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[Valerie Jeanneret Lopez](#), *Emory University*  
[Manuel Yepes](#), *Emory University*

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● INVITED REVIEW

# Tissue-type plasminogen activator is a homeostatic regulator of synaptic function in the central nervous system

Valerie Jeanneret<sup>1</sup>, Manuel Yepes<sup>1,2,3,\*</sup>

1 Department of Neurology & Center for Neurodegenerative Disease, Emory University School of Medicine, Atlanta, GA, USA

2 Department of Neurology, Veterans Affairs Medical Center, Atlanta, GA, USA

3 Division of Neurosciences, Yerkes National Primate Research Center, Atlanta, GA, USA

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## Abstract

Membrane depolarization induces the release of the serine proteinase tissue-type plasminogen activator (tPA) from the presynaptic terminal of cerebral cortical neurons. Once in the synaptic cleft this tPA promotes the exocytosis and subsequent endocytic retrieval of glutamate-containing synaptic vesicles, and regulates the postsynaptic response to the presynaptic release of glutamate. Indeed, tPA has a bidirectional effect on the composition of the postsynaptic density (PSD) that does not require plasmin generation or the presynaptic release of glutamate, but varies according to the baseline level of neuronal activity. Hence, in inactive neurons tPA induces phosphorylation and accumulation in the PSD of the Ca<sup>2+</sup>/calmodulin-dependent protein kinase II $\alpha$  (pCaMKII $\alpha$ ), followed by pCaMKII $\alpha$ -induced phosphorylation and synaptic recruitment of GluR1-containing  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors. In contrast, in active neurons with increased levels of pCaMKII $\alpha$  in the PSD tPA induces pCaMKII $\alpha$  and pGluR1 dephosphorylation and their subsequent removal from the PSD. These effects require active synaptic N-methyl-D-aspartate (NMDA) receptors and cyclin-dependent kinase 5 (Cdk5)-induced phosphorylation of the protein phosphatase 1 (PP1) at T320. These data indicate that tPA is a homeostatic regulator of the postsynaptic response of cerebral cortical neurons to the presynaptic release of glutamate via bidirectional regulation of the pCaMKII $\alpha$ /PP1 switch in the PSD.

**Key Words:** tissue-type plasminogen activator (tPA); homeostatic plasticity; Ca<sup>2+</sup>/calmodulin-dependent protein kinase; post-synaptic density; protein phosphatase 1; plasmin

\*Correspondence to:

Manuel Yepes, M.D.,  
myepes@emory.edu.

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## Introduction

Tissue-type plasminogen activator (tPA) is a serine proteinase that in the intravascular space has a fibrinolytic role mediated by its ability to catalyze the conversion of plasminogen into plasmin (Camiolo et al., 1971; Collen and Lijnen, 1991). Earlier studies found that tPA is abundantly expressed in the brain (Sappino et al., 1993), and although it was initially believed that endothelial cells were the only source of this tPA, later work indicated that tPA is also found in glial cells (Siao et al., 2003) and neurons (Nicole et al., 2001; Yepes et al., 2009). Since then tPA has been implicated in a plethora of functions in the brain including the development of synaptic plasticity (Qian et al., 1993; Madani et al., 1999; Oray et al., 2004; Yepes et al., 2016), the detection and adaptation to metabolic stress (Wu et al., 2012), modulation of blood-brain barrier (BBB) permeability (Yepes et al., 2003), and remodeling of the extracellular matrix (ECM) (Berardi et al., 2004).

A further advancement in the understanding of tPA's

function in the brain was attained by the observation that its release from the presynaptic terminal of glutamatergic neurons (Gualandris et al., 1996) triggers the synaptic vesicle cycle in cerebral cortical neurons (Wu et al., 2015). More specifically, it was discovered that membrane depolarization induces the rapid secretion of tPA at extrasynaptic sites, and that by promoting the recruitment of the cytoskeletal protein  $\beta$ II-spectrin to the active zone (AZ), tPA enlarges the synaptic release site. At the same time, it was found that tPA also induces the phosphorylation of synapsin I, a protein that clusters glutamate-containing synaptic vesicles (SVs) in the reserve pool of the presynaptic terminal. This effect is of pivotal importance for neurotransmission, because phosphorylation of synapsin I at serine 9 allows SVs to translocate from the reserve pool of synaptic vesicles to the synaptic release site, already enlarged by tPA, where they release their load of excitatory neurotransmitters into the synaptic cleft (Wu et al., 2015). Remarkably, this effect does not lead to depletion of SVs

from the presynaptic terminal, because tPA also promotes their endocytic retrieval from the presynaptic membrane, *via* calcineurin-mediated dynamin I dephosphorylation and the formation of the actin scaffold necessary for the newly formed SVs to re-enter the synaptic vesicle cycle (Wu et al., 2015).

These observations originated two important questions about the synaptic effect of the release of tPA from the presynaptic terminal of cerebral cortical neurons: first, if tPA promotes the exocytosis and endocytic retrieval of glutamate-containing SVs, then is tPA an inductor of excitotoxicity by perpetuating the synaptic release of glutamate? Second, does tPA released from the axonal bouton have an effect on the postsynaptic terminal?

### Effect of tPA on the Composition of the Post-Synaptic Density

To address these questions, we used electron microscopy to study the effect of tPA on the postsynaptic density (PSD), an electron-dense structure attached to the postsynaptic terminal that undergoes rapid changes in molecular composition, structure, and function in response to variations in synaptic activity (Dosemeci et al., 2001). We found that either treatment with recombinant tPA (rtPA) or the release of neuronal tPA is followed by a rapid increase in the thickness of the PSD (Jeanerret et al., 2016). Then we used phosphoproteomics and biochemical studies with extracts of the PSD to identify the mechanism underlying this effect. Surprisingly, we found that treatment with rtPA *in vitro* or the endogenous release of neuronal tPA prompted by a brief episode of cerebral ischemia *in vivo*, causes a rapid and sustained increase in the expression of Ca<sup>2+</sup>/Calmodulin-dependent protein kinase II $\alpha$  phosphorylated at T286 (pCaMKII) in the PSD.

CaMKII $\alpha$  is a serine-threonine kinase that is highly abundant in the PSD (Petersen et al., 2003). The influx of calcium into the post-synaptic terminal of the active synapse leads to calcium/calmodulin-mediated CaMKII $\alpha$  activation that is followed by its translocation from the postsynaptic actin cytoskeleton to the PSD where it binds to N-methyl-D-aspartate receptors (NMDARs). Intriguingly, upon its phosphorylation at T286, CaMKII $\alpha$  remains active even after calcium concentrations fall to baseline levels (Miller and Kennedy, 1986; Lisman et al., 2012). This “autonomy” has led many to postulate CaMKII $\alpha$  as a “memory molecule” with a pivotal role in learning and the development of synaptic plasticity (Lisman et al., 2012). Importantly, it was shown that tPA-induced CaMKII $\alpha$  phosphorylation and accumulation in the PSD is independent of its effect on the presynaptic release of glutamate (Jeanerret et al., 2016).

Despite the relevance of these events for the development

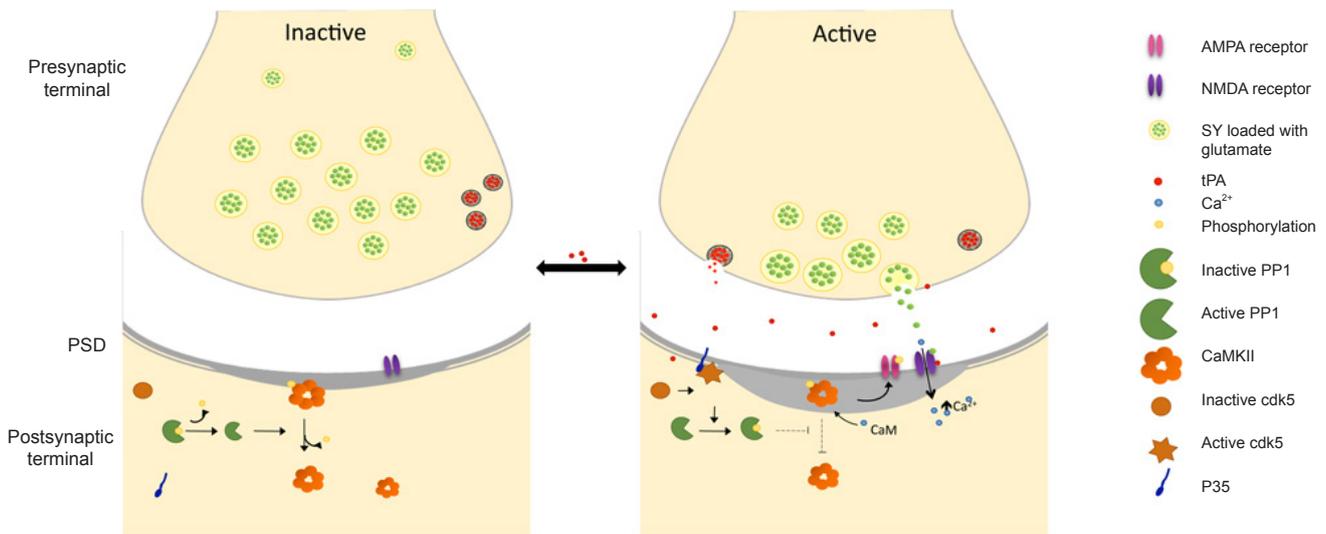
of synaptic plasticity under physiological conditions, it is also important to note that several groups have reported that cerebral ischemia causes pCaMKII $\alpha$  phosphorylation, and that its accumulation in the PSD leads to neuronal death (Coultrap et al., 2011; Liu et al., 2012; Lu et al., 2013). Thus, as stated above, it is conceivable to propose that by inducing its accumulation in the PSD, tPA has a neurotoxic effect. To study this possibility, we used a model in which either exogenous (rtPA), or endogenous (neuronal tPA), was added to neurons in which pCaMKII $\alpha$  was already accumulated in the PSD. Surprisingly, we found that tPA does not cause a further increase in pCaMKII $\alpha$  expression in the PSD of neurons with high baseline levels of the kinase, but that instead it decreases them to those found under baseline, non stimulated conditions (Jeanerret et al., 2016). In summary, these findings indicate that tPA has a bidirectional effect on the phosphorylation and accumulation of pCaMKII $\alpha$  in the PSD that depends on the baseline levels of the kinase. Thus, while in previously inactive neurons tPA increases pCaMKII $\alpha$  expression in the PSD, in those that are already active tPA has the opposite effect, decreasing its accumulation in the postsynaptic terminal. Importantly, although the effect of tPA on pCaMKII $\alpha$  is mediated by synaptic NMDARs, it does not require the conversion of plasminogen into plasmin (Jeanerret et al., 2016).

### tPA Regulates the CamKIIalpha/PP1 Switch in the Postsynaptic Density

CaMKII phosphorylation is tightly regulated by a family of serine/threonine protein phosphatases (PP) (Colbran, 2004). There are different PPs, with PP1, 2A, 2B and 2C accounting for the most of the phosphatase activity in the brain. PP1 is the PP of the PSD, and our data indicate that tPA induces its phosphorylation at T320 *via* Cdk5 activation. Together, these results show that tPA has a bidirectional effect on the PP1/CaMKII $\alpha$  switch.

### tPA is a Homeostatic Regulator of Synaptic Activity

To understand the physiological importance of this effect we used a proteomics approach to study the effect of tPA-induced CaMKII $\alpha$  phosphorylation on synaptic function. We found that tPA induces CaMKII $\alpha$ -mediated phosphorylation at S831 and its subsequent recruitment to the PSD of the  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (AMPA) subunit GluR1. Again, as previously noted, this is a bidirectional effect in which tPA induces GluR1 phosphorylation (pGluR1) in previously inactive neurons and pGluR1 dephosphorylation in neurons with high baseline levels of synaptic activity (Jeanerret et al., 2016). The physiological importance of



**Figure 1** Tissue-type plasminogen activator (tPA) induces homeostatic plasticity in cerebral cortical neurons.

tPA induces bidirectional changes in the structure and protein composition of the postsynaptic density (PSD) *via* its ability to regulate the protein phosphatases (PP) 1/ Calmodulin-dependent protein kinase II (pCaMKII) switch. (A) In inactive neurons with low baseline expression of pCaMKII $\alpha$ , tPA activates the kinase by inducing its phosphorylation at T286 in a Ca<sup>2+</sup>- and N-methyl-D-aspartate receptor (NMDAR)-dependent manner, followed by its subsequent translocation to the postsynaptic density where it induces the phosphorylation and synaptic recruitment of  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors, thus converting silent into active synapses. When activated by p35, cyclin-dependent kinase 5 (Cdk5) induces the PP1 phosphorylation at T320, an event that inactivates PP1 and thereby allows the phosphorylation and synaptic accumulation of pCaMKII. (B) In overactive neurons, tPA prevents p35-induced Cdk5 activation and Cdk5-induced PP1 inactivation by T320 phosphorylation. The active phosphatase is then able to dephosphorylate pCaMKII $\alpha$  preventing its accumulation in the postsynaptic density (PSD) and abrogating the effect of pCaMKII $\alpha$  on the synaptic recruitment of GluR1-containing AMPA receptors.

these findings is underscored by the observation that glutamatergic synapses that contain NMDA but not AMPA receptors are “silent”. However, they become “active” following pCaMKII $\alpha$ -induced phosphorylation and synaptic recruitment of AMPA receptors containing GluR1 subunits (Liao et al., 2001). Together, these data indicate that while tPA decreases the activity of those synapses that are already active, it increases it in those that are previously inactive. These results confirm our initial observation that tPA is a homeostatic regulator of synaptic function.

Homeostatic plasticity is the process whereby neural circuits maintain their activity levels within a constant range (Turrigiano and Nelson, 2000; Turrigiano, 2008). This is a mechanism that allows networks to develop compensatory mechanisms aimed at maintaining the homeostatic balance of a population of synapses (Turrigiano, 2008). For instance, to maintain the equilibrium of the system, a prolonged decrease in neuronal activity activates compensatory mechanisms that increase the population of AMPA receptors and spine size on the postsynaptic terminal. The results discussed here indicate that tPA is an effective inducer of homeostatic plasticity in cerebrocortical neurons during physiological and pathological conditions.

In summary, the data currently available allow to propose a model in which tPA is an inducer of homeostatic plasticity *via* its ability to bidirectionally regulate the PP1/

CaMKII $\alpha$  switch (**Figure 1**). This is of particular importance for our understanding of the mechanisms underlying the development of synaptic plasticity and for the potential therapeutic use of rtPA to protect the synapse of patients with neurological diseases that such as cerebral ischemia are characterized by the presence of severe synaptic dysfunction.

**Author contributions:** VJ wrote the manuscript. MY conceived the idea and wrote the manuscript.

**Conflicts of interest:** None declared.

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