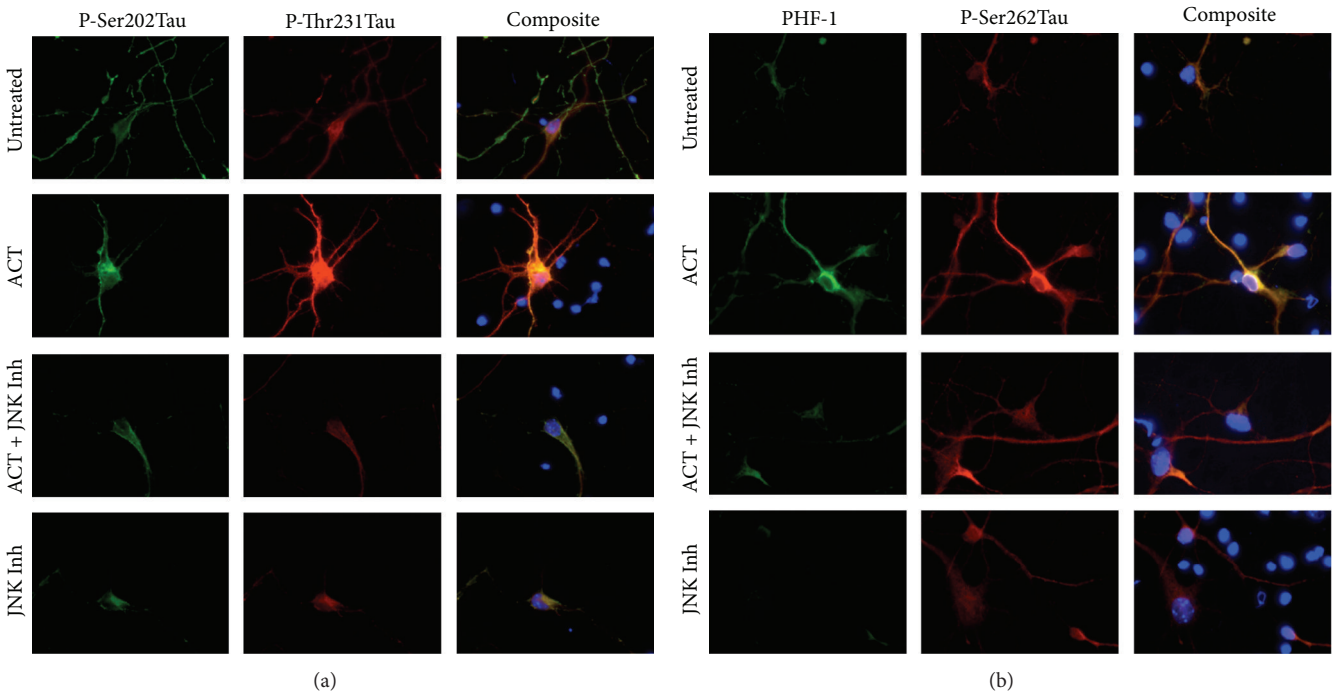

Figure 4: ACT-mediated tau phosphorylation at p-Ser202, p-Thr231, PHF-1, and p-Ser262 in cortical neurons is inhibited by JNK inhibitor: mouse cortical neurons were cultured in 8-chamber slides and preincubated with JNK Inhibitor (10 μM, 1 hour). The cells were then treated with or without 1 mg/mL ACT for 8 hours and probed with (a) p-Ser202 tau monoclonal and p-Thr231 tau polyclonal, (b) PHF-1 tau monoclonal and P-Ser262 tau polyclonal antibodies. Anti-mouse Alexa fluor 488 (green) and anti-rabbit Alexa Flour 594 (red) were used as secondary antibodies, and Hoechst was used to visualize the nuclei. Staining was visualized under a Zeiss fluorescent microscope and analyzed using AxioVision Rel. 4.8 software.

tau phosphorylation (Figure 3(b)). There was no significant change in P-JNK levels in the striatum or hippocampal region from ACT/mTau or ACT/mTau/hTau mice (Figures 3(a) and 3(c)) suggesting that the tau phosphorylation observed in these regions is brought about by kinases other than JNK. Our earlier studies in cultured neurons have shown that ACT-mediated tau hyperphosphorylation is associated with activation of GSK3$\alpha\beta$ and ERK [23]. Here we found that JNK kinases are activated in response to ACT which in turn induces tau hyperphosphorylation. Although initial *in vivo* analysis did not indicate any activation of GSK3$\alpha\beta$ or ERK, further analysis is necessary to confirm these results.

3.4. Effect of JNK Inhibitor on ACT-Induced Tau Phosphorylation in Primary Cortical Neurons. To investigate our hypothesis that ACT enhances tau hyperphosphorylation through a JNK-dependent mechanism, we decided to investigate the effect of JNK inhibitor on tau phosphorylation in primary neurons cultured *in vitro* and treated with ACT. Towards this, we pretreated cortical neurons with 10 μM of JNK inhibitor (SP600125) for 1 hour prior to addition of ACT and continued the incubation for 8 hours in the presence of ACT. The result showed an induction in phosphorylation of tau at different sites, which included PHF-1, p-Ser202, p-Thr231, and p-Ser262, upon ACT treatment (Figures 4(a) and 4(b)). Treatment with JNK inhibitor (SP600125) abolished the ACT-induced tau phosphorylation, indicating the regulatory role of JNK in the process.

4. Discussion

The involvement of ACT in AD is well documented, and its role in Aβ oligomerization has been established. In the study presented here we sought to elucidate the mechanisms of ACT-induced tau phosphorylation, focusing on the role of JNK kinase, in order to increase our understanding of how this relevant kinase influences tau hyperphosphorylation and development of pathology in AD. Our studies illustrate that ACT by itself or in conjunction with IL-1β induces tau phosphorylation and this is, at least in part, mediated by JNK activation. Mixed cultures of human glia from amyloid-prone cortical tissue show activation and expression of IL-1 and ACT, whereas mixed glial cultures from cerebellum failed to show such inflammatory response, suggesting that the regional specificities of amyloid deposition in AD may reflect on basic differences in inflammatory capacity between different brain regions [34]. For this reason, in the present study we decided to choose the brain regions that show pathological modifications in AD and examined them for changes in tau.

The microglia surrounding the plaques in AD brain have been shown to express high levels of the cytokine IL-1β and correlate with the extent of the pathology associated with