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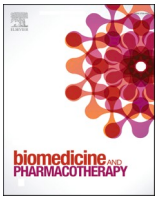

Effects of maternal drinking patterns and epigallocatechin-3-gallate treatment on behavioural and molecular outcomes in a mouse model of fetal alcohol spectrum disorders

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A B S T R A C T

Prenatal alcohol exposure (PAE) impairs fetal development leading to fetal alcohol spectrum disorders (FASD). Antioxidants like epigallocatechin-3-gallate (EGCG) may mitigate alcohol-induced oxidative stress, a major contributor to FASD. This study assessed the effects of PAE on cognition and behaviour under two drinking patterns and the role of postnatal EGCG therapy in a FASD-like mouse model. C57BL/6J mice were divided into five groups: control, moderate drinking (Mod), binge drinking (Bin), Mod+EGCG, and Bin+EGCG. Cognitive and behavioural performance were assessed using Rotarod test, T-Maze, and Morris Water Maze (MWM). Western blot analyses evaluated brain and cerebellum biomarkers related to neuronal plasticity, maturation, differentiation, transport, and proliferation. PAE impaired motor coordination, significantly reducing rotarod walking time in both drinking patterns. Spatial learning and memory were also disrupted, decreasing T-maze success rate. It also decreased time in the platform area and distance travelled in MWM. Both drinking patterns affected neuronal plasticity (BDNF, DYRK1A) and maturation (NeuN), astrocyte differentiation (GFAP, s100β), neuronal transport (MBP) and proliferation (GDNF, Wnt-3) via oxidative stress (Nrf2). Our results show how EGCG treatment significantly improved behavioural tests results and restored most brain and cerebellum biomarkers, reaching levels similar to control. These findings highlight the impact of PAE on cognition and behaviour and how EGCG may counteract its effects by reducing oxidative stress and enhancing brain plasticity. Our findings open the door to future studies on the mechanism of action of this antioxidant in order to use it as a therapeutic tool in this vulnerable population.

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1. Introduction

Alcohol is the most commonly consumed and socially accepted teratogenic substance [1]. Excessive consumption of alcohol is directly linked to the development of adverse health outcomes including cancer, cardiovascular events, brain and hepatic injuries [2–6], as well as mental health conditions as depression or dementia [7,8].

Drinking patterns show variability within different cultures [9], being the chronic moderate and binge drinking the world's most widespread consumption patterns [10]. The National Institute on Alcohol Abuse and Alcoholism (NIAAA) defines moderate drinking as up to one drink per day for women and up to two drinks per day for men, being observed in regions surrounding the Mediterranean Sea [11]. In contrast, the phenomenon of binge drinking, a common occurrence among Eastern European and US population, entails the rapid consumption of substantial quantities of alcohol resulting in intoxication. The NIAAA characterizes binge drinking as a pattern of alcohol consumption that results in a blood alcohol concentration (BAC) of 0.08 g/dL. This phenomenon typically occurs when an individual consumes four to five drinks within a period of approximately two hours [11]. Globally, alcohol consumption is a significant public health concern. In 2019, approximately 2.4 billion individuals reported alcohol consumption, with adult women accounting for 35 %. Crucially, in pregnant women, alcohol consumption poses a unique threat, with Prenatal Alcohol Exposure (PAE) emerging as a leading cause of neurodevelopmental disorders [12]. According to recent studies conducted in Europe, up to 65.7 % of pregnant women drink alcohol either chronically or occasionally at some point during pregnancy [13]. Ethanol freely crosses the placenta and may affect fetal development [14]. PAE can lead to a range of conditions collectively known as Fetal Alcohol Spectrum Disorder (FASD). The Institute of Medicine (IOM) has defined four diagnostic categories for FASD: Alcohol-Related Birth Defects (ARBD), Alcohol-Related Neurodevelopmental Disorder (ARND), partial Fetal Alcohol Syndrome (pFAS), and Fetal Alcohol Syndrome (FAS) [15,16]. FAS is the condition that encompasses the widest range of impairments, including growth delays, craniofacial anomalies and abnormalities in the development of the Central Nervous System (CNS). Disorders in brain processes are associated with various cognitive difficulties, such as poor problem-solving skills, limited language comprehension, memory problems, and difficulty grasping concepts. Furthermore, there are behavioural issues, including impulsivity, hyperactivity, difficulty concentrating, and increased anxiety [17–20]. The global prevalence of FASD among children and youth was 7.7 per 1000 people [18], being the main cause of preventable developmental disabilities worldwide [19]. Although the prevalence of binge drinking has declined from 22.6 % in 2000 to 18.2 % in 2016 among the total population worldwide, nations in the WHO European Region still have high rates of alcohol consumption [21]. Specifically, it remains particularly high in Eastern Europe. Russia, for instance, recorded alcohol consumption per capita of 15.8 litres per person, the fourth highest in Europe [21]. This pattern correlates with the region's elevated prevalence of individuals affected by FASD, reaching 198.2 cases per 10,000 individuals [21].

Recent research has shifted focus towards understanding the molecular mechanisms underlying the neurodevelopmental effects of alcohol exposure. These mechanisms vary depending on the quantity and frequency of alcohol consumed, as well as the specific gestational stage during which exposure occurs. Animal models, particularly murine models, have proven invaluable for elucidating these effects and studying potential interventions. Evidence from murine studies has shown that PAE induces growth restriction and craniofacial anomalies [22]. Most notably, PAE disrupts neurodevelopmental processes including maturation, differentiation, plasticity, gliosis, neuronal death and loss of myelination in the prefrontal cortex and hippocampus [22–26]. These disruptions are accompanied by increased pro-inflammatory signalling, alterations in brain network connectivity [27] and a range of neurobehavioral impairments, including cognitive deficits, increased anxiety-like behaviour, motor coordination impairments, and age-dependent locomotor activity alterations [28–30].

Since the identification of FASD in the 1960s, numerous strategies have been proposed to mitigate the CNS damage caused by PAE. However, abstinence from alcohol during pregnancy remains the only proven preventive measure. In recent years, however, the use of antioxidants and other natural molecules has shown promising results in dementia [31] and in the treatment of neurological disorders such as Down's syndrome [32]. Epigallocatechin-3-gallate (EGCG), a bioactive compound found in green tea, has emerged as a potential treatment for FASD [33] through different mechanisms, specifically controlling inflammation, oxidative stress and inducing beneficial epigenetic changes [34–36]. Previous studies have shown that EGCG treatment in FASD children produces epigenetic modifications leading to neurocognitive and behavioural improvements [37,38]. Studies in a FASD-like mouse model have also shown the beneficial effects of prenatal and postnatal EGCG supplementation on the expression of some biomarkers of oxidative stress, neuronal and cardiac damage biomarkers, and on cardiac remodelling induced by PAE [22,39].

The effects of maternal alcohol consumption on the developing CNS are critical because of the profound implications for the offspring's health and cognitive performance. Given the complexity of the CNS, it is crucial to understand the impact of different patterns of PAE on diverse brain regions and cell types. This will allow the identification of potential diagnostic biomarkers and therapeutic targets. Therefore, the aim of this study is to provide valuable insights into the impact of moderate and binge maternal alcohol consumption patterns on offspring CNS and the potential benefits of postnatal EGCG treatment in the prevention of FASD-related behavioural disorders.

2. Methods

2.1. Animals, housing and ethical statement

Eight-week-old C57BL/6J mice (30 male and 90 female) were purchased from Charles River (Barcelona, Spain), and housed in the animal facilities of the Sant Joan de Déu Barcelona Hospital (Barcelona, Spain). During the study, the mice were housed under controlled environmental conditions (20–24°C, 55 % ± 10 % relative humidity, 12 h light/dark cycle) and were fed according to a standard chow diet (Teklad Global 14 % Protein Rodent Maintenance Diet, 2014.12; Inotiv). The use of this diet was approved by the animal facility committee under protocol 2022/137. All the procedures were performed in accordance with the ARRIVE guidelines [40], the EU Directive 2010/63/EU and approved by the Animal Experimentation Ethics Committee (CEEA) of the University of Barcelona and registered by Generalitat de Catalunya, Departament de Territori i Sostenibilitat (identification code: 3FF6ZD9TL).

2.2. Experimental design

To establish the crosses, 1 male was housed with 2 females for one night. If a sperm vaginal plug was observed the morning after mating, day 1 of gestation was considered. At this point, the pregnant mice were randomly allocated to one of the five experimental groups and individually housed in plastic cages.

The study design consisted of the prenatal administration of ethanol between day 1 and day 19. Postnatally, both mothers and offspring received EGCG-supplemented water *ad libitum*, which was provided twice daily to minimise EGCG oxidation [41], continuing until day 60. The experimental groups were: (1) Control group: prenatal isocaloric maltodextrin solution (8.4g/kg/day). (2) Chronic moderate alcohol consumption (Mod) group: 1.5g/kg/day of ethanol. (3) Mod+EGCG group: 1.5g/kg/day of ethanol and postnatal EGCG 60mg/kg/day. (4) Binge (Bin) group: 4.5g/kg/day of ethanol. (5) Bin+EGCG group: 4.5g/kg/day of ethanol and postnatal EGCG 60mg/kg/day.

The groups were designed to compare the control group against

different prenatal alcohol concentrations, and these groups with groups prenatally exposed to alcohol but treated postnatally with EGCG. A maltodextrin+EGCG group was not included in the experimental design following ethical committee recommendations, because the beneficial effects of EGCG in healthy subjects have been widely reported in previous literature [42–44], being out of the scope of this study.

The estimated daily concentration of EGCG was 60 mg/kg, in newly weaned mice the average water consumption was 3 mL per day. Previous studies confirm that this is the most effective dose for improving memory acquisition and retention [45]. The pups were raised with their mothers until postnatal day 21 (weaning). During this pre-weaning phase (P1–P21), EGCG exposure in pups occurred primarily through maternal milk, as direct ingestion of EGCG-supplemented water was negligible given the pups' immature developmental stage and limited ability to access water bottles [46,47]. Experimental evidence supports that although EGCG is transferred into maternal milk in murine models, the transfer rate is limited [46,47], resulting in expected milk concentrations of approximately 27–57 nM, as previously described in Souchet *et at.* [46,48]. Upon weaning at postnatal day 21, pups were separated by sex and given ad libitum access to water or EGCG-enriched water via specially adapted bottles with elongated nozzles, ensuring continued exposure during the critical period of early development. After weaning, the mice consumed between 0.15 and 0.25 mL of water per gram of body weight per day (approximately 2 mL per 10 g of body weight), consistent with reported norms [49,50]. Water consumption was monitored daily by measuring the intake relative to the number of pups per cage, confirming that the actual intake corresponded to the average values reported in the literature. Based on this, the estimated EGCG intake post-weaning was approximately 60 mg/kg/day. Importantly, throughout the study, the total EGCG intake never exceeded 200 mg/kg/day, remaining within safe and effective ranges established in prior research [22,39]. At postnatal day 60, two males per litter were selected for behavioural testing, undergoing assessments in the following sequence: Rotarod, T-maze, and Morris water maze, in this sequence. All behavioural testing was performed in a blinded manner. Specifically, a second technician, who was aware of the group assignments, selected and handed each mouse to the tester without revealing the group identity, ensuring unbiased data collection. For Western blot analyses, each lane represented a different mouse, with no animal used more than once per marker; all markers were assessed in each subject.

2.3. Reagents and antibodies

Pure Absolute EtOH solution was obtained from PanReac AppliChem ITW Reagents (Dublin, Ireland), and the maltodextrin solution (Pure Series®) was obtained from Bulk Powders (Essex, UK). The EGCG solution was obtained from a dilution of Teavigo, a highly purified and refined green tea extract with a 94 % of EGCG (Healthy Origins, Pittsburgh, PA,USA).

The antibodies used in this study were as follows: Neuronal Nuclear antigen (NeuN) (ref.ab177487,dil1:1000,48.5 kDa), Doublecortin (DCX) (ref.ab135349,dil1:1000,49 kDa), Glial fibrillary acidic protein (GFAP) (ref.ab7260,dil1:1000,55 kDa), Brain-derived neurotrophic factor (BDNF) (ref.ab226843,dil1:1000,17 kDa), Glial cell line-derived neurotrophic factor (GDNF) (ref.ab18956,dil1:1000,24 kDa) and Myelin Basic Protein (MBP) (ref.ab218011,dil1:1000,14 and 22 kDa) from Abcam (Cambridge,MA,USA); Nuclear factor erythroid 2–related factor 2 (Nrf2) (ref.sc-722,dil1:1000,61 and 68 kDa), Wnt-3 (ref.sc-74537,dil1:1000,43 kDa), and s100β (ref.sc-39391,dil1:1000,21 kDa) from Santa Cruz Biotechnology; Dual specificity tyrosine phosphorylation regulated kinase 1A (DYRK1A) (ref.#8765,dil1:1000,90,68 and 25 kDa) from Cell Signalling (Danvers,Massachusetts,USA); Alfa-tubulin (ref.T8203,dil1:2000,50 kDa), beta-actin (ref.A3854,42 kDa,dil 1:2500), and Anti Rabbit IgG secondary (ref.A0545,dil1:2000) from Sigma-Aldrich (Sant Louis,Missouri,USA), and goat antimouse IgG secondary (ref.G21040,dil1:10,000) from Thermo Fisher Technologies (Waltham, MA,USA).

2.4. Western blot analysis

In this study, brain and cerebellum samples were homogenised separately to evaluate systemic biomarker changes. Whole-brain homogenates were used to ensure sufficient sample volume for robust biochemical analysis and to reduce variability associated with microdissection. Whole protein extracts from brain and cerebellum samples were obtained using an Omni Tissue Homogenizer (TH115) by mechanically disrupting tissue samples in RIPA lysis buffer (ThermoFisher Scientific,89901) with Halt™ Protease Inhibitor Cocktail, EDTA-free (100X) (Thermo Fisher Scientific, 1862209) at 4ºC for 2 min with 30-second intervals. PierceTM BCA Protein Assay Kit (ThermoFisher Scientific,23227) was used to quantify the amount of protein (absorbance 562 nm). Then, 40 μg of protein from each sample was separated by SDS-PAGE and transferred to a polyvinylidene fluoride membrane (Bio-Rad Laboratories SA,162–0177) following manufacturer instructions. The membranes were incubated with the primary antibodies overnight at 4ºC and secondary antibodies for 2 h at room temperature, then they were developed in the dark using an iBright CL1000 equipment (ThermoFisher Scientific,A35284) and the Pierce ECLWB Substrate (ThermoFisher Technologies,32106). Densitometric analysis was performed using the Image J programme to assess the intensity of the bands. The densitometry results were normalised to values derived from the control protein bands, which were expressed at constant levels under all conditions. To ensure the correct use of the housekeeping proteins as loading controls, we performed a validation of the housekeeping proteins by comparing their intensities with total protein staining with Ponceau S [51]. The results showed no significant differences between groups, confirming their stability as loading controls (Supplementary figures 1 and 2).

2.5. Behavioural tests

2.5.1. Rotarod

The Rotarod behavioural test is a widely used method for assessing motor coordination and balance in mice. In this study, adolescent mice were trained on a Rotarod (LE 8205,Panlab s.l.u, Barcelona, Spain) with an acceleration rate of 20 rpm/min for two consecutive days and the performance was recorded. On the third day, we conducted a test with five trials per mouse and 5-minute breaks in between. During the test, we recorded the time it took for each mouse to fall off the rotating rod, along with the rotation speed at the time of the fall. We used the average of the last five trials as the measure of the mouse's motor coordination.

2.5.2. T-maze

T-maze test is a method used to study spatial alternation, learning, and memory in rodents [52]. The experiment consists of five free-choice trials, each lasting up to 50 min, conducted on a single day. Each trial consists of blocking a target arm, recording the time taken for the mouse to reach the open goal arm, confining the mouse to the target arm, and then allowing it to choose an arm. A correct choice is made if the mouse enters the previously closed arm, while an error occurs when it enters the same arm. The test ends when the controls achieve 80 % success, or after 50 min have elapsed. If the established success rate is not achieved, the same procedure is repeated the following day. Mice with hippocampal damage often exhibit a preference and typically score below 50 %, whereas control mice score above 80 %.

2.5.3. Morris water maze

Morris Water Maze (MWM) is used to assess spatial learning and memory in rodents, particularly mice. The MWM test consists of multiple training trials in which a mouse is placed in a pool and allowed to swim until it locates a hidden platform. The starting position is varied in each trial to prevent the mouse from learning a fixed path to the

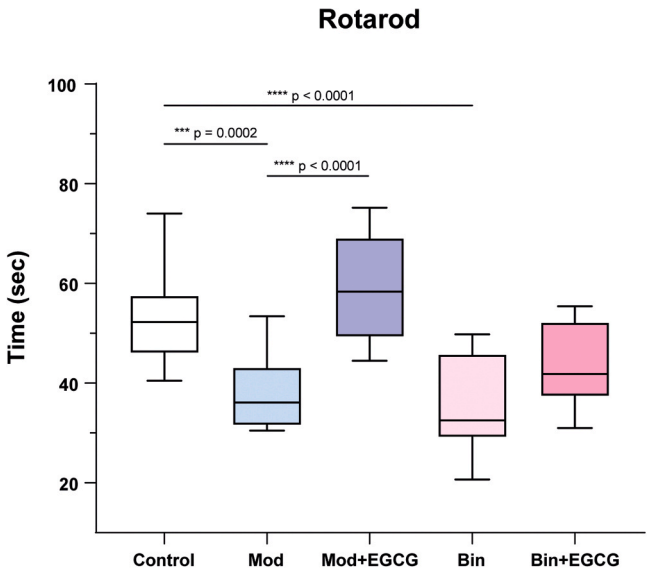

Fig. 1. Motor coordination in the offspring measured with Rotarod after maternal binge and moderate alcohol drinking. The figure displays a box plot representation of the walking time obtained in the last five trials of the rotarod test in control, moderate, binge and after EGCG treatment profiles. The horizontal line within the box indicates the mean. Whiskers extend to the minimum and maximum values within the dataset. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (Kruskall-Wallis $H_{(4)}= 44.61, p < 0.0001$). Mod: moderate; Bin: binge. Level of significance: ***p ≤ 0.001; ****p ≤ 0.0001.

platform [53]. If the mouse fails to find the platform within a predetermined time, it is guided to the platform. The mouse is allowed to rest briefly on the platform before being removed from the maze [53].

The test measures spatial learning in mice, primarily through the time taken to locate a hidden platform. A decrease in escape latency indicates learning. Spatial memory is assessed by a probe trial in which the mouse's preference for the quadrant where the platform was previously located is recorded. Additional parameters such as swimming speed and distance travelled provide further insight. MWM is a reliable tool to study the impact of genetic and environmental factors on these cognitive processes [53]

2.6. Statistical analysis

Statistical analyses were performed using SPSS v.22 (IBM, Chicago, IL, USA) and GraphPad6.0 (Prism,San Diego,CA,USA). The data were presented as means and standard deviations (SD). To evaluate differences in protein expression in brain and cerebellum as well as to evaluate the results of behavioural tests, first we employed the Shapiro-Wilk test to assess the normality of the data in each group. Then, intergroup comparisons were carried out using the non-parametric Kruskal-Wallis test, with Dunn's correction for multiple comparisons. Statistical significance level was set at $p < 0.05$ for all analyses, with additional levels of significance indicated as follows: *p ≤ 0.05;** p ≤ 0.01;***p ≤ 0.001; ****p ≤ 0.0001. At least eight different samples from different litters were used for the statistical analyses.

3. Results

3.1. Behavioural tests

3.1.1. Motor coordination – Rotarod

A total of 120 C57BL/6J mice were included in this study. Blood alcohol concentrations (BAC) were ≥ 0.08 g/dL in Mod and Mod+EGCG groups and ≥ 0.24 g/dL for Bin and Bin+EGCG groups. After birth and until postnatal day 22, the offspring received water supplemented with EGCG at 15 mL/100 g, corresponding to an estimated EGCG concentration of 60 mg/kg/day, in line with the levels observed in previous studies [54].

Walking time for each of the five Rotarod tests were calculated to assess the effect of early alcohol exposure on motor coordination. The average walking time was significantly lower in PAE individuals with binge consumption (t = 35.78 ± 9.625) and in those with moderate consumption (t = 38.16 ± 7.35) compared to the control group (t = 52.17 ± 7.839) (Fig. 1). Interestingly, mice exposed to moderate consumption pattern showed a significant improvement in motor coordination after EGCG treatment (t = 59.28 ± 10.68) compared to

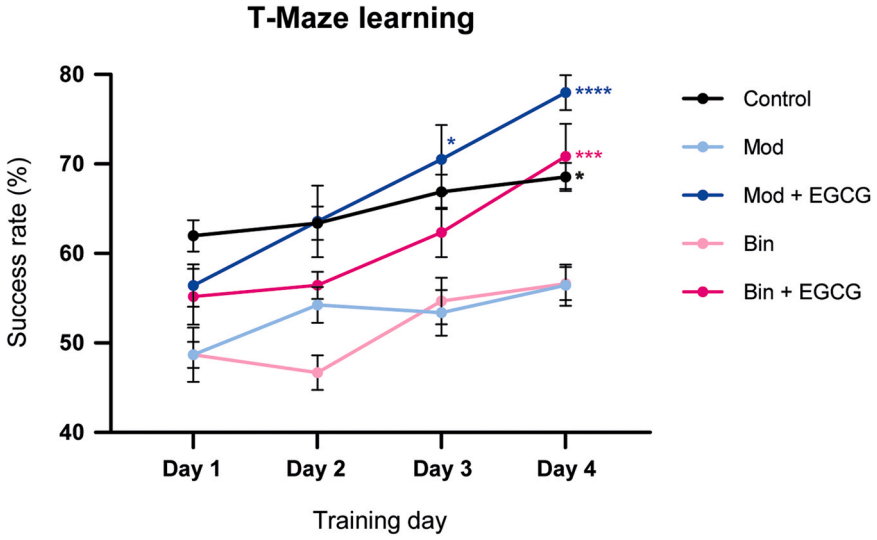

Fig. 2. Learning graph over 4 days of performing the T-maze test. Data are expressed as mean percentage ± SEM of the successful rate percentage in the last four trials of the test in control, moderate, binge and after EGCG treatment groups. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (Kruskall-Wallis $H_{(4)}= 11.21, p = 0.0243$). Mod: moderate; Bin: binge. Pink and blue asterisks denote statistically significant differences between the PAE and PAE+EGCG groups on the same day of the test. The black asterisk indicates a statistically significant difference between the control group and the Mod+EGCG group on the same day of the test: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

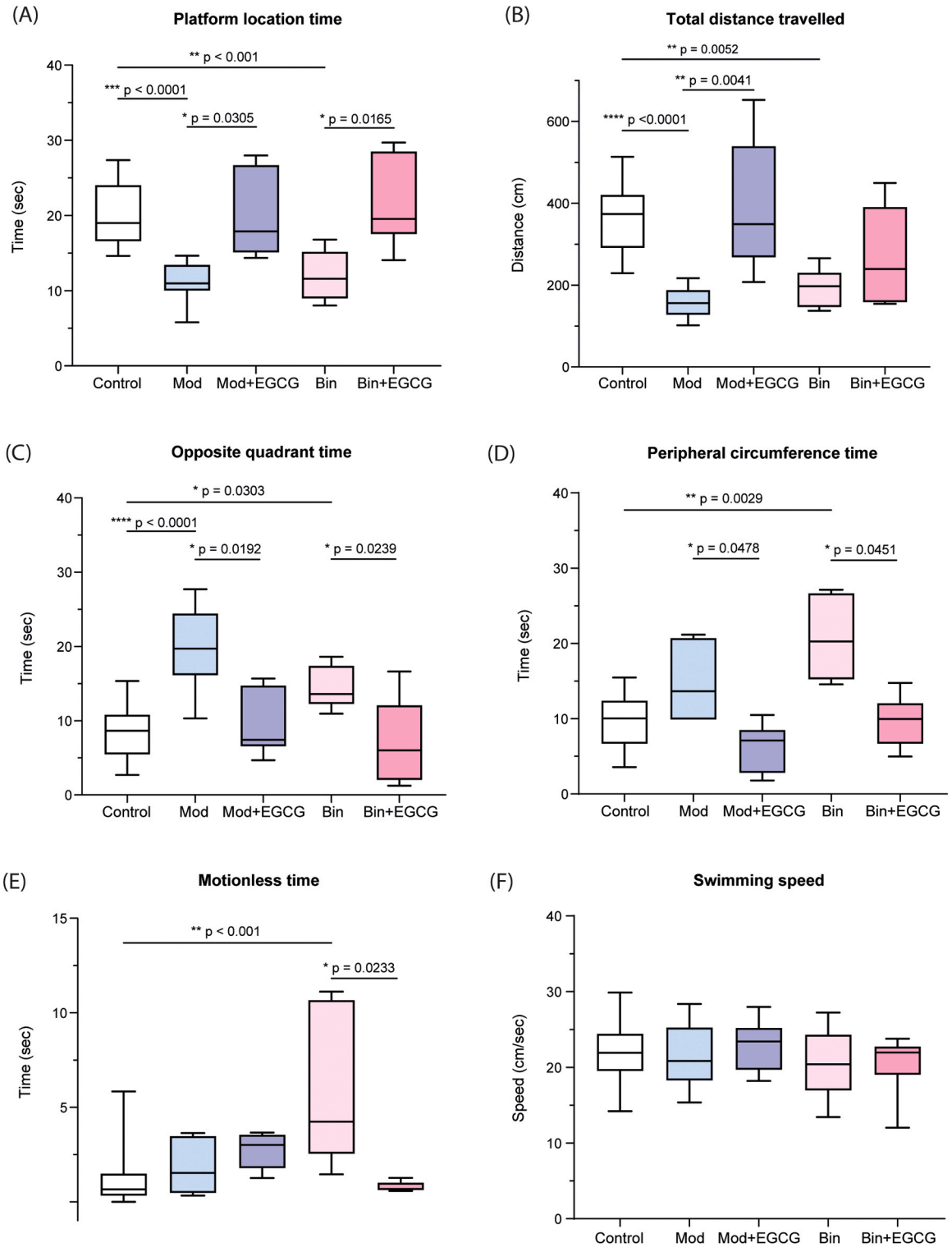

Fig. 3. Morris water maze behavioural test analysis in the offspring after maternal binge and moderate alcohol drinking. (A) Time spent in the location where the platform was previously situated during the learning phase; (B) total distance travelled during the trial; (C) time spent in the opposite quadrant; (D) time spent in the peripheral circumference (15 cm); (E) time spent immobile during the trial; and (F) average swimming speed of the mice. The figure displays a box plot representation of the final test of the MWM test in control, moderate, binge and after EGCG treatment profiles. Mod: moderate; Bin: binge. The horizontal line within the box indicates the mean. Whiskers extend to the minimum and maximum values within the dataset. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(4)}= 31.22$, $p < 0.0001$); (B) (Kruskall-Wallis $H_{(4)}= 30.89$,$p < 0.0001$); (C) (Kruskall-Wallis $H_{(4)}= 29.70$,$p < 0.0001$); (D) (Kruskall-Wallis $H_{(4)}= 22.26$,$p = 0.0002$); (E) (Kruskall-Wallis $H_{(4)}= 20.07$,$p = 0.0005$); (F) (Kruskall-Wallis $H_{(4)}= 2.874$,$p = 0.5791$). Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001; ****p ≤ 0.0001.

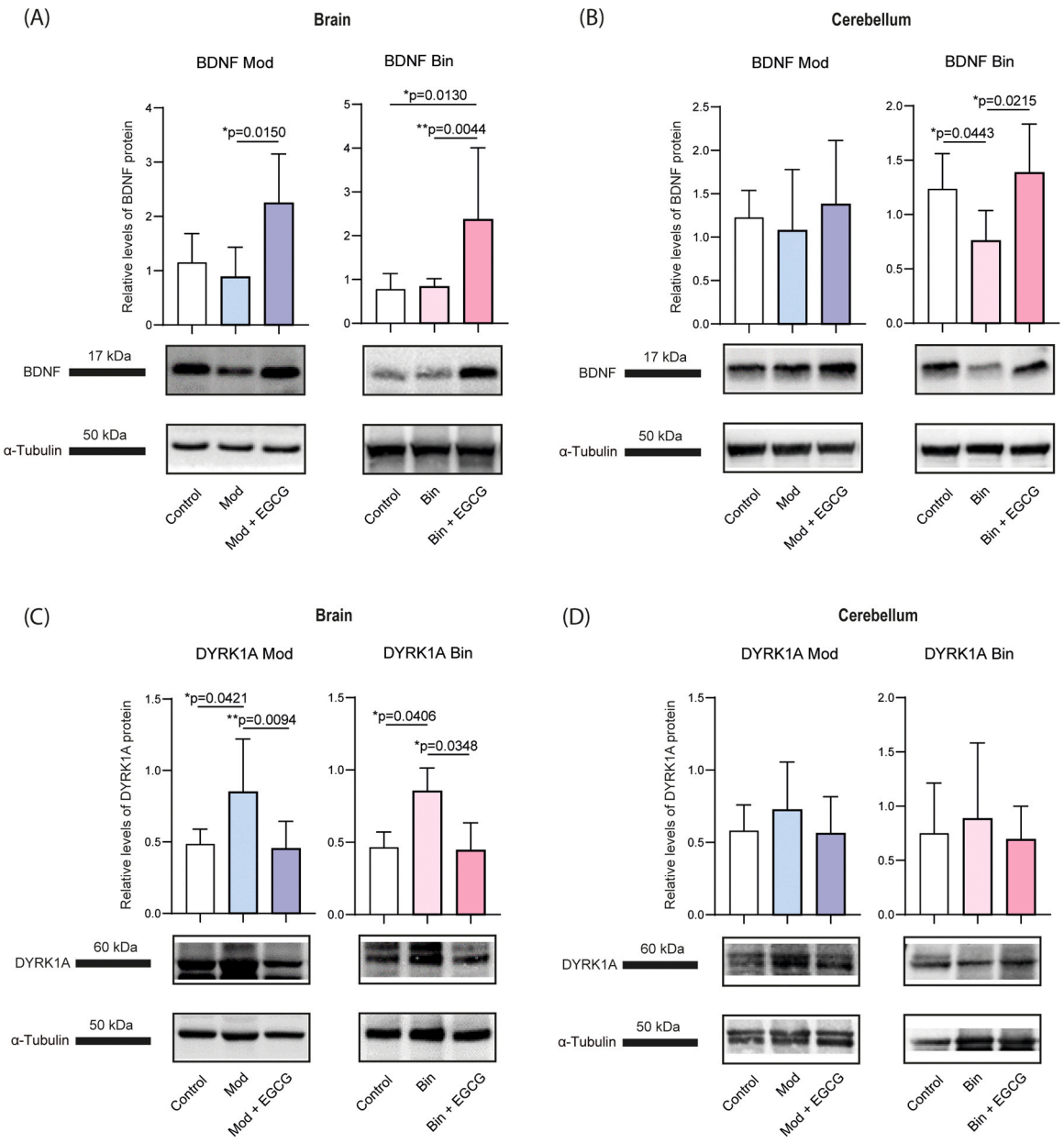

Fig. 4. BDNF (A brain, B cerebellum) and DYRK1A (C brain, D cerebellum) levels in mice tissue to analyse neuronal plasticity after maternal alcohol exposure in moderate and binge patterns. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 8.304$,$p = 0.0090$, $H_{(2)}= 12.60$,$p = 0.0002$); (B) (Kruskall-Wallis $H_{(2)}= 3.145$, $p = 0.2075$, $H_{(2)}= 9.199$,$p = 0.0101$); (C) (Kruskall-Wallis $H_{(2)}= 7.311$,$p = 0.0196$, $H_{(2)}= 7.795$,$p = 0.0116$); (D) (Kruskall-Wallis $H_{(2)}= 1.364$,$p = 0.5258$, $H_{(2)}= 0.006$,$p = 0.9992$). Mod: moderate; Bin: binge. Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001.

moderate consumption pattern.

3.1.2. *Working memory – T-maze*

Adult mice were subjected to the T-maze spontaneous alternation test to assess long-term impact of early alcohol exposure on spatial working memory. On day 1, both moderate and binge groups had a success rate below 50 % (48.69 % ± 9.11 and 48.68 % ± 5.23, respectively), significantly lower than the control group (61.98 % ± 8.37; **p = 0.0075, ***p = 0.0005) (Fig. 2 and supplementary Fig.3A).

After EGCG treatment, both Mod+EGCG and Bin+EGCG groups showed an upward trend in success rates on day 1 (55.00 % ± 6.27 and 55.17 % ± 8.84, respectively), although this increase was not statistically significant (Fig. 2).

The treatment groups, (Mod+EGCG and Bin+EGCG) almost reached the 80 % learning success rate on day 4 (77.98 % ± 5.52 and 70.85 % ± 10.25, respectively) (Fig. 2 and supplementary Fig.3D), which was comparable to the success rate of the control group on day 4 (68.55 % ± 7.17) (Fig. 2). Furthermore, the Bin+EGCG and Mod+EGCG groups showed the greatest improvement in learning (28.42 % and 38.17 % of the total increase, respectively) (Supplementary Fig.3D), while the Binge and Moderate groups had poor improvement rates (16.33 % and 15.96 %, respectively) (Supplementary Fig.3D). Surprisingly, the Mod group following treatment with EGCG, not only showed a significant improvement in their success rate but also surpassed the performance of the control group, highlighting the potential of EGCG to enhance cognitive function beyond baseline levels (Fig. 2).

3.1.3. *Spatial learning and memory - morris water maze (MWM)*

Results indicated that both moderate and binge groups exhibited a

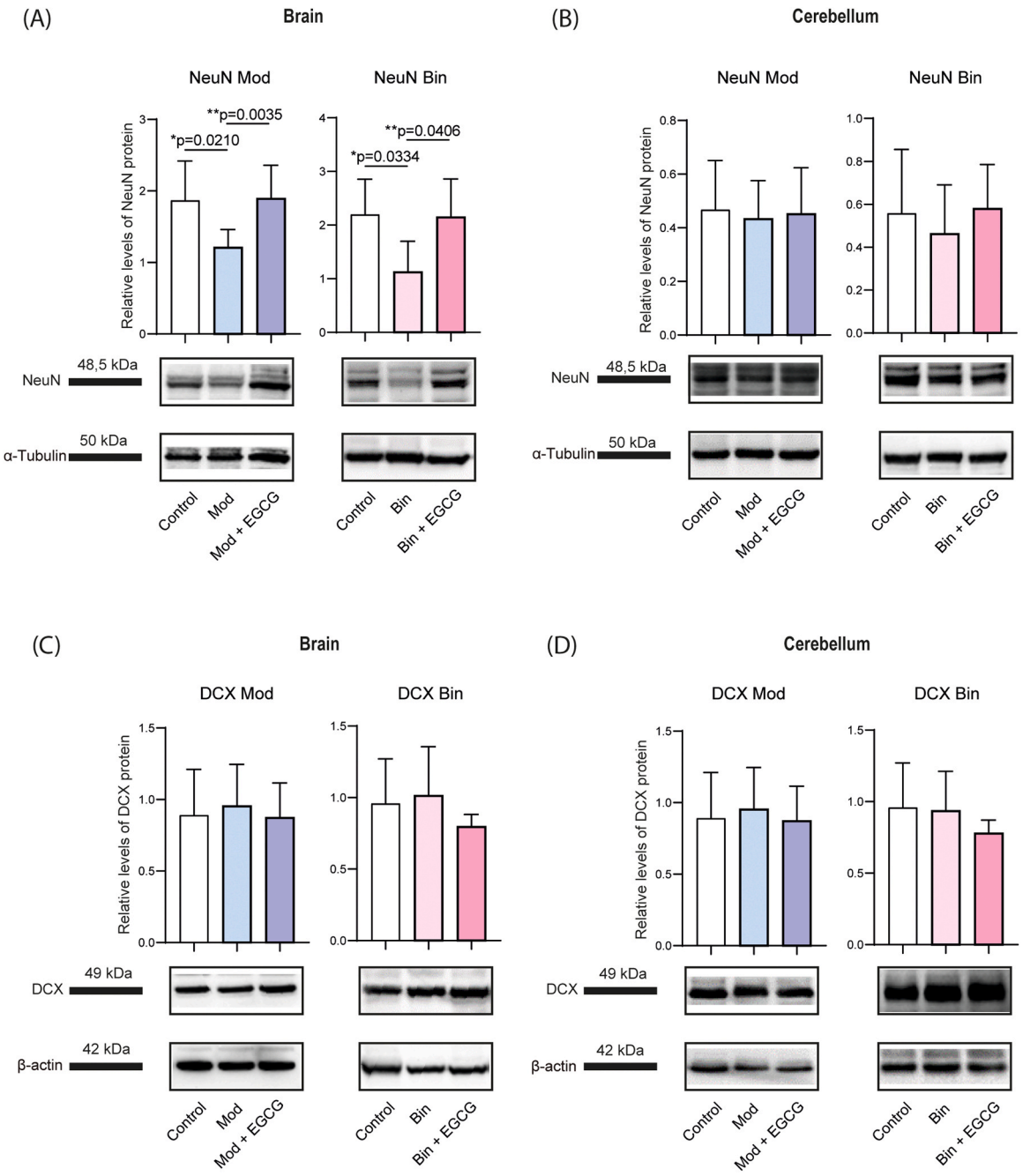

Fig. 5. Brain NeuN (A), cerebellum NeuN (B), brain DCX (C) and cerebellum DCX (D) levels in mice tissue to analyse neuronal maturation after maternal alcohol exposure in moderate and binge patterns. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 12.38, p = 0.0020$, $H_{(2)}= 8.461, p = 0.0145$); (B) (Kruskall-Wallis $H_{(2)}= 0.051, p = 0.9777$, $H_{(2)}= 0.955, p = 0.6429$); (C) (Kruskall-Wallis $H_{(2)}= 0.303, p = 0.8674$, $H_{(2)}= 3.033, p = 0.2245$); (D) (Kruskall-Wallis $H_{(2)}= 0.303$, $p = 0.8674$, $H_{(2)}= 2.701, p = 0.2709$). NeuN: Neuronal Nuclear Antigen; DCX: doublecortin; Mod: moderate; Bin: binge, EGCG: epigallocatechin-3-gallate.Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01.

significant impairment in spatial and working memory. This was observed by the reduced time spent in the location where the platform was previously situated (11.22 s for Mod, 12.09 s for Bin) (Fig. 3A) and the total distance travelled during the trial (158.20 centimetres for Mod, 194.70 centimetres for Bin) (Fig. 3B), as well as the increased time spent in the opposite quadrant (19.75 s for Mod, 14.60 s for Bin)(Fig. 3C) and outside the circle (20.60 s for Bin) compared to control group (Fig. 3D).

Regarding the antioxidant treatment, the EGCG-treated groups showed a statistically significant improvement in the time spent in the platform zone (19.95 s for Mod+EGCG and 21.18 s for Bin+EGCG) (Fig. 3A) and an increase in the distance swum during the test over the moderate (370.30 centimetres for Mod+EGCG) (Fig. 3B). Thus, our results showed a decrease of 9.91 and 7.78 s in the opposite quadrant (Figs. 3C) and 8.42 and 10.94 s in the outer circle (Fig. 3D) in the Mod and Binge group after EGCG treatment compared to control.

In addition, we observed significant differences in the time spent immobile in the water (Fig. 3E). Specifically, the Binge group (5.45 s) spent significantly more time immobile compared to the control group (1.05 s) and the Bin+EGCG group (0.82 s). Surprisingly, there were no significant differences between the groups in terms of speed of movement (Fig. 3F).

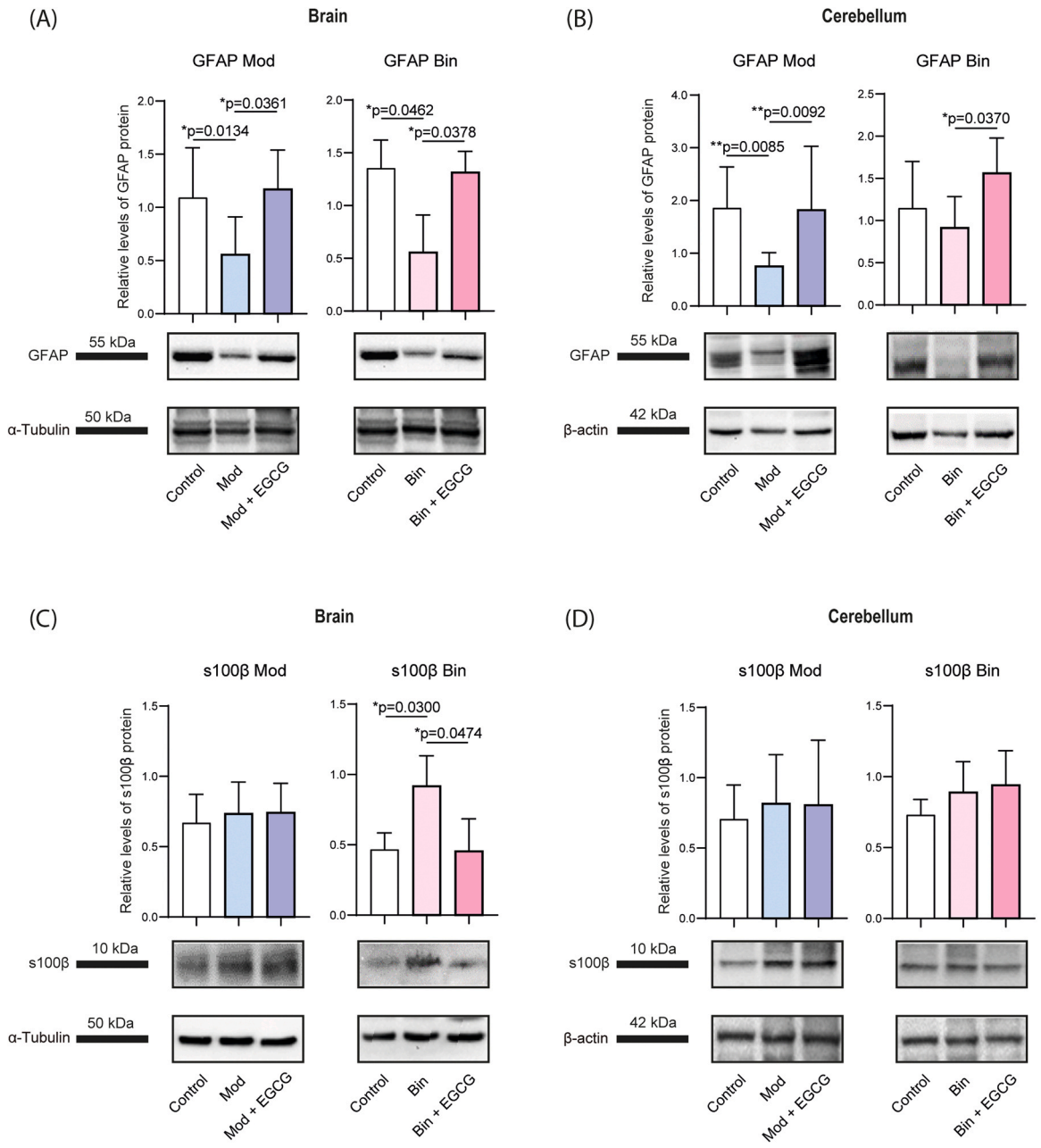

Fig. 6. Brain GFAP (A), cerebellum GFAP (B), brain s100β (C) and cerebellum s100β (D) levels in mice tissue to analyse neuronal differentiation after maternal alcohol exposure in moderate and binge patterns. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 10.15, p = 0.0007$, $H_{(2)}= 8.223, p = 0.0069$); (B) (Kruskall-Wallis $H_{(2)}= 12.24, p = 0.0022$, $H_{(2)}= 7.625, p = 0.0160$); (C) (Kruskall-Wallis $H_{(2)}= 0.572, p = 0.7513$, $H_{(2)}= 7.812, p = 0.0118$); (D) (Kruskall-Wallis $H_{(2)}= 0.2959$, $p = 0.8736$, $H_{(2)}= 2.689, p = 0.2745$). GFAP: Glial Fibrillary Acidic Protein; s100β: s100 Calcium Binding Protein β; Mod: moderate; Bin: binge; EGCG: epigallocatechin-3-gallate.Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01.

3.2. Neuronal plasticity

Despite a trend towards decrease, there were no significant differences in Brain-Derived Neurotrophic Factor (BDNF) levels between control group and Moderate and Binge groups in brain samples (Fig. 4A). Interestingly, we observed a statistically significant increase in BDNF levels in Bin+EGCG group compared to controls and binge group. EGCG also significantly increased BDNF levels in moderate group compared to its untreated counterpart (Fig. 4A). On the other hand, Binge group exhibited a significant reduction relative to control and EGCG treatment groups in cerebellum. Interestingly, after EGCG treatment, the binge group did not show significantly different BDNF levels compared to the control group. No differences in BDNF levels were observed in the moderate group (Fig. 4B). Parallel analyses revealed a significant elevation in Dual specificity Tyrosine(Y) Regulated Kinase 1 (DYRK1A) expression in brain samples of both moderate and binge groups compared to control and EGCG-treated groups (Fig. 4C). In contrast, cerebellar DYRK1A levels remained unchanged in all groups (Fig. 4D).

3.3. Neuronal maturation

Levels of Neuronal Nuclear Antigen (NeuN) were significantly reduced in both moderate and binge groups compared to the controls in

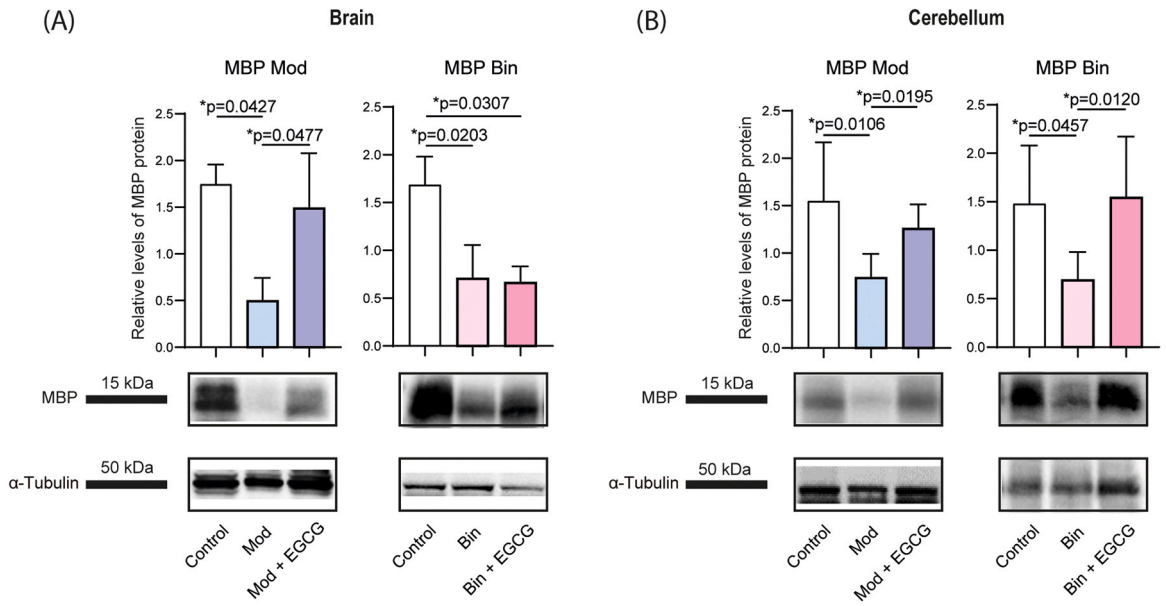

Fig. 7. Brain MBP (A), cerebellum MBP (B) levels in mice tissue to analyse neuronal transport after maternal alcohol exposure. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 7.744, p = 0.0107$, $H_{(2)}= 8.679, p = 0.0056$); (B) (Kruskall-Wallis $H_{(2)}= 10.42, p = 0.0055$, $H_{(2)}= 10.25, p = 0.0022$). MBP: Myelin Basic Protein; Mod: moderate; Bin: binge; EGCG: epigallocatechin-3-gallate. Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01.

brain samples. After EGCG treatment, NeuN levels recovered to physiological levels in both alcohol-exposed groups (Fig. 5A). No changes were observed in cerebellum in either group (Fig. 5B).

For doublecortin (DCX), no differences were observed between the groups studied (Fig. 5C-5D).

3.4. Neuronal differentiation

In brain, Glial Fibrillary Acidic Protein (GFAP) levels were significantly lower in both moderate and binge groups compared to the control (Fig. 6A). After the treatment with EGCG, the levels of these proteins reached the control group in both alcohol-exposed groups (Fig. 6A). In the cerebellum, GFAP levels were significantly reduced in the moderate group compared to the control group, but returned to physiological levels after EGCG treatment, no significant differences were observed with respect to the control group (Fig. 6B). GFAP levels in the cerebellum of the binge group showed a slight tendency to decrease, although without significant results. In contrast, after treatment with the antioxidative agent, we observed a significant increase in the presence of GFAP, with levels similar to those of the control group (Fig. 6B).

Notably, the levels of s100 Calcium Binding Protein β (s100β) in binge group showed significant increased levels relative to the control in brain samples, demonstrating a recovery to physiological levels after EGCG treatment (Fig. 6C). Moderate group in brain and cerebellum samples showed a non-significant trend towards an increase (Fig. 6C-D). No significant differences were observed for the binge group in cerebellum in either group (Fig. 6D).

3.5. Neuronal transport

Myelin Basic Protein (MBP) levels in the brain were significantly lower in both the moderate and binge groups compared to the control (Fig. 7A). The administration of EGCG to the moderate group led to the recovery of physiological levels (Fig. 7A). In contrast, the binge group treated with this antioxidant was unable to recover to the levels observed in the control group (Fig. 7A). In fact, no statistically significant differences were observed between the treated group and the binge group (Fig. 7A). In the same line, a significant reduction in MBP levels was noted in the cerebellum of both PAE groups compared to control and EGCG treatment groups, recovering control levels after EGCG treatment (Fig. 7B).

3.6. Neuronal proliferation

A significant increase in Glial-cell-line Derived Neurotrophic factor (GDNF) was observed in the brain of both Moderate and Binge groups compared to the control group (Fig. 8A). Treatment with EGCG significantly decreased the levels of this protein, reaching values similar to those of the control group (Fig. 8A). In cerebellum samples, GDNF levels showed a significant and tending decreased in the moderate and binge groups compared to the control group (Fig. 8B). Additionally, cerebellum showed a significant increase in GDNF levels in both alcohol-exposed groups after EGCG treatment, reaching similar levels to the control group (Fig. 8B).

Regarding Wnt family member 3a (Wnt-3a), this protein was significantly reduced in the brain of the ethanol-exposed groups. After EGCG treatment, the levels were increased and reached levels similar to the control group (Fig. 8C). In the cerebellum, Wnt-3a levels were significantly reduced in binge group compared to controls and increased after EGCG treatment, without achieving significant differences with respect to the control group. No differences were observed in moderate group (Fig. 8D).

3.7. Oxidative stress

No changes in nuclear factor erythroid 2-related factor 2 (Nrf2) levels were observed in brain and cerebellum for the moderate group (Fig. 9A-B). A significant increase in Nrf2 compared to control was only observed in the binge group in the brain, which decreased significantly after EGCG treatment, to levels similar to the control group (Fig. 9A).

4. Discussion

The present study examines the deleterious effects of PAE on adult cognitive function according to two human drinking patterns, focusing on the potential therapeutic role of EGCG. Nowadays, several studies

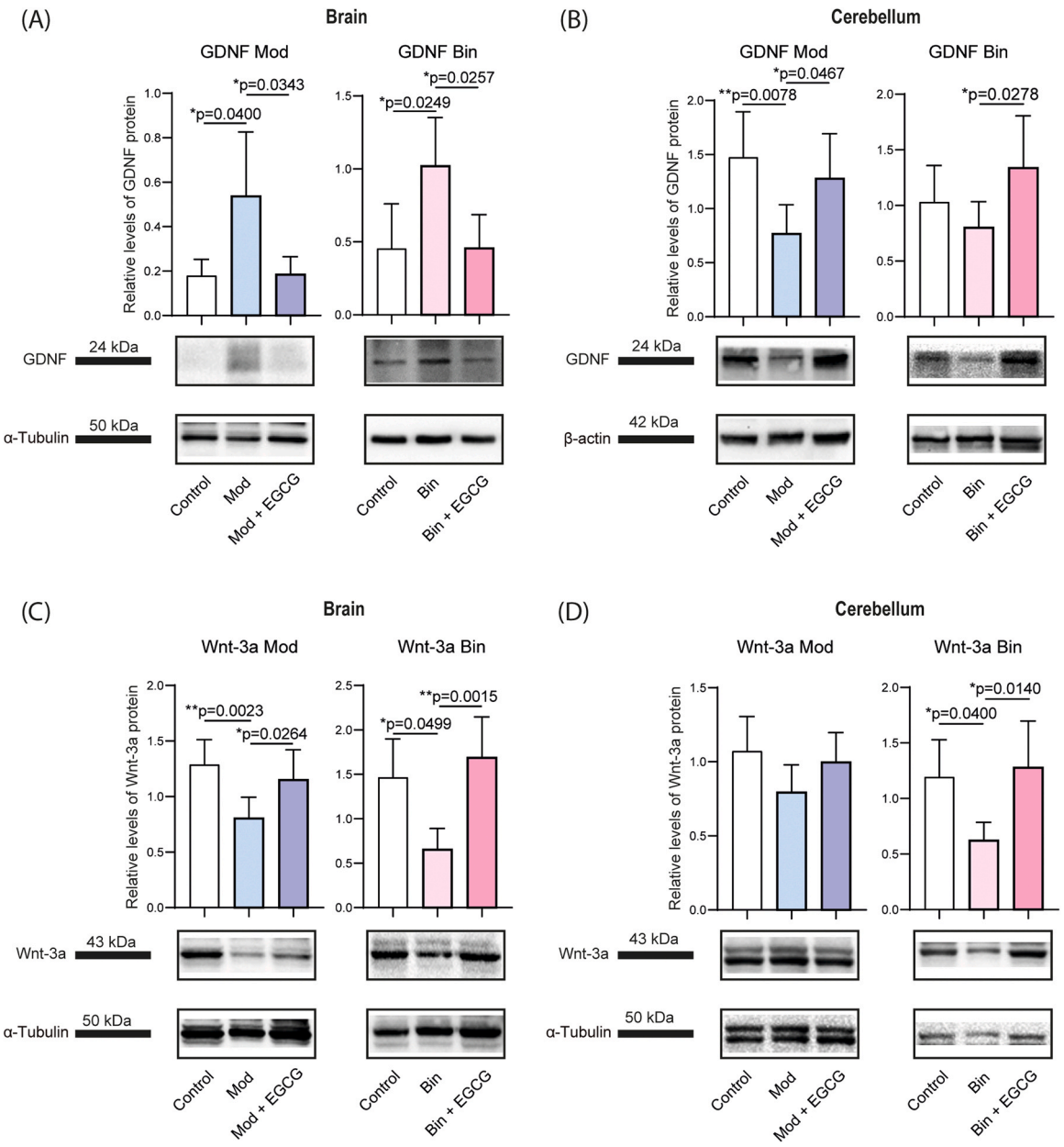

Fig. 8. Brain GDNF (A), cerebellum GDNF (B), brain Wnt-3a (C), cerebellum Wnt-3a (D) levels in mice tissue to analyse neuronal proliferation after maternal alcohol exposure in moderate and binge patterns. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 8.103, p = 0.0101$, $H_{(2)}= 9.070, p = 0.0107$); (B) (Kruskall-Wallis $H_{(2)}= 10.55, p = 0.0051$, $H_{(2)}= 6.801, p = 0.0334$); (C) (Kruskall-Wallis $H_{(2)}= 12.84, p = 0.0016$, $H_{(2)}= 12.78, p = 0.0002$); (D) (Kruskall-Wallis $H_{(2)}= 5.310$, $p = 0.0658$, $H_{(2)}= 9.500, p = 0.0021$). GDNF: Glial-cell-line Derived Neurotrophic factor; Wnt-3a: Wnt family member 3a; Mod: moderate; Bin: binge; EGCG: epigallocatechin-3-gallate. Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01.

have evaluated the effects of PAE on cognitive function [55,56], but few have analysed the consequences according to different maternal drinking patterns. Furthermore, the results provide the first evidence that postnatal EGCG treatment may be a feasible therapy to ameliorate the FASD-like neurodevelopmental disorders caused by PAE.

Alcohol impairs motor coordination, as it was observed in the Rotarod results, where PAE groups had a significantly shorter average walking time compared to control mice. These findings are aligned with previous research [12,57] demonstrating persistent deficits in motor coordination (particularly balance and fine motor control) in PAE subjects. Similarly, biomarker analysis of DYRK1A, a general inhibitor of neuronal plasticity, provided insight into the underlying mechanisms of motor alterations [58]. DYRK1A significantly increased in PAE groups in brain samples, suggesting alterations in neuronal plasticity [59,60] and synaptic maturation [61–63]. Previous research has linked DYRK1A to altered motor function and hyperactivity in Down syndrome mouse models [58,64], indicating that PAE can modify offspring's gene expression and promote various neurological and cognitive alterations [62,65]. NeuN, a widely recognised marker of neuronal maturity, has been implicated not only in the processes of neuronal maturation but also in various behavioural alterations, including hyperactivity [66,67]. Our findings revealed reduced NeuN levels in PAE groups, which is consistent with previous research observing a loss of mature neurons in the dentate gyrus following alcohol exposure [66,68,69]. The Rotarod test results reflect an increase in hyperactivity, which may contribute to the observed alterations in PAE mice. PAE also causes demyelination in various brain regions [70] and alters gamma-aminobutyric acid glutamate ($GABA_A$) and N-methyl-D-aspartate (NMDA) neurotransmission,

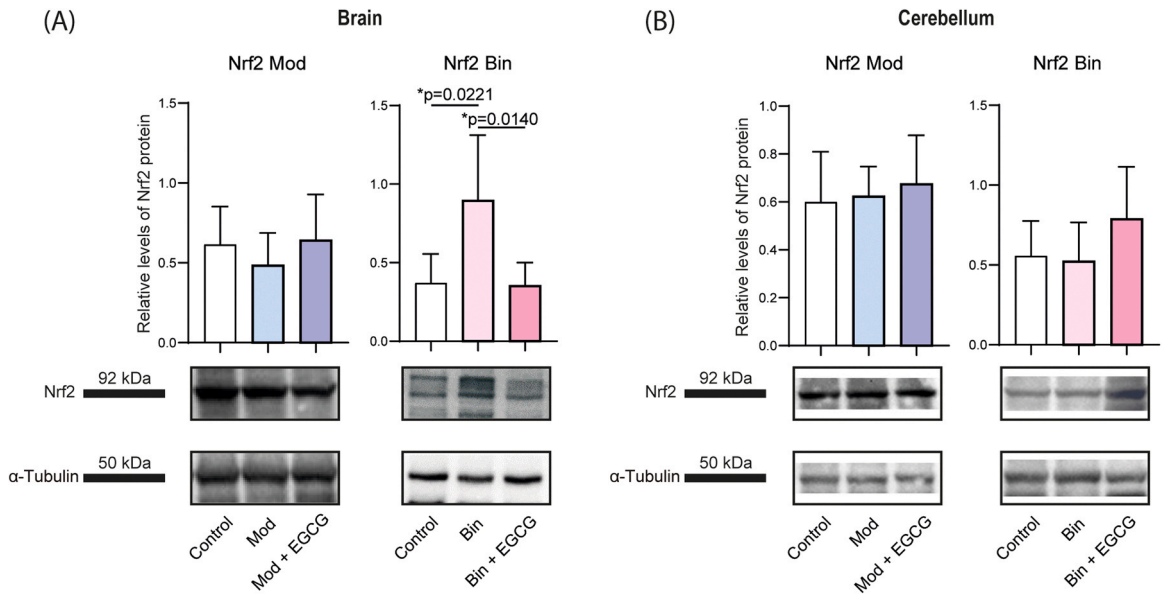

Fig. 9. Brain Nrf2 (A) and cerebellum Nrf2 (B) levels in mice tissue to analyse oxidative stress after maternal alcohol exposure. Shapiro-Wilk test was performed to assess the normality of the data in each group. Kruskal–Wallis test (Dunn's test for multiple comparisons) was performed for inter-group comparisons (A) (Kruskall-Wallis $H_{(2)}= 1.415, p = 0.5174$, $H_{(2)}= 10.33, p = 0.0019$); (B) (Kruskall-Wallis $H_{(2)}= 0.131, p = 0.9453$, $H_{(2)}= 1.557, p = 0.4784$). Nrf2: Nuclear factor Erythroid 2-related factor 2; Mod: moderate; Bin: binge; EGCG: epigallocatechin-3-gallate. Asterisks denote the level of significance: *p ≤ 0.05; **p ≤ 0.01.

affecting the function and structure of neurons [71]. Recent studies have shown that reduced MBP and myelination deficits are linked to long-term susceptibility to neurobehavioural abnormalities [72], indicative of depression, anxiety, and hyperactivity in later life [73–79]. The results of the present study showed that MBP levels were significantly lower in binge and moderate groups in both brain and cerebellum, suggesting potential disruptions in myelination and hence motor coordination disorders.

EGCG produced a significant improvement in motor coordination in those groups in which PAE under moderate exposure caused reversible damage, indicating that this antioxidant has potential therapeutic effects in mitigating motor impairments induced by PAE [80–82]. This is consistent with the existing literature on EGCG, which highlights its neuroprotective, antioxidant, and anti-inflammatory properties, which help to mitigate neural damage in neurodegenerative conditions such as Alzheimer's and Parkinson's disease [83,84]. Furthermore, NeuN and DYRK1A recovered their physiological levels after EGCG treatment. Our findings suggest that in the case of MBP, recovery to physiological levels is only achieved under conditions of moderate alcohol exposure. However, in cases of binge drinking, the extent of damage to neuronal transport mechanisms is so profound that MBP levels remain resistant to restoration, even with EGCG treatment. This suggests a threshold effect, where high-intensity alcohol exposure may irreversibly compromise myelination pathways, underscoring the limits of therapeutic intervention in cases of severe prenatal alcohol damage. Voluntary movement control and hyperactivity are controlled by specific brain regions such as the primary motor cortex and a neuronal circuit involving the frontal cortex and thalamic motor nuclei that controls motor function [85,86]. The striatum, which coordinates signals from different parts of the brain, plays a key role in hyperactivity [87]. The association of motor function and hyperactivity with cerebral regions, rather than the cerebellum, could explain the observed lack of significant changes in biomarker levels within the cerebellum [88–91].

Long-term impact of PAE on learning, immediate and spatial working memory was assessed by T-maze spontaneous alternation test [52]. On the final testing day, both PAE groups achieved success rates of around 50 %, significantly lower than controls, showing greater difficulties in working memory, continuous spatial alternation, and spatial memory, indicating comparable damage in the hippocampus in both consumption patterns [92,93].

Biomarkers involved in processes of plasticity, maturation, and proliferation provide information about processes related to learning and memory [94–97]. PAE groups exhibit problems with working memory, continuous spatial alternation, and spatial memory, suggesting hippocampus damage [98,99]. The reduced levels of BDNF explain the impairment of immediate and spatial memory and the disorders in the learning processes in PAE mice due to its involvement in neurogenesis [100–102] and synaptic plasticity [103,104]. Primarily areas affected are located in the hippocampus [105]. Interestingly, BDNF reductions were more pronounced in the binge drinking group, suggesting a higher susceptibility to severe memory impairment with this drinking pattern.

Nevertheless, BDNF effects in the cerebellum are only observed in cases of severe alcohol impairment, such as binge drinking, as previously observed in schizophrenia patients, particularly affecting immediate memory, dementia and cognitive decline [106]. Early life stress also impairs spatial memory and reduces neurogenesis and BDNF expression in adult mice [107]. Similarly, studies on mouse models with Down Syndrome have shown that overexpression of DYRK1A results in deficits in visuospatial learning and memory [108]. This suggests dysfunction in the hippocampus and prefrontal cortex [109], which is consistent with our results in T-maze. In terms of neuronal maturation processes, mature neurons were reduced. This suggests that alcohol impact significantly the number of mature neurons in the hippocampus of adult mice. Previous studies have shown that under conditions of alcohol exposure, alcohol primarily affects neuronal progenitor cells, with immature neurons being secondarily affected [110–112]. In contrast, another study observed effects on NeuN, a biomarker used to detect mature neurons [67], indicating that PAE leads to a decrease in mature neurons in mice in both consumption patterns. Neuronal maturation, a critical process for brain development and function, influences immediate memory [113]. Impaired maturation can disrupt memory due to inadequate synaptic connections and myelination, slowing down signal transmission [114] and impact immediate memory and overall cognitive ability [115]. GDNF plays a critical role in memory, by controlling cell loss in the hippocampus and protecting against behavioural seizures that occur as a result of brain damage [116]. In the presence of the highest dose of ethanol, GDNF induces the phosphorylation of Shc, which activates intracellular pathways for neuroprotection [117]. Our results

show that in cases of severe brain damage, GDNF levels increase, as has also been shown in Parkinson's disease [118,119] and rodent brain damage [116]. On the other hand, PAE leads to significant neuronal loss in several regions of the CNS; in particular, differentiating Purkinje cells of the cerebellum are particularly susceptible to ethanol exposure, leading to a reduction in GDNF in this area [117].

Interestingly, mice from both groups exposed prenatally to ethanol and treated postnatally with EGCG showed higher success rates in T-maze learning compared to untreated PAE mice. These differences became more noticeable over time, suggesting that EGCG treatment may improve working and spatial memory impairments induced by prenatal alcohol exposure, consistent with previous studies [120]. In addition, the benefits observed in neurobehavioral testing with EGCG treatment were also seen in biomarkers associated with altered neurological processes. EGCG significantly restores BDNF levels, critical for neurogenesis and synaptic plasticity, supporting the observed improvements in memory and learning. NeuN expression was also increased in brain after EGCG treatment, indicating a protection against PAE-induced neuronal loss. Additionally, DYRK1A overexpression decreased, aligning with improved visuospatial learning and memory, reinforcing the potential of EGCG to mitigate hippocampal and prefrontal cortex dysfunction in PAE.

Hippocampal damage caused by PAE impairs spatial recognition and memory [121], as evidenced by the results of the MWM test in both PAE groups. PAE mice showed a reduced time at the platform location and distance travelled during the test, as well as increased time spent in the opposite quadrant and outside the circle compared to control mice. The hippocampus plays a crucial role in long-term memories [122], sorting new information and linking it to similar information already stored in the brain [123]. Therefore, any changes in neuronal maturation can affect this process, leading to memory problems, as observed in MWM and T-maze tests. The binge group spent more time immobile in the water. The low levels of NeuN seen in this work could explain this behaviour by generating alterations in neuronal maturation. This would lead to a reduction in serotonin [124–126], resulting in symptoms of anxiety and depression [127–129] in this consumption pattern. Alternatively, it could indicate that this binge group may be employing less efficient search strategies [130]. However, it is important to note that moderate alcohol exposure still produced observable neurodevelopmental deficits and behavioural alterations, highlighting that even lower levels of exposure can lead to cognitive and emotional impairments. EGCG-treated mice spent significantly less time immobile compared to binge group, suggesting that EGCG treatment may have a positive effect not only on spatial and working memory, but also on motor skills or motivation [131].

Regarding neuronal differentiation, PAE reduced GFAP levels, a protein found in CNS astrocytes that is critical for their differentiation into glial cells and neurodevelopment [23,132]. Our results are consistent with previous studies that demonstrated the same effects after PAE exposure [22,132,133]. Reduced levels of GFAP have been associated with depressive behaviours and dementia [134–136], which is consistent with the findings of our MWM test, highlighting a significant decline in memory capacity. S100β, a neurotrophic factor that modulates neuronal plasticity, is a marker for astrocytes in the CNS and brain damage [137–140]. Increased S100β levels in binge drinking group, indicated brain damage [141–146] and anxiolytic behaviour [144]. Previous studies in mouse model demonstrated a correlation among brain damage and anxious behaviour and memory impairment when performing the MWM test [147]. Hippocampal neurogenesis is regulated by Wnt signalling, which has been suggested to be a key regulator of adult and embryonic neurogenesis [148–150]. The Wnt signalling pathway controls neuronal differentiation, axon outgrowth and guidance, dendritic development and neuronal plasticity [151,152]. The decrease in Wnt-3a levels in brain and cerebellar tissues after prenatal alcohol exposure can result in cognitive memory impairment and neurotoxicity in the hippocampus[153]. This is consistent with the memory deficits in MWM associated with the reduced time spent in the platform area and distance taken during the test in both prenatally exposed groups. On the other hand, we have previously discussed the effects of BDNF, NeuN and MBP biomarkers on memory, cognition, and hyperactivity processes in the context of the rotarod and T-maze tests. These biomarkers are key factors for memory and cognition [70,100–104,154] and are involved in neuronal differentiation and maturation [155–157]. As reflected in the MWM results, the deficits in these biomarkers are also associated with disruptions in neurodevelopment and significant increases in behaviours that are indicative of depression, anxiety and hyperactivity later in life [73,75–79,158].

PAE, which is associated with a number of developmental disorders such as FASD, induces oxidative stress, damaging fetal cellular structures [159,160]. Nrf2, a transcription factor in the CNS, maintains intracellular redox homeostasis [161]. Increased levels of Nrf2 lead to an impaired antioxidant response and exacerbate the oxidative damage caused by PAE [162,163] as reflected in the neurodevelopmental deficits shown here. The binge drinking induced a greater increase in Nrf2 expression compared to moderate drinking, underscoring the enhanced oxidative stress associated with higher levels of alcohol exposure. This pronounced Nrf2 upregulation suggests a response where elevated alcohol intake overwhelms cellular antioxidant defences, potentially exacerbating long-term neurodevelopmental vulnerability.

Moreover, in non-stress situations, most of the Nrf2 protein is ubiquitinated and degraded by the proteasomal machinery. However, under oxidative stress, such as that produced by binge drinking, the interaction between Nrf2 and its regulatory complex Keap1 is disrupted [164,165]. This leads to a reduction in Nrf2 degradation and an increase in its translocation to the nucleus [166,167]. Perturbations in Nrf2 expression due to PAE could potentially lead to cognitive and behavioural abnormalities, as shown in our T-maze and MWM results [162,168–171].

After postnatal EGCG treatment, PAE mice showed marked improvement in performance in the MWM test, improving their ability to locate the platform zone, as they spent more time in this area and traversed a significantly longer distance than their respective PAE groups. They also spent less time in the quadrant opposite to the platform zone and outside the designated circle. This is indicative of their improved spatial navigation skills and memory recall, suggesting a beneficial effect of EGCG treatment. Impairments in spatial navigation and memory, typically caused by PAE, appear to be alleviated by EGCG treatment, aligning with recent studies that support the beneficial impact of EGCG on neuronal health and function, suggesting that EGCG improves spatial navigation and memory [84,172–174].

5. Conclusion

This study demonstrates the impact of two distinct maternal drinking patterns during pregnancy on cognitive function and behaviour in young adult mice, revealing impairments in motor coordination, learning, and immediate and spatial memory. These behavioural deficits are associated with disruptions in biomarkers that are essential for neurodevelopmental processes. Specifically, PAE leads to significant reductions in BDNF, NeuN, MBP, GDNF and Wnt-3, as well as dysregulation of DYRK1A, GFAP, s100b and Nrf2 signalling pathways. These alterations affect synaptic plasticity, myelination, neuronal differentiation and proliferation, and oxidative stress responses, thereby highlighting the broad neurobiological basis of PAE-induced cognitive and behavioural abnormalities. Importantly, postnatal treatment with EGCG, an antioxidant known for its neuroprotective effects, demonstrates marked therapeutic potential by restoring the affected biomarker levels in cases of moderate alcohol exposure, where structural and functional damage to neural pathways remains reparable. EGCG also reduces the overexpression of DYRK1A, a kinase involved in impaired plasticity and hyperactivity, and regulates Nrf2 expression, ameliorating oxidative stress by restoring redox homeostasis within the CNS. Nevertheless, a threshold effect is observed, as binge drinking induces severe,

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potentially irreversible damage to myelination and neuronal transport pathways. This suggests that high levels of alcohol exposure may exceed the reparative capacity of EGCG. Through these mechanisms, EGCG treatment effectively alleviates the motor coordination, working memory, and spatial learning deficits associated with PAE, supporting its utility as a therapeutic agent to counteract cognitive and behavioural impairments.

6. Limitations

This study uses a controlled FASD-like mouse model, which effectively minimises confounding variables and environmental factors that often introduce bias in human studies. One of the major strengths of the present study is the detailed analysis of both behavioural outcomes and molecular biomarkers, providing comprehensive insights into the effects of PAE and the potential therapeutic effects of EGCG. On the other hand, the use of oral gavage for alcohol administration mimics the human oral route and avoids the inaccuracies of other routes of administration.

However, the study has some limitations. In particular, the alcohol exposure model used only simulates the first and second trimesters of human pregnancy, leaving the effects of equivalent exposure in the third trimester unexplored. Future research should focus on extending these findings by including models of third-trimester alcohol exposure and investigating the long-term effects of EGCG treatment on cognition. Moreover, all behavioural assessments were performed in male mice only, which precludes the evaluation of potential sex-specific effects of PAE and EGCG treatment. Future studies should include both sexes to better characterise differential responses.

At the molecular level, we separated the brain from the cerebellum, and each was homogenised. While whole-brain homogenates allow the detection of systemic changes in key biomarkers and provide sufficient sample volume for robust biochemical analyses, this approach precludes region-specific analysis that may have revealed differential effects across brain areas. This is a relevant consideration for future investigations aimed at dissecting the neuroanatomical specificity of PAE-induced changes and therapeutic effects of EGCG.

A maltodextrin+EGCG group was not included in this study due to it was not ethically justified given the study's objectives, in accordance with institutional guidelines and the 3Rs principle of reduction. However, future studies could consider a maltodextrin+EGCG group to evaluate its role on specific and subtle effects on healthy tissues, taking into account these effects to compare the effects of this antioxidant in alcohol-treated and untreated individuals.

Clinical trials are also essential to assess the translatability of these promising preclinical results to human populations, particularly children with FASD, in order to evaluate the full potential of EGCG as a therapeutic intervention. Future research would greatly benefit from a detailed exploration of the molecular mechanisms linking these altered biomarker levels to the cognitive impairments observed in behavioural assessments such as the T-maze and MWM. Such investigations hold great promise for advancing our understanding of the precise pathways by which PAE induces neurodevelopmental deficits. Uncovering these pathways could provide critical insights into the neurobiological basis of PAE-related cognitive decline and offer new avenues for targeted intervention.

CRediT authorship contribution statement

Algar Óscar García: Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Marchei Emilia:** Writing – review & editing, Validation, Resources, Formal analysis. **Gómez-Roig María D.:** Writing – review & editing, Project administration, Investigation, Funding acquisition, Conceptualization. **Muñoz-Lozano Concha:** Methodology, Formal analysis. **Martínez Leopoldo:** Writing – review & editing, Resources, Project administration, Methodology, Funding acquisition. **Navarro-Tapia Elisabet:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Ramos-Triguero Anna:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Almeida-Toledano Laura:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Conceptualization. **Serra-Delgado Mariona:** Writing – original draft, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. **Andreu-Fernandez Vicente:** Writing – review & editing, Writing – original draft, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Conceptualization. **Vieiros Melina:** Writing – original draft, Software, Resources, Methodology, Investigation.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.biopha.2025.118138.

References

[1] K. Dejong, A. Olyaei, J.O. Lo, Alcohol use in pregnancy, Clin. Obstet. Gynecol. 62 (2019) 142, https://doi.org/10.1097/GRF.0000000000000414.

[2] M.G. Griswold, N. Fullman, C. Hawley, N. Arian, S.R.M. Zimsen, H.D. Tymeson, et al., Alcohol use and burden for 195 countries and territories, 1990-2016: a systematic analysis for the global burden of disease study 2016, Lancet 392 (2018) 1015–1035, https://doi.org/10.1016/S0140-6736(18)31310-2.

[3] J. Rehm, G.E. Gmel, G. Gmel, O.S.M. Hasan, S. Imtiaz, S. Popova, et al., The relationship between different dimensions of alcohol use and the burden of disease-an update, Addict. (Abingdon, Engl.) 112 (2017) 968–1001, https://doi.org/10.1111/ADD.13757.

[4] A.M. Wood, S. Kaptoge, A. Butterworth, P.J. Nietert, S. Warnakula, T. Bolton, et al., Risk thresholds for alcohol consumption: combined analysis of individual-participant data for 599 912 current drinkers in 83 prospective studies, Lancet 391 (2018) 1513–1523, https://doi.org/10.1016/S0140-6736(18)30134-X.

[5] N.A. Osna, T.M. Donohue, K.K. Kharbanda, Alcoholic liver disease: pathogenesis and current management, Alcohol Res 38 (2017) 147.

[6] Z.M. Weil, J.D. Corrigan, K. Karelina, Alcohol use disorder and traumatic brain injury, Alcohol Res 39 (2018) 171.

[7] C. Wiegmann, I. Mick, E.J. Brandl, A. Heinz, S. Gutwinski, Alcohol and dementia – what is the link? a systematic review, Neuropsychiatr. Dis. Treat. 16 (2020) 87, https://doi.org/10.2147/NDT.S198772.

[8] Mchugh R. Kathryn, R.D. Weiss, Alcohol use disorder and depressive disorders, Alcohol Res 40 (2019) e1–e8, https://doi.org/10.35946/ARCR.V40.1.01.

[9] A.R.C.R.E. Staff, Drinking patterns and their definitions, Alcohol Res 39 (2018) 17.

[10] B.D. Jani, R. McQueenie, B.I. Nicholl, R. Field, P. Hanlon, K.I. Gallacher, et al., Association between patterns of alcohol consumption (beverage type, frequency and consumption with food) and risk of adverse health outcomes: a prospective cohort study, BMC Med 19 (2021) 1–14, https://doi.org/10.1186/S12916-020-01878-2/TABLES/4.

[11] Drinking Levels Defined | National Institute on Alcohol Abuse and Alcoholism (NIAAA) n.d. https://www.niaaa.nih.gov/alcohol-health/overview-alcohol-consumption/moderate-binge-drinking (accessed January 26, 2024).

[12] S.N. Mattson, G.A. Bernes, L.R. Doyle, Fetal alcohol spectrum disorders: a review of the neurobehavioral deficits associated with prenatal alcohol exposure, Alcohol Clin. Exp. Res 43 (2019) 1046, https://doi.org/10.1111/ACER.14040.

[13] M.D. Gomez-Roig, E. Marchei, S. Sabra, F.P. Busardò, L. Mastrobattista, S. Pichini, et al., Maternal hair testing to disclose self-misreporting in drinking and smoking behavior during pregnancy, Alcohol 67 (2018) 1–6, https://doi.org/10.1016/J.ALCOHOL.2017.08.010.

[14] L. Burd, J. Blair, K. Dropps, Prenatal alcohol exposure, blood alcohol concentrations and alcohol elimination rates for the mother, fetus and newborn, J. Perinatol. 32 (9) (2012) 652, https://doi.org/10.1038/JP.2012.57.

[15] H.E. Hoyme, P.A. May, W.O. Kalberg, P. Kodituwakku, J.P. Gossage, P. M. Trujillo, et al., A practical clinical approach to diagnosis of fetal alcohol spectrum disorders: clarification of the 1996 institute of medicine criteria, Pediatrics 115 (2005) 39–47, https://doi.org/10.1542/PEDS.2004-0259.

[16] S. Popova, M.E. Charness, L. Burd, A. Crawford, H.E. Hoyme, R.A.S. Mukherjee, et al., Fetal alcohol spectrum disorders, Nat. Rev. Dis. Prim. 9 (2023), https://doi.org/10.1038/S41572-023-00420-X.

[17] S. Maya-Enero, S.M. Ramis-Fernández, M. Astals-Vizcaino, Ó. García-Algar, Neurocognitive and behavioral profile of fetal alcohol spectrum disorder (Engl Ed), Pediatr 95 (2021) 208.e1–208.e9, https://doi.org/10.1016/J.ANPEDE.2020.12.012.

[18] S. Popova, S. Lange, C. Probst, G. Gmel, J. Rehm, Global prevalence of alcohol use and binge drinking during pregnancy, and fetal alcohol spectrum disorder, Biochem Cell Biol. 96 (2018) 237–240, https://doi.org/10.1139/BCB-2017-0077.

[19] H.E. Hoyme, W.O. Kalberg, A.J. Elliott, J. Blankenship, D. Buckley, A.S. Marais, et al., Updated clinical guidelines for diagnosing fetal alcohol spectrum disorders, Pediatrics 138 (2016), https://doi.org/10.1542/PEDS.2015-4256.

[20] A. Ramos-Triguero, E. Navarro-Tapia, M. Vieiros, A. Mirahi, M. Astals Vizcaino, L. Almela, et al., Machine learning algorithms to the early diagnosis of fetal alcohol spectrum disorders, Front Neurosci. 18 (2024) 1400933, https://doi.org/10.3389/FNINS.2024.1400933/BIBTEX.

[21] World Health Organization, Global status report on alcohol and health. WhoInt, World Health Organization, Geneva, Switzerland, 2018, p. 478, 2018.

[22] L. Almeida-Toledano, V. Andreu-Fernández, R. Aras-López, Ó. García-Algar, L. Martínez, M.D. Gómez-Roig, Epigallocatechin gallate ameliorates the effects of prenatal alcohol exposure in a fetal alcohol spectrum disorder-like mouse model, Int J. Mol. Sci. 22 (2021) 1–24, https://doi.org/10.3390/IJMS22020715.

[23] G. Brolese, P. Lunardi, N. Broetto, D.S. Engelke, F. Lírio, C. Batassini, et al., Moderate prenatal alcohol exposure alters behavior and neuroglial parameters in adolescent rats, Behav. Brain Res. 269 (2014) 175–184, https://doi.org/10.1016/J.BBR.2014.04.023.

[24] E.T. Costa, D.D. Savage, C.F. Valenzuela, A review of the effects of prenatal or early ethanol exposure on brain ligand-gated ion channels, Alcohol Clin. Exp. Res 24 (2000) 706–715, https://doi.org/10.1111/J.1530-0277.2000.TB02043.X.

[25] M. Naassila, M. Daoust, Effect of prenatal and postnatal ethanol exposure on the developmental profile of mRNAs encoding NMDA receptor subunits in rat hippocampus, J. Neurochem. 80 (2002) 850–860, https://doi.org/10.1046/J.0022-3042.2002.00755.X.

[26] S.G. Evrard, M.D. Vega, A.J. Ramos, P. Tagliaferro, A. Brusco, Altered neuron-glia interactions in a low, chronic prenatal ethanol exposure, Dev. Brain Res. 147 (2003) 119–133, https://doi.org/10.1016/j.devbrainres.2003.09.004.

[27] L. Cantacorps, H. González-Pardo, J.L. Arias, O. Valverde, N.M. Conejo, Altered brain functional connectivity and behaviour in a mouse model of maternal alcohol binge-drinking, Prog. Neuropsychopharmacol. Biol. Psychiatry 84 (2018) 237–249, https://doi.org/10.1016/J.PNPBP.2018.03.006.

[28] B. Petrelli, J. Weinberg, G.G. Hicks, Effects of prenatal alcohol exposure (PAE): insights into FASD using mouse models of PAE, Biochem Cell Biol. 96 (2018) 131–147, https://doi.org/10.1139/BCB-2017-0280.

[29] H.M. Hwang, Y.I. Kawasawa, A. Basha, S. Mohammad, M. Ito, K. Hashimoto-Torii, Fatty acid metabolism changes in association with neurobehavioral deficits in animal models of fetal alcohol spectrum disorders, Commun. Biol. 6 (2023), https://doi.org/10.1038/S42003-023-05127-Z.

[30] T.R. Chaudoin, S.J. Bonasera, A. Dunaevsky, R. Padmashri, Exploring behavioral phenotypes in a mouse model of fetal alcohol spectrum disorders, Dev. Neurobiol. 83 (2023) 184–204, https://doi.org/10.1002/DNEU.22922.

[31] C. Secker, A.Y. Motzny, S. Kostova, A. Buntru, L. Helmecke, L. Reus, et al., The polyphenol EGCG directly targets intracellular amyloid-β aggregates and promotes their lysosomal degradation, J. Neurochem. 166 (2023) 294–317, https://doi.org/10.1111/JNC.15842.

[32] J.M. Starbuck, S. Llambrich, R. Gonzàlez, J. Albaigès, A. Sarlé, J. Wouters, et al., Green tea extracts containing epigallocatechin-3-gallate modulate facial development in Down syndrome, 2021;11, Sci. Rep. 11 (1) (2021) 1–13, https://doi.org/10.1038/s41598-021-83757-1.

[33] L. Long, Y. Li, Y.D. Wang, Q.Y. He, M. Li, X.D. Cai, et al., The preventive effect of oral EGCG in a fetal alcohol spectrum disorder mouse model, Alcohol Clin. Exp. Res 34 (2010) 1929–1936, https://doi.org/10.1111/J.1530-0277.2010.01282.X.

[34] A. Payne, E. Taka, G.M. Adinew, K.F.A. Soliman, Molecular mechanisms of the anti-inflammatory effects of epigallocatechin 3-gallate (EGCG) in LPS-activated BV-2 microglia cells, Brain Sci. 13 (2023) 632, https://doi.org/10.3390/BRAINSCI13040632/S1.

[35] D. Mokra, M. Joskova, J. Mokry, Therapeutic effects of green tea polyphenol-epigallocatechin-3-gallate (EGCG) in relation to molecular pathways controlling inflammation, oxidative stress, and apoptosis, Int J. Mol. Sci. 24 (2023), https://doi.org/10.3390/IJMS24010340.

[36] A. Agarwal, V. Kansal, H. Farooqi, R. Prasad, V.K. Singh, Epigallocatechin gallate (EGCG), an active phenolic compound of green tea, inhibits tumor growth of head and neck cancer cells by targeting DNA hypermethylation, Biomedicines 11 (2023), https://doi.org/10.3390/BIOMEDICINES11030789.

[37] N. Kaminen-Ahola, A. Ahola, M. Maga, K.A. Mallitt, P. Fahey, T.C. Cox, et al., Maternal ethanol consumption alters the epigenotype and the phenotype of offspring in a mouse model, PLoS Genet 6 (2010), https://doi.org/10.1371/JOURNAL.PGEN.1000811.

[38] M. Ungerer, J. Knezovich, M. Ramsay, In utero alcohol exposure, epigenetic changes, and their consequences, Alcohol Res 35 (2013) 37.

[39] V. Andreu-Fernández, M. Serra-Delgado, L. Almeida-Toledano, À. García-Meseguer, M. Vieiros, A. Ramos-Triguero, et al., Effect of postnatal epigallocatechin-gallate treatment on cardiac function in mice prenatally exposed to alcohol, Antioxid. (Basel) 12 (2023), https://doi.org/10.3390/ANTIOX12051067.

[40] N.P. du Sert, A. Ahluwalia, S. Alam, M.T. Avey, M. Baker, W.J. Browne, et al., Reporting animal research: explanation and elaboration for the arrive guidelines 2.0, PLoS Biol. 18 (2020), https://doi.org/10.1371/JOURNAL.PBIO.3000411.

[41] D. Wang, Q. Gao, T. Wang, Z. Kan, X. Li, L. Hu, et al., Green tea polyphenols and epigallocatechin-3-gallate protect against perfluorodecanoic acid induced liver damage and inflammation in mice by inhibiting NLRP3 inflammasome activation, Food Res Int 127 (2020), https://doi.org/10.1016/J.FOODRES.2019.108628.

[42] R. Sharma, R. Kumar, A. Sharma, A. Goel, Y. Padwad, Long-term consumption of green tea EGCG enhances murine health span by mitigating multiple aspects of cellular senescence in mitotic and post-mitotic tissues, gut dysbiosis, and immunosenescence, J. Nutr. Biochem. 107 (2022), https://doi.org/10.1016/J.JNUTBIO.2022.109068.

[43] Y. Sugita, T. Furukawa, Effect of green tea and tea catechin on the visual motion processing for optokinetic responses in mice, Neuroscience 501 (2022) 42–51, https://doi.org/10.1016/J.NEUROSCIENCE.2022.08.013.

[44] F. Li, C. Gao, P. Yan, M. Zhang, Y. Wang, Y. Hu, et al., EGCG reduces obesity and white adipose tissue gain partly through AMPK activation in mice, Front Pharm. 9 (2018), https://doi.org/10.3389/FPHAR.2018.01366.

[45] K. Unno, M. Pervin, K. Taguchi, T. Konishi, Y. Nakamura, Green tea catechins trigger immediate-early genes in the hippocampus and prevent cognitive decline and lifespan shortening, Molecules 25 (2020) 1484, https://doi.org/10.3390/MOLECULES25071484.

[46] B. Souchet, A. Duchon, Y. Gu, J. Dairou, C. Chevalier, F. Daubigney, et al., Prenatal treatment with EGCG enriched green tea extract rescues GAD67 related developmental and cognitive defects in Down syndrome mouse models, 2019;9, Sci. Rep. 9 (1) (2019) 1–13, https://doi.org/10.1038/s41598-019-40328-9.

[47] Fernández, V.A. Toledano LA, N.P. Lozano, E.N. Tapia, M.D.G. Roig, R.D.L. T. Fornell, et al., Bioavailability of epigallocatechin gallate administered with different nutritional strategies in healthy volunteers, Antioxid. (Basel) 9 (2020), https://doi.org/10.3390/ANTIOX9050440.

[48] B. Souchet, F. Guedj, Z. Penke-Verdier, F. Daubigney, A. Duchon, Y. Herault, et al., Pharmacological correction of excitation/inhibition imbalance in down syndrome mouse models, Front Behav. Neurosci. 9 (2015) 162500, https://doi.org/10.3389/FNBEH.2015.00267/BIBTEX.

[49] H. Nagasawa, K. Amano, M. Araki, Relationship between pup growth, mother weight, food or water intake in four strains of mice, Vivo (Brooklyn) (1992).

[50] A.A. Bachmanov, D.R. Reed, G.K. Beauchamp, M.G. Tordoff, Food intake, water intake, and drinking spout side preference of 28 mouse strains, Behav. Genet 32 (2002) 435, https://doi.org/10.1023/A:1020884312053.
[51] Q. Wang, W. Han, C. Ma, T. Wang, J. Zhong, Western blot normalization: time to choose a proper loading control seriously, Electrophoresis 44 (2023) 854–863, https://doi.org/10.1002/ELPS.202200222.
[52] R. d'Isa, G. Comi, L. Leocani, Apparatus design and behavioural testing protocol for the evaluation of spatial working memory in mice through the spontaneous alternation T-maze, 2021;11, Sci. Rep. 11 (1) (2021) 1–13, https://doi.org/10.1038/s41598-021-00402-7.
[53] C.D. Barnhart, D. Yang, P.J. Lein, Using the morris water maze to assess spatial learning and memory in weanling mice, PLoS One 10 (2015) e0124521, https://doi.org/10.1371/JOURNAL.PONE.0124521.
[54] J.W. Gu, K.L. Makey, K.B. Tucker, E. Chinchar, X. Mao, I. Pei, et al., EGCG, a major green tea catechin suppresses breast tumor angiogenesis and growth via inhibiting the activation of HIF-1α and NFκB, and VEGF expression, Vasc. Cell 5 (2013), https://doi.org/10.1186/2045-824X-5-9.
[55] E. Muggli, J. Halliday, S. Hearps, T.N.N. Nguyen, A. Penington, D.K. Thompson, et al., Low to moderate prenatal alcohol exposure and neurodevelopment in a prospective cohort of early school aged children, 2024;14, Sci. Rep. 14 (1) (2024) 1–11, https://doi.org/10.1038/s41598-024-57938-7.
[56] J.L. Jacobson, T. Akkaya-Hocagil, L.M. Ryan, N.C. Dodge, G.A. Richardson, H. C. Olson, et al., Effects of prenatal alcohol exposure on cognitive and behavioral development: findings from a hierarchical meta-analysis of data from six prospective longitudinal U.S. cohorts, Alcohol Clin. Exp. Res 45 (2021) 2040–2058, https://doi.org/10.1111/ACER.14686.
[57] P.D. Connor, P.D. Sampson, A.P. Streissguth, F.L. Bookstein, H.M. Barr, Effects of prenatal alcohol exposure on fine motor coordination and balance: a study of two adult samples, Neuropsychologia 44 (2006) 744–751, https://doi.org/10.1016/J.NEUROPSYCHOLOGIA.2005.07.016.
[58] J. Ortiz-Abalia, I. Sahún, X. Altafaj, N. Andreu, X. Estivill, M. Dierssen, et al., Targeting Dyrk1A with AAVshRNA attenuates motor alterations in TgDyrk1A, a mouse model of Down syndrome, Am. J. Hum. Genet 83 (2008) 479–488, https://doi.org/10.1016/J.AJHG.2008.09.010.
[59] V. Brault, T.L. Nguyen, J. Flores-Gutiérrez, G. Iacono, M.C. Birling, V. Lalanne, et al., Dyrk1a gene dosage in glutamatergic neurons has key effects in cognitive deficits observed in mouse models of MRD7 and Down syndrome, PLoS Genet 17 (2021), https://doi.org/10.1371/JOURNAL.PGEN.1009777.
[60] F.J. Tejedor, B. Hämmerle, MNB/DYRK1A as a multiple regulator of neuronal development, FEBS J. 278 (2011) 223–235, https://doi.org/10.1111/J.1742-4658.2010.07954.X.
[61] W. Becker, W. Sippl, Activation, regulation, and inhibition of DYRK1A, FEBS J. 278 (2011) 246–256, https://doi.org/10.1111/J.1742-4658.2010.07956.X.
[62] F. Guedj, P.L. Pereira, S. Najas, M.J. Barallobre, C. Chabert, B. Souchet, et al., DYRK1A: a master regulatory protein controlling brain growth, Neurobiol. Dis. 46 (2012) 190–203, https://doi.org/10.1016/J.NBD.2012.01.007.
[63] M.L. Arbones, A. Thomazeau, A. Nakano-Kobayashi, M. Hagiwara, J.M. Delabar, DYRK1A and cognition: a lifelong relationship, Pharm. Ther. 194 (2019) 199–221, https://doi.org/10.1016/J.PHARMTHERA.2018.09.010.
[64] G. Arque, A. Casanovas, M. Dierssen, Dyrk1A is dynamically expressed on subsets of motor neurons and in the neuromuscular junction: possible role in down syndrome, PLoS One 8 (2013) 54285, https://doi.org/10.1371/JOURNAL.PONE.0054285.
[65] S. García-Cerro, P. Martínez, V. Vidal, A. Corrales, J. Flórez, R. Vidal, et al., Overexpression of Dyrk1A is implicated in several cognitive, electrophysiological and neuromorphological alterations found in a mouse model of down syndrome, PLoS One 9 (2014), https://doi.org/10.1371/JOURNAL.PONE.0106572.
[66] A S, T S, T K, T M, S K, M M, et al., Identification of Sox2 and NeuN double-positive cells in the mouse hypothalamic arcuate nucleus and their reduction in number with aging, Front Aging Neurosci. 12 (2021), https://doi.org/10.3389/FNAGI.2020.609911.
[67] V.V. Gusel'nikova, D.E. Korzhevskiy, NeuN as a neuronal nuclear antigen and neuron differentiation marker, Acta Nat. 7 (2015) 42, https://doi.org/10.32607/20758251-2015-7-2-42-47.
[68] B. Elibol-Can, I. Dursun, I. Telkes, E. Kilic, S. Canan, E. Jakubowska-Dogru, Examination of age-dependent effects of fetal ethanol exposure on behavior, hippocampal cell counts, and doublecortin immunoreactivity in rats, Dev. Neurobiol. 74 (2014) 498–513, https://doi.org/10.1002/DNEU.22143.
[69] A.Y. Klintsova, J.L. Helfer, L.H. Calizo, W.K. Dong, C.R. Goodlett, W. T. Greenough, Persistent impairment of hippocampal neurogenesis in young adult rats following early postnatal alcohol exposure, Alcohol Clin. Exp. Res 31 (2007) 2073–2082, https://doi.org/10.1111/J.1530-0277.2007.00528.X.
[70] F. Guo, Y.F. Zhang, K. Liu, X. Huang, R.X. Li, S.Y. Wang, et al., Chronic exposure to alcohol inhibits new myelin generation in adult mouse brain, Front Cell Neurosci. 15 (2021) 732602, https://doi.org/10.3389/FNCEL.2021.732602/BIBTEX.
[71] R.W. Olsen, J. Liang, Role of GABAA receptors in alcohol use disorders suggested by chronic intermittent ethanol (CIE) rodent model, 2017;10, Mol. Brain 10 (1) (2017) 1–20, https://doi.org/10.1186/S13041-017-0325-8.
[72] S. Kuhn, L. Gritti, D. Crooks, Y. Dombrowski, Oligodendrocytes in development, myelin generation and beyond, Cells 8 (2019), https://doi.org/10.3390/CELLS8111424.
[73] Z. Wang, A. Baharani, Z. Wei, D. Truong, X. Bi, F. Wang, et al., Low field magnetic stimulation promotes myelin repair and cognitive recovery in chronic cuprizone mouse model, Clin. Exp. Pharm. Physiol. 48 (2021) 1090–1102, https://doi.org/10.1111/1440-1681.13490.
[74] A.A. McKendry, H.K. Palliser, D.M. Yates, D.W. Walker, J.J. Hirst, The effect of betamethasone treatment on neuroactive steroid synthesis in a foetal Guinea pig model of growth restriction, J. Neuroendocr. 22 (2010) 166–174, https://doi.org/10.1111/J.1365-2826.2009.01949.X.
[75] G.A. Bennett, H.K. Palliser, B. Saxby, D.W. Walker, J.J. Hirst, Effects of prenatal stress on fetal neurodevelopment and responses to maternal neurosteroid treatment in Guinea pigs, Dev. Neurosci. 35 (2013) 416–426, https://doi.org/10.1159/000354176.
[76] G.A. Bennett, H.K. Palliser, J.C. Shaw, D. Walker, J.J. Hirst, Prenatal stress alters hippocampal neuroglia and increases anxiety in childhood, Dev. Neurosci. 37 (2015) 533–545, https://doi.org/10.1159/000437302.
[77] G.A. Bennett, H.K. Palliser, D. Walker, J. Hirst, Severity and timing: how prenatal stress exposure affects glial developmental, emotional behavioural and plasma neurosteroid responses in guinea pig offspring, Psychoneuroendocrinology 70 (2016) 47–57, https://doi.org/10.1016/J.PSYNEUEN.2016.04.011.
[78] A.L. Cumberland, H.K. Palliser, G.K. Crombie, D.W. Walker, J.J. Hirst, Increased anxiety-like phenotype in female guinea pigs following reduced neurosteroid exposure in utero, Int. J. Dev. Neurosci. 58 (2017) 50–58, https://doi.org/10.1016/J.IJDEVNEU.2017.02.001.
[79] G.K. Crombie, H.K. Palliser, J.C. Shaw, D.M. Hodgson, D.W. Walker, J.J. Hirst, Effects of prenatal stress on behavioural and neurodevelopmental outcomes are altered by maternal separation in the neonatal period, Psychoneuroendocrinology 124 (2021), https://doi.org/10.1016/J.PSYNEUEN.2020.105060.
[80] O. Afzal, M.H. Dalhat, A.S.A. Altamimi, R. Rasool, S.I. Alzarea, W.H. Almalki, et al., Green tea catechins attenuate neurodegenerative diseases and cognitive deficits, Molecules 27 (2022), https://doi.org/10.3390/MOLECULES27217604.
[81] Q. Xu, M. Langley, A.G. Kanthasamy, M.B. Reddy, Epigallocatechin gallate has a neurorescue effect in a mouse model of parkinson disease, J. Nutr. 147 (2017) 1926–1931, https://doi.org/10.3945/JN.117.255034.
[82] M. Chen, T. Wang, F. Yue, X. Li, P. Wang, Y. Li, et al., Tea polyphenols alleviate motor impairments, dopaminergic neuronal injury, and cerebral α-synuclein aggregation in MPTP-intoxicated parkinsonian monkeys, Neuroscience 286 (2015) 383–392, https://doi.org/10.1016/J.NEUROSCIENCE.2014.12.003.
[83] A. Payne, S. Nahashon, E. Taka, G.M. Adinew, K.F.A. Soliman, Epigallocatechin-3-gallate (EGCG): new therapeutic perspectives for neuroprotection, aging, and neuroinflammation for the modern age, Biomolecules 12 (2022), https://doi.org/10.3390/BIOM12030371.
[84] N.A. Singh, A.K.A. Mandal, Z.A. Khan, Potential neuroprotective properties of epigallocatechin-3-gallate (EGCG), Nutr. J. 15 (2016), https://doi.org/10.1186/S12937-016-0179-4.
[85] R. Muñoz-Castañeda, B. Zingg, K.S. Matho, X. Chen, Q. Wang, N.N. Foster, et al., Cellular anatomy of the mouse primary motor cortex, Nature 598 (2021) 159, https://doi.org/10.1038/S41586-021-03970-W.
[86] N. Takahashi, S. Moberg, T.A. Zolnik, J. Catanese, R.N.S. Sachdev, M.E. Larkum, et al., Thalamic input to motor cortex facilitates goal-directed action initiation, Curr. Biol. 31 (2021) 4148–4155.e4, https://doi.org/10.1016/J.CUB.2021.06.089.
[87] Y. Wang, C. Zuo, Q. Xu, L. Hao, Y. Zhang, Attention-deficit/hyperactivity disorder is characterized by a delay in subcortical maturation, Prog. Neuropsychopharmacol. Biol. Psychiatry 104 (2021) 110044, https://doi.org/10.1016/J.PNPBP.2020.110044.
[88] A. Albajara Sáenz, T. Villemonteix, P. Van Schuerbeek, S. Baijot, M. Septier, P. Defresne, et al., Motor abnormalities in attention-deficit/hyperactivity disorder and autism spectrum disorder are associated with regional grey matter volumes, Front Neurol. 12 (2021) 666980, https://doi.org/10.3389/FNEUR.2021.666980/BIBTEX.
[89] A.F.T. Arnsten, The emerging neurobiology of attention deficit hyperactivity disorder: the key role of the prefrontal association cortex, J. Pedia 154 (2009) I, https://doi.org/10.1016/J.JPEDS.2009.01.018.
[90] I.M.H. Hollund, A. Olsen, J. Skranes, A.M. Brubakk, A.K. Håberg, L. Eikenes, et al., White matter alterations and their associations with motor function in young adults born preterm with very low birth weight, Neuroimage Clin. 17 (2018) 241, https://doi.org/10.1016/J.NICL.2017.10.006.
[91] R. Schulz, P. Koch, M. Zimerman, M. Wessel, M. Bönstrup, G. Thomalla, et al., Parietofrontal motor pathways and their association with motor function after stroke, Brain 138 (2015) 1949–1960, https://doi.org/10.1093/BRAIN/AWV100.
[92] L.E. Baquedano, E.G. Bernal, D.J. Carrion, A.D. Delgado, C.M. Gavidia, D. E. Kirwan, et al., Impaired spatial working memory and reduced hippocampal neuronal density in a rat model of neurocysticercosis, Front Cell Neurosci. 17 (2023) 1183322, https://doi.org/10.3389/FNCEL.2023.1183322.
[93] S.M. Kim, L.M. Frank, Hippocampal lesions impair rapid learning of a continuous spatial alternation task, PLoS One 4 (2009) 5494, https://doi.org/10.1371/JOURNAL.PONE.0005494.
[94] C. La Rosa, R. Parolisi, L. Bonfanti, Brain structural plasticity: from adult neurogenesis to immature neurons, Front Neurosci. 14 (2020) 512123, https://doi.org/10.3389/FNINS.2020.00075/BIBTEX.
[95] T. Klingberg, Training and plasticity of working memory, Trends Cogn. Sci. 14 (2010) 317–324, https://doi.org/10.1016/J.TICS.2010.05.002.
[96] E. Bruel-Jungerman, S. Davis, S. Laroche, Brain plasticity mechanisms and memory: a party of four, Neuroscientist 13 (2007) 492–505, https://doi.org/10.1177/1073858407302725.
[97] P. Marzola, T. Melzer, E. Pavesi, J. Gil-Mohapel, P.S. Brocardo, Exploring the role of neuroplasticity in development, aging, and neurodegeneration, 2023;13:1610, Brain Sci. 13 (2023) 1610, https://doi.org/10.3390/BRAINSCI13121610.
[98] A.S. Solomina, L.G. Kolik, A.V. Rodina, I.O. Logvinov, V.G. Kon'kov, V.S. Kudrin, et al., Chronic ethanol consumption by rats before mating affects working

memory in the offspring, Bull. Exp. Biol. Med 175 (2023) 633–637, https://doi.org/10.1007/S10517-023-05916-W.
[99] J.A. Baker, M.K. Mulligan, K.M. Hamre, Effects of genetics and sex on adolescent behaviors following neonatal ethanol exposure in BXD recombinant inbred strains, Front Neurosci. 17 (2023), https://doi.org/10.3389/FNINS.2023.1197292.
[100] A.K. McAllister, D.C. Lo, L.C. Katz, Neurotrophins regulate dendritic growth in developing visual cortex, Neuron 15 (1995) 791–803, https://doi.org/10.1016/0896-6273(95)90171-X.
[101] M.M. Hofer, Y.A. Barde, Brain-derived neurotrophic factor prevents neuronal death in vivo, Nature 331 (1988) 261–262, https://doi.org/10.1038/331261A0.
[102] B.A.A. Bus, M.L. Molendijk, B.J.W.H. Penninx, J.K. Buitelaar, G. Kenis, J. Prickaerts, et al., Determinants of serum brain-derived neurotrophic factor, Psychoneuroendocrinology 36 (2011) 228–239, https://doi.org/10.1016/J.PSYNEUEN.2010.07.013.
[103] C.R. Bramham, E. Messaoudi, BDNF function in adult synaptic plasticity: the synaptic consolidation hypothesis, Prog. Neurobiol. 76 (2005) 99–125, https://doi.org/10.1016/J.PNEUROBIO.2005.06.003.
[104] V. Pencea, K.D. Bingaman, S.J. Wiegand, M.B. Luskin, Infusion of brain-derived neurotrophic factor into the lateral ventricle of the adult rat leads to new neurons in the parenchyma of the striatum, septum, thalamus, and hypothalamus, J. Neurosci. 21 (17) (2001) 6706, https://doi.org/10.1523/JNEUROSCI.21-17-06706.2001.
[105] E.T. Rolls, Memory systems in the brain, Annu Rev. Psychol. 51 (2000) 599–630, https://doi.org/10.1146/ANNUREV.PSYCH.51.1.599.
[106] X.Y. Zhang, J. Liang, D.C. Chen, M.H. Xiu, F. De Yang, T.A. Kosten, et al., Low BDNF is associated with cognitive impairment in chronic patients with schizophrenia, Psychopharmacol. (Berl.) 222 (2012) 277–284, https://doi.org/10.1007/S00213-012-2643-Y.
[107] Y.X. Sun, Y.A. Su, Q. Wang, J.Y. Zheng, C.C. Zhang, T. Wang, et al., The causal involvement of the BDNF-TrkB pathway in dentate gyrus in early-life stress-induced cognitive deficits in male mice, 2023;13, Transl. Psychiatry 13 (1) (2023) 1–12, https://doi.org/10.1038/s41398-023-02476-5.
[108] V. Fotaki, M. Dierssen, S. Alcántara, S. Martínez, E. Martí, C. Casas, et al., Dyrk1A haploinsufficiency affects viability and causes developmental delay and abnormal brain morphology in mice, Mol. Cell Biol. 22 (2002) 6636, https://doi.org/10.1128/MCB.22.18.6636-6647.2002.
[109] X. Altafaj, M. Dierssen, C. Baamonde, E. Martí, J. Visa, J. Guimerà, et al., Neurodevelopmental delay, motor abnormalities and cognitive deficits in transgenic mice overexpressing Dyrk1A (minibrain), a murine model of Down's syndrome, Hum. Mol. Genet 10 (2001) 1915–1923, https://doi.org/10.1093/HMG/10.18.1915.
[110] S.A. Morris, D.W. Eaves, A.R. Smith, K. Nixon, Alcohol inhibition of neurogenesis: a mechanism of hippocampal neurodegeneration in an adolescent alcohol abuse model, Hippocampus 20 (2010) 596, https://doi.org/10.1002/HIPO.20665.
[111] T.W. Le Maître, G. Dhanabalan, N. Bogdanovic, K. Alkass, H. Druid, Effects of alcohol abuse on proliferating cells, stem/progenitor cells, and immature neurons in the adult human hippocampus, 2017;43, Neuropsychopharmacology 43 (4) (2018) 690–699, https://doi.org/10.1038/npp.2017.251.
[112] K. Nixon, F.T. Crews, Binge ethanol exposure decreases neurogenesis in adult rat hippocampus, J. Neurochem. 83 (2002) 1087–1093, https://doi.org/10.1046/J.1471-4159.2002.01214.X.
[113] B. Kolb, R. Gibb, Brain plasticity and behaviour in the developing brain, J. Can. Acad. Child Adolesc. Psychiatry 20 (2011) 265.
[114] W. Xin, J.R. Chan, Myelin plasticity: sculpting circuits in learning and memory, Nat. Rev. Neurosci. 21 (2020) 682, https://doi.org/10.1038/S41583-020-00379-8.
[115] K. Minatohara, M. Akiyoshi, H. Okuno, Role of immediate-early genes in synaptic plasticity and neuronal ensembles underlying the memory trace, Front Mol. Neurosci. 8 (2016) 172311, https://doi.org/10.3389/FNMOL.2015.00078/BIBTEX.
[116] Y.M. Yoo, C.J. Lee, U. Lee, Y.J. Kim, Neuroprotection of adenoviral-vector-mediated GDNF expression against kainic-acid-induced excitotoxicity in the rat hippocampus, Exp. Neurol. 200 (2006) 407–417, https://doi.org/10.1016/J.EXPNEUROL.2006.02.132.
[117] R.E. McAlhany, R.C. Miranda, R.H. Finnell, J.R. West, Ethanol decreases glial derived neurotrophic factor (GDNF) protein release but not mRNA expression and increases GDNF-stimulated Shc phosphorylation in the developing cerebellum, Alcohol Clin. Exp. Res 23 (1999) 1691–1697, https://doi.org/10.1111/J.1530-0277.1999.TB04062.X.
[118] S.Y. Tong, R.W. Wang, Q. Li, Y. Liu, X.Y. Yao, D.Q. Geng, et al., Serum glial cell line-derived neurotrophic factor (GDNF) a potential biomarker of executive function in Parkinson's disease, Front Neurosci. 17 (2023) 1136499, https://doi.org/10.3389/FNINS.2023.1136499/BIBTEX.
[119] P.A. Kambey, K. Kanwore, A.A. Ayanlaja, I. Nadeem, Y.Z. Du, W. Buberwa, et al., Failure of glial cell-line derived neurotrophic factor (GDNF) in clinical trials orchestrated by reduced NR4A2 (NURR1) transcription factor in parkinson's disease. a systematic review, 645583, Front Aging Neurosci. 13 (2021) 645583, https://doi.org/10.3389/FNAGI.2021.645583.
[120] J. Ajarem, G. Rashedi, Al, M. Mohany, A. Allam, Neurobehavioral changes in mice offspring exposed to green tea during fetal and early postnatal development, Behav. Brain Funct. 13 (2017) 1–14, https://doi.org/10.1186/S12993-017-0128-1/FIGURES/10.
[121] K.L. Kaemingk, P.T. Halverson, Spatial memory following prenatal alcohol exposure: more than a material specific memory deficit, Child Neuropsychol. 6 (2000) 115–128, https://doi.org/10.1076/CHIN.6.2.115.7058.
[122] Almaraz-Espinoza A., Grider M.H. Physiology, Long Term Memory. StatPearls 2023.
[123] V.I. Kryukov, The role of the hippocampus in long-term memory: is it memory store or comparator? J. Integr. Neurosci. 7 (2008) 117–184, https://doi.org/10.1142/S021963520800171X.
[124] N. Alenina, F. Klempin, The role of serotonin in adult hippocampal neurogenesis, Behav. Brain Res. 277 (2015) 49–57, https://doi.org/10.1016/J.BBR.2014.07.038.
[125] W.C. Spencer, E.S. Deneris, Regulatory mechanisms controlling maturation of serotonin neuron identity and function, Front Cell Neurosci. 11 (2017) 269128, https://doi.org/10.3389/FNCEL.2017.00215/BIBTEX.
[126] S. Brummelte, E. Mc Glanaghy, A. Bonnin, T.F. Oberlander, Developmental changes in serotonin signaling: implications for early brain function, behavior and adaptation, Neuroscience 342 (2017) 212, https://doi.org/10.1016/J.NEUROSCIENCE.2016.02.037.
[127] L. Micheli, M. Ceccarelli, G. D'Andrea, F. Tirone, Depression and adult neurogenesis: Positive effects of the antidepressant fluoxetine and of physical exercise, Brain Res Bull. 143 (2018) 181–193, https://doi.org/10.1016/J.BRAINRESBULL.2018.09.002.
[128] A.J. Eisch, D. Petrik, Depression and hippocampal neurogenesis: a road to remission? Science 338 (2012) 72, https://doi.org/10.1126/SCIENCE.1222941.
[129] J. Moncrieff, R.E. Cooper, T. Stockmann, S. Amendola, M.P. Hengartner, M. A. Horowitz, The serotonin theory of depression: a systematic umbrella review of the evidence, 2022;28, Mol. Psychiatry 28 (8) (2022) 3243–3256, https://doi.org/10.1038/s41380-022-01661-0.
[130] R. D'Hooge, P.P. De Deyn, Applications of the Morris water maze in the study of learning and memory, Brain Res Rev. 36 (2001) 60–90, https://doi.org/10.1016/S0165-0173(01)00067-4.
[131] O. Weinreb, S. Mandel, T. Amit, M.B.H. Youdim, Neurological mechanisms of green tea polyphenols in Alzheimer's and Parkinson's diseases, J. Nutr. Biochem. 15 (2004) 506–516, https://doi.org/10.1016/j.jnutbio.2004.05.002.
[132] S. Vallès, J. Pitarch, J. Renau-Piqueras, C. Guerri, Ethanol exposure affects glial fibrillary acidic protein gene expression and transcription during rat brain development, J. Neurochem. 69 (1997) 2484–2493, https://doi.org/10.1046/J.1471-4159.1997.69062484.X.
[133] S. Vallés, M. Sancho-Tello, R. Miñana, E. Climent, J. Renau-Piqueras, C. Guerri, Glial fibrillary acidic protein expression in rat brain and in radial glia culture is delayed by prenatal ethanol exposure, J. Neurochem. 67 (1996) 2425–2433, https://doi.org/10.1046/J.1471-4159.1996.67062425.X.
[134] W. Li, T. Ali, K. He, Z. Liu, F.A. Shah, Q. Ren, et al., Ibrutinib alleviates LPS-induced neuroinflammation and synaptic defects in a mouse model of depression, Brain Behav. Immun. 92 (2021) 10–24, https://doi.org/10.1016/J.BBI.2020.11.008.
[135] M. Brenner, Role of GFAP in CNS injuries, Neurosci. Lett. 565 (2014) 7–13, https://doi.org/10.1016/J.NEULET.2014.01.055.
[136] K.Y. Kim, K.Y. Shin, K.A. Chang, GFAP as a potential biomarker for alzheimer's disease: a systematic review and meta-analysis, Cells 12 (2023), https://doi.org/10.3390/CELLS12091309.
[137] D.B. Zimmer, E.H. Cornwall, A. Landar, W. Song, The S100 protein family: history, function, and expression, Brain Res Bull. 37 (1995) 417–429, https://doi.org/10.1016/0361-9230(95)00040-2.
[138] F. Michetti, N. D'Ambrosi, A. Toesca, M.A. Puglisi, A. Serrano, E. Marchese, et al., The S100B story: from biomarker to active factor in neural injury, J. Neurochem. 148 (2019) 168–187, https://doi.org/10.1111/JNC.14574.
[139] C. Oris, S. Kahouadji, J. Durif, D. Bouvier, V. Sapin, S100B, actor and biomarker of mild traumatic brain injury, Int J. Mol. Sci. 24 (2023), https://doi.org/10.3390/IJMS24076602.
[140] A. Goyal, M.D. Failla, C. Niyonkuru, K. Amin, A. Fabio, R.P. Berger, et al., S100b as a prognostic biomarker in outcome prediction for patients with severe traumatic brain injury, J. Neurotrauma 30 (2013) 946–957, https://doi.org/10.1089/NEU.2012.2579.
[141] B. Sun, H. Liu, S. Nie, S100B protein in serum is elevated after global cerebral ischemic injury, World J. Emerg. Med 4 (2013) 165, https://doi.org/10.5847/WJEM.J.ISSN.1920-8642.2013.03.001.
[142] C. Gahm, O. Beharier, S100B: potential biomarker for CNS insult and injury, Gen. Methods Biomark. Res. Their Appl. (2014) 1–21, https://doi.org/10.1007/978-94-007-7740-8_5-1.
[143] F.T. Crews, Alcohol-related neurodegeneration and recovery: mechanisms from animal models, Alcohol Res. Health 31 (2008) 377.
[144] A.M.S. Kindlundh-Högberg, X. Zhang, P. Svenningsson, S100B overexpressing mutant mice exhibit prolonged behavioural and biochemical responses towards repeated intermittent binge treatments with MDMA, Int J. Neuropsychopharmacol. 12 (2009) 201–215, https://doi.org/10.1017/S1461145708009437.
[145] R.L. Sacco, M. Elkind, B. Boden-Albala, I.F. Lin, D.E. Kargman, W.A. Hauser, et al., The protective effect of moderate alcohol consumption on ischemic stroke, JAMA 281 (1999) 53–60, https://doi.org/10.1001/JAMA.281.1.53.
[146] C.J. Chen, W.M. Brown, C.J. Moomaw, C.D. Langefeld, J. Osborne, B.B. Worrall, et al., Alcohol use and risk of intracerebral hemorrhage, Neurology 88 (2017) 2043, https://doi.org/10.1212/WNL.0000000000003952.
[147] P.S. Brocardo, F. Boehme, A. Patten, A. Cox, J. Gil-Mohapel, B.R. Christie, Anxiety- and depression-like behaviors are accompanied by an increase in oxidative stress in a rat model of fetal alcohol spectrum disorders: Protective effects of voluntary physical exercise, Neuropharmacology 62 (2012) 1607–1618, https://doi.org/10.1016/J.NEUROPHARM.2011.10.006.

[148] S.B. Arredondo, D. Valenzuela-Bezanilla, M.D. Mardones, L. Varela-Nallar, Role of Wnt signaling in adult hippocampal neurogenesis in health and disease, Front Cell Dev. Biol. 8 (2020), https://doi.org/10.3389/FCELL.2020.00860.
[149] S.M.K. Lee, S. Tole, E. Grove, A.P. McMahon, A local Wnt-3a signal is required for development of the mammalian hippocampus, Development 127 (2000) 457–467, https://doi.org/10.1242/DEV.127.3.457.
[150] P. Nagu, V. Sharma, T. Behl, A.K.A. Pathan, V. Mehta, Molecular insights to the Wnt signaling during alzheimer's disorder: a potential target for therapeutic interventions, J. Mol. Neurosci. 72 (2022) 679–690, https://doi.org/10.1007/S12031-021-01940-5/METRICS.
[151] S.B. Rosso, N.C. Inestrosa, WNT signaling in neuronal maturation and synaptogenesis, Front Cell Neurosci. 7 (2013), https://doi.org/10.3389/FNCEL.2013.00103.
[152] C.W. He, C.P. Liao, C.L. Pan, Wnt signalling in the development of axon, dendrites and synapses, Open Biol. 8 (2018), https://doi.org/10.1098/RSOB.180116.
[153] X. Zhang, J. Zhao, T. Chang, Q. Wang, W. Liu, L. Gao, Ketamine exerts neurotoxic effects on the offspring of pregnant rats via the Wnt/β-catenin pathway, Environ. Sci. Pollut. Res Int 27 (2020) 305–314, https://doi.org/10.1007/S11356-019-06753-Z.
[154] A. Planas-Ballvé, L. Grau-López, R.M. Morillas, R. Planas, Neurological manifestations of excessive alcohol consumption, Gastroenterol. ía Y. Hepatol. ía (Engl. Ed.) 40 (2017) 709–717, https://doi.org/10.1016/J.GASTRE.2017.11.001.
[155] C.F. Yang, Y.J. Zhong, Z. Ma, L. Li, L. Shi, L. Chen, et al. NOX4/ROS mediate ethanol-induced apoptosis via MAPK signal pathway in L-02 cells, Int J. Mol. Med 41 (2018) 2306–2316, https://doi.org/10.3892/IJMM.2018.3390.
[156] L. Wang, L. Song, J. Ma, H. Wang, yingzhi Li, D. Huang, Alcohol induces apoptosis and autophagy in microglia BV-2 cells, Food Chem. Toxicol. 177 (2023) 113849, https://doi.org/10.1016/J.FCT.2023.113849.
[157] Sushma, S. Mishra, S. Kanchan, A. Divakar, G. Jha, D. Sharma, et al., Alcohol induces ER stress and apoptosis by inducing oxidative stress and disruption of calcium homeostasis in glial cells, Food Chem. Toxicol. 182 (2023), https://doi.org/10.1016/J.FCT.2023.114192.
[158] S. Montagud-Romero, L. Cantacorps, O. Valverde, Histone deacetylases inhibitor trichostatin A reverses anxiety-like symptoms and memory impairments induced by maternal binge alcohol drinking in mice, J. Psychopharmacol. 33 (2019) 1573–1587, https://doi.org/10.1177/0269881119857208.
[159] M. Derme, M.G. Piccioni, R. Brunelli, A. Crognale, M. Denotti, P. Ciolli, et al., Oxidative stress in a mother consuming alcohol during pregnancy and in her newborn: a case report, Antioxidants 12 (2023), https://doi.org/10.3390/ANTIOX12061216.
[160] S.A. Amini, R.H. Dunstan, P.R. Dunkley, R.N. Murdoch, Oxidative stress and the fetotoxicity of alcohol consumption during pregnancy, Free Radic. Biol. Med 21 (1996) 357–365, https://doi.org/10.1016/0891-5849(96)00027-5.
[161] A.T. Dinkova-Kostova, A.Y. Abramov, The emerging role of Nrf2 in mitochondrial function, Free Radic. Biol. Med 88 (2015) 179, https://doi.org/10.1016/J.FREERADBIOMED.2015.04.036.
[162] Q. Ma, Role of Nrf2 in oxidative stress and toxicity, Annu Rev. Pharm. Toxicol. 53 (2013) 401, https://doi.org/10.1146/ANNUREV-PHARMTOX-011112-140320.
[163] V. Ngo, M.L. Duennwald, Nrf2 and oxidative stress: a general overview of mechanisms and implications in human disease, Antioxidants 11 (2022), https://doi.org/10.3390/ANTIOX11122345.
[164] L. Baird, M. Yamamoto, The molecular mechanisms regulating the KEAP1-NRF2 pathway, Mol. Cell Biol. 40 (2020) e00099-20, https://doi.org/10.1128/MCB.00099-20.
[165] T. Nguyen, P.J. Sherratt, H.C. Huang, C.S. Yang, C.B. Pickett, Increased protein stability as a mechanism that enhances Nrf2-mediated transcriptional activation of the antioxidant response element, Degrad. Nrf2 26 S Protea J. Biol. Chem. 278 (2003) 4536–4541, https://doi.org/10.1074/JBC.M207293200.
[166] F. Pouremamali, A. Pouremamali, M. Dadashpour, N. Soozangar, F. Jeddi, An update of Nrf2 activators and inhibitors in cancer prevention/promotion, 2022; 20, Cell Commun. Signal. 20 (1) (2022) 1–16, https://doi.org/10.1186/S12964-022-00906-3.
[167] N.F. Villeneuve, A. Lau, D.D. Zhang, Regulation of the Nrf2–Keap1 antioxidant response by the ubiquitin proteasome system: an insight into cullin-ring ubiquitin ligases, Antioxid. Redox Signal 13 (2010) 1699, https://doi.org/10.1089/ARS.2010.3211.
[168] C. Branca, E. Ferreira, T.V. Nguyen, K. Doyle, A. Caccamo, S. Oddo, Genetic reduction of Nrf2 exacerbates cognitive deficits in a mouse model of Alzheimer's disease, Hum. Mol. Genet 26 (2017) 4823, https://doi.org/10.1093/HMG/DDX361.
[169] Z. Tang, B. Hu, F. Zang, J. Wang, X. Zhang, H. Chen, Nrf2 drives oxidative stress-induced autophagy in nucleus pulposus cells via a Keap1/Nrf2/p62 feedback loop to protect intervertebral disc from degeneration, 2019;10, Cell Death Dis. 10 (7) (2019) 1–12, https://doi.org/10.1038/s41419-019-1701-3.
[170] J.A. Zweig, M. Caruso, M.S. Brandes, N.E. Gray, Loss of NRF2 leads to impaired mitochondrial function, decreased synaptic density and exacerbated age-related cognitive deficits, Exp. Gerontol. 131 (2020) 110767, https://doi.org/10.1016/J.EXGER.2019.110767.
[171] N.E. Gray, M. Farina, P. Tucci, L. Saso, The role of the NRF2 pathway in maintaining and improving cognitive function, Biomedicines 10 (2022), https://doi.org/10.3390/BIOMEDICINES10082043.
[172] Q. Xu, M. Langley, A.G. Kanthasamy, M.B. Reddy, Epigallocatechin gallate has a neurorescue effect in a mouse model of parkinson disease, J. Nutr. 147 (2017) 1926, https://doi.org/10.3945/JN.117.255034.
[173] J.Y. Choi, C.S. Park, D.J. Kim, M.H. Cho, B.K. Jin, J.E. Pie, et al., Prevention of nitric oxide-mediated 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced Parkinson's disease in mice by tea phenolic epigallocatechin 3-gallate, Neurotoxicology 23 (2002) 367–374, https://doi.org/10.1016/S0161-813X(02)00079-7.
[174] Y. Levites, O. Weinreb, G. Maor, M.B.H. Youdim, S. Mandel, Green tea polyphenol (-)-epigallocatechin-3-gallate prevents N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced dopaminergic neurodegeneration, J. Neurochem. 78 (2001) 1073–1082, https://doi.org/10.1046/J.1471-4159.2001.00490.X.