

# Deciphering ciprofloxacin-induced neurotoxicity: behavioral and molecular profiling of ciprofloxacin treatment in rats

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## Introduction

The neurotoxicity is a major cause of discontinuation of the drug development programs, but commonly is poorly predictable in preclinical phase.

Ciprofloxacin is a fluoroquinolone antibiotic commonly used to treat various bacterial infections, with a potential to induce adverse mood effects in patients.

**Aims:** We aimed to identify **behavioral changes** and corresponding **neurotransmitter pattern** after prolonged ciprofloxacin administration in rats; screened for untoward effects of ciprofloxacin on **locomotor activity**, **despair**, **anhedonia**, **object recognition memory**, and **anxiety**, as behavioral domains affected in various psychiatric diseases.

## Methodology

Three-month old male Sprague-Dawley rats were orally gavaged with ciprofloxacin (20 or 100 mg/kg) or solvent (0.5% methyl cellulose solution) each day for 4 weeks (n=80).

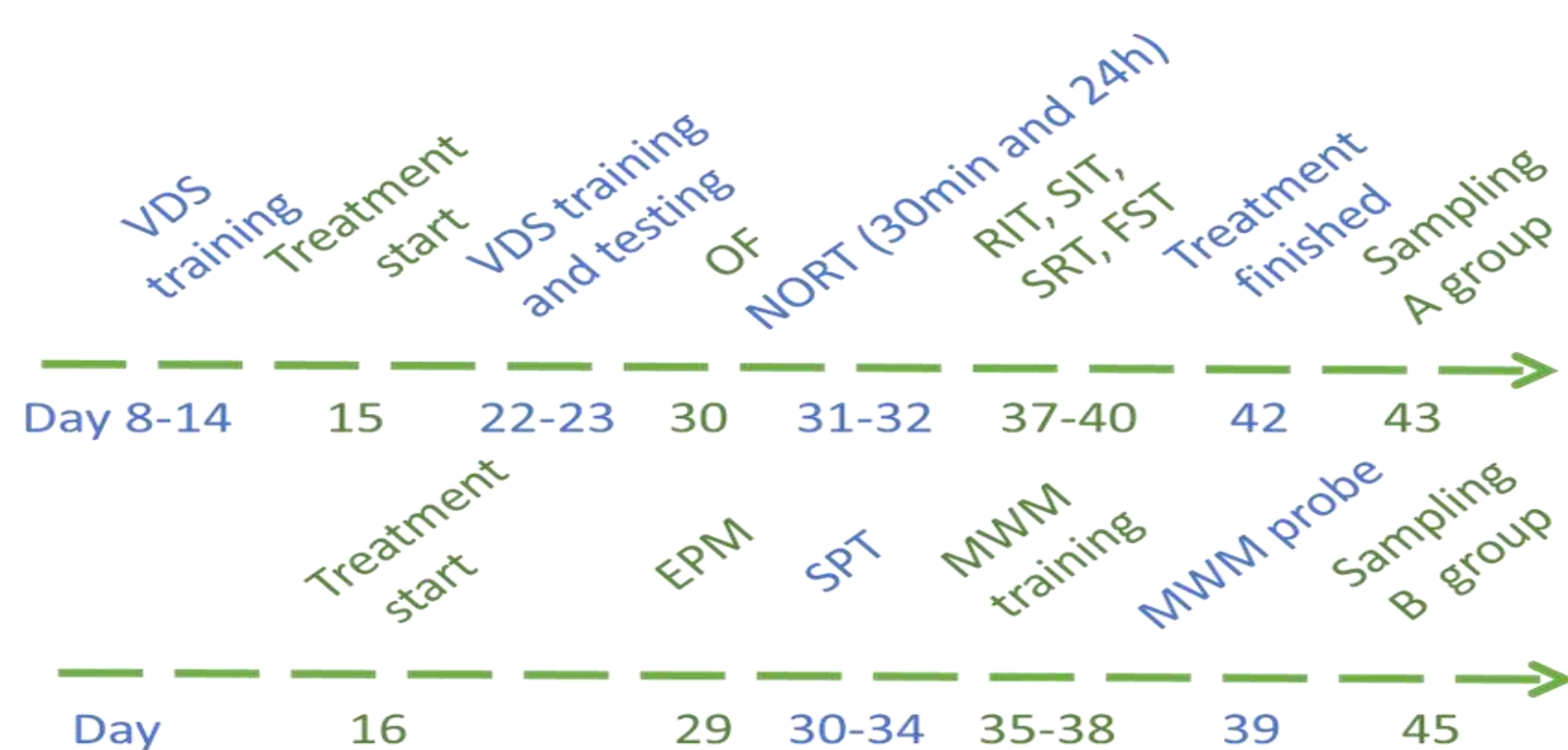
One group of animals (n=40) passed the **open field (OF)**, **novel object recognition test (NORT)**, and **forced swimming test (FST)**.



Another group (n=40) underwent **elevated plus maze (EPM)** and **sucrose preference test (SPT)**.

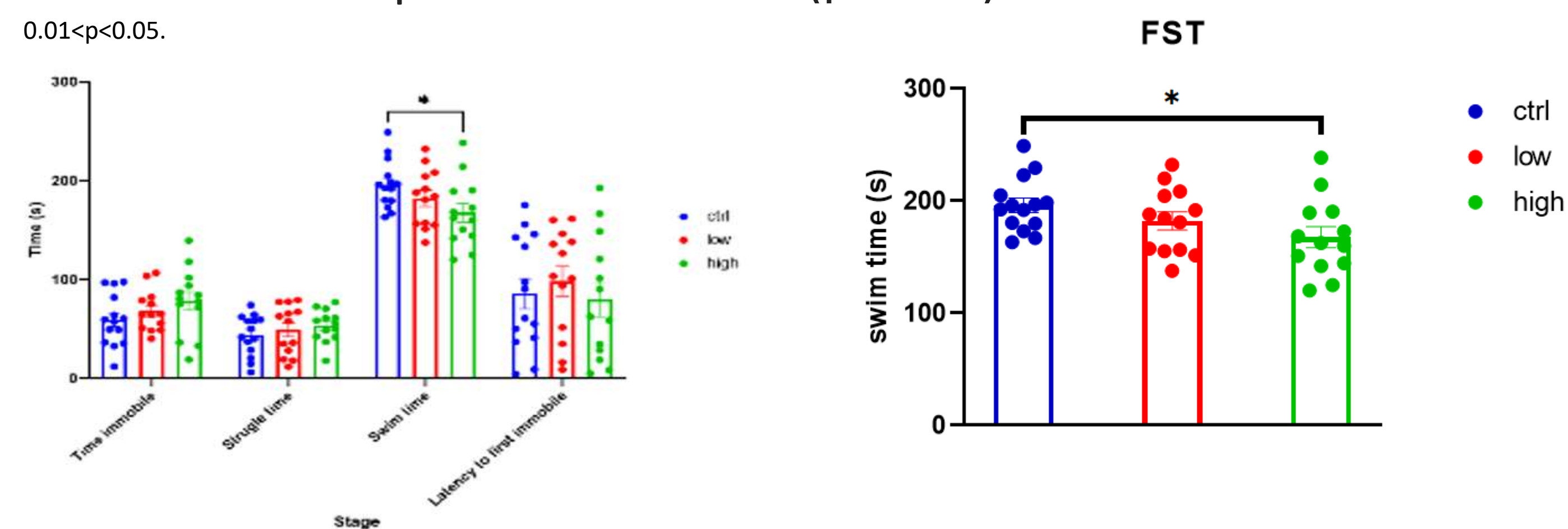


After the completion of behavioral battery, the **prefrontal cortex** and **cerebrospinal fluid (CSF)** were collected. The **neurotransmitters** and **metabolites of the kynurenine pathway** were determined in the prefrontal cortex (PFC) through HPLC-MS/MS. Additionally, levels of **interleukin-2 (IL-2)** in CSF were quantified with Luminex. Behavioral and molecular parameters were analyzed by one-way ANOVA followed by Dunnett post hoc test in GraphPad Prism 9.

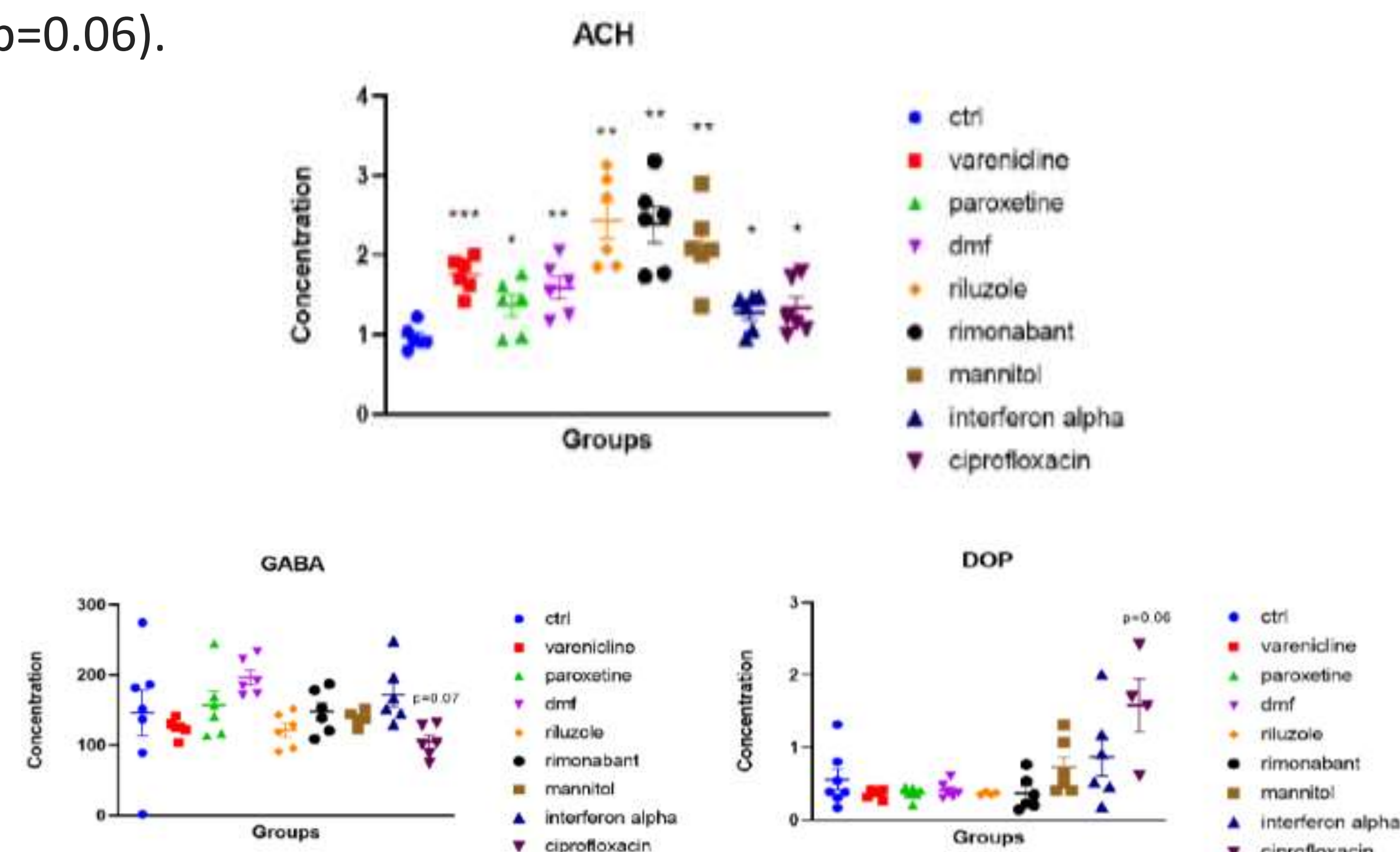


## Results

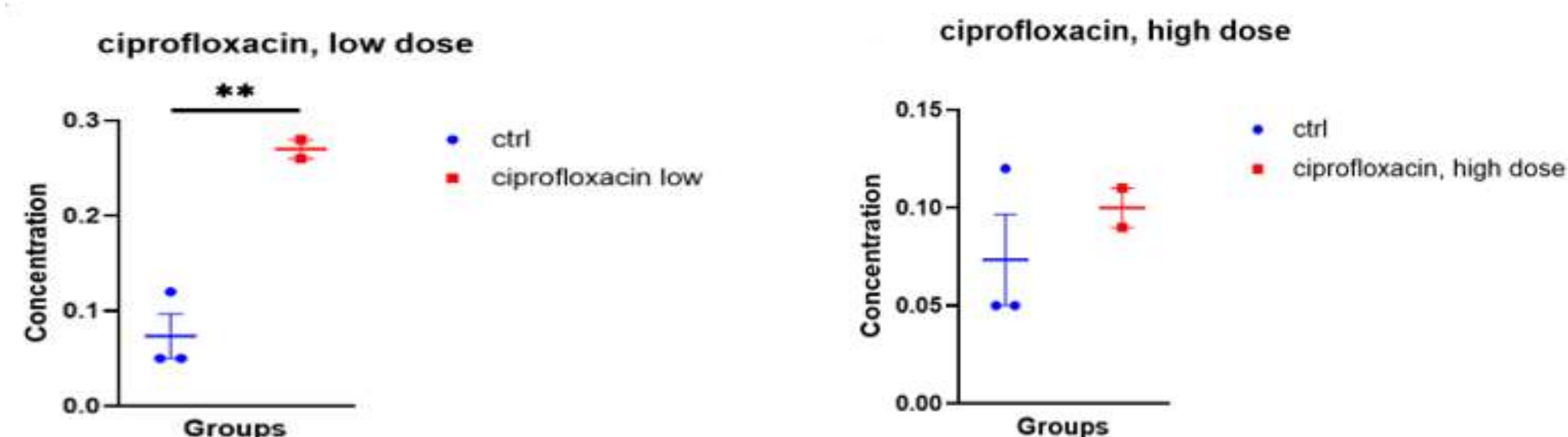
In FST, the treatment with high dose of ciprofloxacin decreased the swim time compared to control ( $p < 0.05$ ). The statistical significances are shown on graphs as \* for  $0.01 < p < 0.05$ .



The acetylcholine levels in PFC were increased after ciprofloxacin treatment ( $p < 0.05$ ) in comparison with controls. In line with that, high dose of ciprofloxacin treatment showed the tendency to decrease and increase levels of GABA and dopamine, respectively, but without reaching the statistical significance ( $p = 0.07$  and  $p = 0.06$ ).



The IL-2 concentration in CSF was increased after prolonged administration of low dose of ciprofloxacin treatment compared to the control levels ( $p < 0.05$ ). The statistical significances are shown on graphs as \* for  $0.01 < p < 0.05$ , and \*\* for  $0.001 < p < 0.01$ .



## Conclusion

The despair behavior after treatment with high dose of ciprofloxacin was accompanied by increased levels of acetylcholine in PFC. Furthermore, the high dose of ciprofloxacin treatment showed tendency to decrease GABA levels, and increase dopamine levels in PFC, which could be connected to psychiatric adverse effects. Nonetheless, further studies are essential to confirm these neurotransmitter changes. On the other hand, the low dose of ciprofloxacin treatment elicited the increase of IL-2, which could be a marker of neuroinflammation-related neurotoxicity. In the future, efforts should be made to examine the role of IL-2 in the interaction of the immune system and the central nervous system, as its potential significance as a biomarker. In FST, the treatment with high dose of ciprofloxacin decreased the swim time compared to control, which could be related to induction of despair-like behavior.

This research was supported by the Science Fund of the Republic of Serbia, Grant No.7749108, Neuroimmune aspects of mood, anxiety and cognitive effects of leads/drug candidates acting at GABAA and/or  $\alpha 2$  receptors: In vitro/in vivo delineation by nano- and hiPSC-based platforms - NanoCellEMoCog.

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