Supplemental data

Fig 1 – Effects of glutamate on tau phosphorylation in cultured hippocampal slices at different times. Rat hippocampal brain slices were incubated with 100 μM glutamate for 0, 20 min, 40 min, or 60 min. Tau phosphorylation levels at Ser396, Ser404, and Thr231 were detected by Western blotting. Treatment for 20 min reaches the highest level of tau phosphorylation at Ser396 and Thr231, prolonged incubation for 60 min does not further increase tau phosphorylation.

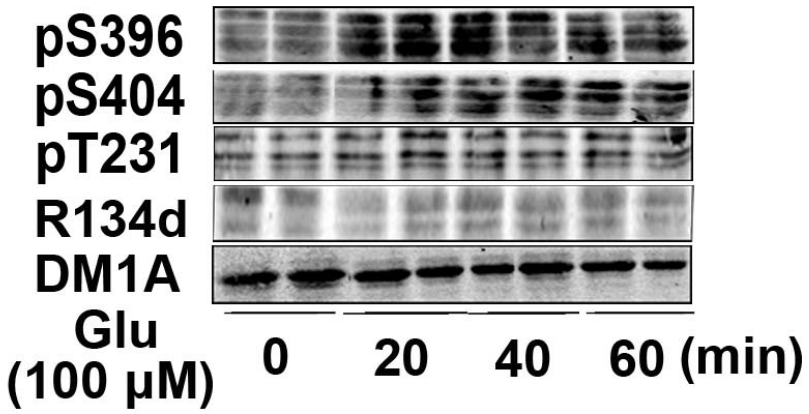

Fig 2 – Effects of glutamate at different concentrations on tau phosphorylation in cultured hippocampal primary neurons. Hippocampal primary neurons were incubated with 0, 10, 20, 50 μM glutamate for 20 min. Tau phosphorylation levels at Ser396, Ser404, and Thr231 were detected by Western blotting. No significant difference was detected between the concentrations 10 to 50 μM of glutamate.

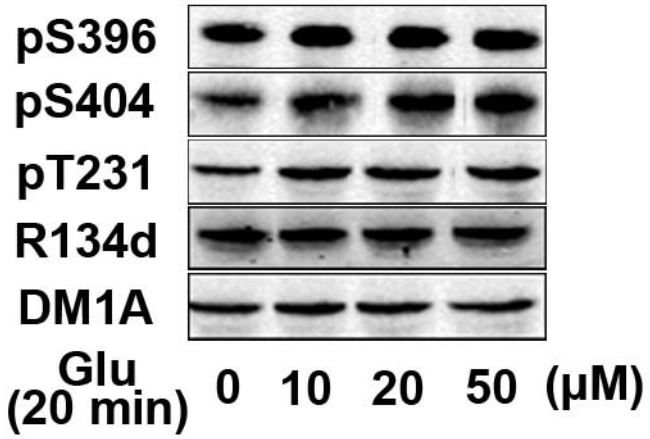

Fig 3 - Extracellular zinc causes tau hyperphosphorylation through voltage-gated calcium channels (VGCC). (A) Rat hippocampal brain slices were incubated with 300 μM $ZnSO_4$ for 60 min, with or without pretreatment with APV (NMDA receptor antagonist), CNQX (AMPA receptor antagonist), and nimodipine (VGCC blocker), tau phosphorylation levels at different sites were detected by Western blotting. (B) Quantitative analysis of blots in (A), * $p<0.05$, ** $p<0.01$ versus untreated control slices; # $p<0.05$, ## $p<0.01$ versus $ZnSO_4$ treated slices.

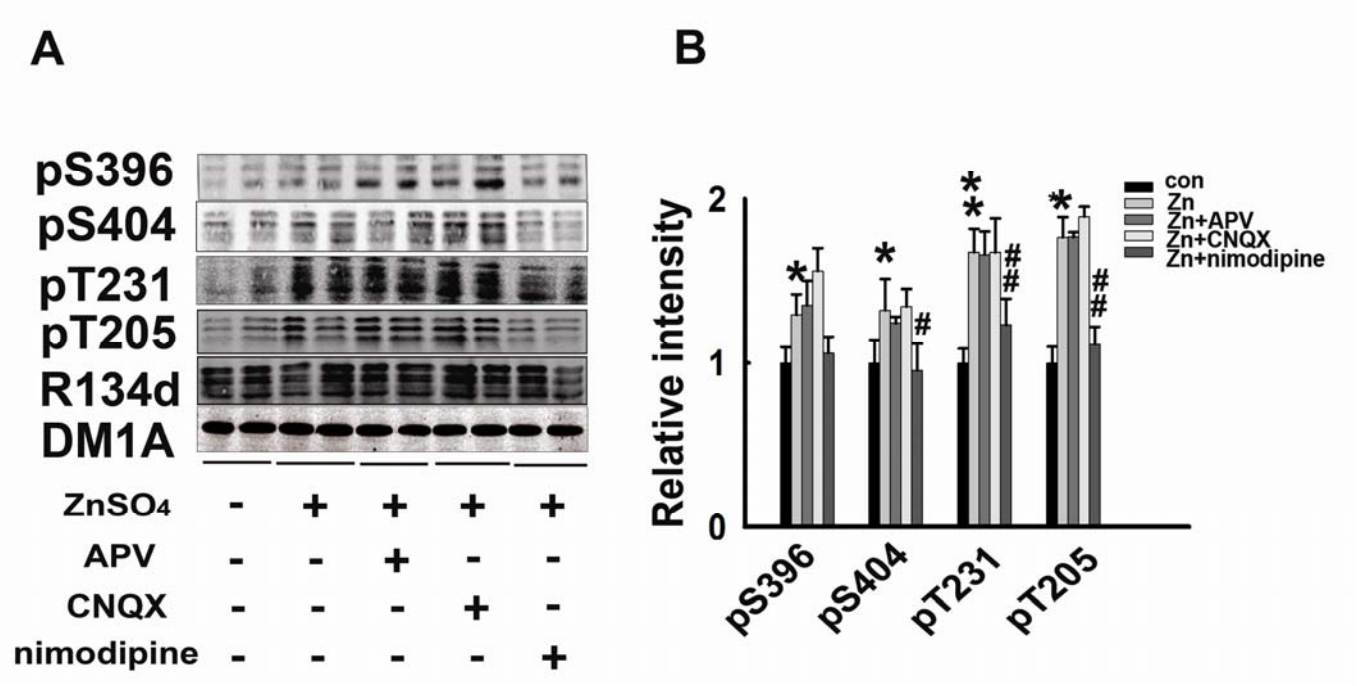

Fig 4 - Pre-incubation of kinase inhibitors does not prevent glutamate induced tau hyperphosphorylation. (A) Rat hippocampal brain slices were treated with 100 μM glutamate for 20 min, with or without pre-incubation of different kinase inhibitors: SB216763 (inhibitor of GSK-3), PD98059 (inhibitor of Erk1/2), SP600125 (inhibitor of JNK) or SB202190 (inhibitor of p38) for 30 min, tau phosphorylation levels at different sites were detected by Western blotting. (B) Quantitative analysis of blots in (A), * $p<0.05$, ** $p<0.01$ versus untreated control slices.