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# Tissue-Type Plasminogen Activator: A Multifaceted Modulator of Neurotransmission and Synaptic Plasticity

Minireview

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**For over a decade, tissue-type plasminogen activator (t-PA), a serine protease classically known for its profibrinolytic role in the vasculature, has been implicated in numerous aspects of the synaptic plasticity process. But despite being the most intensively studied protease of the CNS, the mechanisms and molecular mediators behind the action of t-PA on synaptic efficacy remain largely undefined. Rather than examine the role of t-PA in proteolytic remodeling of the synaptic extracellular matrix, this review will focus on the evidence that defines t-PA as a direct modulator of neurotransmission and synaptic plasticity by impacting on glutamatergic and dopaminergic pathways.**

On the basis of spatial and temporal localization, tissue-type plasminogen activator (t-PA) presents as a candidate modulator of neurotransmission. Within the CNS, the t-PA transcript is regulated in an immediate-early manner. Both intracellular t-PA protein and mRNA deposits have been localized to the synapse. Moreover, various depolarization agents promote exocytosis of t-PA from neuronal stores. Once in the extracellular space, t-PA can convert the proenzyme plasminogen into its active form, plasmin. Extracellular t-PA activity is controlled by inhibitors including neuroserpin and plasminogen activator inhibitor (PAI)-1 and targeted to cell surfaces by association with Annexin II, LDL receptor-related protein-1 and -1b. Such precise regulation of t-PA expression and activity, in conjunction with its well-documented role in long-term potentiation (LTP), memory formation, and seizure, implicate t-PA as an important modulator of neurotransmission.

## Participation of t-PA in Glutamatergic Transmission

Glutamate is the main excitatory neurotransmitter of the mammalian brain. The ionotrophic glutamate receptors are classified according to their selectivity for the glutamate analogs: NMDA, kainate, and AMPA. The efficacy of glutamatergic transmission is not fixed, but can vary in an activity-dependent manner—a phenomenon known as synaptic plasticity that underlies learning and memory formation. The NMDA receptor (NMDAR) plays a central role in these events. A recent wave of research has strongly implicated t-PA as a modulator of NMDAR function, although the mechanism by which t-PA influences this receptor has been the subject of much debate. Accordingly, several models explaining the ability of t-PA to alter NMDAR-mediated processes have been put forward.

### *Cleavage of the NR1 Subunit of the NMDA Receptor*

The first demonstration of a modulatory role for t-PA in NMDAR function was provided by Nicole et al., (2001). In this study, t-PA was shown to enhance NMDA-evoked rise in free intracellular calcium ($\Delta[Ca^{2+}]_i$) and NMDA-induced cell death in vitro. A physical interaction between t-PA and the NR1 subunit of the NMDAR was demonstrated by the coimmunoprecipitation of these agents from embryonic neuronal membrane proteins. Moreover, these authors showed that incubation of t-PA with membrane proteins resulted in cleavage of the NR1 subunit. It was hypothesized that t-PA, via plasmin-independent proteolysis of NR1 could increase NMDAR calcium permeability and thereby aggravate NMDA-induced events (Figure 1). This notion was advanced by the demonstration that t-PA could cleave a recombinant NR1 fragment (Fernandez-Monreal et al., 2004). Mass spectrometry analysis identified Arginine260 as the t-PA-sensitive cleavage site within this NR1 fragment. It was subsequently shown that mutation of Arginine260 within this fragment abrogated the ability of t-PA to cause cleavage (Fernandez-Monreal et al., 2004).

At the functional level, t-PA was shown to augment the NMDA-induced $\Delta[Ca^{2+}]_i$ in HEK293 cells expressing recombinant NMDARs, whereas mutation of Arginine260 abrogated the ability of t-PA to exacerbate NMDA-evoked $\Delta[Ca^{2+}]_i$. This work formed the first direct link between t-PA and the NMDAR and argued strongly for a t-PA-NR1 interaction that impinges on NMDAR currents.

Although there is now substantial evidence to indicate a relationship between t-PA and the NMDAR (see below), controversy surrounds the mechanistic basis of this association. In particular, contention surrounds the capacity of t-PA to cleave the NR1 subunit. Although one study has corroborated the physical association of t-PA with the NR1 subunit (Kvajo et al., 2004), three reports have failed to detect cleavage of NR1 by t-PA (Liu et al., 2004; Matys and Strickland, 2003; Kvajo et al., 2004). It is hard to reconcile these inconsistencies in the field. Salient details may rest in the experimental system used or the developmental stage during which the experiments were performed. The research which defined NR1 as a substrate for t-PA utilized material from embryonic neuronal cultures, whereas the research that failed to detect the t-PA-mediated cleavage of NR1 utilized lysates prepared from adult mouse brain. Another possibility may be that the cleavage of NR1 by t-PA is sensitive to parameters such as ionic strength (Mars et al., 2005).

That proteolytic activity is a prerequisite for the effect of t-PA on the NMDAR was concluded from the finding that t-PA-STOP (a t-PA inhibitor) could attenuate the t-PA enhancement of NMDA-induced $\Delta[Ca^{2+}]_i$ (Liot et al., 2004). This dependence on proteolytic activity implies the existence of a substrate. Notwithstanding the controversy over NR1 cleavage, the other most likely substrate for t-PA is plasminogen. At the biochemical level, a role for

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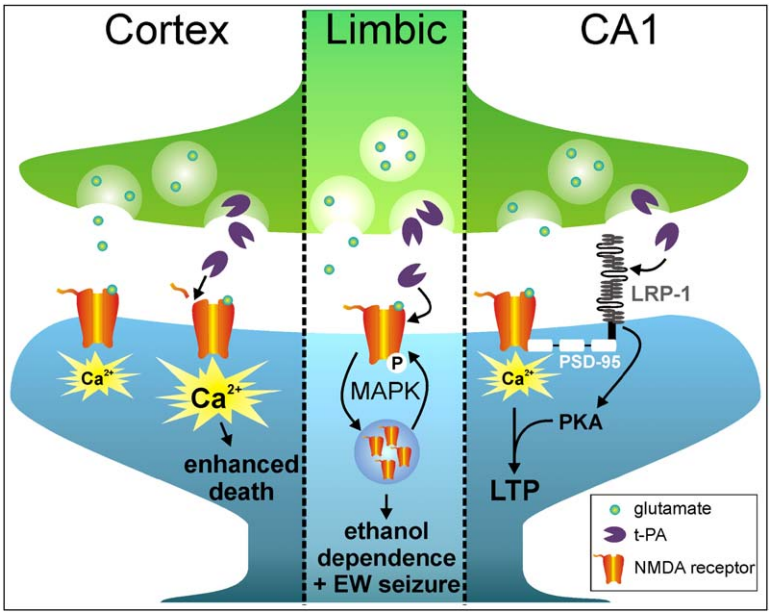


Figure 1. Mechanisms by which t-PA Influences Glutamatergic Transmission

Left: t-PA cleavage of the NR1 subunit increases NMDAR ion permeability. In embryonic cortical neuronal culture, this cleavage event augments NMDAR-dependent calcium flux and excitotoxic cell death. Middle: limbic system seizure resulting from ethanol withdrawal (EW) in alcohol-dependent mice. t-PA potentiates the development of ethanol dependency and EW-induced seizure via a nonproteolytic interaction with the NR2B subunit. This potentiation involves increased NR2B phosphorylation, MAPK-ERK1/2 activation, and a rise in the number NR2B-containing NMDARs. Right: LRP-1 is tethered to the NMDAR via PSD-95. t-PA facilitates NMDAR-dependent L-LTP at the CA1 synapse by binding to LRP-1 and transducing a signal that elevates PKA levels. For simplicity, only the exocytosis of t-PA from presynaptic stores is depicted. However, t-PA stores within the postsynapse have also been demonstrated (Shin et al., 2004).

plasmin in t-PA-mediated NR1 proteolysis was considered unlikely because the incubation of a recombinant NR1 fragment or full-length native NR1 with plasmin led to their complete degradation (Fernandez-Monreal et al., 2004; Matys and Strickland, 2003). Nonetheless, at the functional level, a role for plasmin should be fully investigated because plasminogen has already been shown to enhance NMDA-induced $\Delta[Ca^{2+}]_i$ (Inoue et al., 1994).

Even if NR1 is a bona fide t-PA substrate, it remains to be seen whether this cleavage event can affect the ion permeability of the NMDAR. As acknowledged by Traynelis and Lipton (2001), direct evidence that NR1 cleavage has an electrophysiological consequence is lacking. Indeed, a study by Centonze et al. (2002) demonstrated that t-PA$^{-/-}$ mice displayed unaltered NMDAR-mediated excitatory postsynaptic currents (EPSCs), suggesting that endogenous t-PA does not influence ion flux through the NMDAR. However, because changes in free intracellular calcium levels are not a direct reflection of NMDAR-mediated EPSCs, it is conceivable that the potentiation of NMDA-induced $\Delta[Ca^{2+}]_i$ may lie downstream of other receptor-mediated signaling events, e.g., intracellular store-operated calcium release. As a result, other routes by which t-PA might influence NMDA-evoked events cannot be excluded.

***Upregulation of the NR2B Subunit by t-PA***

A relationship between t-PA and the NMDAR has also been connoted in the setting of alcoholism. Ethanol directly interacts with the NMDAR suppressing ion flux through this channel. Continued suppression of NMDAR function because of chronic alcohol consumption leads to a compensatory rise in NMDAR expression and activity. Such neuroadaptation underlies the development of ethanol tolerance and dependence. The heightened NMDAR number and activity as a result of chronic alcohol abuse triggers the onset of seizure upon the withdrawal of ethanol from the diet.

Pawlak et al. (2005) demonstrated that ethanol-dependent t-PA$^{-/-}$ mice displayed attenuated seizure severity relative to their wild-type and plasminogen$^{-/-}$ counterparts upon ethanol withdrawal (EW). In addition, the administration of t-PA into t-PA$^{-/-}$ mice augmented EW-induced seizure severity. Because plasminogen$^{-/-}$ mice displayed no change in seizure severity, the role of t-PA in this setting is plasmin independent. Interestingly, intracerebral administration of t-PA-STOP had no impact on seizure severity in wild-type mice, indicating that t-PA was acting in a nonproteolytic fashion.

Clues to the underlying mechanism were derived from the use of the NR2B-specific antagonist, ifenprodil. The administration of ifenprodil abrogated the enhancing effect of t-PA on seizure severity. Moreover, the acquisition of ethanol dependence and EW-induced seizure was correlated with increased total and phosphorylated NR2B levels in wild-type, but not t-PA$^{-/-}$ mice. It was also shown that t-PA and NR2B could be reciprocally coimmunoprecipitated. Consistent with a nonproteolytic mechanism, cleavage of NR2B by t-PA was not observed. Collectively, this data implies that the alteration of EW-induced seizure by t-PA is not only NR2B-dependent but also mediated by a direct interaction between NR2B and t-PA (Figure 1).

Based on the knowledge that activation of NR2B-containing NMDARs triggers ERK1/2 phosphorylation, Pawlak and colleagues correlated EW-induced seizure with ERK1/2 activation in wild-type, but not t-PA$^{-/-}$ mice. An independent study also reported the ability of t-PA to trigger ERK1/2 phosphorylation in a manner dependent on NMDAR activation but independent of proteolysis (Medina et al., 2005). Importantly, the "cause or effect" relationship between phosphorylated ERK1/2 and NR2B upregulation during the acquisition of ethanol dependence is yet to be delineated. Altogether, the observation that t-PA physically interacts with NR2B, and that t-PA-deficiency ablates the NR2B-dependent rise in phosphorylated ERK1/2 upon seizure induction, raises the prospect of a t-PA-triggered intracellular signal that is transduced through the NR2B subunit.

At first glance, the finding that t-PA aggravates EW-induced seizure is not surprising as t-PA is known to

promote kainate-induced seizure spread from the amygdala to the hippocampus in a plasmin-independent manner (Yepes et al., 2002). However, as the natural t-PA inhibitor, neuroserpin, blunts this seizure propagation, the role of t-PA in kainate-induced seizure spread depends on proteolysis and is therefore distinct from its role in EW-induced seizure.

***Low-Density Lipoprotein Receptor-Related Protein-1***

Lipoprotein receptor-related protein-1 (LRP-1) is a member of the low-density lipoprotein receptor (LDLR) family of endocytic receptors. LRP-1 is widely expressed throughout the CNS where it mediates the signaling and catabolism of many ligands. Indeed, LRP-1 endocytoses free t-PA and its inhibitory complexes (Makarova et al. [2003] and references therein). Hence, an alternative mechanism by which t-PA might impact on NMDA-mediated events is via its ability to signal through LRP-1 (Figure 1).

An important study by Huang et al. (1996) demonstrated that t-PA$^{-/-}$ mice exhibited impaired late-phase LTP (L-LTP) along both the Schaffer collateral (CA1) and Mossy fiber (CA3) neural tracts. Zhuo et al. (2000) extended these findings by showing that in t-PA$^{-/-}$ mice, t-PA application restored LTP along the CA1 pathway. Intriguingly, coapplication of t-PA with the LDLR panligand blocker, RAP (receptor-associated protein), ablated the t-PA-mediated restoration of CA1 LTP. Although other LDLR family members are known to be crucial for CA1 LTP (May et al., 2004), LRP-1 is the most likely LDLR given its known association with t-PA. Zhuo and colleagues went on to show that addition of t-PA increased protein kinase A (PKA) activity in hippocampal neurons and that this increase could also be attenuated by RAP. Interestingly, a precedent for t-PA-mediated intracellular signal transduction through LRP-1 exists (Wang et al., 2003). Might a LRP-1-mediated rise in cAMP/PKA levels underlie the ability of t-PA to enhance NMDA-induced $\Delta[Ca^{2+}]_i$? At least with regards to NMDAR ion permeability, this does not appear to be the case because t-PA application failed to alter NMDAR excitatory postsynaptic potentials (EPSPs) (Zhuo et al., 2000). But as changes in electrophysiological properties and changes in free intracellular calcium are not necessarily coupled events, it remains to be seen whether a LRP-1-mediated rise in cAMP/PKA underlies the ability of t-PA to enhance NMDA-induced $\Delta[Ca^{2+}]_i$.

Circumstantial evidence for the participation of LRP-1 during t-PA modulation of NMDAR function can be inferred from the findings that other LRP-1 ligands—$\alpha$-2-macroglobulin, lactoferrin, apo$\varepsilon$4—also modulate NMDA-evoked $\Delta[Ca^{2+}]_i$ (Qiu et al. [2003] and references therein). LRP-1 is tethered to the NMDAR via the intracellular scaffold protein, PSD-95. Moreover, this association is activity dependent (May et al., 2004). As a result of this physical association, LRP-1 and its ligands are ideally situated to be NMDAR modulators.

***Conclusion***

Several reports have highlighted the NMDAR as the cell-surface receptor through which t-PA exerts its neuromodulatory effects. Direct cleavage of the NR1 subunit and a nonproteolytic interaction with the NR2B subunit are two currently proposed models. In addition, signaling crosstalk between the NMDAR and LRP-1 may underlie the ability of t-PA to facilitate NMDA-evoked events. Whether the NMDAR can accommodate all three of these scenarios remains to be seen. It should be noted, however, that the experiments that led to these three postulates were performed on different regions of the CNS, utilized different experimental systems, and were performed during different stages of murine development. As in the setting of LTP and LTD, the mechanisms by which t-PA operates may change depending on the neural compartment and on the developmental stage. Nonetheless, the number of independent reports demonstrating a relationship between t-PA and the NMDAR define t-PA as a direct modulator of glutamatergic transmission.

## Participation of t-PA in Dopaminergic Transmission

Dopamine is a slow-acting neuromodulatory transmitter that exerts its effects via two classes of receptors: D1-like receptors that couple to Gs proteins and elevate cAMP and D2-like receptors that couple to Gi proteins and suppress cAMP. Although dopaminergic neurons are relatively few in number, they exercise diverse and dramatic control on many neural circuits. Substantial experimental evidence for t-PA involvement in dopaminergic transmission has been obtained in the synaptic plasticity paradigms of LTP and drug addiction.

***Long-Term Potentiation***

The striatum is heavily innervated by dopaminergic neurons. Centonze et al. (2002) demonstrated that striatal spiny neurons from t-PA$^{-/-}$ mice, despite unaltered physiological and pharmacological EPSP properties, harbored a severe impairment in corticostriatal LTP (Figure 2). In an attempt to mimic D1-receptor activation, it was shown that pharmacological elevation of cAMP rescued corticostriatal LTP in t-PA$^{-/-}$ mice. Next, it was shown that the striatal interneurons of t-PA$^{-/-}$ mice were refractory to dopamine-mediated depolarization via D1 receptors. No change in the number of dopaminergic afferents could be detected in the t-PA$^{-/-}$ striatum. It was postulated that t-PA deficiency hinders striatal dopamine D1 signaling; a deficiency that can be overcome by elevating cAMP levels. In support of the hypothesis that t-PA facilitates D1-mediated signaling, a reduced level of D2 receptor was detected in the t-PA$^{-/-}$ striatum. As D2 and D1 receptors have opposing actions on cAMP generation, this reduced D2 receptor level was posited as an adaptation to attenuated D1-mediated signaling.

In line with LTP being a molecular correlate for learning and memory formation, cognitive deficits have been documented in the t-PA$^{-/-}$ mouse. Indeed, striatal defects in dopaminergic processes has been hypothesized to be the basis for the reduced capacity of t-PA$^{-/-}$ mice to learn an active avoidance task—a behavior that is sensitive to striatal lesion. In this context it was shown that after a conditioned stimulus, t-PA$^{-/-}$ mice were worse than wild-type mice at learning to actively avoid an aversive electrical foot shock (Huang et al., 1996; Calabresi et al., 2000). It must be noted, however, that aversion-related memory paradigms are confounded by the fact that t-PA$^{-/-}$ mice display reduced anxiety and fear (Pawlak et al., 2003).

Ties between t-PA and dopaminergic transmission have also been made in LTP along the CA1 and CA3 pathways. These two forms of hippocampal LTP can

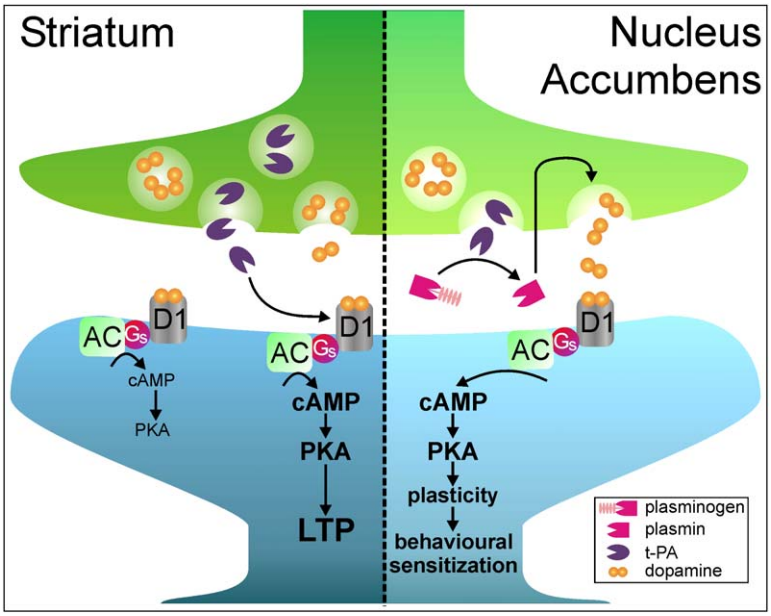


Figure 2. Mechanisms by which t-PA Influences Dopaminergic Transmission

The D1 class of dopamine receptors couples to Gs proteins and elevates cAMP/PKA levels upon activation of adenylate cyclase (AC). Left: in the striatum, t-PA$^{-/-}$ mice have impaired D1 receptor-mediated signaling that impedes corticostriatal LTP induction. Right: in the nucleus accumbens, t-PA-mediated generation of plasmin triggers dopamine release upon morphine injection. This plasmin-induced dopamine release underlies the plasticity-related behavioral sensitization brought on by morphine administration.

be evoked by the pharmacological elevation of cAMP. Huang et al. (1996) showed that D1 agonist-induced CA1 LTP was abolished in t-PA$^{-/-}$ mice. Similarly, forskolin-induced CA3 L-LTP maintenance was attenuated in t-PA$^{-/-}$ mice. Consistent with a proteolytic role for t-PA in hippocampal LTP, the maintenance of forskolin-induced CA3 LTP in wild-type mice could be diminished by exogenous PAI-1 (Baranes et al., 1998). Huang and colleagues speculated that a dopamine-induced rise in cAMP might initiate new t-PA gene transcription and protein synthesis—a step that dramatically facilitates the long-lasting potentiation of synaptic efficacy in the hippocampus. This idea is supported by the observation that dopamine receptor antagonists block methamphetamine-induced immediate-early t-PA gene transcription (Hashimoto et al., 1998) and that forskolin triggers immediate-early t-PA gene transcription in hippocampal neurons (Baranes et al., 1998).

Based upon the marked deficits in hippocampal LTP that exist in the t-PA$^{-/-}$ mouse, it might be expected that these mice would harbor a severe impairment in spatial learning and memory (a hippocampus-dependent response). However, no such impairment has been demonstrated in t-PA$^{-/-}$ mice (Huang et al., 1996). Consequently, in the t-PA$^{-/-}$ mouse, the observed dopaminergic deficiencies in hippocampal LTP do not strictly translate into defects in spatial learning and memory. Rather, these defects may impact on other aspects of memory or even other plasticity-related behavioral processes such as anxiety or addiction. Notably, the fact that t-PA deficiency produces no change in spatial learning capabilities does not preclude a role for t-PA in this cognitive process. Indeed, overexpression of t-PA within the murine CNS enhances both hippocampal LTP and spatial learning and memory performance (Madani et al., 1999). Whether an alteration in dopamine transmission exists within the hippocampus of these t-PA-overexpressing mice is yet to be assessed.

### *Drug Addiction*

Analogous to the setting of LTP and natural memory formation, drug abuse triggers cellular and behavioral changes that are experience dependent, long lasting, and strengthened by repetition. It is widely hypothesized that the synaptic plasticity process more commonly associated with memory, also underlies the establishment of drug addiction. Furthermore, psychomotor stimulants, including cocaine, morphine, and methamphetamine are known to rely on dopaminergic transmission.

Given the participation of t-PA in dopaminergic transmission and plasticity-related events, it seems hardly surprising that recent investigations point to a prominent role for t-PA in stimulant abuse. Nagai et al. (2004) demonstrated that t-PA can acutely regulate the cellular and behavioral morphine-induced events in the nucleus accumbens (NAc) (Figure 2). In this example, contrary to the aforementioned situation in the striatum, no alteration in dopamine signaling was found. Rather, t-PA$^{-/-}$ mice exhibited reduced dopamine release in the NAc after morphine injection. This deficit in dopamine release was reversed in t-PA$^{-/-}$ mice by microinjection of t-PA or plasmin into the NAc prior to morphine administration. At the behavioral level, t-PA$^{-/-}$ mice displayed attenuated morphine-induced hyperlocomotion. Accordingly, microinjection of t-PA or plasmin into the t-PA$^{-/-}$ NAc increased morphine-induced hyperlocomotion. t-PA$^{-/-}$ and plasminogen$^{-/-}$ mice were then shown to be partially resistant to the rewarding effects of morphine as assessed by conditioned place preference (a NAc-dependent task). Altogether, the work of Nagai and colleagues demonstrates that t-PA, via the generation of plasmin within the NAc, participates in both the cellular (dopamine release) and behavioral (hyperlocomotion) responses to morphine administration.

### *Conclusion*

Defects in D1 signaling within the striatum have been correlated with t-PA deficiency, whereas in the NAc, defects in dopamine release have been discovered in

t-PA$^{-/-}$ mice. In the setting of morphine abuse, t-PA appears to function in a plasmin-dependent manner, although the plasmin-sensitive target remains unknown. With regards to hippocampal LTP, it is clear that t-PA requires proteolytic activity to promote LTP along both CA1 and CA3 circuits. Curiously, with CA1 LTP, the facilitatory influence of t-PA has been ascribed to LRP-1-mediated signaling (Zhuo et al., 2000), to dopamine-triggered t-PA gene transcription (Huang et al., 1996), and to plasmin-mediated generation of neurotrophins (Pang et al., 2004). How these critical events can coexist at the same synapse is a question yet to be addressed. Furthermore, whether t-PA-mediated plasmin generation is central to LTP and LTD along other neural tracks remains to be determined. Indeed, roles for t-PA in hippocampal-dependent learning that are reliant upon proteolytic capacity but independent of plasminogen are likely to exist because the acquisition of passive avoidance was diminished by the intrahippocampal infusion of t-PA-STOP into wild-type mice, whereas infusion of urokinase (that also activates plasminogen) could not substitute for t-PA in the acquisition of passive avoidance in t-PA$^{-/-}$ mice (Pawlak et al., 2002).

Although no mechanistic commonality has been assigned to the t-PA-mediated plasticity paradigms of LTP and drug addiction, shifts in dopaminergic transmission are a recurrent theme. As with the t-PA modulation of NMDA-induced events, the participation of t-PA in dopaminergic transmission, LTP, and memory formation awaits further delineation.

## Concluding Remarks

Extravascular roles for the plasminogen activator/plasmin system have recently arisen. The generation of the t-PA$^{-/-}$ mouse has focused attention on the participation of t-PA in CNS function and dysfunction. Although not addressed in this review, numerous studies have defined t-PA as a deleterious contributor to excitotoxicity and other neurodegenerative paradigms; the effect of t-PA on zinc-induced toxicity is a notable exception (Strickland [2001] and references therein). In more physiological settings, the neurological activities of t-PA are contextual with synaptic plasticity processes.

As a protease that undergoes stimulus-dependent exocytosis from neuronal stores, t-PA also participates in the proteolytic remodeling of cell-cell and cell-matrix molecules that arises during synaptic plasticity. This role for t-PA is exemplified within the visual cortex during monocular deprivation-induced plasticity (Berardi et al., 2004). In addition to this role for t-PA in shaping the synaptic extracellular scaffold, a growing number of observations are demonstrating that t-PA can also impact on neurotransmission in a more direct and receptor-mediated fashion. To this end, the research demonstrating the participation of t-PA in the plasticity-related paradigms of seizure, LTP, and drug addiction has been highlighted in this review.

An alteration of ion channel properties (NR1 cleavage), an increase in cell-surface receptor activity and number (NR2B activation), intracellular signaling cascades (MAPK or PKA), and shifts in modulatory transmission (changes in dopaminergic properties) are all ways in which t-PA might alter synaptic efficacy. Several neurological outcomes have been attributed to the t-PA-mediated generation of plasmin; however, the pertinent plasmin substrates remain largely unknown. Similarly, a number of proteolytic yet plasmin-independent roles for t-PA have also been described, implicating the existence of other currently unidentified non-plasminogen substrates for t-PA within the CNS.

Despite the fact that the molecular mediators and mechanisms behind the neurobiology of t-PA remain elusive, the vast array of experimental evidence clearly denotes t-PA as a multifaceted modulator of neurotransmission and the plasticity process. This action of t-PA is clearly distinct from its classical role in haemostasis and fibrinolysis.

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