ERβ and GPER in the anterior cingulate cortex contribute to pain-related aversion

Kaikai Zang¹, Xiao Xiao¹, Liqiang Chen¹, Yulong Tang¹, Hong Cao¹, Ling Zhang² and Yuqiu Zhang¹* 

¹Institute of Neurobiology, Institutes of Brain Science and State Key Laboratory of Medical Neurobiology, Collaborative Innovation Center for Brain Science, Fudan University, Shanghai 200032, China.

²Department of Anatomy and Neurobiology, Tongji University School of Medicine, Shanghai 200092, China

*Corresponding author

E-mail: yuqiu.zhang@fudan.edu.cn

Abstract: Objective Estrogen has been proven to participate in pain-related negative emotion in the rostral anterior cingulate cortex (rACC). It is not clear which subtypes of estrogen receptors (ERs) are involved in this function. In present study, we aimed to explore the contributions of diverse ERs to pain-related negative emotion in rACC.

Methods We used formalin-induced conditioned place aversion (F-CPA) to reflect the pain-related negative emotion. Histological, molecular biological, electrophysiological and optogenetic methods were also used in this study. Results (1) All the three types of ERs (ERα, ERβ and GPER) were abundantly distributed in the rACC, especially in excitatory pyramidal neurons. (2) Intra-rACC injection of ERβ antagonist PHTPP (4-[2-Phenyl-5,7-bis(trifluoromethyl)pyrazolo[1,5-a]pyrimidin-3-yl]phenol, 0.5 ng /hemisphere) and GPER agonist G15 (3aS*,4R*,9bR*)-4-((6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-3H-cyclopenta[c]quinoline, 0.9 ug/hemisphere), rather than ERα antagonist MPP (1,3-Bis(4-hydroxyphenyl)-4-methyl-5-[4-(2-piperidinylethoxy)phenol]-1H-pyrazole dihydrochloride, 0.5ng /hemisphere) significantly blocked the F-CPA. (3) ERβ agonist DPN (2,3-bis(4-Hydroxyphenyl)-propionitrile, 0.01 ng /hemisphere) and GPER agonist G1
(±)-1-[(3aR*,4S*,9bS*)-4-(6-Bromo-1,3-benzodioxol-5-yl)-3a,4,5,9b-tetrahydro-3H-cyclopenta[c]quinolin-8-yl]-ethanone, 0.1 ug/hemisphere) into the rACC directly induced conditioned place aversion in the absence of a formalin noxious stimulation. (4) ERβ agonist DPN and GPER agonist G1 but not ERα agonist PPT (4,4',4''-(4-Propyl-[1H]-pyrazole-1,3,5-triyl)trisphenol) induced a NMDA-long-term potentiation (LTP). (5) photo-inhibited excitatory pyramidal neurons prevented the F-CPA, whereas photo-activated excitatory pyramidal neurons elicit CPA.

**Conclusion** We propose that estrogen in the rACC via ERβ and GPER but not ERα mediated the pain-related aversion.

**Keywords:** Excitatory pyramidal neurons, Estrogen receptor alpha (ERα), Estrogen receptor beta (ERβ), G protein-coupled estrogen receptor 1 (GPER), Pain-related aversion, Rostral anterior cingulate cortex (rACC)