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Pro-inflammatory effects of placebo neurosurgery in rats: age-related features

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Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most prevalent neurodegenerative diseases, affecting millions of people globally and causing significant disability and mortality. Animal models are the final step in completing preclinical studies and the most appropriate approach for gaining a thorough understanding of disease pathophysiology. Modeling of idiopathic AD and PD in rodents requires stereotactic injections of disease-triggering substances. The placebo surgery group is an integral component of the design of these experiments in order to diminish study bias as a result of animal stress and non-specific surgical impact. Inflammation is the most commonly reported non-specific post-surgery phenomenon, which can manifest in different ways in animals of different ages used in these experiments.

Objective — to compare the long-term pro-inflammatory effects of placebo surgery, commonly employed for PD and AD modeling, in rats of different ages.

Materials and methods. Adult male Wistar rats aged 4 and 14 months were used in the study. The placebo surgery consisted of a stereotactic unilateral intracerebral infusion of buffer solution. Before the placebo surgery, animals were anaesthetized using ketamine or xylazine administered intraperitoneally. Intact animals of both ages were used as a control. The evaluation of pro-inflammatory effects of placebo surgery was conducted using biomarkers of local and systemic inflammation: metabolic polarization of phagocytes (microglia, peripheral blood cells), C-reactive protein (CRP) plasma level, and systemic inflammation indexes calculated from the hemogram study.

Results. In young lesioned animals, a pronounced pro-inflammatory functional shift of microglia and signs of the resolution of systemic inflammation (an anti-inflammatory skew of circulating phagocyte metabolism as compared to age-matched intact controls) were observed in the long term after the placebo neurosurgery. In old intact animals, hematological and immunological markers of low-grade systemic inflammation were observed. In lesioned old rats, residual neuroinflammation along with pronounced systemic inflammatory responses (leukocytosis, substantially increased SIRI and SII values, pro-inflammatory metabolic shift of peripheral blood phagocytes as compared to age-matched intact controls) were registered.

Conclusions. The effects of placebo neurosurgical manipulations in rats depend on age. Meta-inflammation inherent to aged rats is aggravated by non-specific post-surgery inflammation, leading to pronounced, persistent systemic inflammatory responses.

Keywords
placebo surgery, phagocytes, neuroinflammation, systemic inflammation, inflammaging.

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Alzheimer's disease (AD) and Parkinson's disease (PD) are the two most prevalent neurodegenerative diseases, affecting millions of people globally and causing significant disability and mortality. Because of the increasing prevalence of these diseases in an ageing population, as well as lack of therapeutics, AD and PD have become two of the leading causes of disability, burdening patient families and healthcare systems [7, 8]. Pharmaceuticals and/or surgery are accessible for symptomatic alleviation. However, nothing is available for the pathogenic treatment of these diseases. The absence of therapeutics for the treatment of AD and PD is in large part due to the lack of reliable information on the etiology and pathogenesis of sporadic forms of these diseases.

Animal models are the final step in completing preclinical studies and the most appropriate approach for gaining a thorough understanding of disease pathophysiology [4, 12]. The development of animal models for sporadic neurodegenerative diseases entails stereotactic surgical manipulations for the intracerebral introduction of disease-triggering substances, as well as the mandatory use of placebo-operated animals to control for non-specific effects elicited by the surgical procedure itself.

Surgical interventions are associated with intrinsic side effects, including local tissue damage, acute inflammation, wound healing, and temporary post-surgery immunosuppression. In addition, some psychological variables affect early surgical recovery [3, 25, 27]. All of the preceding emphasizes the critical importance of placebo surgery control groups in removing the impact of these non-specific effects on the evaluation of study results [26].

Immune-dependent consequences of surgical interventions (inflammation, immunosuppression, tissue remodeling, etc.) are age-dependent phenomena. Ageing is linked to the development of chronic low-grade inflammation, also known as 'inflammaging'. Prolonged inflammaging will unavoidably result in immune system exhaustion and the development of secondary immunodeficiency. These are two sides of immunosenescence [2, 22]. Inflammaging indicates age-associated impairment of the resolution of inflammation, including post-surgical inflammation [23]. Inflammaging and age-related immune suppression are also inherent to laboratory animals, including those used in the modeling of PD and AD [9, 10, 31].

Different research groups successfully use rats of different ages for non-transgenic PD and AD models. All these models involve placebo surgery controls. Nonetheless, there is a scarcity of data on the comprehensive evaluation of non-specific post-surgical immunomodulatory effects of placebo surgery in rats of various ages.

Objective — to compare the long-term pro-inflammatory effects of placebo surgery, commonly employed for PD and AD modeling, in rats of different ages.

Materials and methods

Laboratory animals and study design. The experiments were performed on adult male, young (4 months, 220—250 g) and old (14 months, 450—500 g) Wistar rats bred in the vivarium of the ESC «Institute of Biology» of Taras Shevchenko National University of Kyiv, Ukraine. Animals were housed in the animal facility within a constant ambient temperature (22 °C), humidity, and a normal photoperiod (12 : 12 h light/dark cycle, lights on at 7:30). Rats were given standard laboratory feed and water ad libitum. Animal protocol was approved by the University Ethics Committee according to Animal Welfare Act guidelines. All procedures involving animals were carried out in accordance with the principles of humanity outlined in the «General Principles of Animal Experimentation», which was approved by the National Congress on Bioethics (Kyiv, 2001—2007), as well as the Council directive of November 24, 1986 on the approximation of laws, regulations, and administrative provisions of the Member States governing the protection of animals used for experimental and other scientific purposes (86/609/EEC). In total, forty rats were used in this study: twenty young animals and twenty old animals. Animals of the same age were randomly divided into two groups prior to all experiments: intact animals and animals that had been lesioned (sham-operated). In total, four groups were formed: young intact (group 1, n = 10) and lesioned (group 2, n = 10) rats, old intact (group 3, n = 10) and lesioned (group 4, n = 10) animals. Randomization was conducted using the «RAND ()» function in Microsoft Excel. Each animal was allocated to a separate cage. For each animal, six different investigators were involved as follows: a first investigator (the only person aware of the treatment group allocation) administered the treatment based on the randomization table; a second investigator performed anaesthetic, surgical, and euthanasia procedures; a third investigator was responsible for the sample collection and cell isolation; a fourth investigator assessed hematological indices; a fifth investigator performed flow cytometry for phagocyte metabolic profile assessment; and a sixth investigator was responsible for the statistical analysis. Immediately before surgery, animals from groups 2 and 4 were anesthetized with a mixture of ketamine (75 mg/kg diluted in sterile water for injection, Sigma, USA) and 2 % Xylazine

(400 μl /kg, Alfasan International BV, Netherlands) i.p. at a volume of 1 ml. After this, animals from group 2 were placed in a stereotaxic instrument (SEJ-4, Ukraine), scalped, and injected unilaterally (left side) with 2 μl of 0.9 % sodium chloride directly into the substantia nigra (Ap = –5.3; ML = ±2.0; DV = –7.2) according to the protocol for PD modeling based on the bacterial lipopolysaccharide intracerebral introduction, which was developed by Hoban et al., 2013 [11]. NaCl was injected into the brain tissue at a rate of 1 μl/min (every 15s). In order to allow for liquid diffusion into the brain tissue and to prevent reflux, the injector was left in place for 5 minutes before slowly withdrawing. Placebo surgery in group 3 was conducted according to the protocol developed for modeling unilateral AD, induced by the intrahippocampal infusion of human amyloid beta 1—40 [1, 15]. Animals from group 4 were scalped from the point of intersection of the sagittal suture with the bregma (zero point) at 2 mm distally, 2 mm laterally, and 3.5 mm deep, and a burr hole was made with an injection needle directly into the hippocampus. The microinjector tip was lowered into the burr hole, and 10 μl of sterile water for the injection were slowly infused at a rate of 0.5 μl/min. After infusion, the tip of the microinjector remained in the brain tissue for 4 minutes, and was then removed, and the soft tissues of the head were sutured. The pro-inflammatory effects of placebo surgery were estimated at day 29 after the manipulation. Cervical dislocation was used to sacrifice the animals because it minimizes cell death in brain structures and allows for a high viability rate in microglia cells after the isolation [1]. Peripheral blood and brain tissue specimens were sampled immediately after the rat's euthanasia.

Hematological assay. Hematological parameters were detected using an analyzer, «Particle Counter Model PCE 210» (ERMA, Japan), which was adapted for the experiments with the use of blood cells in rats and mice. A differential leukocyte count was conducted. Systemic inflammation indices were also calculated. The neutrophil to lymphocyte ratio (NLR) was figured out as the absolute neutrophil count/absolute lymphocyte count. The lymphocyte to monocyte ratio was calculated as the absolute lymphocyte count/absolute monocyte count. Platelet count r NLR was used to calculate the systemic immune-inflammation index (SII) [14]. The systemic inflammation response index (SIRI) was defined as (N x M)/L, where N, M, and L represent the counts of peripheral neutrophils, monocytes, and lymphocytes, respectively [13].

Plasma CRP Level Assay. C-reactive protein (CRP) levels in plasma were determined using an ELISA Kit (Labcare Diagnostics India Pvt Ltd) and the manufacturer's recommendations.

Microglia cell isolation. Microglial cells were isolated from whole brain tissue homogenates as described earlier [18]. For this purpose, animal brains were rapidly excised on ice and homogenized in ice-cold phosphate buffered saline (PBS) supplemented with 0.2 % glucose using a Potter homogenizer. Tissue homogenates were then filtered and centrifuged at room temperature. Microglia cells were isolated in the Percoll density gradient. The purity of isolated microglial cells was assessed by flow cytometry using a FACSCalibur flow cytometer and CellQuest Pro software (Beckton Dickinson, USA), and using fluorescein isothiocyanate (FITC) murine anti-rat CD11b (BD PharmingenTM) and phycoerythrin (PE) murine anti-rat CD45 (BD PharmingenTM). The percentage of $CD11b+CD45^{+}$ cells was ≥ 88 %. Cell viability, detected by the Trypan Blue exclusion test, was ≥ 93 %.

Phagocytic metabolic polarization assay. Metabolic polarization of brain microglia and circulating phagocytes was characterized by their phagocytic activity, reactive oxygen species (ROS) generation, and phenotypic marker expression, which were assayed using flow cytometry, as described earlier [21]. FITC-labeled, thermally inactivated cells of *Staphylococcus aureus* Cowan I were used as a phagocytosis object. Data are presented as the percentage of phagocytizing cells and the phagocytosis index (the average fluorescence per phagocytic cell). ROS was determined using 2'7'-dichlorodihydrofluorescein diacetate (H2DCFDA, Invitrogen) as described previously. FITC-labeled anti-CD80/86 and phycoerythrin (PE)-labeled anti-CD206 antibodies (Becton Dickinson, Pharmingen, USA) were used for phagocyte phenotyping. Results were assessed using a FACSCalibur flow cytometer and CellQuest software (Becton Dickinson, USA). Granulocytes and monocytes were gated according to forward and side scatter.

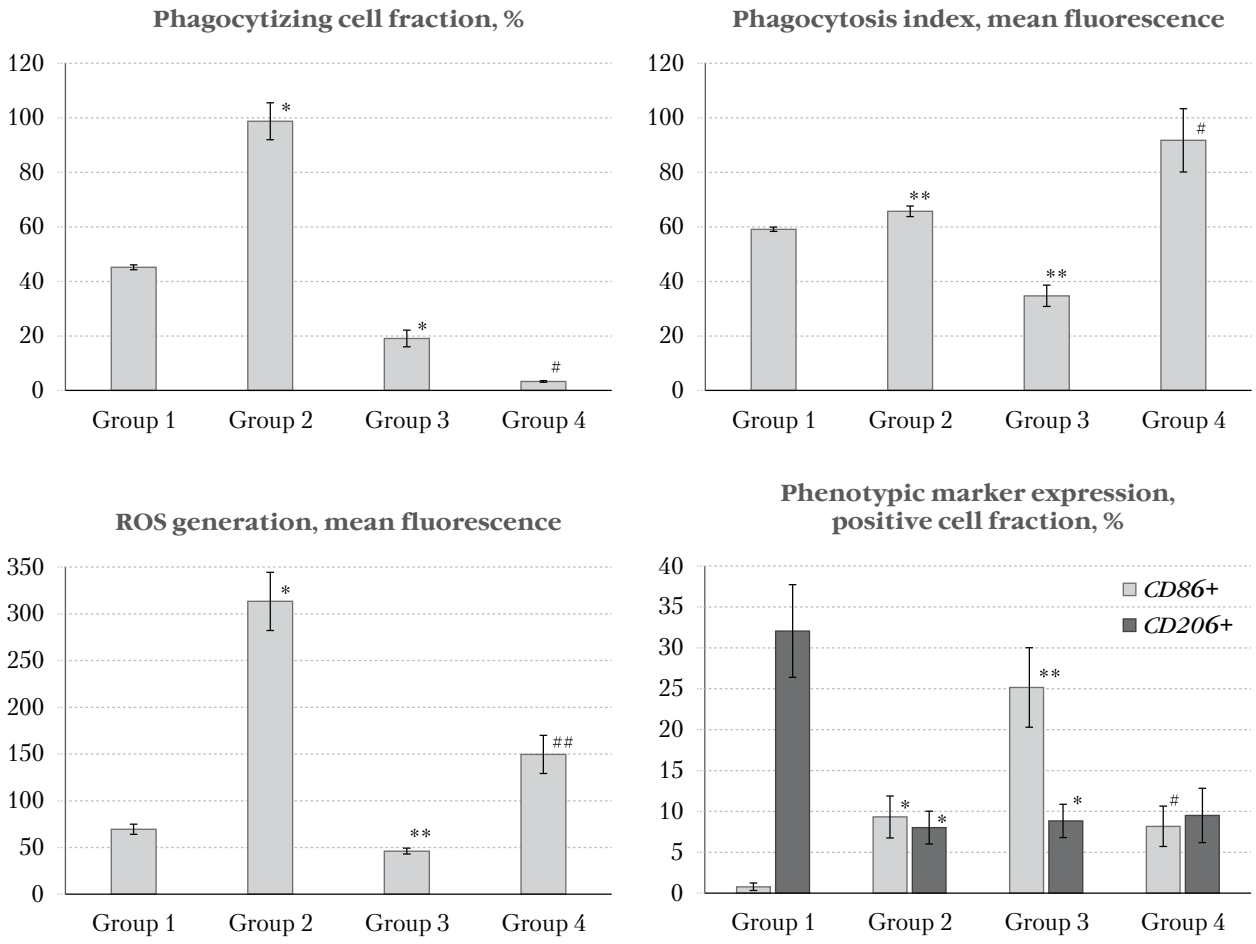
Statistical analysis. All data are presented as mean ± SD. Statistical differences were calculated using an ANOVA with Tukey's post-hoc test. At $p \leq 0.05$, differences were considered significant.

Results and discussion

Acute post-surgical inflammation, comprising two successive stages (systemic inflammatory response syndrome, SIRS, and compensatory anti-inflammatory response syndrome), is widely described in the literature [17]. However, much less is known about long-term post-surgical inflammation after the interventions. For this reason, we studied markers of

inflammation 28 days after the surgical manipulations. In order to conduct a comprehensive comparative assessment of post-surgery inflammation, we used both commonly accepted inflammation biomarkers (leukocytosis, CPR plasma level, hematological inflammation coefficients) and the metabolic profile of phagocytes from different locations (microglia, peripheral blood). Tissue-resident and circulating phagocytes are key players in the initiation, progression, and resolution of inflammation, including that after the surgery [24]. Tissue-resident phagocytes are responsible for the course of local inflammation, whereas circulating cells are key players in systemic inflammatory responses. Phagocytes participating in different phases of the inflammatory process are characterized by different metabolic polarizations. Pro-inflammatory phagocytes (M1 macrophages and monocytes, N1 neutrophilic granulocytes) participate in the initiation of inflammatory responses, whereas cells with an anti-inflammatory metabolic profile are involved in the resolution of inflammation and tissue repair [6].

Placebo surgery in animal models of PD and AD primarily causes local neuroinflammation, where microglial cells are key drivers [5]. In our studies, we found different patterns of microglia metabolic profile in young and old rats in the long term after placebo neurosurgery. Microglial cells from intact old animals were characterized by decreased phagocytic activity and oxidative metabolism as well as an increased number of $CD86^+$ cells along with a reduced fraction of $CD206^+$ cells as compared to young intact rats (Fig. 1). Weakened microglia metabolic activity indicates immune senescence. A significantly higher proportion of cells expressing the pro-inflammatory metabolic shift CD86 marker, as well as a lower proportion of anti-inflammatory CD206-positive cells, indicate local brain meta-inflammation, which is a common feature of the ageing brain [29].



The difference as compared to group 1 is statistically significant: * $p < 0.01$; ** $p < 0.05$.
The difference as compared to group 3 is statistically significant: # $p < 0.01$; ## $p < 0.05$.
Statistical differences are calculated using an ANOVA with Tukey's post-hoc test.

Figure 1. **Functional and phenotypic characteristics of microglial cells in young and old rats in the long term after placebo neurosurgery**

Differences in the basal state of microglial cells in young and old animals were combined with their different responses to placebo surgery. 28 days after the surgical intervention, microglia from young rats demonstrated highly stimulated oxidative metabolism along with a shift in the balance in the proportions of $CD86^+$ and $CD206^+$ cells in favour of cells with a pro-inflammatory phenotypic marker as compared to age-matched intact controls. Altogether, these microglial characteristics certify residual neuroinflammation with the involvement of both resident macrophages and recruited circulating phagocytes [19].

In old animals subjected to placebo neurosurgery, the proportion of phagocytizing (i.e., activated) microglial cells was substantially lowered in comparison with an age-matched intact control. Both phagocytic activity and oxidative metabolism increased significantly along with the decrease in the $CD86^+$ cell fraction. These characteristics also point to residual neuroinflammation, which nevertheless differs from that in young lesioned rats. Deep insight into these differences requires separate assessment of resident and recruited phagocytes in the complex microglia population, considering their distinct responses to environmental stimuli [20].

Peripheral blood biomarkers, which were used to reveal the systemic inflammation, demonstrated different patterns in young and old animals too. indicated Inflammaging was indicated by hematological indices in old intact rats: leukocytosis with significantly increased SIRI and SII values (Table).

SIRI mirrors the status of systemic inflammation, displaying an increased proportion of the two myeloid cell populations involved in this process: neutrophils and monocytes. SII contains an additional variable in the formula that characterizes systemic inflammation: platelet count. Because platelets are a rich source of pro-inflammatory cytokines, SII is regarded as a systemic inflammation index that comprehensively reflects the balance of the host immune (adaptive immunity) and inflammatory (innate immunity) conditions [16, 30]. When compared to an age-matched intact control, placebo neurosurgery caused aggravated low-grade systemic inflammation in old animals, with exacerbated leukocytosis and increased SII values.

The CRP basal plasma level was higher in old intact rats (Fig. 2A). It is consistent with the literature and indicates inflammaging. CRP values didn't differ significantly in both young and old rats 28 days after the placebo neurosurgery as compared to their intact age-matched counterparts, owing to the acute nature of the CRP surge in the inflammation.

Peripheral blood phagocytes in old intact animals had decreased endocytic activity (Fig. 2B) and oxidative metabolism (Fig. 2C) as compared to young intact rats, indicating age-related metabolic exhaustion of these cells. Metabolic characteristics of circulating phagocytes from young lesioned rats were as follows: decreased monocyte phagocytic activity (Fig. 2B) and ROS generation (Fig. 2C), increased proportion of $CD86^+$ neutrophils (Fig. 2D). CD86 is a co-stimulatory molecule involved in antigen presentation by innate immunity cells, including neutrophilic granulocytes [6]. In addition, CD86 is overexpressed by granulocytic myeloid-derived suppressor cells participating in the resolution of inflammation [28]. So, we tend to assume that, taken together, the metabolic characteristics of circulating phagocytes in young rats indicate resolution of systemic inflammation.

In old lesioned rats, an increased proportion of $CD86^+$ granulocytes (Fig. 2D) and enhanced ROS generation by these cells (Fig. 2C) rather indicate persistent systemic inflammation.

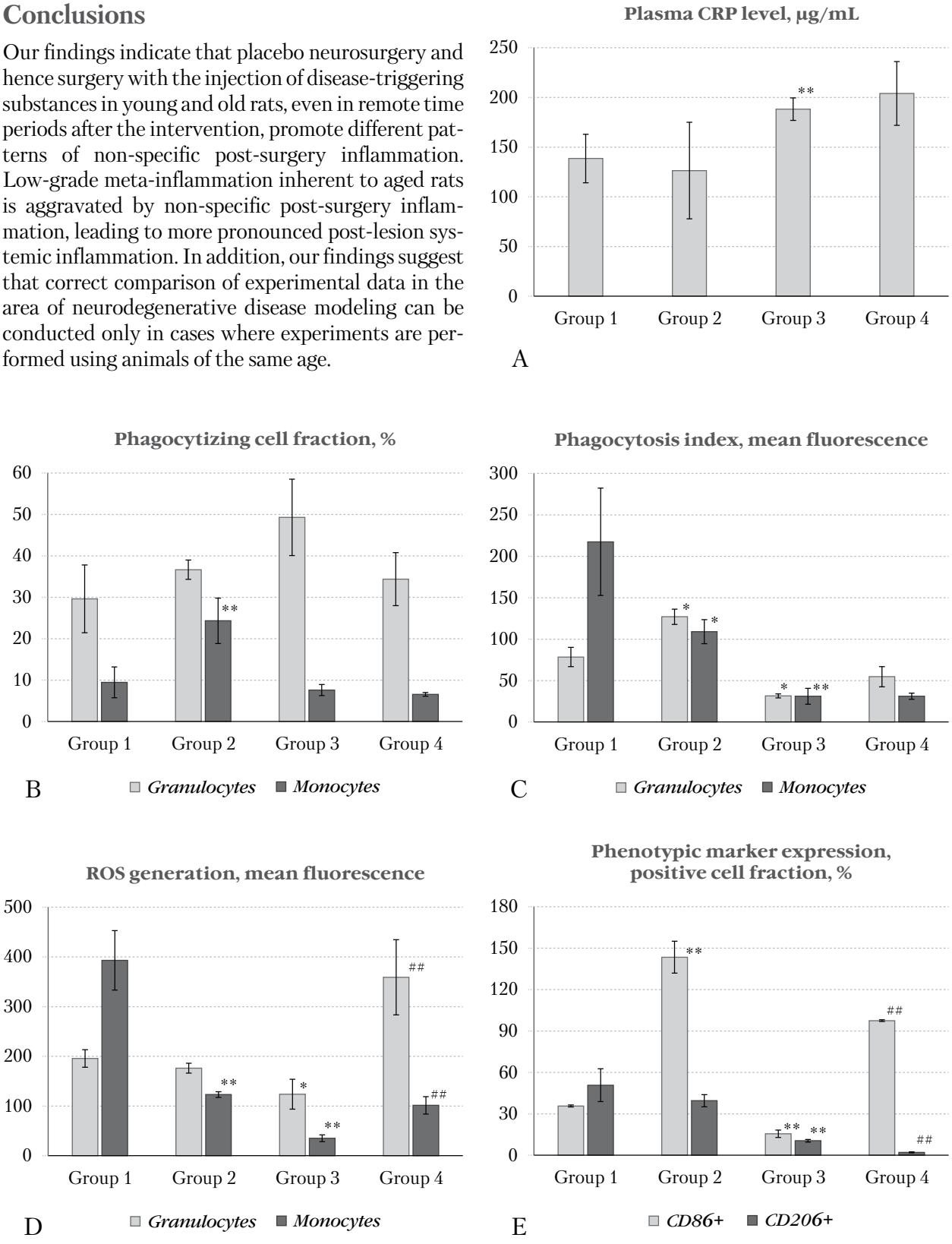
Table. **Hematological indices of peripheral inflammation in young and old rats in the long term after placebo neurosurgery**

Indicator	Group 1	Group 2	Group 3	Group 4
WBC count, $10^3/\mu L$	6,11 ± 0,56	5,93 ± 0,68	11,01 ± 1,53*	19,37 ± 2,57#
NLR	0,32 ± 0,03	0,25 ± 0,06	0,41 ± 0,09	0,39 ± 0,05
LMR	11,0 ± 2,44	5,52 ± 2,28*	4,77 ± 1,51*	5,32 ± 1,82
SIRI	0,11 ± 0,06	0,22 ± 0,09	1,23 ± 0,65*	1,43 ± 0,58
SII	33.73 ± 9.09	41,91 ± 7,83	88,56 ± 21,52*	141,17 ± 27,55#

Note. * Significant ($p < 0.05$) differences in comparison with group 1.
Significant ($p < 0.05$) differences in comparison with group 3.
Statistical differences are calculated using an ANOVA with Tukey's post-hoc test.
NLR — neutrophil to lymphocyte ratio; LMR — lymphocyte to monocyte ratio;
SIRI — systemic inflammation response index; SII — systemic immune inflammation index.

Conclusions

Our findings indicate that placebo neurosurgery and hence surgery with the injection of disease-triggering substances in young and old rats, even in remote time periods after the intervention, promote different patterns of non-specific post-surgery inflammation. Low-grade meta-inflammation inherent to aged rats is aggravated by non-specific post-surgery inflammation, leading to more pronounced post-lesion systemic inflammation. In addition, our findings suggest that correct comparison of experimental data in the area of neurodegenerative disease modeling can be conducted only in cases where experiments are performed using animals of the same age.



The difference as compared to group 1 is statistically significant: * $p < 0.01$; ** $p < 0.05$.
The difference as compared to group 3 is statistically significant: # $p < 0.01$; ## $p < 0.05$.
Statistical differences are calculated using an ANOVA with Tukey's post-hoc test.

Figure 2. **Immunological markers of systemic inflammation in young and old rats in the long term after placebo neurosurgery**

Declaration of interests

The authors have no conflict of interest to declare.

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Authors contributions

A. Nefodova: surgical manipulations, cell isolation; M. Rudyk: immunophenotyping, data collection, and statistical analysis; M. Pasichnichenko: ELISA, hematological assays; R. Dovhyi: intracellular ROS assay, phagocytosis assay, data collection, and analysis; T. Dovbynchuk: surgical manipulations, data collection, and statistical analysis; G. Tolstanova: designed the study, reviewed the manuscript; L. Skivka: conception and design, analysis and interpretation of data, drafting the article, critical revision of the article.

References

1. Aguwa US, Eze CE, Obinwa BN, et al. Comparing the effect of methods of rat euthanasia on the brain of wistar rats: Cervical dislocation, chloroform inhalation, diethyl ether inhalation and formalin inhalation. Journal of Advances in Medicine and Medical Research. 2020:8-16. doi: 10.9734/jammr/2020/v32i1730636.
2. Aiello A, Farzaneh F, Candore G, et al. Immunosenescence and its hallmarks: How to oppose aging strategically? A review of potential options for therapeutic intervention. Frontiers in Immunology. 2019;10. doi: 10.3389/fimmu.2019.02247.
3. Arias J-I, Aller M-A, Arias J. Surgical inflammation: A pathophysiological rainbow. Journal of Translational Medicine. 2009;7(1). doi: 10.1186/1479-5876-7-19.
4. Banerjee R, Rai A, Iyer SM, Narwal S, Tare M. Animal models in the study of alzheimer's disease and parkinson's disease: A historical perspective. Animal Models and Experimental Medicine. 2022;5(1):27-37. doi: 10.1002/ame2.12209.
5. Buckley MW, McGavern DB. Immune Dynamics in the CNS and its barriers during homeostasis and disease*. Immunological Reviews. 2022;306(1):58-75. doi: 10.1111/imr.13066.
6. Cassetta L, Cassol E, Poli G. Macrophage polarization in health and disease. The Scientific World JOURNAL. 2011;11:2391-2402. doi: 10.1100/2011/213962.
7. Cui L, Hou N-N, Wu H-M, et al. Prevalence of alzheimer's disease and parkinson's disease in China: An updated systematical analysis. Frontiers in Aging Neuroscience. 2020;12. doi: 10.3389/fnagi.2020.603854.
8. Dorsey ER, Elbaz A, Nichols E, et al. Global, regional, and national burden of parkinson's disease, 1990-2016: A systematic analysis for the global burden of disease study 2016. The Lancet Neurology. 2018;17(11):939-953. doi: 10.1016/s1474-4422(18)30295-3.
9. El-naseery NI, Mousa HSE, Noreldin AE, El-Far AH, Elewa YHA. Aging-associated immunosenescence via alterations in splenic immune cell populations in rat. Life Sciences. 2020;241:117168. doi: 10.1016/j.lfs.2019.117168.
10. Gordon CJ, Rowsey PJ, Bishop BL, Ward WO, MacPhail RC. Serum biomarkers of aging in the Brown Norway rat. Experimental Gerontology. 2011;46(11):953-957. doi: 10.1016/j.exger.2011.07.006.
11. Hoban DB, Connaughton E, Connaughton C, et al. Further characterisation of the LPS model of parkinson's disease: A comparison of intra-nigral and intra-striatal lipopolysaccharide administration on motor function, microgliosis and nigrostriatal neurodegeneration in the rat. Brain, Behavior, and Immunity. 2013;27:91-100. doi: 10.1016/j.bbi.2012.10.001.
12. Janowski M, ed. Experimental Neurosurgery in Animal Models. New York: Springer; 2016. doi: 10.1007/978-1-4939-3730-1.
13. Jin Z, Wu Q, Chen S, et al. The associations of two novel inflammation indexes, sii and Siri with the risks for cardiovascular diseases and all-cause mortality: A ten-year follow-up study in 85,154 individuals. Journal of Inflammation Research. 2021;Volume 14:131-140. doi: 10.2147/jir.s283835.
14. Karimi A, Shobeiri P, Kulasinghe A, Rezaei N. Novel systemic inflammation markers to predict COVID-19 prognosis. Frontiers in Immunology. 2021;12. doi: 10.3389/fimmu.2021.741061.
15. Kasza Á, Penke B, Frank Z, et al. Studies for improving a rat model of alzheimer's disease: ICV administration of well-characterized β-amyloid 1-42 oligomers induce dysfunction in spatial memory. Molecules. 2017;22(11):2007. doi: 10.3390/molecules22112007.
16. Ludwig N, Hilger A, Zarbock A, Rossaint J. Platelets at the crossroads of pro-inflammatory and resolution pathways during inflammation. Cells. 2022;11(12):1957. doi: 10.3390/cells11121957.
17. Margraf A, Ludwig N, Zarbock A, Rossaint J. Systemic inflammatory response syndrome after surgery: Mechanisms and protection. Anesthesia & Analgesia. 2020;131(6):1693-1707. doi: 10.1213/ane.0000000000005175.
18. Pjanova D, Hurmach Y, Rudyk M, et al. Effect of bacteriophage-derived double stranded RNA on rat peritoneal macrophages and microglia in normoxia and hypoxia. Proceedings of the Latvian Academy of Sciences Section B Natural, Exact, and Applied Sciences. 2021;75(5):343-349. doi: 10.2478/prolas-2021-0050.
19. Qu L, Matz AJ, Karlinsey K, Cao Z, Vella AT, Zhou B. Macrophages at the Crossroad of Meta-Inflammation and Inflammaging. Genes (Basel). 2022 Nov 9;13(11):2074. doi: 10.3390/genes13112074.
20. Ritzel RM, Patel AR, Grenier JM, et al. Functional differences between microglia and monocytes after ischemic stroke. Journal of Neuroinflammation. 2015;12(1). doi: 10.1186/s12974-015-0329-1.
21. Rudyk MP, Pozur VV, Voieikova DO, et al. Sex-based differences in phagocyte metabolic profile in rats with monosodium glutamate-induced obesity. Scientific Reports. 2018;8(1). doi: 10.1038/s41598-018-23664-0.
22. Salminen A. Activation of immunosuppressive network in the aging process. Ageing Research Reviews. 2020;57:100998. doi: 10.1016/j.arr.2019.100998.
23. Sendama W. The effect of ageing on the resolution of inflammation. Ageing Research Reviews. 2020;57:101000. doi: 10.1016/j.arr.2019.101000.
24. Soehnlein O, Lindbom L. Phagocyte partnership during the onset and resolution of inflammation. Nature Reviews Immunology. 2010;10(6):427-439. doi: 10.1038/nri2779.
25. Sokolenko VL. Impact of emotional stress on the immune system indices among residents of radiation contaminated areas. Fiziolohichnyĭ zhurnal. 2016;62(4):53-59. doi: 10.15407/fz62.04.053.
26. Swift T. 'Sham surgery' control groups: Etics and context. Research Ethics. 2011;7(4):148-155. doi: 10.1177/174701611100700405.
27. Torrance HD, Longbottom ER, Vivian ME, et al. Post-operative immune suppression is mediated via reversible, interleukin-10 dependent pathways in circulating monocytes following major abdominal surgery. PLOS ONE. 2018;13(9). doi: 10.1371/journal.pone.0203795.
28. Tucker SL, Sarr D, Rada B. Granulocytic myeloid-derived suppressor cells in cystic fibrosis. Frontiers in Immunology. 2021;12. doi: 10.3389/fimmu.2021.745326H.
29. Walker KA, Basisty N, Wilson DM 3rd, Ferrucci L. Connecting aging biology and inflammation in the omics era. J Clin Invest. 2022 Jul 15;132(14):e158448. doi: 10.1172/JCI158448.
30. Wang Q, Zhu D. The prognostic value of systemic immune-inflammation index (SII) in patients after radical operation for carcinoma of stomach in gastric cancer. Journal of Gastrointestinal Oncology. 2019;10(5):965-978. doi: 10.21037/jgo.2019.05.03.
31. Yousefzadeh MJ, Flores RR, Zhu Y, et al. An aged immune system drives senescence and ageing of solid organs. Nature. 2021;594(7861):100-105. doi: 10.1038/s41586-021-03547-7.

Прозапальні ефекти плацебо-нейрохірургії у щурів: вікові особливості

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Хвороба Альцгеймера (ХА) і хвороба Паркінсона (ХП) — найпоширеніші нейродегенеративні захворювання. Вони вражають мільйони людей у світі та спричиняють значну інвалідизацію і смертність. Останнім етапом для завершення доклінічних досліджень і найадекватнішим підходом до глибокого розуміння патофізіології цих захворювань є використання тваринних моделей. Моделювання ідіопатичних ХА та ХП потребує ін'єкції речовин, які спричиняють захворювання, з використанням стереотаксичної хірургії. Групу плацебо-хірургії, що є невід'ємним компонентом дизайну цих експериментів, використовують як контроль неспецифічних побічних ефектів, спричинених хірургічними маніпуляціями. Запалення як найчастіший неспецифічний післяопераційний феномен може по-різному виявлятися у тварин різного віку, яких використовують в експериментах.

Мета — порівняти довгострокові прозапальні ефекти плацебо-хірургічного втручання, яке зазвичай використовують для моделювання ХА та ХП, у щурів різного віку.

Матеріали та методи. У дослідженні використано дорослих самців щурів лінії Wistar віком 4 та 14 міс. Операція плацебо полягала у стереотаксичній унілатеральній внутрішньомозковій інфузії буферного розчину. Перед операцією тварин анестезували внутрішньочеревно кетаміном/ксилазином. Як контроль використовували інтактних тварин такого самого віку. Оцінку прозапальних ефектів плацебо-хірургії проводили за допомогою біомаркерів місцевого та системного запалення: метаболічної поляризації фагоцитів (мікроглії, периферичної крові), за рівнем С-реактивного білка у плазмі крові, а також індексів системного запалення, розрахованих за результатами дослідження гемограми.

Результати. У молодих прооперованих тварин у віддалені терміни після плацебо-нейрохірургічної маніпуляції спостерігали виразні прозапальні функціональні зміни мікроглії та відсутність ознак системного запалення (протизапальний метаболічний зсув циркулюючих фагоцитів порівняно з групою вікового інтактного контролю). У старших інтактних тварин були наявні гематологічні та імунологічні ознаки системного запалення низького ступеня (мета-запалення). У прооперованих старших щурів зареєстрували залишкове нейрозапалення у поєднанні з виразними системними запальними реакціями (лейкоцитоз, суттєво підвищені показники індексу системної запальної відповіді (SIRI) та індексу системного імунного запалення (SII), прозапальний метаболічний зсув фагоцитів периферичної крові) порівняно з інтактним контролем.

Висновки. Наслідки проведення плацебо-нейрохірургічних маніпуляцій у щурів залежать від віку. Мета-запалення, характерне для старших щурів, посилюється неспецифічним післяопераційним запаленням, що призводить до виразного стійкого персистентного системного запального процесу.

Ключові слова: хірургія плацебо, фагоцити, нейрозапалення, системне запалення, хронічне запалення.

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