THE ROLE OF FRACTALKINE IN THE NEURON-MICROGLIA CROSSTALK – A LINK TO DEPRESSION

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INTRODUCTION: Microglia are now recognized as crucial neuromodulators responsible for brain development and its functioning in adult life. The disturbances in microglia and neurons interactions may be potentially involved in the pathogenesis of psychiatric disorders, such as depression [1]. The key role in this communication is played by chemokine: fractalkine (CX3CL1) and its receptor (CX3CR1) [2]. Complementary expression of CX3CL1 on neurons and CX3CR1 on microglial cells establishes a unique system which may be involved in regulation of microglial activation. In our previous studies, adult rats after prenatal stress procedure – an animal model of depression – showed enhanced production of cytokines in frontal cortex and hippocampus. Moreover, in primary cell cultures we discovered that prenatal stress leads to activation of microglia.

AIM: Therefore this project is aimed at investigating whether the activation of microglia can be linked to the disturbances in CX3CL1-CX3CR1 system and if the administration of exogenous fractalkine could counteract aberrant activation found in primary microglial cell cultures.

METHODS: Pregnant Sprague-Dawley rats were subjected to three stress sessions every day from the 14th day of pregnancy until delivery. Control pregnant females were left undisturbed in their homecages. Primary cell cultures were prepared from the cortices of 1–2 day old offspring. The exogenous fractalkine (FKN) in the concentration of 50-200 ng/ml was added to the microglial cultures and after 24 hours cell supernatants were collected. In *in vivo* studies, adult rats (3-months of age) were first tested behaviorally (verification of the used model of depression) and next the activation of microglial cells in hippocampus and frontal cortex were evaluated. Moreover, fractalkine and its receptor expression were estimated.

Statistics analysis: One-way or Two-way ANOVA, post-hoc: Duncan test, $p<0.05$

Primary cultures of microglial cells

Fig 5

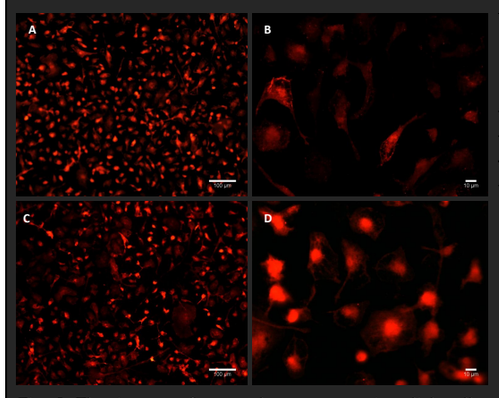


Fig 5 The impact of prenatal stress on microglial cells morphology.; immunofluorescent staining A, B- control cultures; C, D – cultures obtained from prenatally stressed rats

Fig 6

gene	control	control + FKN100	control + FKN200	stress	stress + FKN100	stress + FKN200
IL-1β	1,02±0,11	1,04±0,22	1,00±0,09	1,45±0,11*	1,10±0,21#	1,09±0,23#
IL-18	0,98±0,09	1,02±0,28	0,89±0,16	1,38±0,15*	1,11±0,10#	1,12±0,21#
TNF-α	0,89±0,09	0,88±0,16	0,80±0,25	1,55±0,16*	1,01±0,12#	0,99±0,25#
IL-6	0,79±0,13	0,88±0,15	0,79±0,23	1,39±0,14*	1,31±0,12	0,99±0,25#

Fig 6 The influence of prenatal stress and exogenous fractalkine (100 and 200 ng/ml) on pro-inflammartory cytokines expression in microglial cells in primary cultures

Fig 7

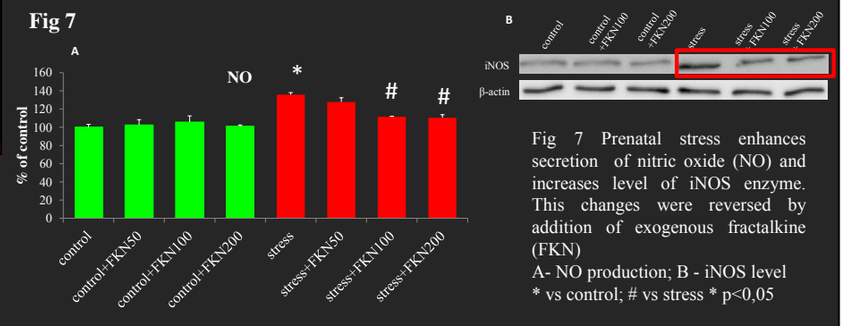


Fig 7 Prenatal stress enhances secretion of nitric oxide (NO) and increases level of iNOS enzyme. This changes were reversed by addition of exogenous fractalkine (FKN)
A- NO production; B - iNOS level
* vs control; # vs stress * $p<0,05$

Fig 8

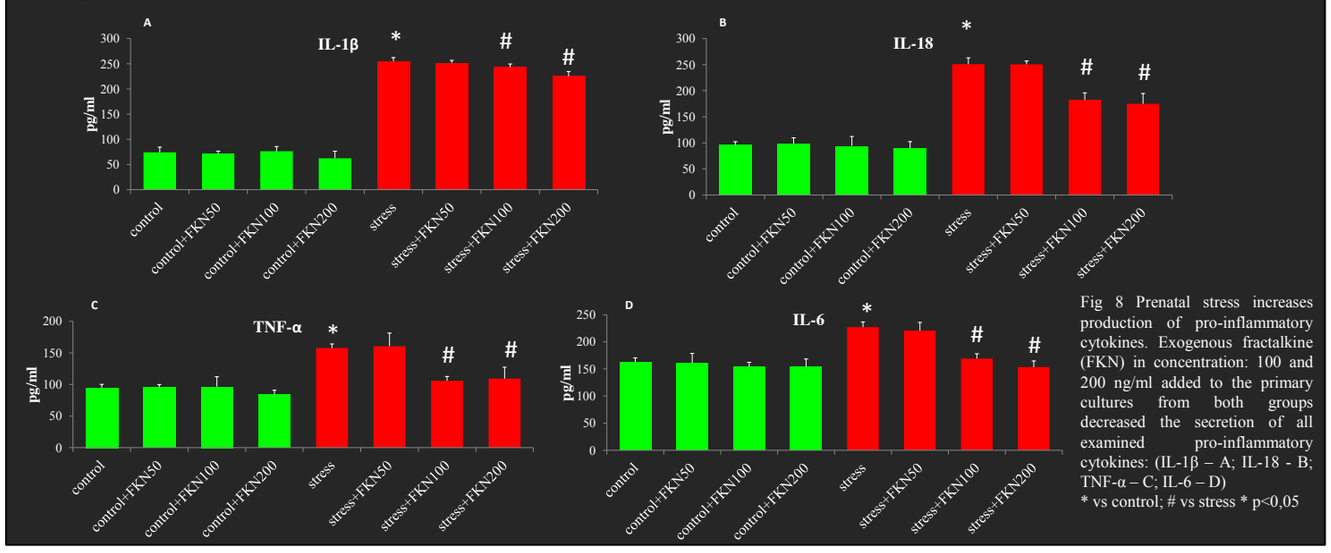


Fig 8 Prenatal stress increases production of pro-inflammatory cytokines. Exogenous fractalkine (FKN) in concentration: 100 and 200 ng/ml added to the primary cultures from both groups decreased the secretion of all examined pro-inflammatory cytokines: (IL-1β – A; IL-18 - B; TNF-α – C; IL-6 – D)
* vs control; # vs stress * $p<0,05$

Behavioral tests – adult animals

Fig 1

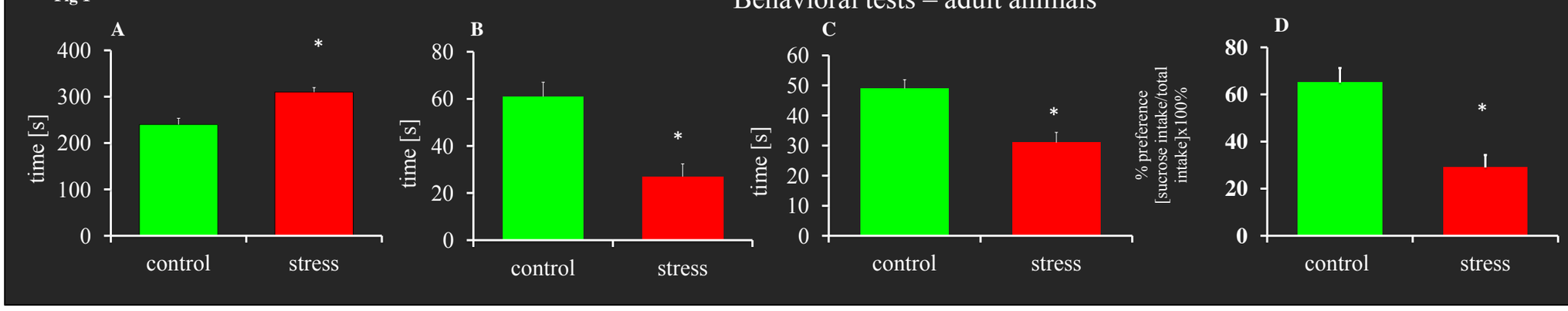
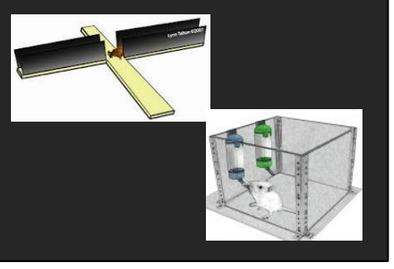


Fig 1 The impact of prenatal stress on immobility (A), climbing time (B) in Porsolt test; time spent in the open arm in the elevated plus-maze test (C); and sucrose intake (D)



Biochemical studies – adult rats

Fig 2

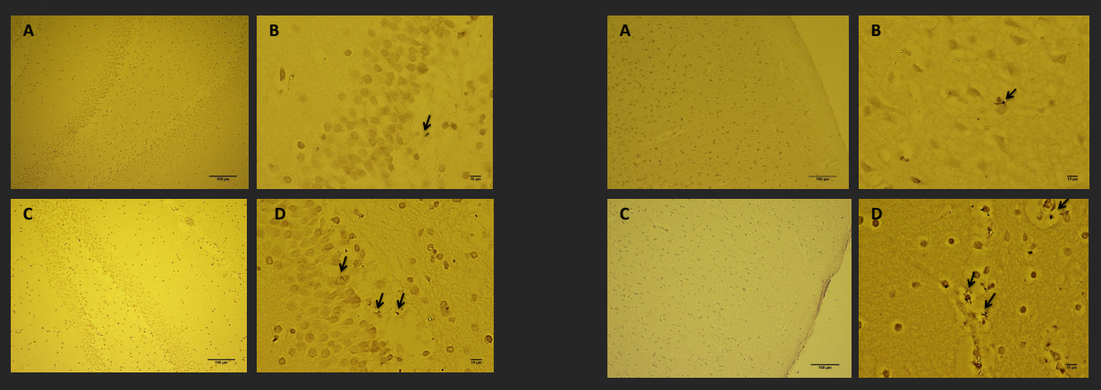


Fig 2 Iba-1 immunohistochemical staining in adult rats – A,B control; C,D prenatally stressed rats

Fig 3

Fig 3 The impact of prenatal stress on microglial activation marker C1q expression in hippocampus (left) and frontal cortex (right)

Fig 4

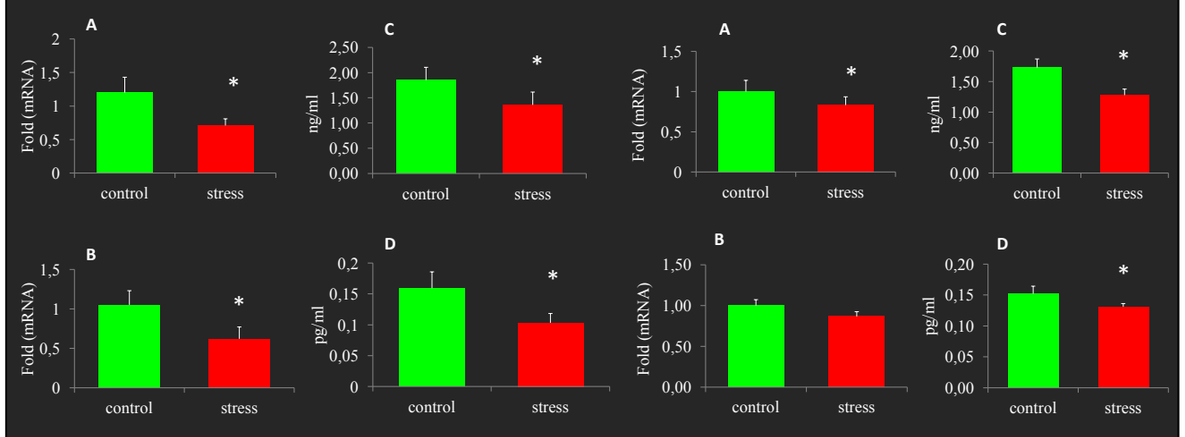


Fig 4 The impact of prenatal stress on fractalkine and fractalkine receptor levels in hippocampus (left) and frontal cortex (right) (A, B- expression of fractalkine and fractalkine receptor; C,D – levels of fractalkine and fractalkine receptor)

RESULTS: Prenatal stress caused a significant increase of IL-1β, IL-18, IL-6, and TNF-α mRNA expression and protein release in primary microglial cells. Additionally, prenatal stress enhanced the production of NO and increased expression and concentration of iNOS enzyme. Administration of exogenous fractalkine inverted the changes observed in microglial cells obtained from prenatally stressed rats. Fractalkine significantly down-regulated mRNA and protein expression of all measured pro-inflammatory cytokines. Besides, fractalkine treatment attenuated nitric oxide production and suppressed iNOS activation. In adult rats behavioral disturbances occured together with increased activation of microglial cells and changes in mRNA and protein levels of CX3CL1 and its receptor in both examinated structures.

CONCLUSIONS: In summary, our study showed that prenatal stress procedure intensifies the activation of microglial cells in primary cultures. Fractalkine administration attenuated the pro-inflammatory cytokines release and suppressed production of other potentially neurotoxic factors. It may be suggested that microglial activation observed in our study may result from the disturbances in CX3CL1-CX3CR1 system function. It is possible that prenatal stress by impact on the chemokine and its receptor functions disturbed the brain communication between neurons and microglial cells, and in this way contributes to the pathogenesis of depression.

REFERENCES:

1. Stuart, M.J., Baune, B.T., Chemokines and chemokine receptors in mood disorders, schizophrenia, and cognitive impairment: A systematic review of biomarker studies. Neurosci Behav Res 2014 Feb 8;42C:93–115
2. Sheridan, G.K., Murphy, K.J., Neuron-glia crosstalk in health and disease: fractalkine and CX3CR1 take centre stage. Open Biol. 2013 Dec 18; 3(12): 130181

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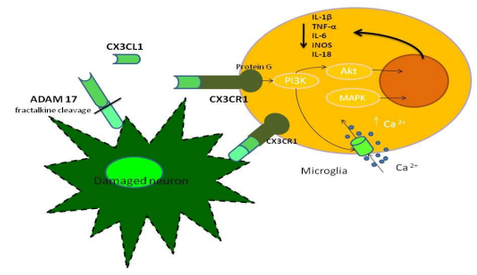

Neuron-microglia crosstalk: role of fractalkine and fractalkine receptor CX3CR1

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Therefore this project is aimed at investigating whether the activation of microglia can be linked to the disturbances in CX3CL1-CX3CR1 system and if the administration of dexogenous fractalkine could counteract aberrant activation found in primary microglial cell cultures.

Pregnant Sprague-Dawley rats were subjected to three (at 9.00, 12.00, 17.00) stress sessions every day from the 14th day of pregnancy until delivery. Control pregnant females were left undisturbed in their homecages. Primary cell cultures were prepared from the cortices of 1–2 day old offspring. After 8 days cells were plated onto 24-well or 96-well plates. Adherent cells were incubated for 48h in culture medium before being used for the study. The exogenous fractalkine in the concentration of 100ng/ml was added to the microglia cultures and after 24 hours cell supernatants were collected. The mRNA expression and protein levels of pro-inflammatory cytokines were measured by quantitive RT-PCR and ELISA methods. Moreover the iNOS enzyme activation and nitric oxide (NO) release were evaluated using Western blot method and Griess reaction, respectively. Statistics analysis: Two-way ANOVA, post-hoc: Duncan test, $p<0.05$.

Prenatal stress caused a significant increase of IL-1β, IL-18, IL-6, and TNF-α mRNA expression and protein release in microglial cells. Additionally, prenatal stress enhanced the production of NO and increased expression and concentration of iNOS enzyme. Administration of exogenous fractalkine inverted the changes observed in microglial cells obtained from prenatally stressed rats. Fractalkine significantly down-regulated mRNA and protein expression of all measured pro-inflammatory cytokines. Besides, fractalkine treatment attenuated nitric oxide production and suppressed iNOS activation.

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Keywords

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