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# tPA promotes cortical neuron survival *via* mTOR-dependent mechanisms

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ABSTRACT

Tissue plasminogen activator (tPA) is a thrombolytic agent commonly used in the treatment of ischemic stroke. While the thrombolytic effects of tPA have been well established, the impact of this blood–brain barrier (BBB) crossing drug on neurons is not known. Given the widespread use of tPA in the clinical setting and the strict therapeutic window established for effective use of the drug, we examined the molecular mechanisms mediating the impact of tPA on postnatal cortical neurons isolated from the mouse brain. Dissociated postnatal primary cortical neurons were treated with tPA and the effects on neuron survival were evaluated. Pharmacological inhibitors of several signaling pathways previously implicated in neuroprotection (mTOR, JAK/STAT, MAPK and PKA-dependent mechanisms) were used to pinpoint the mechanistic effectors of tPA on neuron survival *in vitro*. We report here that tPA treatment results in a time-dependent neuroprotective effect on postnatal cortical neurons that relies predominantly on Janus kinase (JAK) and mammalian target of rapamycin (mTOR) signaling mechanisms. Taken together, these data suggest that tPA promotes neuroprotection in a temporally-regulated manner and that both JAK and mTOR signaling effectors are critical mediators of this neuroprotective effect. The results suggest the possibility of targeting these defined mechanisms to potentially expand the therapeutic window for tPA.



## 1. Introduction

Stroke is a leading cause of death and disability in North America, with limited options for therapeutic intervention. Tissue plasminogen activator (tPA) is a well-known FDA-approved drug used in the treatment of ischemic stroke; however, due to the short therapeutic window of this drug, there are major limitations in its efficacy within a clinical setting. Although the thrombolytic effects of tPA are well characterized, the direct impact of tPA on neurons in the brain remains unknown. Previous research has suggested that tPA can contribute to either neuroprotective or neurotoxic effects in neurons (Yepes et al., 2009). In this regard, embryonic cortical neurons are protected from cell death by treatment with tPA *in vitro* (Echeverry et al., 2010; Haile et al., 2012; Liot et al., 2006; Wu et al., 2013; Wu et al., 2012); however, the mechanisms mediating the effects of tPA on neurons still remain unclear. Furthermore, there is currently no evidence regarding the impact of tPA on postnatal cortical neurons. The effect of tPA in postnatal neurons is of particular importance, as it is well established that these neurons react differentially to external stimuli (Kole et al., 2013). Indeed, a converging hypothesis suggesting a developmental loss of neuronal survival responses in the postnatal brain is well characterized in both *in vitro* and *in vivo* experimental paradigms (Aubert et al., 1995; Chen et al., 1997; Wu et al., 2012). An emerging body of evidence suggests a clear correlation between increased neuronal cell death in postnatal compared to embryonic neurons (Chen et al., 1997; Goldberg et al., 2002; Haile et al., 2012; Wu et al., 2012). Postnatal cortical neurons rapidly and naturally undergo neurodegeneration when dissociated from the brain. This dissociation process is a well characterized stressor that naturally induces cell death mechanisms in postnatal neurons (Beaudoin et al., 2012); this is in striking contrast to embryonic neurons which can survive in culture for days to weeks following the dissociation process. While it is not feasible to culture adult cortical neurons for the purposes of this study, we suggest that using postnatal cortical neurons more closely mimics neurodegenerative events that may occur in the mature brain. Indeed, previous work has shown a dramatic difference in neuronal viability responses soon after birth (Goldberg et al., 2002), suggesting a possible switch in molecular and cellular signaling cascades at the time of birth that likely persists well into adulthood. These signaling cascades likely limit overgrowth of neurons in the brain at the time of birth. Interestingly, previous work has shown a striking difference in regenerative abilities in embryonic *versus* postnatal CNS neurons, pointing to differential expression and regulation of molecules intrinsic to embryonic and postnatal neurons or the postnatal CNS environment (Chen et al., 1997; Goldberg et al., 2002).

Several mechanisms have been proposed as potential mediators of cell death in central nervous system (CNS) neurons. Of particular note,

*Abbreviations:* DIV, days *in vitro*; JAK, Janus kinase; mTOR, mammalian target of rapamycin; p2, postnatal day 2; PDL, poly-D-lysine; p-S6, phospho-S6; p-STAT3, phosphorylated signal transducer and activator of transcription 3; tPA, tissue plasminogen activator.

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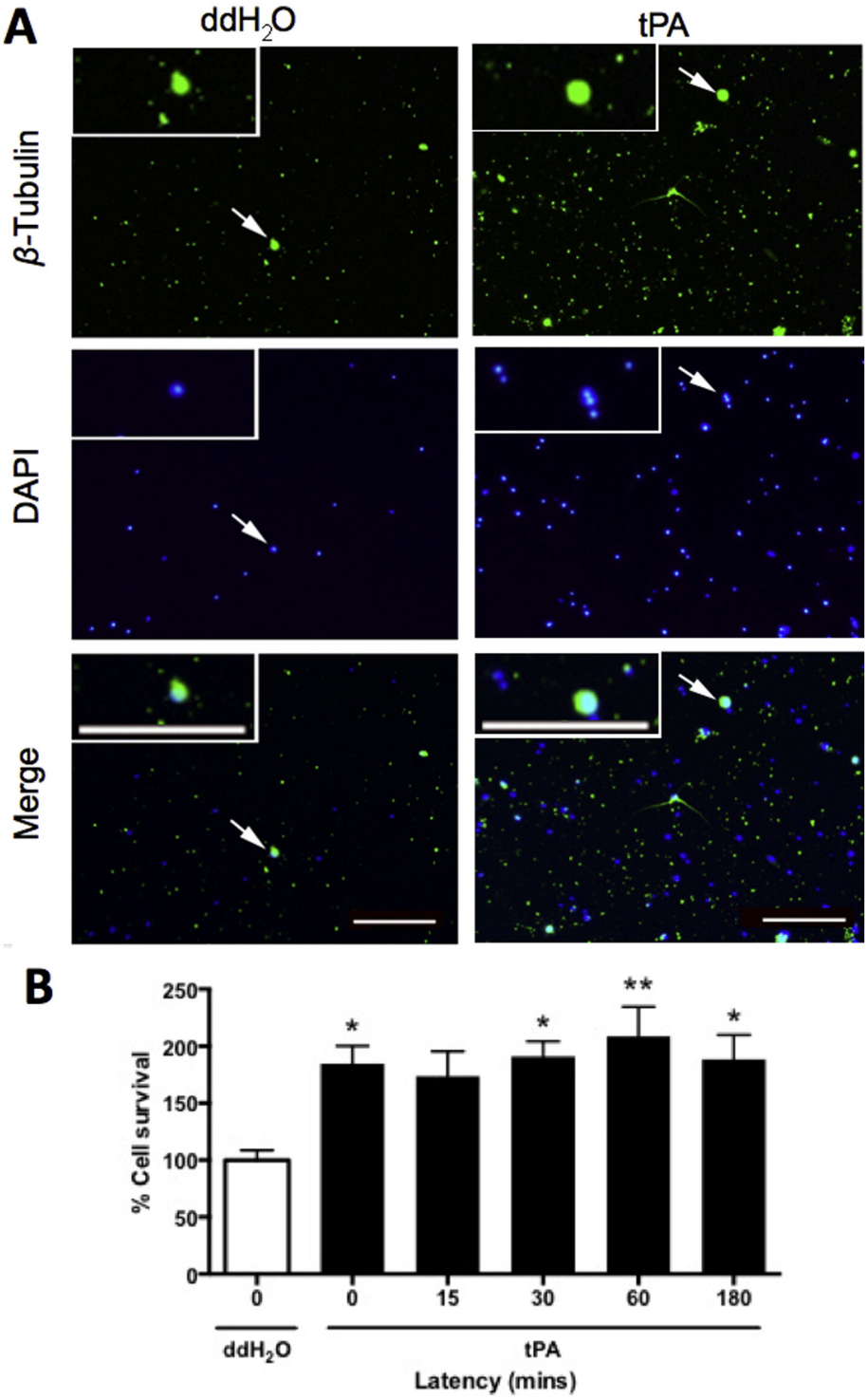


**Fig. 1.** tPA promotes primary cortical neuron survival within a 3-hour time interval. (A) Representative fluorescence micrographs at 0 min time latency (immediately after treatment) illustrating the expression of β-Tubulin (green), DAPI (blue) and merge of β-Tubulin and DAPI (green + blue) in postnatal primary cortical neurons following treatment with $ddH_2O$ (vehicle) or 5 nM tPA. Arrows indicate neuronal puncta, which is highlighted in the boxed insets. (B) Percent cell survival (neurons positively-stained with β-Tubulin and co-labeled with DAPI) of neuronal cells treated with tPA during time course experiment. Data are presented as the mean percentage of the control ± SEM of n = 6 pooled biological samples per group. $^{*}p < 0.05$; $^{**}p < 0.01$ denotes a statistically significant Bonferroni post-hoc between the $ddH_2O$-treated control and the tPA-treated groups. Magnification 10×, scale bar 100 μm; inset magnification 40×, scale bar 25 μm. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

the Janus kinase/Signal transducer and activator of transcription (JAK/STAT), mammalian target of rapamycin (mTOR), MEK, and Protein Kinase A (PKA) signaling pathways have all been implicated in neuroprotection (Dziennis and Alkayed, 2008; Park et al., 2004). In addition, previous work has shown that activation of JAK/STAT signaling is correlated with increased neuroprotective effects in several types of CNS neurons *in vitro* (Kretz et al., 2005; Shyu et al., 2008) and *in vivo* (Smith et al., 2009). Furthermore, activation of the mTOR signaling pathway has been previously shown to be neuroprotective in both *in vitro* and *in vivo* paradigms (Ma and Blenis, 2009; Park et al., 2008; Smith et al., 2009). MEK and PKA signaling mechanisms have also been suggested to play a role in promoting neuroprotection in the CNS