**TITLE IN FONT 14 (TIMES NEW ROMAN) AND IN BOLD CAPITAL LETTERS**

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**ABSTRACT**

These Instructions should serve as a guide for preparing camera-ready papers for the 16th International Conference on Fundamental and Applied Aspects of Physical Chemistry, PHYSICAL CHEMISTRY 2022. The recommended word processor is MS Word. The abstract should contain not more than 1,000 characters (including spaces) and should not include references. The use of special characters and formulas in the abstract should be avoided.

**INTRODUCTION**

The authors are kindly asked to respect the all proposition of the paper. ***Otherwise, the paper will be immediately returned for technical revision before sending to reviewer***. The proposition includes strict four-page limit for the regular papers and 8 pages limit for the invited papers (including abstract, introduction, results and discussion, and conclusion) in A4 format singly spaced. ***Margins of 3 cm on the top and bottom should be left and 2 cm on both right and left of the text.*** The paragraphs should be indented 5 mm, except the first one in each heading. The title should be centered at the first page of the manuscript and written in font 14 (Times New Roman) and in bold capital letters. The rest of the text should be in 12- point fonts (Times New Roman) justified. The author’s names should be given as initials followed by surname: P. Petrović, I. N. Bubanja, C. B. Delgado, J. P. Hsu. Name of the corresponding author should be underlined and his/her e-mail address should be given with other affiliation details. The author’s address (with indication of the corresponding University, where it is appropriate) should be given in italics. The title and names of authors should be followed by the abstract of the paper. The section titles of the paper should be typed in 12-point bold capital letters. References to the literature within text should be placed in square brackets: [1], [2], …, and cited at the end of the text. (***references should be given as in the example at the end of the text***)

Illustration (tables, figures, photographs, etc.) must be inserted into the text as a picture with at least 300 dpi and no larger than 12.5 cm width and 18 cm height including the caption (please allow 12 pts for each line of caption text). Tables must stay within text margins, too. Table caption and number should be given above each table, centered. Figure captions should be given at the bottom of each figure. The style of table and figure captions should be identical. Figure lettering should be done in a character not smaller than 12 pt. in final version of figure.

Submission of paper to our Conference assumes that all coauthors are informed and that these results are not submitted or published in any other Conference or scientific journal in the same form. It is full responsibility of the corresponding author.

**METHODS**

The papers should be submitted by e-mail (paper16@socphyschemserb.org) before May 31, 2022. The papers received after that deadline cannot be guaranteed the publication in the Conference Proceedings. The authors are limited to no more than three regular contributions and one invited lecture. Invited lectures will be printed in full-length (eight pages maximum).

Participants from Serbia are kindly recommended to include one line of the Acknowledgement including the Project numbers.

File title should be based on the surname of the first author, and if you submit more than one paper with the same first author, than add some ordinal number, next, add preferred type of presentation (poster or oral) and finally a letter identifying one preferred section (eg. Petrovic\_3\_oral\_J.doc).

***Rest of the Template is sample text with some examples of figure and table given to fill-in four pages.***

Samples were loaded onto 10 % SDS polyacrylamide gel, and electrophoresis was carried out using a mini protein system (Bio-Rad Laboratories, Inc., USA). The proteins were electrophoretically transferred onto PVDF membranes (Imobilion-P membrane, Millipore, USA), blocked for 1h with 5 % BSA in TBST (50 mM Tris-HCl, pH 7.4, 150 mM NaCl, 0.1 % Tween 20), and incubated with primary and secondary antibodies (**Table 1**.).

**Table 1.** List of primary and secondary antibodies used for Western blot analysis

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| **Antigen** | **Manufacturer and Catalog No** | **Species** |
| PSD 95 | MAB1598, Merck Millipore Corporation, USA | mouse monoclonal |
| NR2B | ab93610, Abcam, UK | mouse monoclonal |
| vGlut1 | ab134283, Abcam, UK | mouse monoclonal |
| β-actin | sc-1615, Santa Cruz Biotechnology Inc., USA | goat polyclonal |
| mouse IgG | sc-2318, Santa Cruz Biotechnology Inc., USA | donkey |
| goat IgG | sc-2033, Santa Cruz BiotechnologyInc., USA | donkey |

Following incubation with antibodies, an enhanced chemiluminescence (ECL) system (Immobilon Western Chemiluminescent HRP Substrate, Millipore, USA) was poured on the membranes. Immunoreactive bands were detected on X-ray films in the dark chamber. β-actin as quantified and used as a loading control. The signal intensity was evaluated using the Image J software package, and results were expressed relative to the control value (set as 100%).

All data were expressed as means ± S.E.M. Results were analyzed using One-way ANOVA followed by a posthoc Tukey's test. p < 0.05 was defined as statistically significant.

#### Results and Discussion

In the HIP, a significant effect of d-gal treatment was detected only on vGlut1 protein level (F = 3.835, p < 0.05), and *post-hoc* analysis revealed a significantly lower vGlut1 level in 500 mg/kg compared to the 200 mg/kg (p < 0.05). In the PFC, One-way ANOVA revealed a significant effect of d-gal treatment on vGlut1 (F = 5.823, p < 0.01) and NR2B protein level (F = 18.29, p < 0.001), while *post-hoc* test showed a statistically significant decrease of vGlut1 (p < 0.05) and NR2B (p < 0.01, p < 0.001, respectively) in both treated groups compared to Con. D-gal treatment did not affect the PSD-95 protein level in the PFC and HIP (**Figure 1.**).

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| Chart, bar chart  Description automatically generated |
| Figure 1*.* Effect of D-galactose treatment applied in two doses (200 mg/kg and 500 mg/kg) on components of glutamatergic signaling in the hippocampus (HIP) and prefrontal cortex (PFC). Data are presented as the mean ± SEM, and the values of the control group were set as 100 %. \* symbols indicate significant difference control *vs.* d-gal groups (\*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001) while #represents the difference between two d-gal groups (#p < 0.05). |

Detected region-specific changes of vGlut1 and NR2B proteins suggest compromised glutamatergic synapses homeostasis in the PFC but not in the HIP. Herein reported a decreased level of vGlut1 in the PFC indicates jeopardized glutamate import into synaptic vesicles, resulting in diminished glutamate content in the synaptic cleft. Our findings are similar to those published by Dawson and co-workers who reported a functionally intact glutamate pool in the frontal cortex but age-dependent changes in presynaptic modulation of glutamate release [1]. Furthermore, the literature emphasizes that the most consistent age-related change in the glutamatergic system is the loss of glutamate receptors [7]. The observed decrease of NR2B subunit in the PFC indicate region-specific NMDAR modulation. Indeed, studies suggest that the NMDA receptors in the cerebral cortex are more vulnerable to aging changes than those in the hippocampus [3].

Interestingly, the protein level of PSD-95 stayed unaltered regardless of group and brain region. Considering that PSD-95 may be crucial for the synapse interaction with downstream signaling, its preserved level may prevent further propagation of the potential detrimental signal. Still, the exact effect will be determined in future research.

**CONCLUSION**

To our knowledge, we are the first to report changes in the expression of synapse-located proteins in rats following chronic oral d-gal treatment. Our results represent valuable data guiding us into further research on region-specific modulation of NMDA receptors in the aging brain.

***Acknowledgment***

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