

TEMPORAL AND SPATIAL EXPRESSION DYNAMICS OF PERSEPHIN IN THE DEVELOPING RAT BRAIN

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Received on January 10, 2026

Presented by N. Atanassova, Corresponding Member of BAS, on April 28, 2026

Abstract

The aim of this paper was to examine the developmental profile as well as the temporal and spatial distribution of neurotrophic factor persephin, using enzyme-linked immunosorbent assay (ELISA) in the prefrontal cortex (PFC), striatum, hypothalamus, thalamus, cerebellum, and hippocampus of male Wistar Albino rats at 1, 3, 6, and 12 weeks of age. The study was conducted in the Department of Physiology, Faculty of Medicine, Balıkesir University, Balıkesir, Türkiye, from 01/03/2024 to 01/04/2025. Male Wistar Albino rats; 1, 3, 6, and 12 weeks old, were used in this study. Persephin levels at the PFC, striatum, hypothalamus, thalamus, cerebellum, and hippocampus were determined using enzyme-linked immunosorbent assay (ELISA). The Shapiro–Wilk test was used to assess the normality of the data. Data that were not found to be normally distributed were analyzed using Kruskal–Wallis test and Dunn’s as post hoc test. The data that were found to be normally distributed were assessed using One-Way Analysis of Variance (ANOVA) followed by Tukey’s multiple comparisons post hoc test. Statistical analyses were conducted using the GraphPad Prism version 10.5. A biphasic temporal expression pattern for persephin was detected, with peak levels primarily observed at 1 and 6 weeks of age independently within the prefrontal cortex, striatum, cerebellum, hypothalamus, thalamus, and hippocampus. Our results demonstrate a temporal association between persephin levels and critical neurodevelopmental processes, suggesting

Balıkesir University Scientific Research Projects Directorate supported this research with project number 2023/211.

<https://doi.org/10.7546/CRABS.2026.05.05>

a possible supportive role in neurogenesis, synaptic pruning, and maturation during the early postnatal and adolescent periods. The expression profile of persephin that we detected in our work highlights its potential involvement in the localized development of higher cognitive and sensory systems. So, dysregulation of persephin, as a member of the glial cell line-derived neurotrophic factor (GDNF) family, during these developmental phases may contribute to the formation of various neurodevelopmental disorders.

Key words: neurotrophic factors, persephin, GDNF, rat brain, neurodevelopment

Introduction. The proper development of the nervous system is crucial for the well-being of the species. Neuronal development in the central nervous system follows conserved mechanisms among mammals, including neurogenesis, myelination, synaptogenesis, and neuronal and synaptic pruning. While these mechanisms are primarily complete in humans at birth, the same processes continue or even begin in neonatal rodents [1].

The postnatal period is critical for the appropriate development of the rodent nervous system. Rodents' neurogenesis remains active throughout the first two postnatal weeks. Myelination and synapse formation progress rapidly after birth, condensed into a brief 3 to 4-week developmental period that concludes by sexual maturity [2].

Neurotrophic factors support the growth, survival, and differentiation of neurons. They are indispensable for the development and maintenance of neural circuits, which are essential for the proper functioning of the nervous system [3]. Moreover, their dysregulation is proposed to cause the development of various diseases, including Alzheimer's, Parkinson's, depression, and Huntington's disease [4].

Persephin, one of the four trophic factors in the Glial Cell Line-Derived Neurotrophic Factor (GDNF) protein subfamily, is structurally related to its counterparts, neurturin (NTN) and GDNF. Persephin uses tyrosine kinase receptor RET system of the GDNF family receptor alpha ($GFR\alpha$), specifically $GFR\alpha4$ [5]. In addition to $GFR\alpha4$ receptors, persephin has also been found to bind to $GFR\alpha1$ receptors, promoting neuritogenesis [6]. Like other members of the GDNF protein subfamily, persephin not only exerts neuroprotective and neuroreparative functions but has also been shown to regulate neuronal maintenance, development, and differentiation [7].

NTN, initially found to support the survival of various peripheral neurons, is structurally 40% identical to GDNF. The presence of the NTN mRNA in developing striatum also suggests its alternative role in the development of midbrain dopaminergic neurons [8]. Another member of the GDNF subfamily, ART exerts its function through $GFR\alpha3$ receptors and has been shown to support the survival of sensory neurons in vitro [9].

This study aims to examine the developmental profile as well as the temporal and spatial distribution of persephin.

Methodology. Forty male Wistar Albino rats aged 1, 3, 6, and 12 weeks, with 10 animals in each age group were used in the study. The animals were group housed under controlled conditions, with $50 \pm 5\%$ relative humidity, $24 \pm 2^\circ\text{C}$ temperature, and proper ventilation. Animals were maintained on a 12-hour light/dark cycle and had ad libitum access to water and food. All experimental procedures were conducted with the approval of the Balikesir University Local Ethics Board for Animal Experimentation (Approval Reference No.: 2024-3-2).

After the intraperitoneal administration of ketamine/xylazine combination (50/10 mg/kg) cocktail, animals were sacrificed via cardiac exsanguination. Following this procedure, the skull was carefully opened, and the entire brain was removed. Brain tissues (PFC, striatum, hypothalamus, thalamus, cerebellum, and hippocampus) were dissected on a cold cutting block using razor blades. All collected samples were immediately frozen and stored at -80°C until analysis.

To ensure the accuracy of the ELISA measurements, the homogenization process was standardized. Dissected brain tissues were weighed and homogenized on ice at a constant 1:10 (weight/volume) ratio, utilizing 0.1 g of wet tissue per 0.9 mL of ice-cold extraction buffer (0.01 M PBS, pH 7.4) mixed with a protease inhibitor cocktail to prevent proteolytic degradation. Homogenization was performed using a mechanical homogenizer, followed by centrifugation at 10 000 for 20 min at 4°C . Supernatants were collected and stored at -80°C .

Persephin levels were quantified using an ELISA kit (Catalog no: E3487Ra) according to the manufacturer's instructions. Following the ELISA procedure, the initial concentration results (measured in ng/mL) were normalized to the initial tissue mass to mitigate any variations during the extraction phase. Consequently, all persephin levels are expressed as nanograms of persephin per milligram of wet tissue weight (ng/mg tissue).

All statistical analyses were performed using GraphPad Prism version 10.5 (Boston, MA, USA). To mitigate potential within-subject dependence arising from sampling multiple brain regions from the same biological subjects, statistical comparisons were limited to evaluating the main effect of age within each brain region. No inter-regional statistical comparisons were conducted.

Before analysis, the normality of data sets was assessed for each brain region using the Shapiro-Wilk test. For data exhibiting a normal distribution (Prefrontal Cortex, Thalamus, and Hippocampus), age-dependent differences were analyzed using a One-Way Analysis of Variance (ANOVA) followed by Tukey's multiple comparisons post hoc test. The aforementioned parametric data are represented as Mean $\pm$ Standard Error of the Mean (SEM) using standard bar charts. For data sets that did not exhibit the normal distribution (Striatum, Cerebellum, and Hypothalamus), the non-parametric Kruskal-Wallis test was employed, followed by Dunn's multiple comparisons post hoc test. To represent this non-parametric data,

results are presented as medians with interquartile ranges (IQR; 25th–75th percentiles) and minimum/maximum values, visualized using box-and-whisker plots. For all analyses, a two-tailed p -value of < 0.05 was considered statistically significant. Exact significance levels are denoted in Fig. 1 as follows: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

Results. Persephin levels in brain regions (PFC, striatum, hypothalamus, thalamus, cerebellum, and hippocampus) of rats aged 1, 3, 6, and 12 weeks were measured and given in Fig. 1. To mitigate for within-subject dependence, the temporal expression dynamics of persephin were evaluated independently within each specific brain region. A general biphasic trend was observed with respect to the age of the animals, with peak persephin levels detected at 1 and 6 weeks of age within the individual regions. Accordingly, all statistical evaluations and developmental comparisons are presented individually for each brain region.

When the brain regions were analyzed individually, the highest levels of persephin in the PFC were measured in animals aged 1 and 6 weeks. Persephin levels in the PFC were lowest at 3 weeks of age, and they were significantly different from those at 1 and 6 weeks of age (Fig. 1A). Similarly, the second lowest levels of persephin were detected at 12 weeks of age, and they were significantly lower than those at 6 weeks of age. For the striatum, the highest levels of persephin were found at 6 weeks of age, and these levels were significantly higher than those at 3 and 12 weeks of age (Fig. 1B). For the cerebellum, the highest level of persephin was measured at 1 week of age, and these levels were significantly higher than those at 3 and 12 weeks of age (Fig. 1C). When the results for the hypothalamus are considered, the lowest levels of persephin were measured at 3 weeks of age, and these values were significantly lower than those in all other age groups (Fig. 1D). A similar trend was observed for the results regarding the thalamus and hippocampus, as persephin levels were lowest at 3 weeks of age, and these values were significantly lower than those in all other age groups (Fig. 1E, F).

Discussion. The present study identifies a biphasic pattern of persephin protein expression in the rat PFC, with peak concentrations observed at 1 and 6 weeks of age. Elevated expression of persephin in the PFC highlights its potential roles during this rapid neurodevelopmental period. In support of this hypothesis, studies show that neurotrophic factors, especially GDNF family members, promote axonal growth and synaptic connectivity, particularly in dopamine-sensitive regions like the PFC [7]. The second peak (6 weeks of age) represents the adolescent phase in rodents, which comprises synaptic pruning, myelination, and refinement of executive functions. While the precise function of persephin in this context remains to be fully elucidated, the elevated persephin levels observed during this phase may be associated with a compensatory or neuroprotective mechanism, potentially stabilizing specific neural circuits in the PFC. These findings are consistent with previous literature indicating that neurotrophic factors can modulate

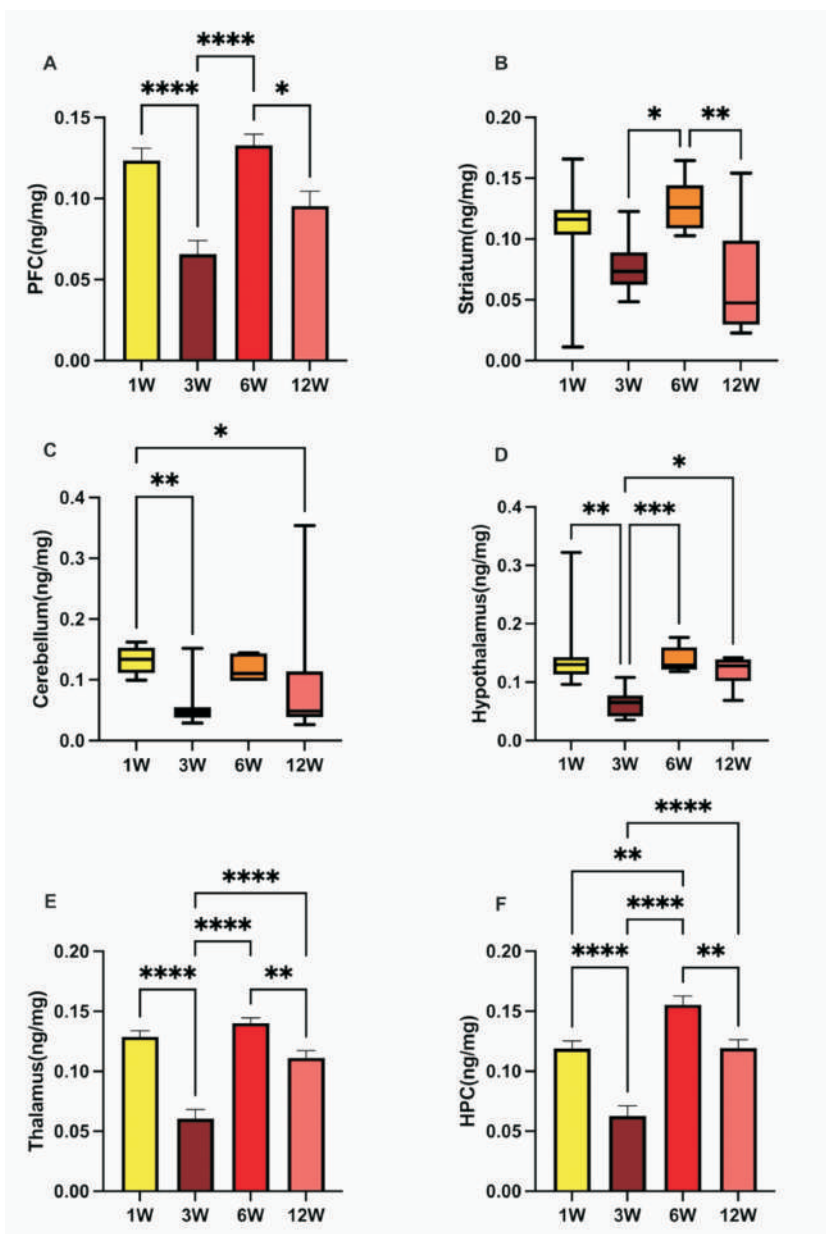


Fig. 1. Temporal expression dynamics of persephin across six brain regions. Panels (A-F) represent persephin protein levels expressed as ng/mg of wet tissue in the PFC (A), striatum (B), cerebellum (C), hypothalamus (D), thalamus (E), and hippocampus (F) at 1, 3, 6, and 12 neonatal weeks. Regions with normal distribution of the dataset (Panels A, E, F) were analyzed via One-Way ANOVA with Tukey's post hoc test and are presented as Mean $\pm$ SEM in bar charts. Regions that have dataset that are not normally distributed (Panels B, C, D) were analyzed via Kruskal-Wallis with Dunn's post hoc test and are presented as box-and-whisker plots showing the median, interquartile range (IQR), and minimum/maximum values. Statistical comparisons were limited to the main effect of age within each region to avoid within-subject dependence. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

synaptic elimination and stabilization, although further studies are required to confirm this relationship [10].

The striatum is a key component of the basal ganglia, which regulates the proper functioning of motor control, the reward system, and learning. The rat's striatum starts its development and neurogenesis prenatally around embryonic days 14–15. Postnatally, the striatum continues to mature, with the dopaminergic system organizing and its distribution becoming more uniform by the third week [11]. The early peak of persephin we detected in our study may represent its possible roles for proper neuronal development in the striatum. The second peak of persephin reported in our study at 6 weeks of age corresponds to adolescence in rats, a phase marked by refinement of striatal circuits, regulation of synaptic plasticity, and dendritic arborization. The biphasic expression pattern of persephin in our study may reflect the possibility that its dysregulation could contribute to improper striatal maturation. In addition to its well-known regulatory functions in motor coordination and balance, the cerebellum also contributes to cognitive processes. Its postnatal development in rodents involves tightly regulated processes: granule cell proliferation, Purkinje cell maturation, synaptogenesis, and pruning of climbing/parallel fibre connections [12]. Thus, the first peak of persephin detected in our study temporally coincides with the survival and differentiation of granule cells or Purkinje neurons, pointing to a possible association. The second peak of persephin detected in the cerebellum in our study at six weeks corresponds to adolescence in rats, which suggests its potential regulatory roles in synaptic stabilization or glial support for myelination [13]. Cerebellar dysfunction is linked with various neurodevelopmental diseases that comprise disrupted synaptic pruning and connectivity.

The hypothalamus is a central regulator of homeostasis, autonomic functions, and the nervous system's neuroendocrine signalling [14]. Our results showed a triphasic expression pattern for persephin with peak protein concentrations at 1, 6, and 12 weeks. The first neonatal week in rats coincides with early postnatal neurogenesis and the differentiation of hypothalamic nuclei. At 6 weeks, which coincides with the onset of puberty, is a period marked by hormonal surges and metabolic reprogramming. Adult hypothalamic plasticity involves glial-neuronal crosstalk and circuit rewiring in response to stressors, diet, or circadian disruptions [15]. The sustained high levels of persephin observed in the hypothalamus of rats at 12 weeks of age suggest a potential homeostatic or supportive function in maintaining hypothalamic integrity. Given the hypothalamus's crucial role in homeostatic regulation and neuroendocrine signalling, the sustained expression of GDNF-family ligands may be necessary to support the structural plasticity of hypothalamic circuits in response to metabolic and environmental stressors. Hypothalamic dysfunction is found to be associated with the pathogenesis of various diseases like obesity, diabetes, sleep disorders, and mood disorders [16].

Regarding the thalamus region, we detected a dynamic expression profile of persephin, with peak concentrations at 1 and 6 weeks of age and a moderate but sustained elevation at 12 weeks. The thalamus is a critical relay station for sensory, motor, and limbic information [17]. High persephin levels detected in our study in the first week signal its possible neurotrophic roles in the differentiation of thalamic neurons and formation of connections with cortical targets. Results from studies reporting the involvement of GDNF family members in promoting axonal growth in sensory pathways support this assumption [18]. We detected a second peak concentration of persephin in the thalamus during the adolescent phase, which comprises the stimulus-dependent remodelling of the thalamus, particularly in sensory nuclei. While the persephin levels at 12 weeks were slightly reduced compared to earlier peaks, they remain elevated, suggesting a role for it in maintaining thalamic integrity or adapting to plasticity [19]. Studies reporting the involvement of GDNF family members in promoting axonal growth in sensory pathways support this assumption [18]. We detected a second peak concentration of persephin in the thalamus during the adolescent phase, which comprises the stimulus-dependent remodelling of the thalamus, particularly in sensory nuclei. While the persephin levels at 12 weeks were slightly reduced compared to earlier peaks, they remain elevated, suggesting a role for it in maintaining thalamic integrity or adapting to plasticity [19].

We detected a similar expression pattern of persephin in the rat hippocampus with the thalamus region. Persephin levels were at peak concentrations at 1 and 6 weeks of age, followed by a moderate yet sustained elevation at 12 weeks. The hippocampus is central to learning, memory, and spatial navigation, and, similar to other brain regions, its postnatal maturation involves neurogenesis, dendritic arborization, and synaptic pruning [20]. The first postnatal week represents the proliferation of granule cells in the dentate gyrus and the establishment of the hippocampal circuitry of the hippocampus. Persephin's elevation at the hippocampus, as we detected in our study at the adolescent phase, may facilitate activity-dependent plasticity, such as long-term potentiation, or protect against oxidative stress during metabolic demands. Since the adult hippocampus retains neurogenic capacity in the dentate gyrus, our study's slightly reduced peak of persephin at 12 weeks may suggest a role for this protein in maintaining adult hippocampal plasticity or neuroprotection.

Limitations. There are several limitations present in this study. First, this study shows a temporal association between persephin levels and developmental milestones in the rat brain but does not point out direct causality. Functional imaging assays utilizing specific binding agents or genetic models would be necessary to associate persephin with the developmental processes, such as myelination or synaptic pruning. Second, due to the fact that the neonatal rat brain offers limited tissue content, normalization of the ELISA results relied on standardized wet tissue mass used in the homogenization process rather than direct colourimetric

total protein quantification (e.g., BCA assay). However, due to the strictly controlled 1:10 (w/v) homogenization ratio across all samples, the reported “ng/mg” values provide a reliable representation of the regional protein content differences.

Conclusion. Our results demonstrate temporal coincidence between peaks in persephin expression and critical neurodevelopmental processes, such as neurogenesis, synaptic pruning, and maturation, during the early postnatal and adolescent phases. The differential expression pattern observed across distinct brain regions suggests a possible supportive association with the development of higher cognitive and sensory systems. Consequently, it can be hypothesized that dysregulation of persephin during these developmental phases may contribute to the formation of various neurodevelopmental disorders.

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