

FRACTALKINE PROFILE IN DIFFERENT REGIONS OF THE BRAIN FROM THE EARLY POSTNATAL PERIOD TO ADOLESCENCE

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Abstract

In the present study, fractalkine levels were investigated in different brain regions and serum from the early postnatal period to adolescence. Thirty Wistar albino male rats aged 15, 30, and 41 days were used ($n = 10$ per group). After sacrifice, the prefrontal cortex, hypothalamus, thalamus, striatum, hippocampus, temporal lobe, and serum were analyzed by ELISA. A cumulative increase was observed in the thalamus, temporal lobe, and serum toward adolescence. In contrast, a cumulative decrease was observed in the hippocampus and striatum toward adolescence. The highest fractalkine levels were measured in the prefrontal cortex of 15-day-old rats, in the thalamus of 30-day-old rats, and in the thalamus and cerebellum of 41-day-old rats. Traumas that may occur during early developmental stages can negatively affect fractalkine production and neuroimmunity.

Key words: fractalkine, neuroanatomy, neurophysiology, pediatric neurodevelopment

Introduction. Fractalkine (FKN) is a type I transmembrane chemokine. This molecule is synthesized as a 371-amino-acid transmembrane protein. It includes a 76-amino acid N-terminal chemokine domain; a 241-amino acid glycosylated C-terminal region; an 18-amino acid hydrophobic transmembrane region; and a 37-amino acid intracellular C-terminal domain [1].

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High levels of FKN protein have been reported in brain, lung, and heart tissues, while lower levels have been found in kidney, pancreas, skeletal muscle, testis, and colon tissues [2]. Fractalkine is found in the central nervous system, including the hippocampus, frontal cortex, amygdala, striatum, globus pallidus, thalamus, and olfactory bulb. Expression levels in these regions may vary during postnatal development and influence processes such as synaptic plasticity and neurogenesis [3]. Additionally, disruption of fractalkine signalling may impair social behaviour and learning processes [4]. This study aims to determine regional and temporal changes in fractalkine secretion levels.

Materials and methods. This study was conducted in the Animal Research Laboratory of Balıkesir University in 2024, following approval from the Balıkesir University Animal Experiments Local Ethics Committee. All experimental procedures were performed within the period.

Animals. The study used male Wistar albino rats (*Rattus norvegicus*) at different postnatal periods. A total of 30 male Wistar albino rats were used ($n = 10$ per group) at 15 days, 30 days, and 41 days of age. This ensured group homogeneity. The 15-day-old rats were fed their mother's milk, the 30-day-old rats were weaned, and the 41-day-old rats were selected because they were in adolescence. Ethical approval was obtained from the Ethics Committee (Approval number: 2024/12-3 Date: 18.12.2024). All experiments were conducted in accordance with the US National Animal Guidelines [5].

Lee index, body weight, and brain weight. The calculation of the Lee index involved the use of the formula $(VA(g))^{1/3}/\text{naso-anal length (cm)}$ [6]. The brain weight, body weight, and Lee index of the subjects were measured under anesthesia.

Molecular analysis. Tissues were homogenized in potassium chloride buffer at a ratio of 1:9 (0.1 g tissue: 0.9 mL, 140 mmol/L) and then centrifuged at 7000 rpm for 5 min at 4°C. Fractalkine levels were then measured using the ELISA method (BT LAB, catalogue number E1968Ra).

Statistical tests. The Shapiro–Wilk test was performed to assess normality. For data showing a normal distribution, a One-Way ANOVA and Tukey's post hoc test were performed. A p -value < 0.05 was considered significant. For data that did not show a normal distribution, the Kruskal–Wallis and Mann–Whitney U post hoc tests were performed. Data were presented as median, interquartile range, and mean $\pm$ SEM (standard error of the mean). A p -value < 0.05 was considered significant [7]. For comparisons using ANOVA, the Holm–Sidak test was used; for comparisons using the Kruskal–Wallis test, Dunn's test was used.

Results. Murinometric result. Body weight increased significantly toward adolescence (Kruskal–Wallis, $p < 0.05$ – 0.0001). Compared with 15-day-old rats, brain weight increased significantly on postnatal days 30 and 41 (Kruskal–Wallis tests, $p < 0.01$ and $p < 0.0001$, respectively). Although there was no significant difference between days 30 and 41, there was a slight increase. The Lee

index was significantly lower in 30- and 41-day-old rats than in 15-day-old rats (Kruskal–Wallis, $p < 0.05$).

Temporal molecular result. There were no significant differences observed among the three periods in the cerebellum, PFC, hypothalamus, hippocampus, or parietal lobe (Kruskal–Wallis, $p > 0.05$). In the thalamus, fractalkine increased significantly toward adolescence (Fig. 1C). Fractalkine levels in 41-day-old rats were significantly higher than in 15-day-old rats (Kruskal–Wallis, $p < 0.05$). Temporal lobe fractalkine levels increased significantly toward adolescence (Fig. 1G). Fractalkine levels in 30-day-old rats were significantly higher than in 15-day-old rats (Kruskal–Wallis, $p < 0.05$). In the striatum, fractalkine decreased significantly toward adolescence (Fig. 1E). Fractalkine levels in 15-day-old rats were significantly higher than in 30-day-old rats (One-way ANOVA, $p < 0.01$). In serum, fractalkine increased significantly toward adolescence (Fig. 1I). Fractalkine levels in 15-day-old rats were significantly lower than in 30-day-old rats and 41-day-old rats (Kruskal–Wallis, $p < 0.005$, $p < 0.001$).

Spatial molecular results. In 15-day-old rats, fractalkine levels were highest in the prefrontal cortex (PFC) (2.003 ± 0.39) and lowest in the temporal lobe (0.89 ± 0.13). Serum levels were significantly lower in all brain regions except the temporal lobe and thalamus (Kruskal–Wallis, $p < 0.05$). There were no significant differences between brain regions (Kruskal–Wallis, $p > 0.05$) (Fig. 2).

In 30-day-old rats, fractalkine levels were highest in the thalamus (1.84 ± 0.20) and lowest in the striatum (0.84 ± 0.09) among brain regions. Fractalkine levels in the thalamus were significantly higher than those in serum and striatum (Kruskal–Wallis, $p < 0.05$, $p < 0.005$). PFC fractalkine levels were significantly higher than those in the striatum (Kruskal–Wallis, $p < 0.05$), (Fig. 3).

In 41-day-old rats, fractalkine levels were highest in the thalamus (2.92 ± 0.71) and cerebellum (2.92 ± 0.75), and lowest in the striatum (1.07 ± 0.05) and hippocampus (1.056 ± 0.09). Fractalkine levels in the thalamus are significantly higher than those in the hippocampus and striatum (Kruskal–Wallis, $p < 0.05$), (Fig. 4).

Discussion. The temporal and spatial expression dynamics of fractalkine were investigated for the first time during the early period of rat brain development. Rats are a commonly used model species in the study of brain diseases and nervous system physiology [8]. They are typically fed mother's milk for up to 21 days [9]. For this purpose, 15-day-old rats were selected to model breastfed human infants. Thirty-day-old rats were selected to model both infants who are not breast-fed and those who have not yet entered puberty. Rats 41 days old were selected as the group entering adolescence [10]. From the gestational period to the onset of adolescence, various cellular processes occur, including cell proliferation and migration, myelination, apoptosis, synaptogenesis, and synaptic pruning [11]. According to our results, when examining the healthy development profile of subjects, we observe increases in body and brain weight as they approach adolescence.

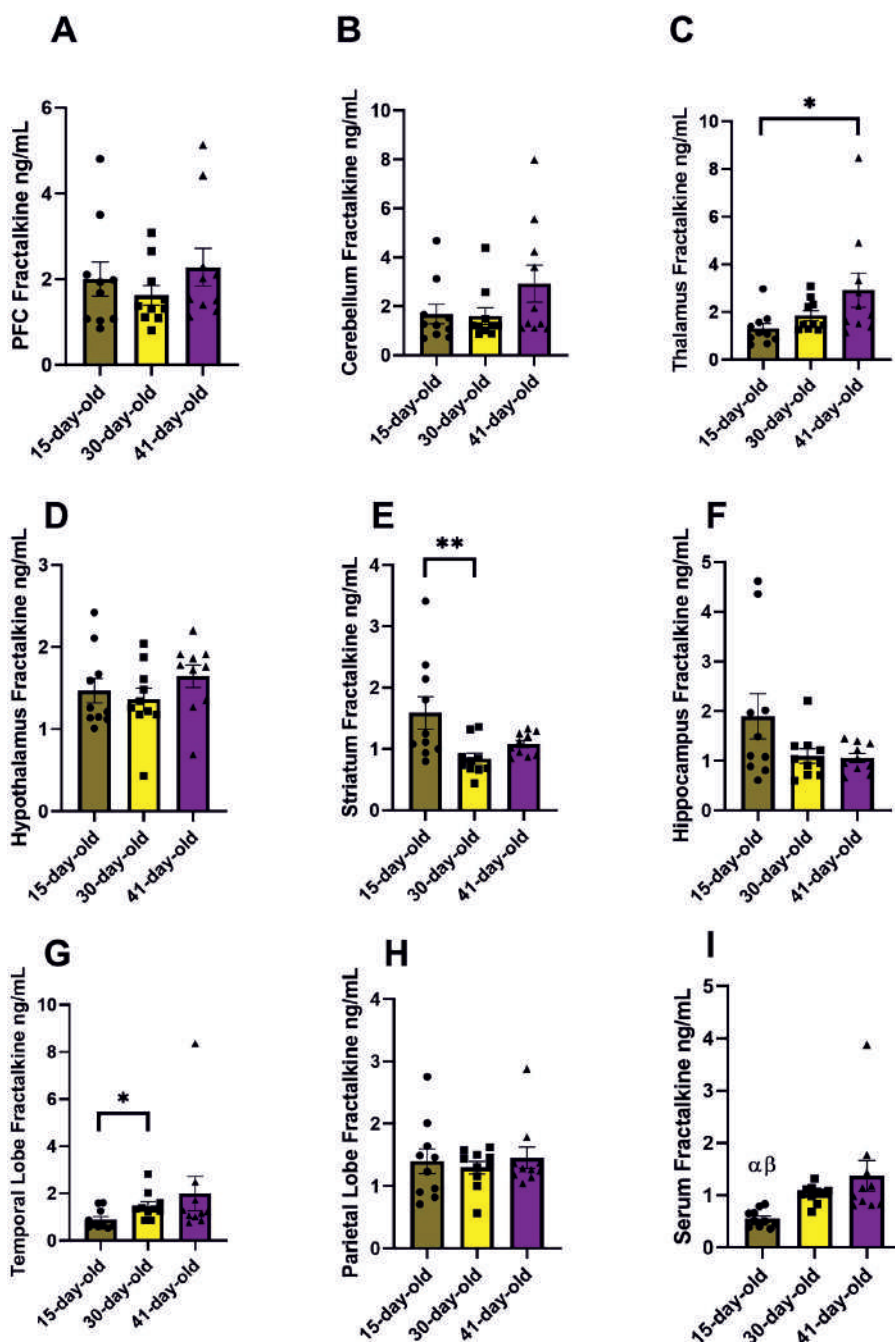


Fig. 1. Temporal molecular findings. Fractalkine levels by age group: (A) PFC, (B) Cerebellum, (C) Thalamus fractalkine, (D) Hypothalamus, (E) Striatum, (F) Hippocampus, (G) Temporal lobe, (H) Parietal lobe, (I) Serum. The significant differences are as follows: * 15-day-old and 41-day-old in thalamus ($p < 0.05$); 15-day-old and 30-day-old in temporal lobe ($p < 0.05$); ** 15-day-old and 30-day-old in striatum ($p < 0.01$); α 15-day-old and 30-day-old in serum ($p < 0.005$); β 15-day-old and 41-day-old in serum ($p < 0.001$), ($n = 10$, each group)

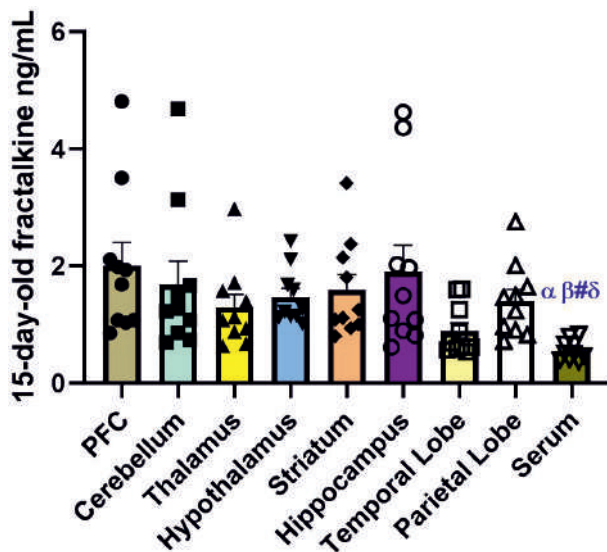


Fig. 2. Spatial molecular findings (15 days old rats): The significant differences are as follows: α serum and hypothalamus ($p < 0.005$); serum and striatum ($p < 0.005$); β serum and cerebellum ($p < 0.05$); serum and parietal lobe ($p < 0.05$); # serum and hippocampus ($p < 0.01$); δ serum and PFC ($p < 0.0005$), ($n = 10$, each group)

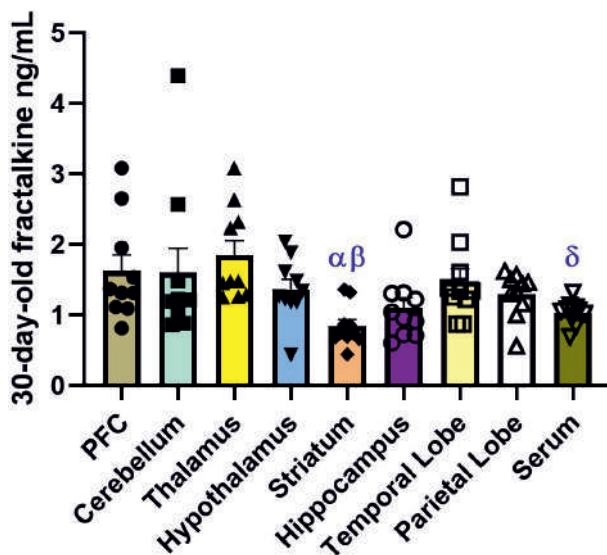


Fig. 3. Spatial molecular findings (30-day-old rats). The significant differences are as follows: α the striatum and PFC ($p < 0.05$); β the striatum and thalamus ($p < 0.005$); δ serum and thalamus ($p < 0.05$)

Additionally, the relationship between height and weight was examined using the Lee index, which was developed for rats in obesity studies [12,13]. Because the

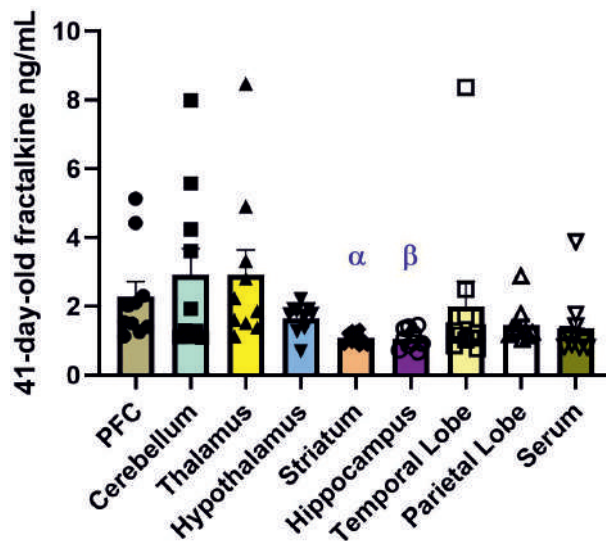


Fig. 4. Spatial molecular findings (41-day-old rats). The significant differences are as follows: α , the thalamus and striatum ($p < 0.05$); β , the thalamus and hippocampus ($p < 0.05$) ($n = 10$, each group)

increase in subjects' height exceeded that in their weight, the Lee index decreased as they approached adolescence.

The thalamus acts as a bridge between the cortex and subcortical structures. The thalamus plays a role in sleep, wakefulness, and primary sensory processing [14]. A time-dependent cumulative increase has been observed in the thalamus. Previous studies have shown that fractalkine and microglia activation in the thalamus and temporal lobe play a role in migraine pathology in an epilepsy animal model [15].

In the striatum, there was a significant decrease in fractalkine levels from 15-day-old rats to 41-day-old adolescent rats. Fractalkine activates microglia via its receptors and facilitates neuron-microglia communication [16]. Damage occurring during the first 15 days, specifically during the weaning period, can have serious effects. While fractalkine decreases during the transition to adolescence in the striatum and hippocampus, an increase was particularly noticeable in the thalamus. This may be related to the embryonic origin and the distribution of gray-white matter ratios across brain regions. Damage occurring in the thalamus before the transition to adolescence may prevent the peak in fractalkine that typically occurs during adolescence. In contrast, early damage in the hippocampus and striatum may lead to a further decrease in fractalkine, which is already decreasing during the transition to adolescence. In addition, the highest fractalkine levels were observed in the thalamus among all brain regions in 30- and 41-day-old rats. This may be because the immunological development of the thalamus differs from that of other brain regions.

In the present study, no specific peak or cumulative decrease-increase was observed in the prefrontal cortex. However, the highest fractalkine expression was observed in the prefrontal cortex during the lactation period, compared to other regions. Connections between the PFC and the striatum are important for cognitive functions [17]. Furthermore, our results showed another cumulative increase in serum fractalkine levels during adolescence. Serum fractalkine levels showed a rise similar to that in the thalamus and temporal lobe. Microglia play a role in synaptic pruning and waste removal during different developmental periods [18]. The distribution of microglia in neural tissue can be as important as their number and function [19]. Since fractalkine is one of the factors that determines microglial distribution, it contributes to neurodevelopment.

Elucidating the roles of fractalkine in neurological disorders of the central nervous system may be crucial. A significant increase in cerebrospinal fluid levels has been observed in patients with severe brain injury during the first four days [20]. In the same study, a slight but statistically insignificant increase in fractalkine levels was observed in the brains of mice subjected to a closed-head trauma model; however, in that study, the entire damaged cerebral hemisphere was examined [20]. This finding once again underscores the importance of the regional distribution of fractalkine in our study. It would be beneficial to investigate the potential roles of fractalkine in other neurological disorders, such as epilepsy, cerebral palsy, migraine, and multiple sclerosis, through clinical and preclinical studies.

Our study has some limitations. The first limitation is the use of male rats only. Experiments with female subjects are also necessary from the perspective of public health and translational medicine. This was not possible in the presented study due to budget and time constraints. Secondly, we focused only on the temporal and regional profiles of fractalkine expression from the early post-natal period to adolescence. Another limitation is the study's budget. Increasing the budget could make it possible to investigate different brain regions and cerebrospinal fluid. In particular, studies should be conducted in adult rodents to examine cerebrospinal fluid in models of neurological diseases.

Our study has raised some new questions. In particular, fractalkine production, receptors, and microglia behaviour can be investigated in brain lesions in specific regions. Another critical point is that fractalkine serum levels increase toward adolescence. Can serum fractalkine levels be used as a marker in pediatric clinics for children's development and the development of specific brain regions?

Conclusions. The present study found that fractalkine levels varied temporally and regionally during early developmental stages. Fractalkine levels were particularly high in the hippocampus and striatum during the lactation period and decreased towards adolescence. In contrast, fractalkine levels increased toward adolescence in the thalamus, temporal cortex, and serum. The highest fractalkine levels were observed in the prefrontal cortex of 15-day-old rats and

in the thalamus of 30- and 41-day-old rats. Fractalkine may be susceptible to damage during early developmental stages.

REFERENCES

- [1] HUNDHAUSEN C., D. MISZTELA, T. A. BERKHOUT et al. (2003) The disintegrin-like metalloproteinase ADAM10 is involved in constitutive cleavage of CX3CL1 (fractalkine) and regulates CX3CL1-mediated cell-cell adhesion, *Blood*, **102**(4), 1186–1195.
- [2] KIM K. W., A. VALLON-EBERHARD, E. ZIGMOND et al. (2011) In vivo structure/function and expression analysis of the CX3C chemokine fractalkine, *Blood*, **118**(22), e156–e167.
- [3] GEMMA C., A. D. BACHSTETTER (2013) The role of microglia in adult hippocampal neurogenesis, *Front. Cell. Neurosci.*, **7**, 229.
- [4] KAWAMURA N., G. KATSUURA, N. YAMADA-GOTO et al. (2021) Impaired brain fractalkine-CX3CR1 signaling is implicated in cognitive dysfunction in diet-induced obese mice, *BMJ Open Diabetes Res. Care*, **9**(1), e001492.
- [5] National Research Council Committee for the Update of the Guide for the Care and Use of Laboratory Animals (2011) *Guide for the Care and Use of Laboratory Animals*, 8th ed., Natl. Acad. Press.
- [6] BERNARDIS L. L., B. D. PATTERSON (1968) Correlation between ‘Lee index’ and carcass fat content in weanling and adult female rats with hypothalamic lesions, *J. Endocrinol.*, **40**(4), 527–528.
- [7] DOĞAN İ., N. DOĞAN (2020) Estimation of sample size with resource equation method in experimental animal studies, *Turk. Klin. Biostat.*, **12**(2), 211–217.
- [8] ARNOUX I., E. AUDINAT (2015) Fractalkine signaling and microglia functions in the developing brain, *Neural Plast.*, **2015**, 689404.
- [9] ELLENBROEK B., J. YOUN (2016) Rodent models in neuroscience research: is it a rat race?, *Dis. Model. Mech.*, **9**(10), 1079–1087.
- [10] SENGUPTA P. (2013) The laboratory rat: relating its age with human’s, *Int. J. Prev. Med.*, **4**(6), 624–630.
- [11] TZANOULINO S., O. RICCIO, M. W. DE BOER et al. (2014) Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved in excitation and inhibition in the amygdala, *Transl. Psychiatry*, **4**(7), e410.
- [12] AGU F. U., M. U. ELENDU, H. K. OKECHUKWU et al. (2022) Obesity related alterations in kidney function and plasma cytokines: Impact of sibutramine and diet in male Wistar rats, *Afr. J. Pharm. Pharmacol.*, **16**(10), 161–172.
- [13] BASTÍAS-PÉREZ M., D. SERRA, L. HERRERO (2020) Dietary options for rodents in the study of obesity, *Nutrients*, **12**(11), 3234.
- [14] PEYRACHE A., A. J. DUSZKIEWICZ, G. VIEJO et al. (2019) Thalamocortical processing of the head-direction sense, *Prog. Neurobiol.*, **183**, 101693.
- [15] ZHOU Y., L. ZHANG, Y. HAO et al. (2022) FKN/CX3CR1 axis facilitates migraine-like behaviour by activating thalamic-cortical network microglia in status epilepticus model rats, *J. Headache Pain*, **23**(1), 42.
- [16] CAMACHO-HERNÁNDEZ N. P., F. PEÑA-ORTEGA (2023) Fractalkine/CX3CR1-dependent modulation of synaptic and network plasticity in health and disease, *Neural Plast.*, **2023**, 4637073.

- [17] ZHANG Y., X. PAN, R. WANG et al. (2016) Functional connectivity between pre-frontal cortex and striatum estimated by phase locking value, *Cogn. Neurodyn.*, **10**(3), 245–254.
- [18] KIM K. H., S. M. SON, I. MOOK-JUNG (2013) Contributions of microglia to structural synaptic plasticity, *J. Exp. Neurosci.*, **7**, 85–91.
- [19] TAN Y. L., Y. YUAN, L. TIAN (2020) Microglial regional heterogeneity and its role in the brain, *Mol. Psychiatry*, **25**(2), 351–367.
- [20] RANCAN M., N. BYE, V. I. OTTO et al. (2004) The chemokine fractalkine in patients with severe traumatic brain injury and a mouse model of closed head injury, *J. Cereb. Blood Flow Metab.*, **24**(10), 1110–1118.

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