Name: Zheng-Xiong Xi Email: zxi@intra.nida.nih.gov

Neuronal *versus* glial CB2 receptor: Findings from a new strain of CB2-KO-EGFP reporter mice

Zheng-Xiong Xi[1], Haiying Zhang[1,2], Omar Soler-Cedeno[1,3], Guo-Hua Bi[1], Pinaki Bhattacharjee[4], Malliga R. Iyer[4], Qing-Rong Liu[5]

[1] NIDA, IRP, Baltimore, MD; [2] NIMH, IRP, Bethesda, MD; [3] NIGMS, IRP, Bethesda, MD; [4] NIAAA, IRP, Bethesda, MD; [5] NIA, IRP, Baltimore, MD

The expression of the CB2 receptor (CB2R) in neurons and glial cells remains a subject of debate. While brain CB2Rs are conventionally thought to be primarily expressed in microglia, direct evidence is limited, and microglial CB2R mechanisms fall short in explaining many CB2R-mediated effects. Despite classical immunostaining and advanced RNAscope assays consistently detecting CB2R expression in neurons, contradictory results arise from CB2-GFP reporter mice studies.

In two existing CB2-GFP reporter mouse strains, the GFP gene is inserted either outside the endogenous CB2 gene or into the 3'-end of the untranslated region of the CB2R gene. These GFP gene knock-in strategies pose problems, as GFP expression may not be controlled by endogenous CB2R during protein translation. To address this issue, we developed a new CB2-KO-EGFP mouse line, replacing the endogenous CB2-coding sequence with the EGFP gene. This new mouse line exhibits clear EGFP-immunostaining in midbrain dopamine neurons, cortical and hippocampal glutamate neurons, and microglia (excluding astrocytes). CB2 mRNA is absent in brain and peripheral tissues, and immunohistochemistry with two commonly used CB2 antibodies (Alomone, Abcam) suggests their lack of CB2R specificity.

Interestingly, CB2R-KO-EGFP mice display higher basal levels of locomotion and body weight than their wild-type littermates. Furthermore, systemic administration of MRI-2594, a novel CB2R agonist, inhibits heroin self-administration in wild-type mice but not in CB2R-KO-GFP mice. In summary, the newly generated CB2-KO-EGFP mice may serve as a valuable tool for studying brain CB2R expression and function, offering potential applications as both a full CB2-KO mouse and a CB2-EGFP reporter mouse.