Modulation of calcium signalling in NMDARs and kainate receptors by knocking down of TSG101

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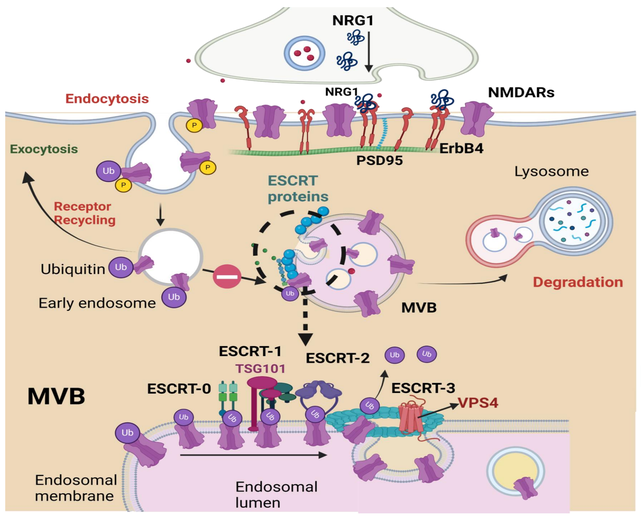
Hypothesis

Hypofunction of NMDARs in neurons is a convergent mechanism of major genetic and/or environmental risks, leading to schizophrenia.

Knocking down of Tsg101 or silencing of Vps4a will decrease the degradation of NMDARs and increase the recycling and signalling of the receptors



Objective

Evaluate the knocking-down effect of TSG101 on the surface expression of NMDARs and KARs in HEK293 cells

Evaluate the stimulation and the blocking action of the overexpressed NMDARs and KARs on its calcium signalling

Figure 1: ESCRT machinery is involved in endosomal maturation and degradation of the receptors

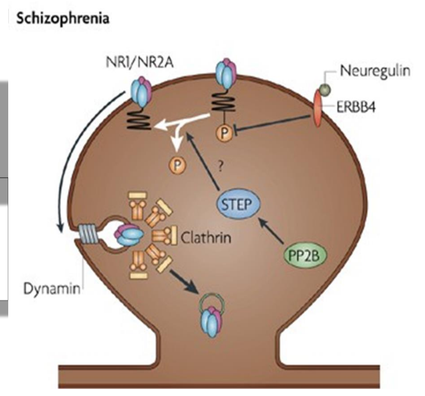


Figure 2: Degradation of NMDARs in Schizophrenia

Results

1 The knocking down of TSG101 increased the calcium signalling of NMDARs and Kainate receptors and alleviated the blocking actions

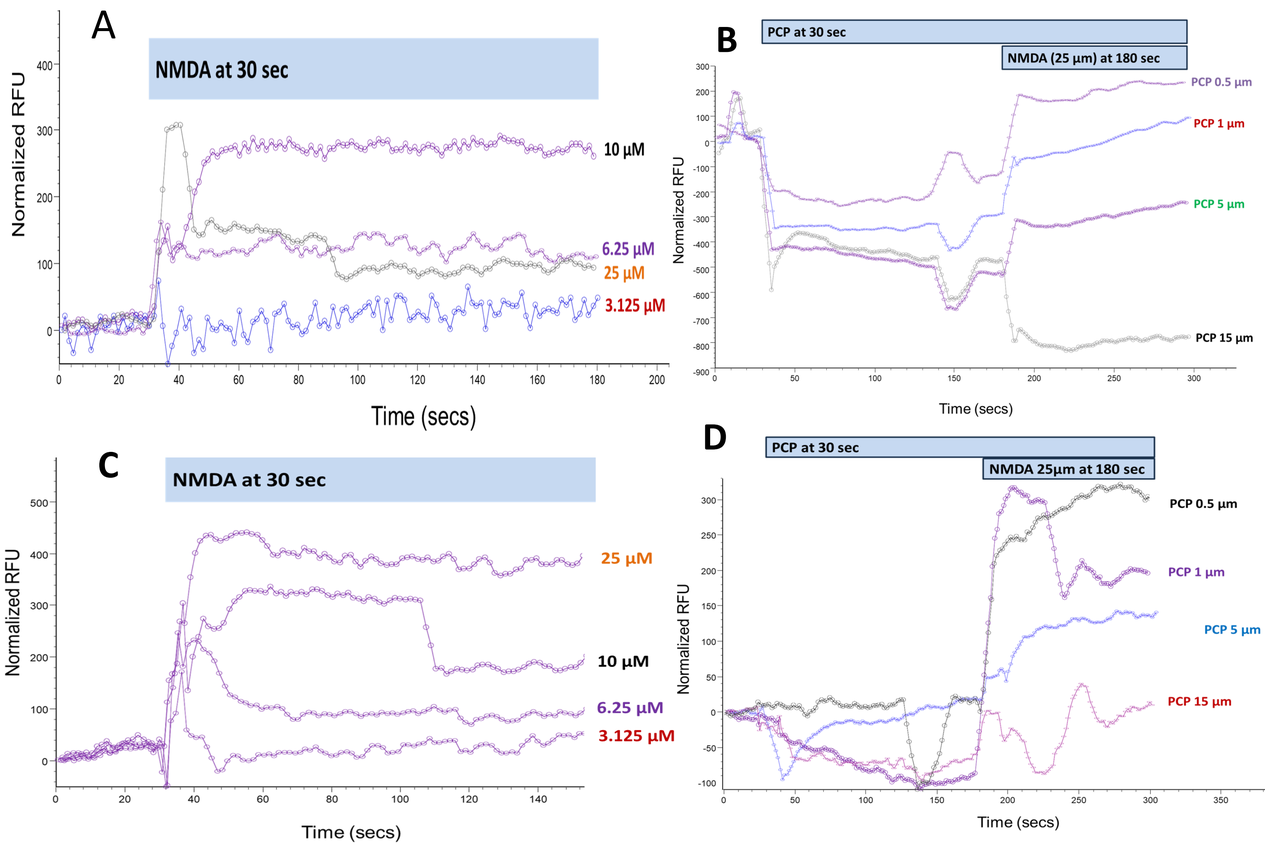


Figure 3: Cytosolic calcium measurements for NMDARs. (A) action of NMDA on NMDARs, (B) action of PCP/NMDA on NMDARs, © action of NMDA on NMDARs +shRNA tsg101, (D) action of PCP/NMDA on NMDARs + shRNA tsg101

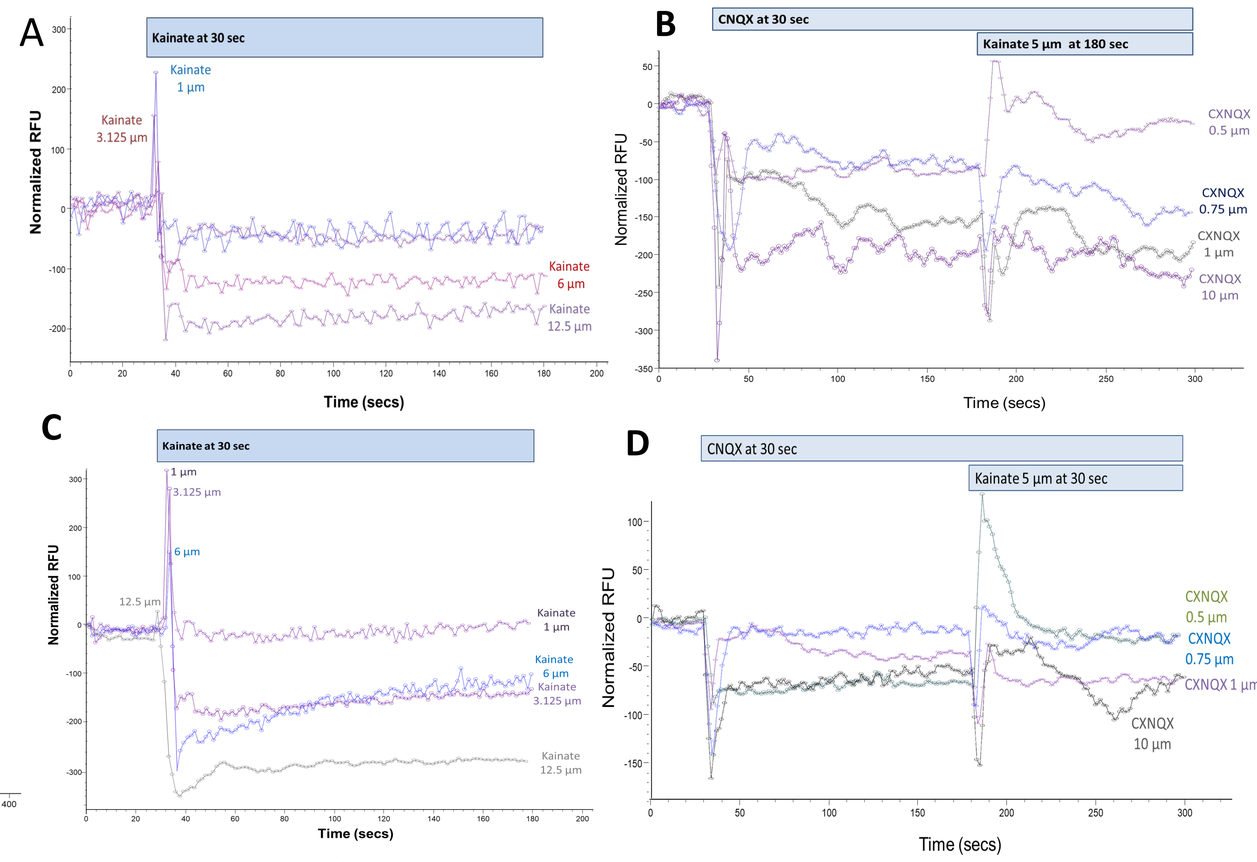


Figure 4: Cytosolic calcium measurements for KARs. (A) action of Kainate on KARs, (B) action of CBQX/kainate on KARs, (C) action of Kainate on KARs +shRNA tsg101, (D) action of CBQX/kainate on KARs + shRNA tsg101

2 The knocking down of TSG101 increased the surface expression of NMDARs subunits (NR1/2a)and KARs subunit (GLUR6)

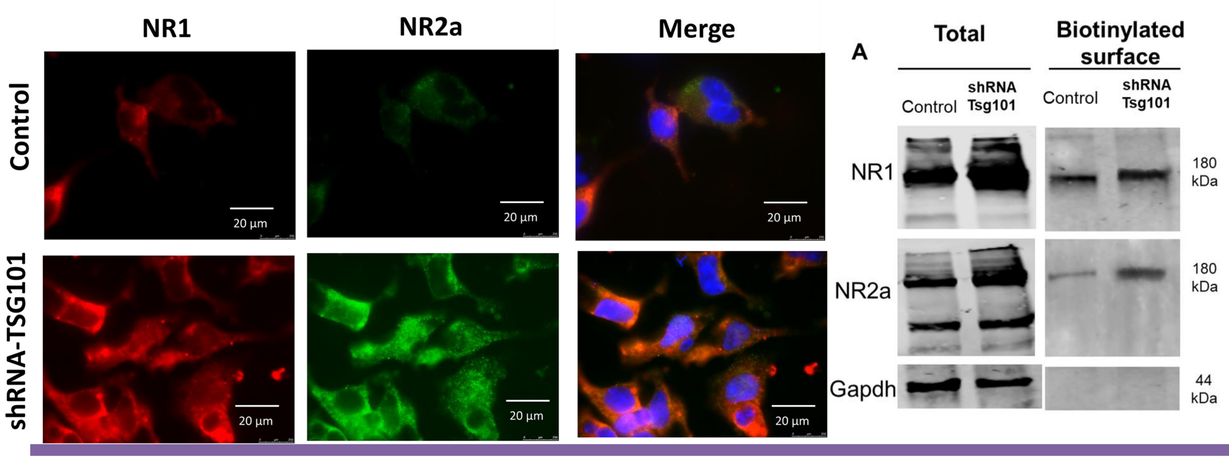


Figure 5: Knocking down of TSG101 increased the surface expression of NMDARs and KARs subunits. The receptors were treated with NMDA 20 µM, glycine 20 µM/kainite 5 um, and glutamate 100 µM for 30 min before fixation or lysis for WB.

Conclusion

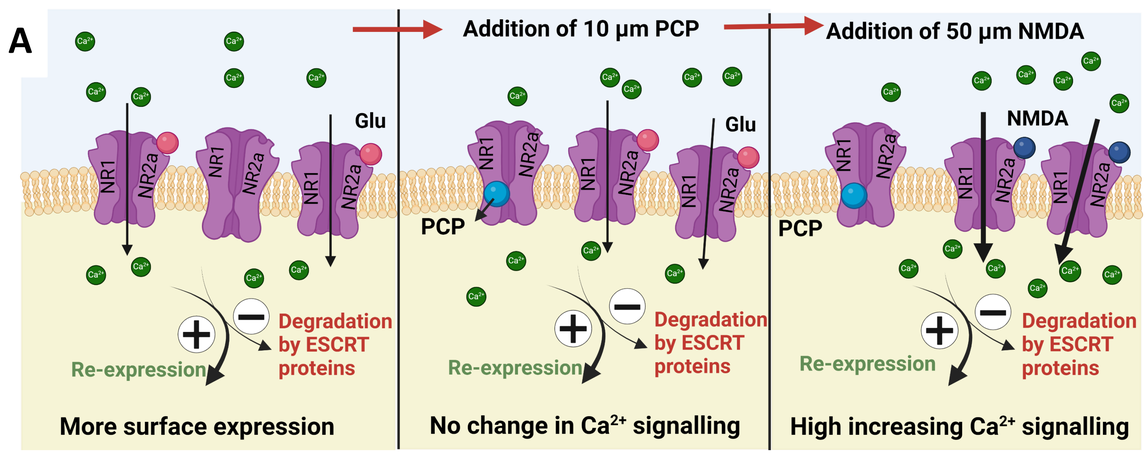


Figure 6: (A) Knocking down of TSG101 showed a marking rise in calcium signaling of glutamate receptors which alleviates the blocking action of PCP and CNQX.

(B) Knocking down of Tsg101 antagonizes the endosomal sorting complex required for transport and the receptors accumulate in the cytoplasm for more recycling and signaling.