

Supplementary Material

Ouabain counteracts retinal ganglion cell death through modulation of BDNF and IL-1 signaling pathways

Amanda Candida da Rocha Oliveira^{1,2}, Camila Saggioro de Figueiredo^{1,2}, Ícaro Raony³, Juliana Salles Von-Held-Ventura¹, Marcelo Gomes Granja^{1,4}, Thalita Mázala-de-Oliveira^{1,2}, Vinicius Henrique Pedrosa Soares¹, Aline Araujo dos Santos^{2,5}, Elizabeth Giestal-de-Araujo^{1,2*}

¹ Department of Neurobiology and Program of Neurosciences, Institute of Biology, Federal Fluminense University, Niteroi, Brazil;

² National Institute of Science and Technology on Neuroimmunomodulation - INCT-NIM, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil;

³ Institute of Medical Biochemistry Leopoldo de Meis, Federal University of Rio de Janeiro, Rio de Janeiro, Brazil;

⁴ Research, Innovation, and Surveillance Center for Covid-19 and Health Emergencies, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil;

⁵ Department of Physiology and Pharmacology and Program of Neurosciences, Biomedical Institute, Federal Fluminense University, Niteroi, Brazil;

* Correspondence: egiestal@id.uff.br

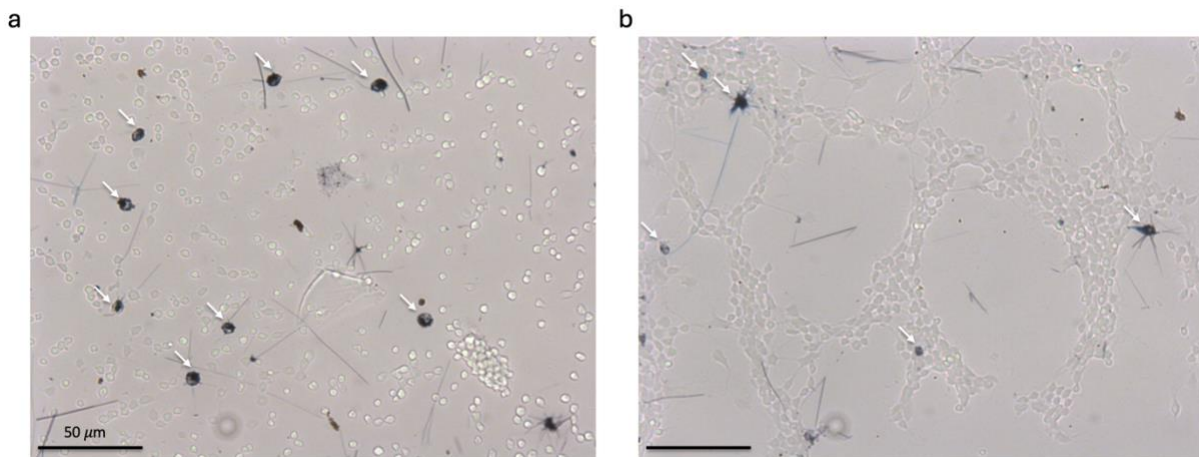


Figure S1. Retrograde labelling of RGCs. Representative images of RGCs labelled with HRP in untreated retinal cell cultures after 4 h (a) and 48 h (b). Scale bars: 50 µm; arrows show HRP-labeled RGCs.

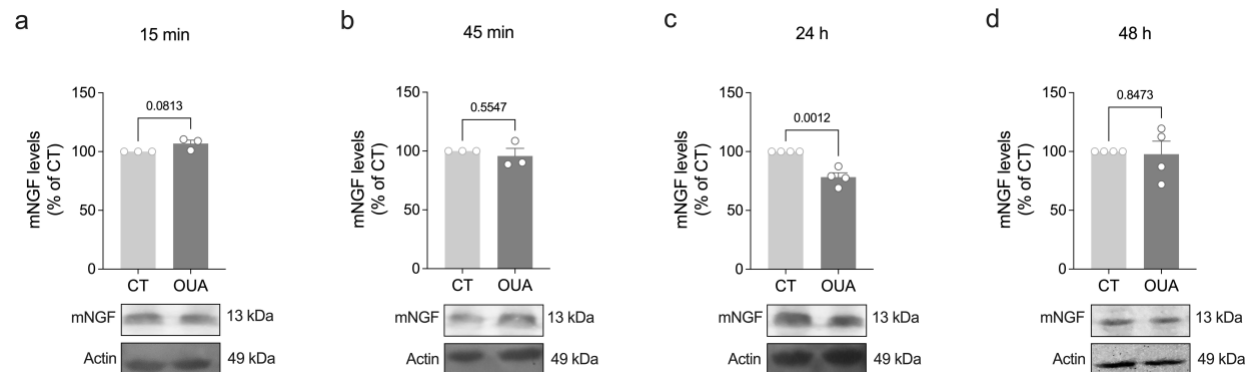


Figure S2. Levels of mature NGF in retinal cells after exposure to ouabain. (a-d) Primary retinal cultures were treated with ouabain (OUA; 3 nM) for the indicated periods to assess levels of mNGF by Western blot. Plots represent mean (\pm SEM) protein optical density (normalized to actin) expressed as a percentage of control (CT) group (set on 100%), with N = 3-4 experiments using independent retinal cultures. Data were analyzed by two-tailed Student t-test.

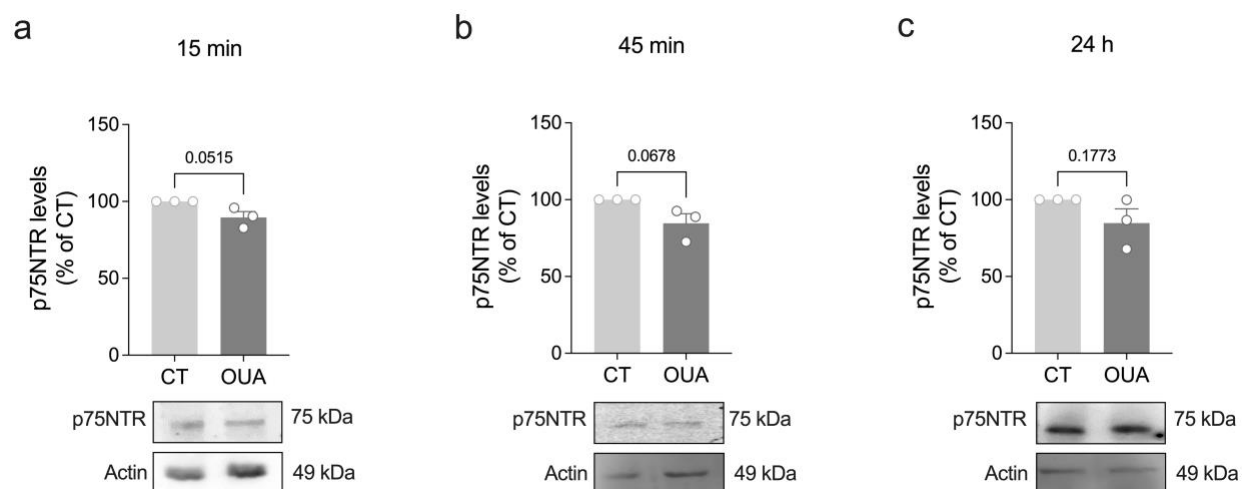


Figure S3. Levels of p75 neurotrophin receptor in retinal cells after exposure to ouabain. (a-c) Primary retinal cultures were treated with ouabain (OUA; 3 nM) for the indicated periods to assess levels of p75NTR by Western blot. Plots represent mean (\pm SEM) protein optical density (normalized to actin) expressed as a percentage of control (CT) group (set on 100%), with N = 3 experiments using independent retinal cultures. Data were analyzed by two-tailed Student t-test.

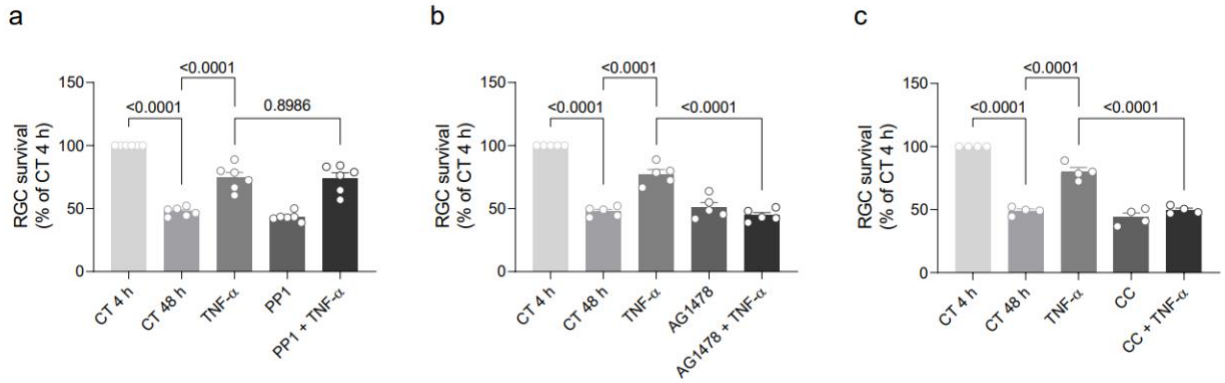


Figure S4. EGFR and PKC signaling pathways mediate TNF- α -induced RGC survival. Primary retinal cultures were treated with ouabain (OUA; 3nM) and/or the following drugs for 48 hours: (a) PP1 (Src inhibitor; 1 μ M), (b) AG-1478 (EGFR inhibitor; 2.5 μ M), or (c) chelerythrine chloride (CC; pan-PKC inhibitor; 1.25 μ M). Plots represent mean (\pm SEM) RGC survival and are expressed as a percentage of control (CT) group at 4 hours in culture (set on 100%), with N = 4-6 experiments using independent retinal cultures. Data were analyzed by one-way ANOVA followed by Holm-Sidak's test.