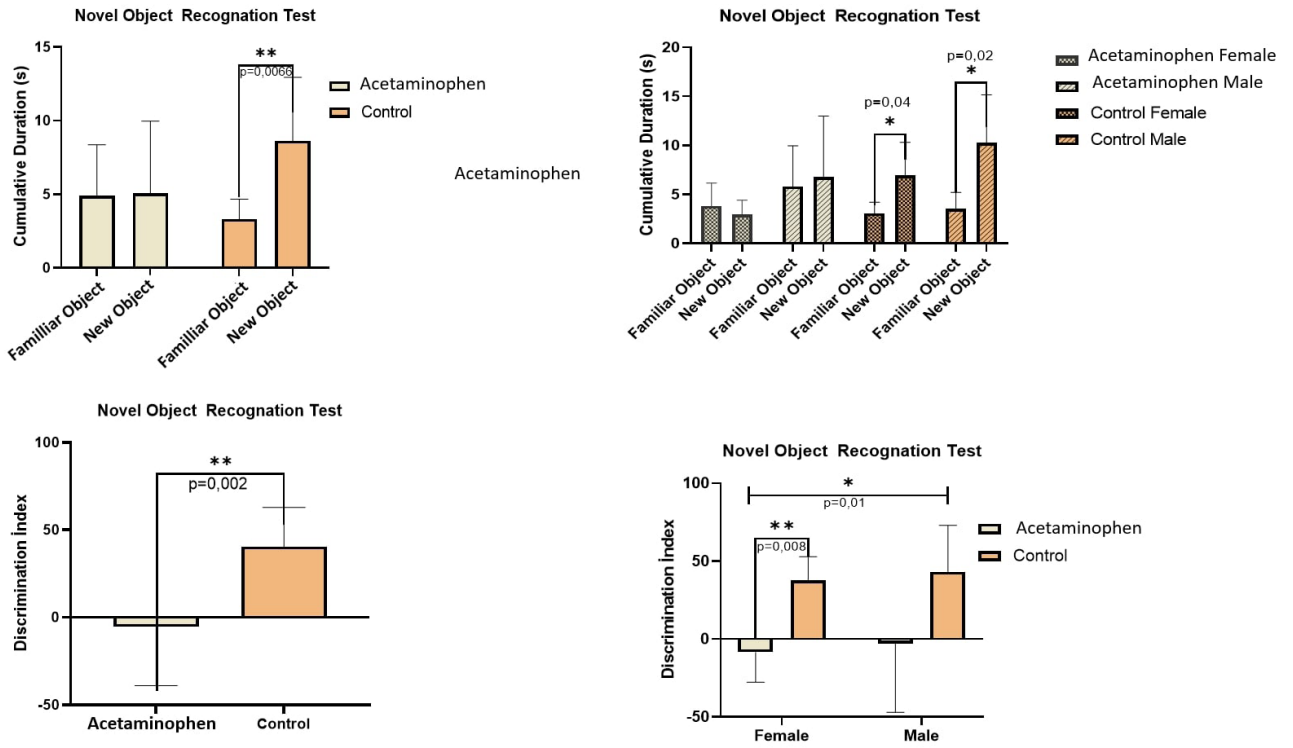

Figure 2a. Novel Object Recognition Test. The Novel Object Test results of the acetaminophen and control groups are shown in the figure.

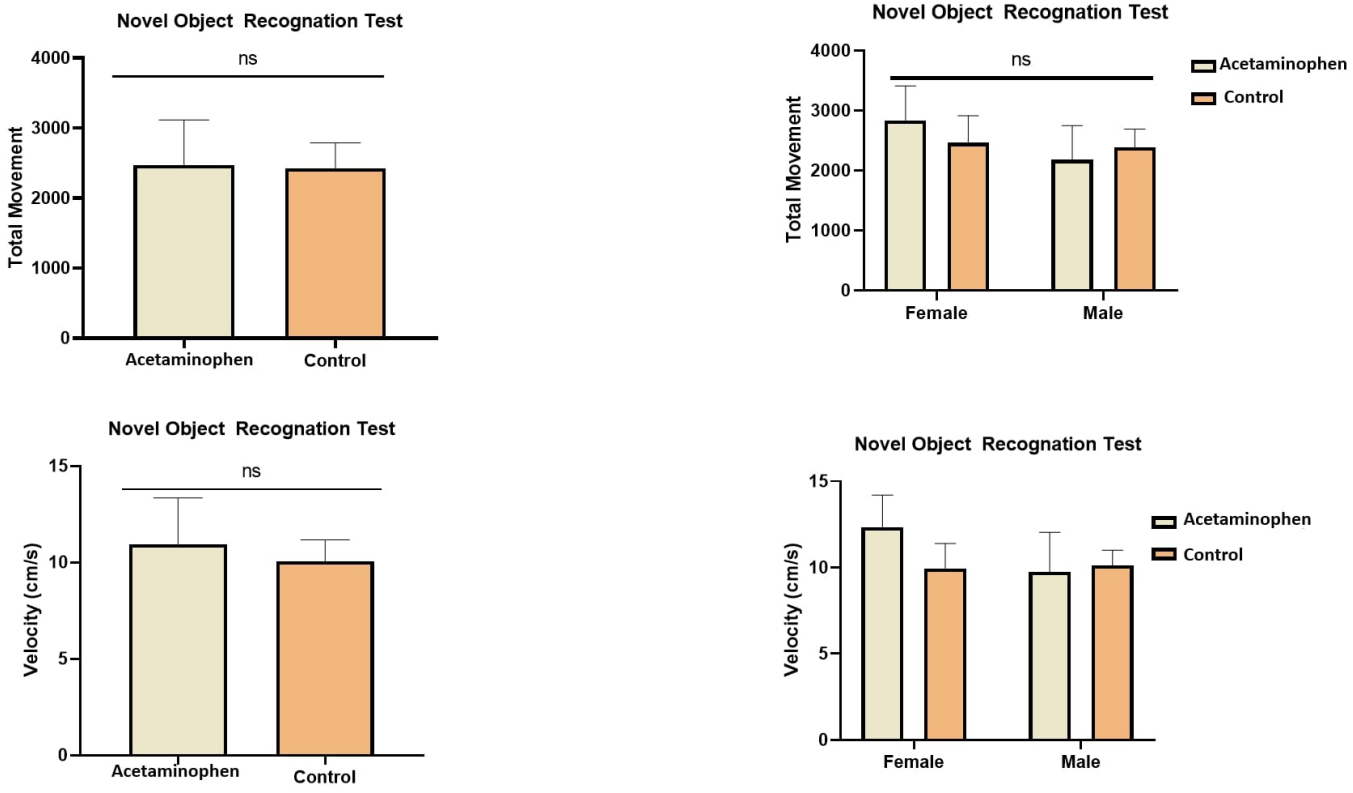

Figure 2b. Novel Object Recognition Test. The Novel Object Test results of the acetaminophen and control groups are shown in the figure.

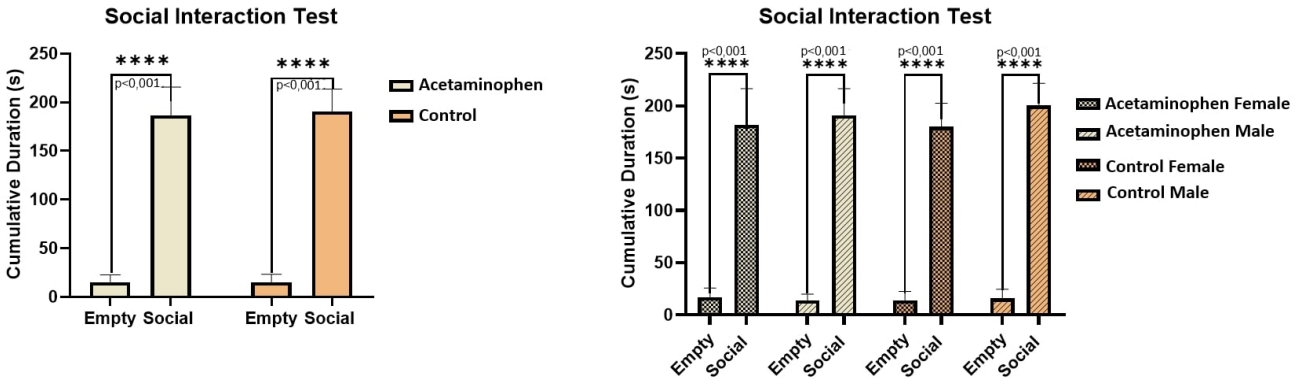

Figure 3. Social Interaction Test. The mean time (± SEM) in the empty side and stranger side chamber (± SEM) in the acetaminophen and control groups is shown in the figure.

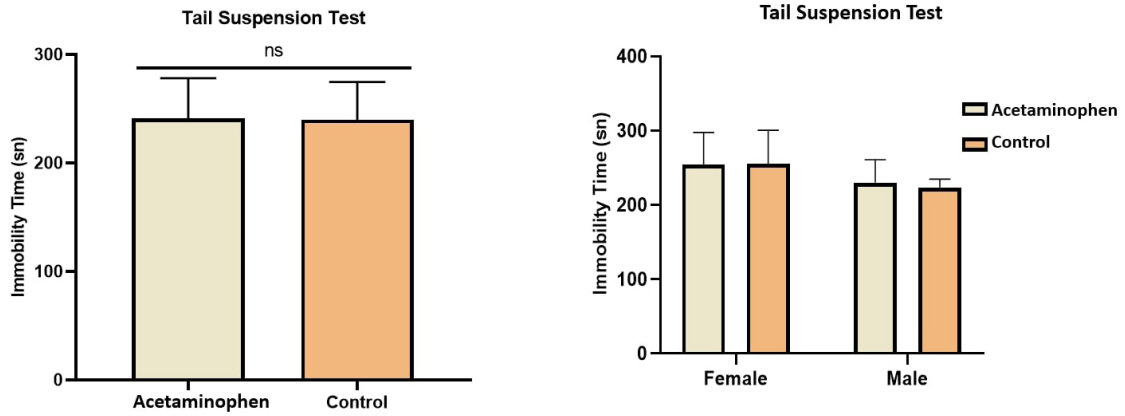

Figure 4. Tail Suspension Test. The immobility time in the tail suspension test in the acetaminophen and control groups is shown in the figure.

mechanisms [8, 9]. This study aimed to explore the potential negative impact of acetaminophen on brain development and cognitive function by examining the transcript levels of 93 genes that are critical for GABAergic receptors, glutamate receptors, and synaptic plasticity.

The impact of prenatal and postnatal acetaminophen exposure on learning, memory, and behavior in laboratory animals remains a significant area of study. Research has shown that administration of acetaminophen to mice on the postnatal 3rd or 10th day led to altered locomotor activity and impaired spatial learning in adulthood [26]. Additionally, repeated doses of acetaminophen administered at 4-hour intervals to mice

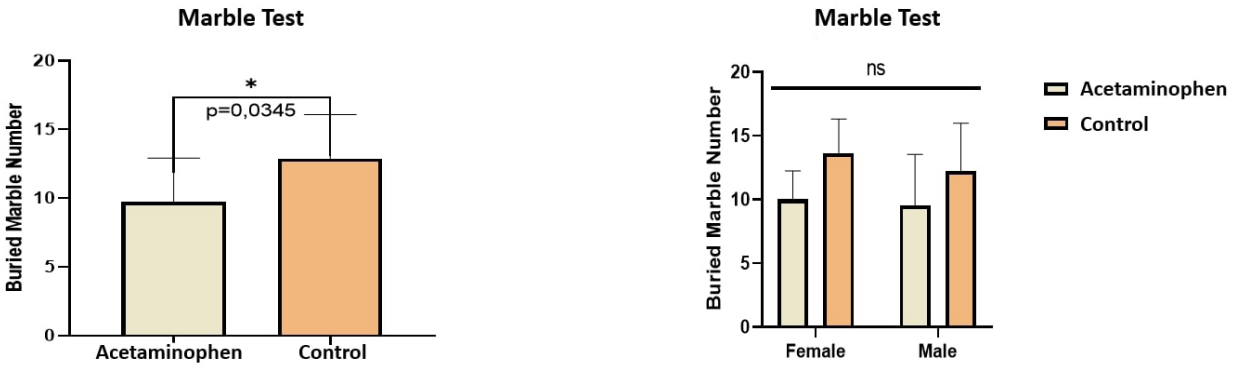

Figure 5. Marble Burying Test. The results of the Marble Burying Test are shown in the figure

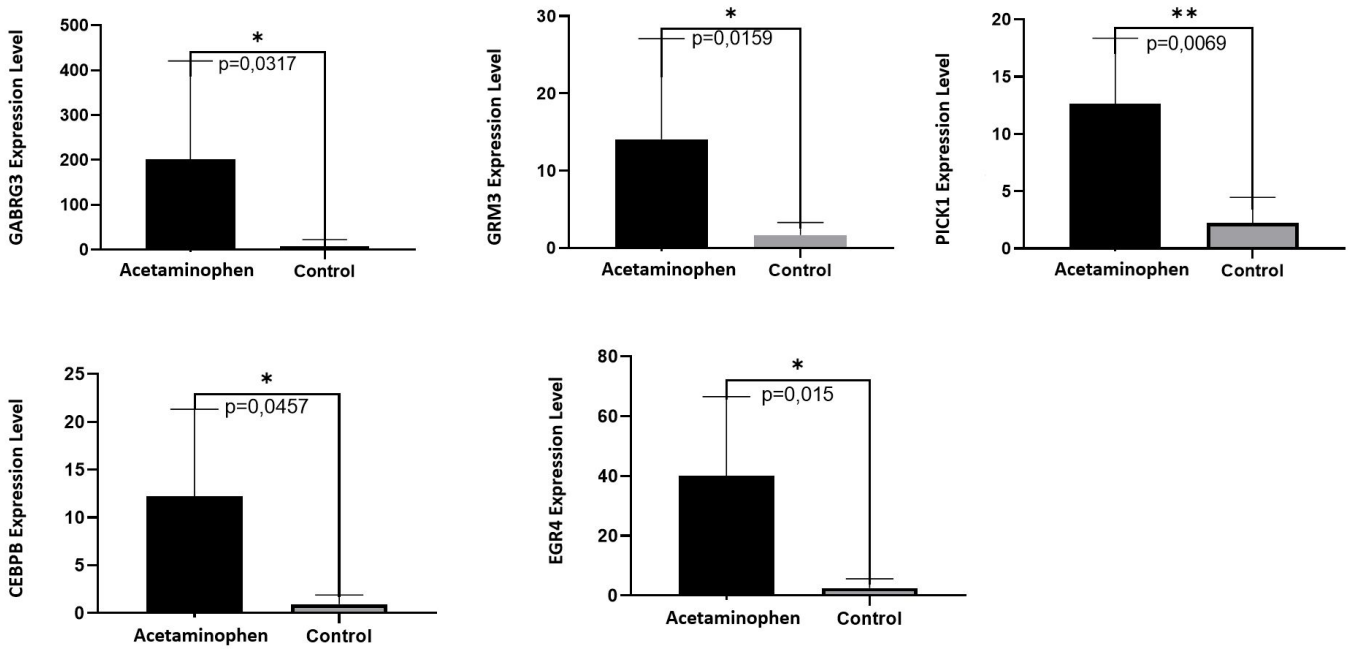

Figure 6. Fold changes of five gene expressions between the acetaminophen and control groups in mice. The figure shows the fold changes of GABRG3, GRM3, PICK, CEBPB, and EGR4 gene expression levels.

on postnatal 10th day resulted in reduced memory-learning ability and altered locomotor and rearing activity in adulthood [27, 28]. Furthermore, a study investigating the effects of prenatal and early postnatal exposure to acetaminophen in rats reported behavioral changes and decreased social interaction in adulthood [29].

Prenatal acetaminophen exposure (150 mg/kg/day) by gavage from day 7 of pregnancy until delivery did not affect locomotor activity [30]. Another study reported that prenatal acetaminophen exposure impaired brain development and decreased sexual behavior in male mice [31]. The neurodevelopmental neurotoxicity of prenatal acetaminophen was evaluated in rats. Pregnant rats were exposed to acetaminophen (350 mg/kg/day) by oral gavage from day 6 of pregnancy until birth. An increase in stereotypy, a decrease in rostral grooming, a decrease in exploratory behavior in three-chamber sociability, and impaired nest-seeking behaviors were found in males [32, 33].

In the present study, mice exposed to acetaminophen in the novel object recognition test spent less time with novel objects than the control group. Mice in both the control and experimental groups spent a longer time on the stranger side than on the empty side in the social interaction test, showing a preference for social intimacy. In the tail suspension test, the immobility time was similar between the control and experimental groups, indicating that prenatal acetaminophen exposure negatively affects the learning and memory functions of mice. No negative effects were detected for social affiliation or memory. However, in the marble burying test, more burying activity was observed in the control group than in the acetaminophen group, suggesting that prenatal acetaminophen affects social curiosity and fear of novel objects. The different results obtained according to the social interaction test can be explained by the fact that the basic structure measured in the marble burying test is not yet clear [21].

GABRB3, which is among the genes encoding GABAA receptor subunits, was reported to play roles in the seizures observed in maternal dup15q syndrome and may contribute to the autism phenotype [34-37], and *GABRB3* was associated with Autism Spectrum Disorders [38]. Decreased Gabrb3 expression was found in brain tissue samples of individuals with Obsessive-Compulsive Disorder [34, 35]. In the present study, *GABRB3* transcript levels were increased in mice exposed to acetaminophen during the prenatal period.

Metabotropic glutamate receptors constitute a family of G protein-coupled receptors, which are subdivided into three groups based on sequence homology, putative signal transduction mechanisms, and pharmacological properties [39]. Also, mGluR3 (encoding the GRM3 gene) receptors sense excessive synaptic glutamate release and modulate glutamate concentrations with a negative feedback mechanism [40]. A meta-analysis reported an association between *GRM3* and schizophrenia risk and suggested that alleles conferring this risk might be population-specific [41]. Lithium has been shown to reduce *mGluR3* expression in the mouse brain [42]. It has been reported in the literature that drugs that increase N-acetylaspartylglutamate have procognitive activity and reverse cognitive deficits in animal models. In addition, *mGluR3* antagonists inhibit this pro-cognitive effect, and mice with *mGluR3* receptor void mutants are insensitive to these pro-cognitive drugs [40]. In this study, *GRM3* transcript levels were increased in mice exposed to acetaminophen.

PICK1 specifically interacts with the GluA2 subunit of the AMPA receptor at neuronal synapses and plays roles in the learning-memory mechanism [43]. In a previous study that examined gene polymorphism and cognitive function in schizophrenia patients, it was shown that *PICK1* might be associated with cognitive functions in schizophrenia [44]. It was shown that *PICK1* inhibitors block long-term depression, highlighting the importance of discovering potent, selective PICK1-GluA2 inhibitors that may be useful for the treatment of neurodegenerative disorders [43]. *PICK1* transcript levels were found to be increased in mice exposed to acetaminophen in this study.

The transcription factor *CEBPB* has been demonstrated to play a role in neurogenesis and the expression of inflammatory genes in the brain [45, 46]. It has been demonstrated that CEBPB is regulated in a cell-specific manner, resulting in alterations to neurogenesis, axonal damage, inflammation, and differentiation, which depend on the cell type in question [45]. *CEBPB* -mediated gene regulation was associated with Alzheimer's disease, amyotrophic lateral sclerosis, and multiple sclerosis [45, 47-49]. However, the specific cell types in which *CEBPB* functions in neurodegenerative diseases are still not known [50]. In a previous study that evaluated HIV-associated neurocognitive disorders by postmortem examination of prefrontal cortices in 33 patients, it was shown that the *CEBPB* expression significantly increased in patients with minor neurocognitive impairment when compared to cognitively normal individuals, which is particularly interesting because of its role in inflammation, autophagy, and neurogenesis [50]. *CEBPB* transcript levels were determined to be increased in mice exposed to acetaminophen in this study.

Early Growth Response (EGR) gene regulates certain aspects of synaptic plasticity associated with learning and memory. The target genes regulated by the EGR are not known [51]. RBFOX3 is the marker of post-mitotic neurons among different species. *EGR4* expression was found to have increased in a study that was conducted in mice in which this gene, which was produced to study brain functions and development, was inactive. It was shown that synaptic transmission and synaptic plasticity are defective in these mice. It was reported that the *RBFOX3* deletion increases seizure susceptibility and causes anxiety [52]. It is already known that *EGR4* functions as a transcriptional repressor and exhibits autoregulatory activities, downregulating its gene expression in a dose-dependent manner [53]. In a previous study, the *EGR4* gene was associated with schizophrenia in male patients [54]. In this study, *EGR4* transcript levels were found to be increased in mice that were exposed to acetaminophen.

It was shown that low-dose acetaminophen (15 mg/kg/d), acute (1 day), and chronic exposure (5 days) in rats during the prenatal period alter the expression of multiple genes in fetal brains [55]. It was observed that the administration of repeated doses of acetaminophen on the postnatal 10th day might induce oxidative stress in the hippocampus, and there was a corresponding increase in the *NRF2/KEAP1* transcription rate at the 6th hour in the hippocampus [28]. In the present study, different genes were evaluated in mice according to literature data, and it was found that the transcript levels of some genes (*GABRG3, GRM3, PICK1, CEBPB, EGR4*) changed, which suggests that prenatal acetaminophen exposure alters the transcript levels of some genes.

The results of epidemiological studies were evaluated in a recently published consensus statement. Prenatal exposure to acetaminophen might cause adverse neurodevelopmental and behavioral outcomes, such as Attention Deficit and Hyperactivity Disorder, Autism Spectrum Disorder, language

delay (in females), and low IQ. It is recommended to minimize exposure by using the lowest dose for the shortest possible duration during pregnancy [56].

The current study is the first to investigate the possible negative effects of acetaminophen on intrauterine brain development and cognitive status and to evaluate the expression of genetic GABAergic receptors, glutamate receptors, and genes that play key roles in synaptic plasticity. It was found in the study that prenatal acetaminophen exposure affected the learning and memory functions of mice negatively. Although acetaminophen exposure had no effects on social memory and social affiliation, it was shown to have effects on social curiosity and fear of new objects. It was found that prenatal acetaminophen exposure had effects at the genetic level, and the expression of *GABR3, GRM3, PICK1, CEBPB*, and *EGR4* was increased. The current study will guide future research to understand the possible effects of prenatal acetaminophen exposure and the pathogenesis of these effects.

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