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Neurovascular disease

The story of an exceptional serine protease, tissue-type plasminogen activator (tPA)

L'histoire d'une sérine protéase exceptionnelle, l'activateur tissulaire du plasminogène

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ABSTRACT

The only acute treatment of ischemic stroke approved by the health authorities is tissue recombinant plasminogen activator (tPA)-induced thrombolysis. Under physiological conditions, tPA, belonging to the serine protease family, is secreted by endothelial and brain cells (neurons, astrocytes, microglia, oligodendrocytes). Although revascularisation induced by tPA is beneficial during a stroke, research over the past 20 years shows that tPA can also be deleterious for the brain parenchyma. Thus, in this review of the literature, after a brief history on the discovery of tPA, we reviewed current knowledge of mechanisms by which tPA can influence brain function in physiological and pathological conditions.

RÉSUMÉ

Le seul traitement aigu autorisé de l'accident vasculaire cérébral ischémique est la thrombolyse par l'activateur tissulaire du plasminogène recombinant (tPA). Le tPA est une sérine protéase sécrétée de manière physiologique par les cellules endothéliales et cérébrales (neurones, astrocytes, microglie, oligodendrocytes). Bien qu'il existe un effet bénéfique du tPA dans le cadre de la revascularisation cérébrale après AVC ischémique, les recherches de ces 20 dernières années montrent que le tPA peut-être également délétère pour le parenchyme cérébral. Ainsi, dans cette revue de la littérature, après un bref rappel historique sur la découverte du tPA, nous passerons en revue les connaissances actuelles des mécanismes par lesquels le tPA peut influencer le fonctionnement cérébral en conditions physiologique et pathologique.

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1. The history of tPA

In ancient Greece, Hippocrate had already observed that blood from dead people was not clotted. It is in 1844 that Andral put forward the idea that clotted blood can become again liquid, giving to the scientific community the first data on the fibrinolytic system [1]. In 1893, Denys & Marbaix and Dastre determined that this process was the consequence of a proteolytic mechanism that they name fibrinolysis (for fibrin and lysis) [2,3]. In 1902, Conradi brought the first observations of degradation of blood clots by adding extracts of different organs [4]. In 1904, Hedin identified a proteolytic activity in the serum globulin fraction, that was later identified as the fraction containing the plasminogen, the precursor of the enzyme responsible of the fibrin clot lysis, plasmin [5]. This inactive precursor plasminogen presents in the circulation can be activated by bacterial extracts (the proteins involved in this process were identified later as streptokinase and staphylokinase). This observation was later confirmed and refined by Fleisher & Loeb in 1915 and by Astrup in 1947, providing the concept of thrombolysis (degradation of pre-formed clots) [6,7]. It was only in 1979 that the effector of this cascade of events, tissue-type plasminogen activator (tPA) was purified and characterized first in circulation and then in the uterus [8,9]. The first use in humans was conducted in 1981 in Rotterdam by W. Weimar on patients with thrombosis of the renal vein. This led, in 1983, to the cloning of tPA and its recombinant production [10]. 32 years later, tPA is now used in clinic to promote fibrinolysis, especially at the acute phase of ischemic stroke either alone [11] or combined with thrombectomy [12]. In parallel of its well-known roles in the circulation, tPA also displays critical functions in the brain parenchyma. Here, we reviewed our present knowledge of the mechanisms and functions of tPA in the central nervous system (CNS), both in physiological and pathological conditions.

2. Structure and functions of tPA

tPA is a glycoprotein belonging to the superfamily of serine proteases and is a member of the chymotryspin family. In 1983, Pennica has cloned and produced a functional recombinant tPA, composed of 527 amino acids, with a molecular mass of 70 kDa. The access to the tPA nucleotide sequence has allowed the identification of potential glycosylation sites and disulfide bridges, and therefore the determination of its three dimensional structure [10]. tPA is a protein composed of five domains, maintained in its conformation thanks to 17 disulfide bridges (Fig. 1A). The N-terminal end of tPA starts by a finger domain (also called fibronectin domain, Fig. 1A–C). This domain is involved in the binding of tPA to fibrin, resulting in the formation of a ternary complex with plasminogen [13]. Through this finger domain tPA can also interacts with several membrane receptors including the Low Density Lipoprotein Receptor-related Protein (LRP) [14] and Annexin II [15]. The second domain, called epidermal growth factor-like domain, due to its homology with the epidermal growth factor (EGF, Fig. 1A–C), allows tPA to activate EGF receptor [16]. The sequence continues by the Kringle 1 and the Kringle 2 domains (respectively: K1 and K2, Fig. 1A–C). These domains are characterized by an active site having a high affinity for lysine (lysine binding site; LBS), which is composed of two hydrophobic aromatic amino acids (Trp^{242} and Trp^{253}) forming a pocket in the tertiary structure of the protein. The precise role of the K1 domain is not well known. Although its LBS domain is not functional, the glycosylation at the Asp^{117} is important for the uptake-clearance of tPA by liver endothelial cells via the mannose receptor [17]. The K2 domain contains a functional LBS. The K2 domain is reported to be involved in the capacity of tPA to bind and activate substrates and/or receptors such as plasminogen, the PDGF-CC (Platelet Derived Growth Factor-CC) [18] and the NMDAR (N-methyl-D-Aspartate Receptor) [19,20]. All these domains (Finger, EGF, K1 and K2) form the heavy chain of tPA (A-chain). The second chain, the light chain (B-chain), consists of a single large domain containing the catalytic activity of the protease (Fig. 1A–C). The catalytic triad is composed of the amino acids His^{322}, Asp^{371} and Ser^{478} [10,21], that enables the activation of plasminogen to plasmin. Like all serine proteases, tPA exists in two forms: single chain tPA (sc-tPA) and two chains (tc-tPA). In contrast to the other known serine proteases, which are inactive under their single chain form, the two forms of tPA are proteolytically active [22] (Fig. 1D). The processing of sc-tPA into tc-tPA is mediated by other proteases such as plasmin [23] or kallikreins [24,25]. In the absence of an allosteric regulator such as fibrin, tc-tPA is catalytically more active than sc-tPA [23,26]. However, in the presence of fibrin, both sc-tPA and tc-tPA display the same catalytic or fibrinolytic activity [27].

3. Cellular localization of tPA in the brain

tPA was primarily identified in the blood circulation [8,28]. In this compartment, it is mainly produced and released by endothelial cells [29]. In the brain parenchyma, tPA has been reported to be expressed and synthesized by most of the cell types: astrocytes [30], neurons [31] (Figs. 2A and 3), oligodendrocytes [16] and microglia [32]. In neurons, the presence of tPA can be observed in dendrites and synapses [33], where tPA is stored in pre-synaptic vesicles [34] and released in the synaptic cleft following a depolarization [35] (Fig. 2A). Astrocytes are capable to regulate the quantity of tPA at the synaptic cleft. Indeed, tPA can be endocytosed by astrocytes involving its Finger domain and mediated by LRP receptors [36] (Fig. 2A). Interestingly, astrocytes are also capable to release the tPA previously recaptured by a mechanism involving PKC through a mechanism dependent of the activation of kainate receptors by the glutamate present at the synaptic cleft [36] (Fig. 2A).

4. tPA inhibition in the brain

Only few studies have correlated the levels of tPA mRNA with its enzymatic activity in the brain parenchyma. For example, although tPA mRNA can be detected in the entire hippocampus, no proteolytic activity can be found in the CA1 region [37]. This strongly suggests the presence and differential expression of

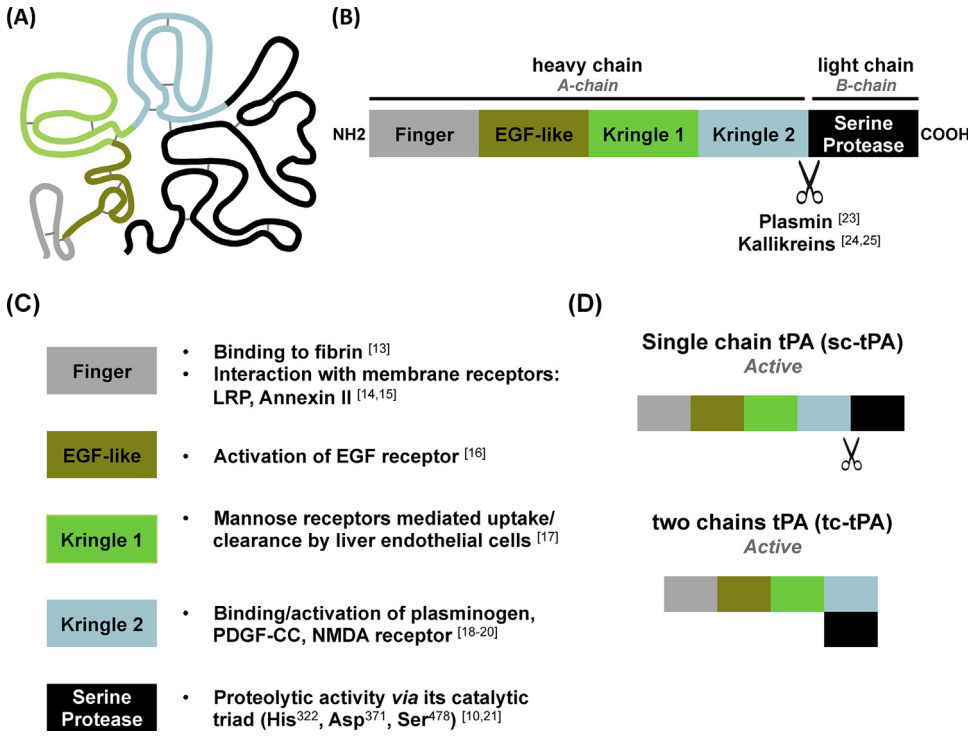

Fig. 1 – Structure and functions of tissue-type Plasminogen Activator (tPA). (A) Structure of tPA. The 527 amino acids sequence of the protein can be subdivided in five domains that are depicted as follow: 'Finger domain' is in light grey, 'EGF-like domain' is in dark green, 'Kringle 1' domain is in light green, 'Kringle 2' domain is in blue, and finally 'serine protease' domain is depicted in black. The 17 disulfure bridges are depicted by grey lines. (B) tPA is composed of two chains: the heavy chain (also called A-chain), starting at the amino-terminal end of the protein and containing the 'Finger', 'EGF-like', 'Kringle 1' and 'Kringle 2' domains, and the light chain (also called B-chain), ending at the carbocyl-terminal end of the protein and containing only the 'serine protease' domain. Plasmin and kallikreins can cleave tPA at the junction between these two chains. (C) Main roles of the five domains. (D) The cleavage of tPA by plasmin and kallikreins allow the conversion of the 'single chain tPA' (sc-tPA) form into a 'two chains tPA' (tc-tPA) form. In contrast of the other serine proteases, the two forms of tPA are proteolytically active. Numbers in brackets refer to references.

inhibitors of tPA proteolytic activity in these regions: the serine proteases inhibitors (serpins). Currently, four serpins have been described to inhibit tPA proteolytic activity: type 1 and 2 plasminogen activator inhibitor (PAI-1 and -2), neuroserpin (NS) and the protease nexin-1 (PN-1) [38,39]. In the brain parenchyma, PAI-1 and NS are the two main inhibitors of tPA (Fig. 2B). PAI-1 is a suicide inhibitor that forms irreversible complexes with tPA [40]. In the brain parenchyma, PAI-1 is expressed at very low levels by neurons and astrocytes under physiological conditions [30,41], but dramatically over-expressed by reactive astrocytes [42,43]. In contrast of PAI-1, NS is highly expressed under physiological conditions by both neurons and astrocytes [38,42]. NS also acts as a substrate of tPA but without formation of an irreversible protease-serpin complex, as previously reported for tPA and PAI-1[44]. In the brain parenchyma, NS mRNA are detected from the early embryonic to adult stages, and are widely distributed in numerous brain structures [45]. Interestingly, accumulation of NS in neuronal bodies is associated with a familial encephalopathy, named FENIB (Familial Encephalopathy with Neuroserpin Inclusion Bodies), dues to the presence of a mutated form of NS [46]. NS have been also described to play a role in the etiology of epilepsy and schizophrenia [47,48].

5. tPA-dependent functions in the brain parenchyma

5.1. *Development and cellular migration*

tPA is highly expressed in embryonic regions undergoing cell migration and tissue remodeling (Fig. 4). For example, Krystosek and Seed evidenced that tPA was secreted by granule neurons in the cerebellum during development [49]. In this structure, the expression of tPA mRNA and tPA proteolytic activity closely correlate with the neuronal migration phase [50,51] (Fig. 4). Accordingly, mice lacking tPA display a delayed neuronal migration when compared with their wild type littermates [52]. A recent study also showed that Pituitary adenylate cyclase-activating polypeptide (PACAP) induces tPA release implicated in the extracellular matrix (ECM) degradation, during the neuronal migration [53].

5.2. *Long-term potentiation*

Chemical or high frequency stimulation of hippocampal neurons led to an increase of synaptic transmission efficiency.

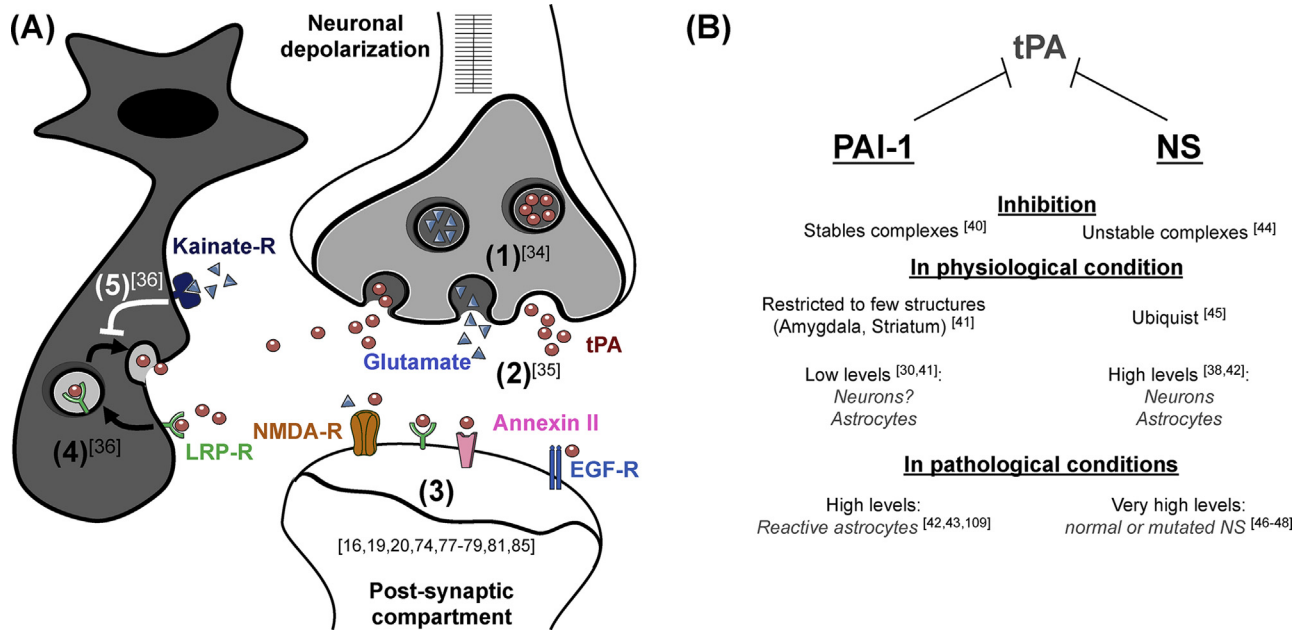

Fig. 2 – Release, post-synaptic interactions, recapture and inhibition of tPA in the brain parenchyma. A. Release and recapture of tPA at the synaptic cleft. tPA is stored in neuronal presynaptic vesicles (1) and released into the synaptic cleft conjointly to glutamate following a neuronal depolarization (2). Once into the synaptic cleft, tPA can mediate its effects by different mechanisms on different postsynaptic targets (3). In addition, astrocytes are capable to regulate the quantity of tPA at the synaptic cleft to avoid an excess of tPA. Indeed, they can endocytose tPA by a mechanism involving its Finger domain and mediated by astrocytic LRP receptors (4), and release it by a mechanism involving PKC signaling pathway and dependent of kainate receptors activation by glutamate (5). B. PAI-1 and NS are the two main inhibitors of tPA in the brain parenchyma: mechanisms of inhibition, cellular and tissular synthesis and modified expression in different brain pathologies. EGF-R: Epidermal Growth Factor receptor; Kainate-R: Kainate receptor; LRP-R: Low Density Lipoprotein Receptor-related Protein receptor; MMPs: matrix metalloproteinases; NMDA-R: N-methyl-D-Aspartate Receptor; PDGFR-α: Platelet-Derived Growth Factor Receptor α; NS: Neuroserpin, PAI-1: Type 1 Plasminogen Activator Inhibitor. Numbers in brackets refer to references.

This phenomenon, initially discovered in the hippocampus and called long-term potentiation [54] (LTP), is considered as the molecular and cellular support of learning and memory processes [55]. The induction of LTP on hippocampal slices leads to a rapid increase of tPA mRNA levels in granular cells of the dentate gyrus [56]. On hippocampal slices, administration of tPA inhibitors (PAI-1 or tPA stop) was reported to inhibit the late phase of LTP either induced by forskolin or a tetanic stimulation [57]. In addition, tPA deficient mice present an altered late LTP in CA1 [58], based on modification of the GABAergic neurotransmission [59]. tPA-dependent ECM degradation [57], its ability to activate the neurotrophic factor proBDNF into mBDNF [60] and to cleave reelin [61], are three putative mechanisms through which tPA could contribute to synaptic plasticity and tissue remodeling. Interestingly, only the sc-tPA is capable to increase the late phase of LTP, a mechanism dependent of NMDAR [62] (Fig. 3).

5.3. *Behavioral processes*

According to its localization in the hippocampus, and to its involvement in synaptic plasticity, tPA has been described as an important actor of spatial learning (results summarized in Fig. 4 and Table 1). When tested in an object recognition task, tPA deficient mice display an impairment to react to a spatial configuration change, without impairment of the detection of non spatial changes [63]. Similarly tPA deficient mice show impaired spatial learning abilities in a two-trial place recognition task in a Y-Maze [64], as well as in a Morris water maze task [65]. In the hippocampus, the interaction between tPA and the NMDA receptor seems to be the key mechanism by which tPA play its role in spatial learning [64,66].

tPA is also expressed in the amygdala and in the Bed Nucleus of the Stria Terminalis (BNST) [67–69]. According to these localizations, tPA plays roles in stress response, anxiety and learned fear (results summarized in Table 1). For instance, tPA is involved in the acquisition and retention of a contextual and/or cued learned fear, even if some results found in the literature are sometimes ambiguous [58,63,70]. tPA is also involved in the mediation of stress effects. Although restraint stress or Corticotrophin-Releasing Factor-induced response lead to an exacerbation of anxiety-like behaviors in wild type animals, all these effects are abrogated in tPA deficient mice [67–69]. Accordingly, NS deficient mice display an increased anxiety-like behavior, assessed using a zero-maze task, compared to their wild-type littermates [71].

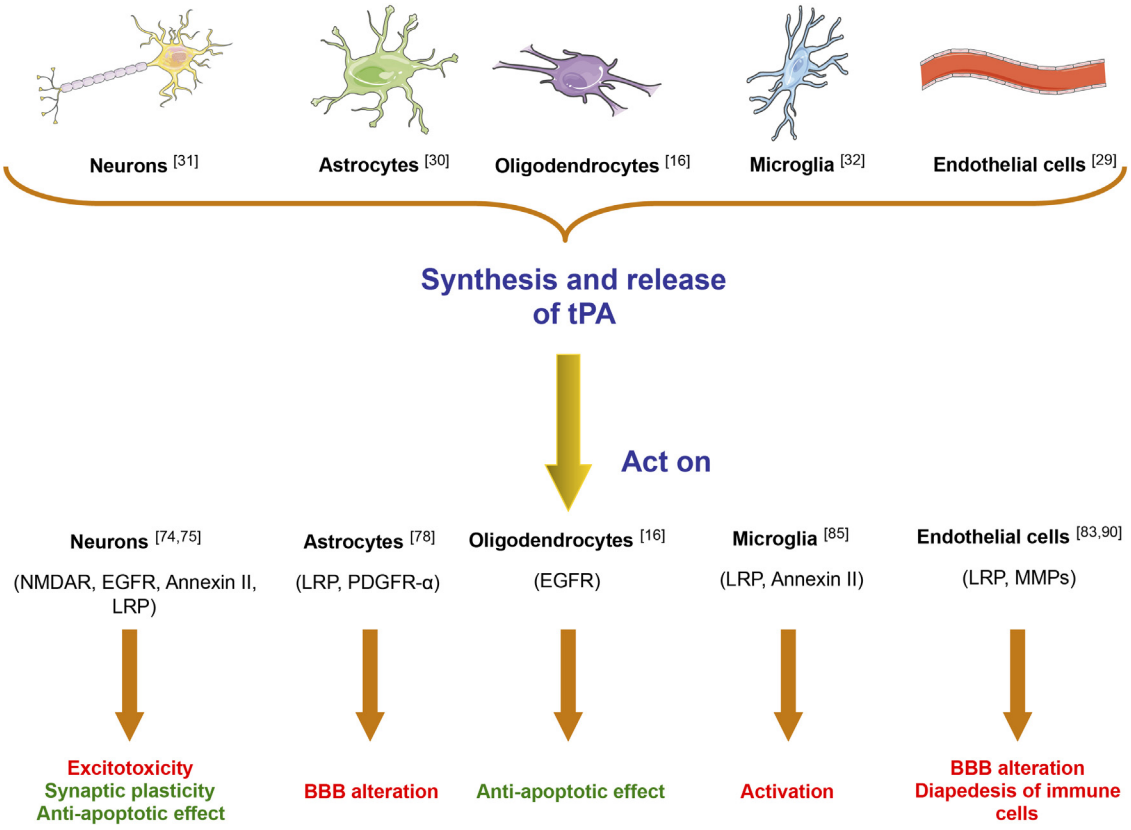

Fig. 3 – Source of tPA in the brain, targets and effects. tPA can be synthesized and released by most of the brain cells. Once released, it can be fixed to these same cells via different receptors (in brackets). The interaction of tPA with these receptors leads to different effects that can be beneficial (in green) or deleterious (in red). The exogenous tPA injected for thrombolysis after a ischaemic stroke is capable of binding to these same cells through the same receptors and have similar effects. EGF-R: Epidermal Growth Factor receptor; LRP-R: Low Density Lipoprotein Receptor-related Protein receptor; NMDA-R: N-methyl-D-Aspartate Receptor. Numbers in brackets refer to references.

5.4. *Neuronal death and survival*

Several studies have reported that neuroserpin and PAI-1 protected neurons against NMDARs over-activation-induced toxicity [30,72,73]. Accordingly, exogenous tPA has been reported to induce excitotoxic neuronal death mediated by over-activation of NMDARs [74,75] (Figs. 3 and 4), by plasmin-dependent or -independent mechanisms [62,74,76]. For instance, tPA is capable to interact with the GluN1 subunit of NMDAR via its LBS localized on the K2 domain [19,20]. This interaction allows the cleavage of the amino-terminal domain of the GluN1 subunit, leading to the enhancement of NMDAR signaling by sc-tPA [74,77]. However, other authors did not detect such tPA-mediated cleavage of the GluN1 subunit, despite enhancement of NMDAR function by exogenous tPA in cortical cultures [78]. In addition, LRP receptors could act as tPA co-receptors, which in turn enhance Ca^{2+} influx through NMDARs [78]. Interestingly, in response to tPA, LRP1 has been described as able to assemble a co-receptor system to initiate cell-signaling; this system is composed of LPR1, NMDAR, and Trk receptors [79].

However, it is interesting to note that, in addition to its role in excitotoxic neuronal death, both in vivo, in vitro and ex vivo studies also suggest that tPA may have pro-survival and anti-apoptotic effects on both neurons and oligodendrocytes [16,80–84] (Fig. 3). Two candidates have been proposed as receptors mediating the pro-survival effects of tPA: Annexin II and EGF receptor (EGFR) [16,81,85] (Fig. 3). Despite the heterogeneity of the paradigms used, most of these studies propose that these trophic effects of tPA occurs independently of its proteolytic activity, with the activation of either PI3 K/Akt-, AMPK- or mTor-HIF-1alpha-dependent signaling pathways [16,86].

Although the deleterious effects of tPA have been extensively reported from animal models (especially its neurotoxicity), it is still a debate in human. Only few papers have reported indirect proofs of this neurotoxicity. For example, in 2015 Alvarez showed that thrombolysis with rt-PA may increase the likelihood of epileptic seizures at the acute phase of ischemic stroke patients, independently of the recanalization rate or of symptomatic intracerebral hemorrhages [87]. This paper supports previous work from Tsirka et al. in 1995 demonstrating in preclinical models that tPA promoted seizures [88]. Tisserand also reported, in human, that a tPA treatment was associated with increased lesion volumes in the grey matter and reduced damages in the white matter following cerebral ischemia [89]. These observations are also supported by data obtained from experimental models of stroke [16].

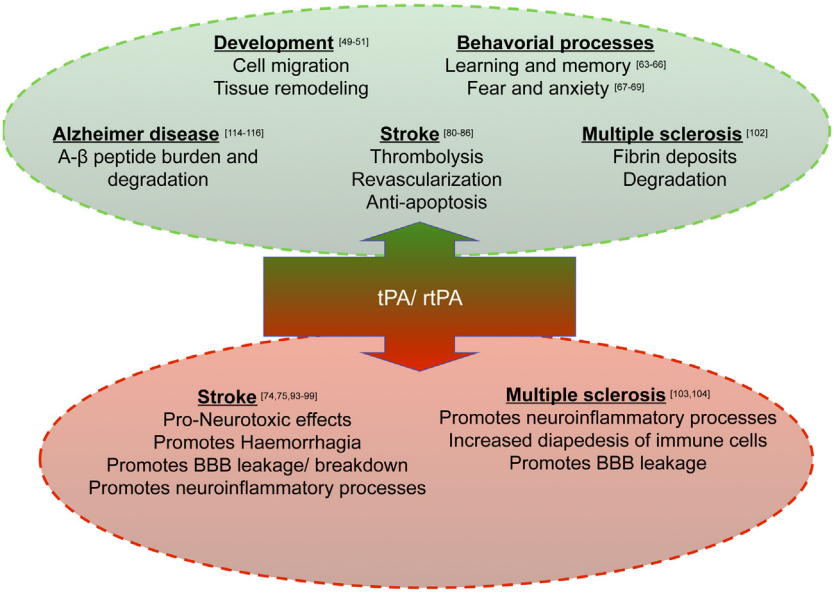

Fig. 4 – tPA, a multifaceted protease with beneficial and deleterious effects. In the green circle are summarized the principal beneficial effects of endogenous/exogenous tPA in physiological situation (on cerebral development and on the regulation of behavorial processes) but also in pathophysiological situation like in stroke, Alzheimer disease or multiple sclerosis. Conversely, in the red circle are summarized the main adverse effects of tPA in pathological conditions such as in stroke and multiple sclerosis. A.β: β-amyloid peptide. BBB: Blood-Brain Barrier; rtPA: recombinant tPA. Numbers in brackets refer to references.

Up to now, there is no clear clinical data to determine, in human, whether tPA is neurotrophic or neurotoxic. Additional studies are needed to further understand the possible differential functions of endogenous versus exogenous tPA on neuronal survival.

5.5. *tPA and the homeostasis of the Blood Brain Barrier*

In experimental models, the intraveinous administration of rtPA 4 hours post-ischemia results in permeabilization of the BBB [73]. The alterations of the integrity of the BBB induced by tPA are due to different molecular mechanisms involving LRP receptors, NMDAR and metalloproteinases (MMPs) (Fig. 3). MMPs, including MMP-9, are overexpressed after a cerebral ischemia [90], leading to an increased degradation of type IV collagen, laminin and fibronectin [91]. In 2008, Cuadrado showed that neutrophils are the main source of MMP-9 following stroke and tPA treatment, a mechanism involved in the permeabilization of the BBB and in the occurrence of hemorrhagic transformations [92]. By interacting with the LRP receptors and subsequent cleavage of their ectodomain, tPA promotes the removal of astrocytic feet from the basal lamina and permeabilization of the BBB [83]. tPA is also capable to interact with and activate PDGF-CC, a mechanism dependent of both its Kringle2 domain and its catalytic activity. Then, cleaved PDGF-CC activates PDGFR-α (Platelet Derived Growth Factor Receptor-alpha) of perivascular astrocytes promoting BBB leakage and bleeding [18,93] (Fig. 3). Accordingly, co-injection of tPA with an inhibitor of PDGFR-a receptors (Imatinib, Glivec) significantly reduced rtPA-induced hemorrhagic transformations [94].

Hemorrhagic transformation induced by rtPA is the most feared complication (Fig. 4). As mentioned above, the state of the BBB and the delay before treatment seems to play a critical role on the intra-cerebral haemorrhage (Fig. 4). Majority of works that have studied the haemorrhagic transformation with intravenous tPA performed within 3 h confirmed the rate of bleeding observed in the NINDS study: 6.4% of patient showed a haemorrhagic transformation [11]. Others important studies showed and confirmed that as compared to placebo, rtPA was more frequently associated with symptomatic intracranial hemorrhage when thrombolysis is performed between 3 and 4.5 hours after the onset of symptoms. [95,96] (Figs. 3 and 4).

5.6. *tPA and inflammation*

In vivo, a late i.v. administration of rtPA (4 hours post-occlusion) is associated, 24 hours post-ischemia, with an overexpression of cellular adhesion molecule E- and P-selectin, ICAM-1 which are involved in leukocyte infiltration (Fig. 4).

Compared to rtPA alone, rtPA administration combined with the ICAM-1 inhibition reduces lesion volume ($28.9 \pm 2.7\%$ versus $39.1 \pm 3.9\%$; $P < 0.05$) [97]. Similarly, the combined injection of statins and rtPA 4 hours post-ischemia inhibits the expression of MMP-9, PAR-1 and ICAM-1 and reduced the lesion volume [98]. The interaction of the finger domain of tPA with annexin II [85] or LRP-1 microglial receptor [99] activates microglia, inducing the production of NO and secretion of tPA by activated microglia [32] (Fig. 3). These processes contribute to the worsening of brain damage. Thus, in ischemic conditions tPA promotes the inflammatory response but is

Table 1 – Roles of tPA in behavioral processes. Numbers in brackets refer to references.

Study by	Species/strains	Task	Results
Spatial learning			
Calabresi et al., 2000 [63]	tPA +/+ and tPA–/–	Object recognition	Hippocampal tPA is needed during spatial learning
Benchenane et al., 2007 [64]	tPA +/+ and tPA–/–	Y-Maze two-trial place recognition	Hippocampal tPA is needed during spatial learning
	tPA +/+ immunized with an antibody blocking the interaction between tPA and NMDAR or with vehicle	Y-Maze two-trial place recognition	Interaction between tPA and NMDAR in the hippocampus is one of the mechanism by which tPA promotes spatial learning
Obiang et al., 2012 [66]	C57BL6/J immunized with an antibody blocking the interaction between tPA and NMDAR or with vehicle	Y-Maze two-trial place recognition	Interaction between tPA and NMDAR in the hippocampus is one of the mechanism by which tPA promotes spatial learning
Oh et al., 2014 [65]	tPA +/+ and tPA –/–	Morris water maze	Hippocampal tPA is needed during spatial learning
Stress response, anxiety and learned fear			
Stress response and anxiety			
Madani et al., 2003 [71]	NS +/+ and –/– mice	Zero-Maze	NS is a determinant of anxiety level, via a mechanism independent of tPA proteolytic activity
Pawlak et al., 2003 [69]	tPA +/+ and tPA –/– mice subjected to acute restraint stress	Elevated Plus Maze	In the amygdala, tPA plays a key role in the development of stress-induced anxiety behavior by promoting synaptic remodeling
Matys et al., 2004 [67]	tPA +/+ and tPA –/– mice injected with stress peptide hormone CRF or with vehicle	Elevated Plus Maze	In the amygdala, tPA plays a key role in the development of stress-induced anxiety behavior by promoting responses to CRF
Matys et al., 2005 [68]	tPA +/+ and tPA –/– mice subjected to acute restraint stress	Acoustic startle	In the BNST, tPA mediates the potentiation of the acoustic startle response by stress and CRF, by promoting neuronal activation
Contextual and/or cued learned fear			
Huang et al., 1996 [58]	tPA +/+ and tPA –/– mice	Contextual and cued fear conditioning	Hippocampal tPA seems to be needed for the learning of both contexual or cued fear, but this is gender-dependent
Calabresi et al., 2000 [63]	tPA +/+ and tPA –/– mice	Contextual and cued fear conditioning	Hippocampal tPA is crucial for the learning of a contextual fear
Barnes and Thomas, 2008 [70]	Lister hooded rats injected with an inhibitor of tPA proteolytic activity (tPA stop) or with vehicle	Contextual fear conditioning	Control of pro/mBNDF ratios in the hippocampus by tPA plays a central role in the development/maintenance of a learned contexual fear

also beneficial for stimulating both macrophage migration on the sites of axonal degeneration and MMP-9 expression, that promotes axonal regrowth [100] (Figs. 3 and 4).

Targeting brain inflammation could be an interesting therapeutic strategy. However, inflammation processes are necessary to eliminate cell debris and to fight against infections, their uncontrolled inhibition could thus be deleterious [101].

5.7. *Role in axonal damage, myelinisation and regeneration*

tPA was reported protective against axonal damage and to favour axonal regeneration through the proteolytic removal of fibrin deposits in inflammatory conditions [102] (Fig. 4). In neuroinflammatory conditions such as multiple sclerosis (MS), efficient fibrin removal is impaired, and this is associated with a decreased expression of tPA and an increased expression of PAI-1 [103,104]. In experimental autoimmune encephalitis (EAE, a mouse model of MS), tPA deficient mice show more severe symptoms and impaired recovery [105,106], whereas PAI-1 deficient mice show a delayed onset and less severe symptoms [107]. More specifically, tPA action on axonal regeneration is thought to involve the degradation of chondroitin sulphate proteoglycans (CSPGs), a set of ECM proteins with inhibitory action on axon regrowth both in vitro and in vivo [108–110]. Thus, the combination of tPA with chondroitinase ABC promotes axonal regeneration in experimental models of spinal cord injury (SCI) [108]. In addition, tPA has been shown to activate the CSPG-degrading protease ADAMTS-4 (a desintegrin and metalloprotease with thrombospondin domains-4) following SCI, thus promoting axonal growth and functional recovery [109]. It is also interesting to note that tPA, through its EGF domain, was reported to display oligotrophic functions, promoting survival of oligodendrocytes, thus reducing white matter damages following cerebral ischemia [16] (Figs. 3 and 4).

5.8. *tPA and aging*

During physiological aging, the cerebral proteolytic activity of tPA decrease [111–113]. Such modifications of the levels of tPA in the brain during physiological aging have functional consequences. For example, in the hippocampus this decrease of tPA levels leads to spatial memory impairments [113]. Interestingly, there is numerous links existing in the literature between the tPA/plasminogen system, tPA inhibitors and Alzheimer Disease (AD; Fig. 4) including activity on A-β peptide burden and degradation (Fig. 4). For instance, plasmin has been shown to promote Aβ degradation [114–116]. In AD, a dramatic increase of PAI-1 was reported, produced by glial cells, thus worsened the decrease of tPA proteolytic activity occurring during physiological aging [111,117,118] (Fig. 2). During cerebral ischemia, the decrease of tPA with physiological aging is associated with reduced ischemic lesion volumes in the grey matter and worsened alterations of the white matter [16,112].

6. Conclusions and perspectives

Tissue-type plasminogen activator (tPA) is an extracellular proteolytic enzyme that was first described for its effects on blood coagulation and extracellular matrix homeostasis. However, during the last twenty years, tPA has been shown to have numerous functions in brain physiology and pathology (Fig. 4). tPA can act on virtually all cell types of the brain, including neurons, endothelial and glial cells. tPA conducts its actions by enzymatic or growth-factor-like effects on various molecular substrates or receptors. Its first described action was the conversion of the zymogen plasminogen into the active enzyme plasmin, but tPA has since been discovered to drive multiple and sometimes even opposite effects by interacting with or activating BDNF, NGF, PDGF-CC Annexin II, LRP, or NMDAR (Fig. 3). It is therefore often referred to as a 'double-edged sword'. A lot of interest has been therefore given in the last years to the potential clinical relevance of targeting tPA for neuroprotection or modulation of neuronal plasticity in different diseases of the brain such as stroke, multiple sclerosis or Alzheimer's disease. Thus, a challenging question is whether these effects of tPA, including neurotoxicity, can be exerted by exogenous recombinant tPA when injected to stroke patients in an attempt to achieve reperfusion. New therapeutic strategies for stroke are in development. For example, safer thrombolytics have been produced [20,62]. Antibodies have been also developed to prevent the interaction of tPA with NMDAR capable to extend the therapeutic window of thrombolysis when injected 4 hours after stroke onset in mice [119]. Other approaches such as a direct intra-arterial endovascular reperfusion of rtPA are also promising [120]. Future therapeutic strategies for AD could emerge *via* the manipulation of tPA and plasmin activity in order to promote the degradation of Aβ. In multiple sclerosis whether tPA can help the axonal regeneration or remyelinisation is also an opened avenue. Overall, further studies should provide a better understanding of the complex effects of tPA and its endogenous regulators. This should enable a better discrimination between the different actions of tPA. With regard to the involvement of tPA in brain diseases, investigation on new treatments for brain pathologies should gain from these future progresses.

Disclosure of interest

The authors declare that they have no competing interest.

REFERENCES

[1] Andral G. Pathological hæmatology: an essay on the blood in disease. Philadephia: Lea and Bl; 1844.
[2] Denys J, de Marbaix H. Les peptonisations provoquées par le chloroforme. Cellule 1889;5:197–251.
[3] Dastre A. Fibrinolyse dans le sang. Arch Physiol Norm Path 1893;5:661.
[4] Conradi H. Über die Beziehung der Autolyse zur Blutgerinnung. Beitr Chem Physiol Path 1902;1:136.
[5] Hedin SG. On the presence of a proteolytic enzyme in the normal serum of the ox. J Physiol 1903;30:195–201.
[6] Fleisher M, Loeb R. On tissue fibrinolysis. J Biol Chem 1915;21:477–501.
[7] Astrup TY, Permin P. Fibrinolysis in the animal organism. Nature 1947;159:681–2.
[8] Binder BR, Spragg J, Austen KF. Purification and characterization of human vascular plasminogen activator derived from blood vessel perfusates. J Biol Chem 1979;254:1998–2003.
[9] Rijken DC, Wijngaards G, Jong MZ, Welbergen J. Purification and partial characterization of plasminogen activator from human uterine tissue. Biochim Biophys Acta 1979;580:140–53.
[10] Pennica D, Holmes WE, Kohr WJ, Harkins RN, Vehar GA, Ward CA, et al. Cloning and expression of human tissue-type plasminogen activator cDNA in *E. coli*. Nature 1983;301:214–21.
[11] The National Institute of Neurological Disorders and Stroke rt-PA Stroke Study Group (NINDS). Tissue plasminogen activator for acute ischemic stroke. N Engl J Med 1995;333:1581–7.
[12] Goyal M, Demchuk AM, Menon BK, Eesa M, Rempel JL, Thornton J, et al. Randomized assessment of rapid endovascular treatment of ischemic stroke. N Engl J Med 2015;372:1019–30. http://dx.doi.org/10.1056/NEJMoa1414905.
[13] Kagitani H, Tagawa M, Hatanaka K, Ikari T, Saito A, Bando H, et al. Expression in *E. coli* of finger-domain lacking tissue-type plasminogen activator with high fibrin affinity. FEBS Lett 1985;189:145–9.
[14] Bu G, Williams S, Strickland DK, Schwartz AL. Low density lipoprotein receptor-related protein/alpha 2-macroglobulin receptor is an hepatic receptor for tissue-type plasminogen activator. Proc Natl Acad Sci U S A 1992;89:7427–31.
[15] Hajjar KA, Jacovina AT, Chacko J. An endothelial cell receptor for plasminogen/tissue plasminogen activator. I. Identity with annexin II. J Biol Chem 1994;269:21191–7.
[16] Correa F, Gauberti M, Parcq J, Macrez R, Hommet Y, Obiang P, et al. Tissue plasminogen activator prevents white matter damage following stroke. J Exp Med 2011;208:1229–42. http://dx.doi.org/10.1084/jem.20101880.
[17] Kuiper J, Otter M, Rijken DC, van Berkel TJ. Characterization of the interaction in vivo of tissue-type plasminogen activator with liver cells. J Biol Chem 1988;263:18220–4.

[18] Fredriksson L, Li H, Fieber C, Li X, Eriksson U. Tissue plasminogen activator is a potent activator of PDGF-CC. EMBO J 2004;23:3793–802. http://dx.doi.org/10.1038/sj.emboj.7600397.
[19] Lopez-Atalaya JP, Roussel BD, Levrat D, Parcq J, Nicole O, Hommet Y, et al. Toward safer thrombolytic agents in stroke: molecular requirements for NMDA receptor-mediated neurotoxicity. J Cereb Blood Flow Metab 2008;28:1212–21. http://dx.doi.org/10.1038/jcbfm.2008.14.
[20] Parcq J, Bertrand T, Baron AF, Hommet Y, Anglès-Cano E, Vivien D. Molecular requirements for safer generation of thrombolytics by bioengineering the tissue-type plasminogen activator A chain. J Thromb Haemost 2013;11:539–46. http://dx.doi.org/10.1111/jth.12128.
[21] Rathore YS, Rehan M, Pandey K, Sahni GA. First structural model of full-length human tissue-plasminogen activator: a SAXS data-based modeling study. J Phys Chem B 2012;116:496–502. http://dx.doi.org/10.1021/jp207243n.
[22] Rijken DC, Hoylaerts M, Collen D. Fibrinolytic properties of one-chain and two-chain human extrinsic (tissue-type) plasminogen activator. J Biol Chem 1982;257:2920–5.
[23] Wallén P, Bergsdorf N, Rånby M. Purification and identification of two structural variants of porcine tissue plasminogen activator by affinity adsorption on fibrin. Biochim Biophys Acta 1982;719:318–28.
[24] Blaber M, Yoon H, Juliano MA, Scarisbrick IA, Blaber SI. Functional intersection of the kallikrein-related peptidases (KLKs) and thrombostasis axis. Biol Chem 2010;391:311–20. http://dx.doi.org/10.1515/BC.2010.024.
[25] Ichinose A, Kisiel W, Fujikawa K. Proteolytic activation of tissue plasminogen activator by plasma and tissue enzymes. FEBS Lett 1984;175:412–8.
[26] Petersen LC, Johannessen M, Foster D, Kumar A, Mulvihill E. The effect of polymerised fibrin on the catalytic activities of one-chain tissue-type plasminogen activator as revealed by an analogue resistant to plasmin cleavage. Biochim Biophys Acta 1988;952:245–54.
[27] Thelwell C, Longstaff C. The regulation by fibrinogen and fibrin of tissue plasminogen activator kinetics and inhibition by plasminogen activator inhibitor 1. J Thromb Haemost 2007;5:804–11. http://dx.doi.org/10.1111/j.1538-7836.2007.02422.x.
[28] Collen D. On the regulation and control of fibrinolysis. Edward Kowalski Memorial Lecture. Thromb Haemost 1980;43:77–89.
[29] Levin EG, Santell L, Osborn KG. The expression of endothelial tissue plasminogen activator in vivo: a function defined by vessel size and anatomic location. J Cell Sci 1997;110:139–48.
[30] Buisson A, Nicole O, Docagne F, Sartelet H, Mackenzie ET, Vivien D. Up-regulation of a serine protease inhibitor in astrocytes mediates the neuroprotective activity of transforming growth factor beta1. FASEB J 1998;12:1683–91.
[31] Salles FJ, Strickland S. Localization and regulation of the tissue plasminogen activator-plasmin system in the hippocampus. J Neurosci 2002;22:2125–34.
[32] Siao C-J, Fernandez SR, Tsirka SE. Cell type-specific roles for tissue plasminogen activator released by neurons or microglia after excitotoxic injury. J Neurosci 2003;23:3234–42.
[33] Shin CY, Kundel M, Wells DG. Rapid, activity-induced increase in tissue plasminogen activator is mediated by metabotropic glutamate receptor-dependent mRNA translation. J Neurosci 2004;24:9425–33. http://dx.doi.org/10.1523/JNEUROSCI.2457-04.2004.
[34] Parmer RJ, Mahata M, Mahata S, Sebald MT, O'Connor DT, Miles L. Tissue Plasminogen Activator (t-PA) is targeted to the regulated secretory pathway: catecholamine storage vesicles as a reservoir for the rapid release of t-PA. J Biol Chem 1997;272:1976–82. http://dx.doi.org/10.1074/jbc.272.3.1976.
[35] Lochner JE, Honigman LS, Grant WF, Gessford SK, Hansen AB, Silverman MA, et al. Activity-dependent release of tissue plasminogen activator from the dendritic spines of hippocampal neurons revealed by live-cell imaging viral infection of primary cultures. J Neurobiol 2006;66:564–77. http://dx.doi.org/10.1002/neu.
[36] Cassé F, Bardou I, Danglot L, Briens A, Montagne A, Parcq J, et al. Glutamate controls tPA recycling by astrocytes, which in turn influences glutamatergic signals. J Neurosci 2012;32:5186–99. http://dx.doi.org/10.1523/JNEUROSCI.5296-11.2012.
[37] Sappino AP, Madani R, Huarte J, Belin D, Kiss JZ, Wohlwend A, et al. Extracellular proteolysis in the adult murine brain. J Clin Invest 1993;92:679–85. http://dx.doi.org/10.1172/JCI116637.
[38] Osterwalder T, Contartese J, Stoeckli ET, Kuhn TB, Sonderegger P. Neuroserpin, an axonally secreted serine protease inhibitor. EMBO J 1996;15:2944–53.
[39] Sprengers ED, Kluft C. Plasminogen activators inhibitors. Blood 1987;69:381–7.
[40] Strömqvist M, Karlsson KE, Björquist P, Andersson JO, Byström M, Hansson L, et al. Characterisation of the complex of plasminogen activator inhibitor type 1 with tissue-type plasminogen activator by mass spectrometry and size-exclusion chromatography. Biochim Biophys Acta 1996;1295:103–9.
[41] Hino H, Akiyama H, Iseki E, Kato M, Kondo H, Ikeda K, et al. Immunohistochemical localization of plasminogen activator inhibitor-1 in rat and human brain tissues. Neurosci Lett 2001;297:105–8.
[42] Docagne F, Nicole O, Marti HH, Mackenzie ET, Buisson A, Vivien D. Transforming growth factor- b1 as a regulator of the serpins/t-PA axis in cerebral ischemia. FASEB J 1999;13:1315–24.
[43] Lemarchant S, Docagne F, Emery E, Vivien D, Ali C, Rubio M. tPA in the injured central nervous system: different scenarios starring the same actor? Neuropharmacology 2012;62:749–56. http://dx.doi.org/10.1016/j.neuropharm.2011.10.020.
[44] Barker-Carlson K, Lawrence DA, Schwartz BS. Acyl-enzyme complexes between tissue-type plasminogen activator and neuroserpin are short-lived in vitro. J Biol Chem 2002;277:46852–7. http://dx.doi.org/10.1074/jbc.M207740200.
[45] Krueger SR, Ghisu GP, Cinelli P, Gschwend TP, Osterwalder T, Wolfer DP, et al. Expression of neuroserpin, an inhibitor of tissue plasminogen activator, in the developing and adult nervous system of the mouse. J Neurosci 1997;17:8984–96.
[46] Davis RL, Holohan PD, Shrimpton E, Tatum H, Daucher J, Collins GH, et al. Familial encephalopathy with neuroserpin inclusion bodies. Am J Pathol 1999;155:1901–13. http://dx.doi.org/10.1016/S0002-9440(10)65510-1.
[47] Vawter MP, Shannon Weickert C, Ferran E, Matsumoto M, Overman K, Hyde TM, et al. Gene expression of metabolic enzymes and a protease inhibitor in the prefrontal cortex are decreased in schizophrenia. Neurochem Res 2004;29:1245–55.
[48] Yepes M, Sandkvist M, Coleman TA, Moore E, Wu J, Mitola D, et al. Regulation of seizure spreading by neuroserpin and tissue-type plasminogen activator is plasminogen-independent. J Clin Invest 2002;109:1529–31. http://dx.doi.org/10.1172/JCI200214308.Introduction.
[49] Krystosek A, Seeds NW. Plasminogen activator secretion by granule neurons in cultures of developing cerebellum. Proc Natl Acad Sci U S A 1981;78:7810–4.

[50] Friedman GC, Seeds NW. Tissue plasminogen activator mRNA expression in granule neurons coincides with their migration in the developing cerebellum. J Comp Neurol 1995;360:658–70. http://dx.doi.org/10.1002/cne.903600410.
[51] Krystosek A, Seeds NW. Plasminogen activator release at the neuronal growth cone. Science 1981;213:1532–4.
[52] Seeds NW, Basham ME, Haffke SP. Neuronal migration is retarded in mice lacking the tissue plasminogen activator gene. Proc Natl Acad Sci U S A 1999;96:14118–23.
[53] Raoult E, Bénard M, Komuro H, Lebon A, Vivien D, Fournier A, et al. Cortical-layer-specific effects of PACAP and tPA on interneuron migration during post-natal development of the cerebellum. J Neurochem 2014;130:241–54. http://dx.doi.org/10.1111/jnc.12714.
[54] Bliss TV, Lomo T. Long-lasting potentiation of synaptic transmission in the dentate area of the anaesthetized rabbit following stimulation of the perforant path. J Physiol 1973;232:331–56.
[55] Nabavi S, Fox R, Proulx CD, Lin JY, Tsien RY, Malinow R. Engineering a memory with LTD and LTP. Nature 2014;511:348–52. http://dx.doi.org/10.1038/nature13294.
[56] Qian Z, Gilbert ME, Colicos MA, Kandel ER, Kuhl D. Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature 1993;361:453–7.
[57] Baranes D, Lederfein D, Huang Y, Chen M, Bailey CH, Kandel ER. Tissue plasminogen activator contributes to the late phase of LTP and to synaptic growth in the hippocampal mossy fiber pathway. Neuron 1998;21:813–25.
[58] Huang YY, Bach ME, Lipp HP, Zhuo M, Wolfer DP, Hawkins RD, et al. Mice lacking the gene encoding tissue-type plasminogen activator show a selective interference with late-phase long-term potentiation in both Schaffer collateral and mossy fiber pathways. Proc Natl Acad Sci U S A 1996;93:8699–704.
[59] Frey U, Müller M, Kuhl D. A different form of long-lasting potentiation revealed in tissue Plasminogen Activator mutant mice. J Neurosci 1996;16:2057–63.
[60] Pang PT, Teng HK, Zaitsev E, Woo NT, Sakata K, Zhen S, et al. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 2004;306:487–91. http://dx.doi.org/10.1126/science.1100135.
[61] Trotter JH, Lussier L, Psilos KE, Mahoney HL, Sponaugle E, Hoe H-S, et al. Extracellular proteolysis of reelin by tissue plasminogen activator following synaptic potentiation. Neuroscience 2014;274:299–307. http://dx.doi.org/10.1016/j.neuroscience.2014.05.046.
[62] Parcq J, Bertrand T, Montagne a, Baron a F, Macrez R, Billard JM, et al. Unveiling an exceptional zymogen: the single-chain form of tPA is a selective activator of NMDA receptor-dependent signaling and neurotoxicity. Cell Death Differ 2012;19:1983–91. http://dx.doi.org/10.1038/cdd.2012.86.
[63] Calabresi P, Napolitano M, Centonze D, Marfia G, Gubellini P, Teule M, et al. Tissue plasminogen activator controls multiple forms of synaptic plasticity and memory. Eur J Neurosci 2000;12:1002–12.
[64] Benchenane K, Castel H, Boulouard M, Bluthé R, Fernandez-Monreal M, Roussel BD, et al. Anti-NR1 N-terminal-domain vaccination unmasks the crucial action of tPA on NMDA-receptor-mediated toxicity and spatial memory. J Cell Sci 2007;120:578–85. http://dx.doi.org/10.1242/jcs.03354.
[65] Oh SB, Byun CJ, Yun J-H, Jo D-G, Carmeliet P, Koh J-Y, et al. Tissue plasminogen activator arrests Alzheimer's disease pathogenesis. Neurobiol Aging 2014;35:511–9. http://dx.doi.org/10.1016/j.neurobiolaging.2013.09.020.
[66] Obiang P, Macrez R, Jullienne A, Bertrand T, Lesept F, Ali C, et al. GluN2D subunit-containing NMDA receptors control tissue plasminogen activator-mediated spatial memory. J Neurosci 2012;32:12726–34. http://dx.doi.org/10.1523/JNEUROSCI. 6202-11.2012.
[67] Matys T, Pawlak R, Matys E, Pavlides C, McEwen BS, Strickland S. Tissue plasminogen activator promotes the effects of corticotropin-releasing factor on the amygdala and anxiety-like behavior. Proc Natl Acad Sci U S A 2004;101:16345–50. http://dx.doi.org/10.1073/pnas.0407355101.
[68] Matys T, Pawlak R, Strickland S. Tissue plasminogen activator in the bed nucleus of stria terminalis regulates acoustic startle. Neuroscience 2005;135:715–22. http://dx.doi.org/10.1016/j.neuroscience.2005.06.076.
[69] Pawlak R, Magarinos AM, Melchor J, McEwen B, Strickland S. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat Neurosci 2003;6:168–74. http://dx.doi.org/10.1038/nn998.
[70] Barnes P, Thomas KL. Proteolysis of proBDNF is a key regulator in the formation of memory. PLoS One 2008;3:e3248. http://dx.doi.org/10.1371/journal.pone.0003248.
[71] Madani R, Kozlov S, Akhmedov A, Cinelli P, Kinter J, Lipp HP, et al. Impaired explorative behavior and neophobia in genetically modified mice lacking or overexpressing the extracellular serine protease inhibitor neuroserpin. Mol Cell Neurosci 2003;23:473–94. http://dx.doi.org/10.1016/S1044-7431(03)00077-0.
[72] Lebeurrier N, Liot G, Lopez-Atalaya JP, Orset C, Fernandez-Monreal M, Sonderegger P, et al. The brain-specific tissue-type plasminogen activator inhibitor, neuroserpin, protects neurons against excitotoxicity both in vitro and in vivo. Mol Cell Neurosci 2005;30:552–8. http://dx.doi.org/10.1016/j.mcn.2005.09.005.
[73] Zhang Z, Zhang L, Yepes M, Jiang Q, Li Q, Arniego P, et al. Adjuvant treatment with neuroserpin increases the therapeutic window for tissue-type plasminogen activator administration in a rat model of embolic stroke. Circulation 2002;106:740–5. http://dx.doi.org/10.1161/01.CIR. 0000023942.10849.41.
[74] Nicole O, Docagne F, Ali C, Margaill I, Carmeliet P, MacKenzie ET, et al. The proteolytic activity of tissue-plasminogen activator enhances NMDA receptor-mediated signaling. Nat Med 2001;7:59–64. http://dx.doi.org/10.1038/83358.
[75] Reddrop C, Moldrich RX, Beart PM, Farso M, Liberatore GT, Howells DW, et al. Vampire bat salivary plasminogen activator (desmoteplase) inhibits tissue-type plasminogen activator-induced potentiation of excitotoxic injury. Stroke 2005;36:1241–6. http://dx.doi.org/10.1161/01.STR.0000166050.84056.48.
[76] Matys T, Strickland S. Tissue plasminogen activator and NMDA receptor cleavage. Nat Med 2003;9:371–2. http://dx.doi.org/10.1038/nm0403-371 [author reply 372–3].
[77] Fernández-Monreal M, López-Atalaya JP, Benchenane K, Cacquevel M, Dulin F, Le Caer J-P, et al. Arginine 260 of the amino-terminal domain of NR1 subunit is critical for tissue-type plasminogen activator-mediated enhancement of N-methyl-D-aspartate receptor signaling. J Biol Chem 2004;279:50850–6. http://dx.doi.org/10.1074/jbc.M407069200.
[78] Samson AL, Nevin ST, Croucher D, Niego B, Daniel PB, Weiss TW, et al. Tissue-type plasminogen activator requires a co-receptor to enhance NMDA receptor function. J Neurochem 2008;107:1091–101. http://dx.doi.org/10.1111/j.1471-4159.2008.05687.x.
[79] Mantuano E, Lam MS, Gonias SL. LRP1 assembles unique co-receptor systems to initiate cell-signaling in response

to tissue-type plasminogen activator and myelin-associated glycoprotein. J Biol Chem 2013. http://dx.doi.org/10.1074/jbc.M113.509133.
[80] Haile WB, Wu J, Echeverry R, Wu F, An J, Yepes M. Tissue-type plasminogen activator has a neuroprotective effect in the ischemic brain mediated by neuronal TNF-α. J Cereb Blood Flow Metab 2012;32:57–69. http://dx.doi.org/10.1038/jcbfm.2011.106.
[81] Lemarchand E, Maubert E, Haelewyn B, Ali C, Rubio M, Vivien D. Stressed neurons protect themselves by a tissue-type plasminogen activator-mediated EGFR-dependent mechanism. Cell Death Differ 2015. http://dx.doi.org/10.1038/cdd.2015.76.
[82] Liot G, Roussel BD, Lebeurrier N, Benchenane K, López-Atalaya JP, Vivien D, et al. Tissue-type plasminogen activator rescues neurones from serum deprivation-induced apoptosis through a mechanism independent of its proteolytic activity. J Neurochem 2006;98:1458–64. http://dx.doi.org/10.1111/j.1471-4159.2006.03982.x.
[83] Polavarapu R, Gongora MC, Yi H, Ranganthan S, Lawrence DA, Strickland D, et al. Tissue-type plasminogen activator–mediated shedding of astrocytic low-density lipoprotein receptor–related protein increases the permeability of the neurovascular unit. Blood 2007;109:3270–9. http://dx.doi.org/10.1182/blood-2006-08-043125.The.
[84] Wu F, Echeverry R, Wu J, An J, Haile WB, Cooper DS, et al. Tissue-type plasminogen activator protects neurons from excitotoxin-induced cell death via activation of the ERK1/2-CREB-ATF3 signaling pathway. Mol Cell Neurosci 2013;52:9–19. http://dx.doi.org/10.1016/j.mcn.2012.10.001.
[85] Siao C-J, Tsirka SE. Tissue plasminogen activator mediates microglial activation via its finger domain through annexin II. J Neurosci 2002;22:3352–8 [doi:20026281].
[86] Wu F, Wu J, Nicholson AD, Echeverry R, Haile WB, Catano M, et al. Tissue-type plasminogen activator regulates the neuronal uptake of glucose in the ischemic brain. J Neurosci 2012;32:9848–58. http://dx.doi.org/10.1523/JNEUROSCI.1241-12.2012.
[87] Alvarez V, Rossetti AO, Papavasileiou V, Michel P. Acute seizures in acute ischemic stroke: does thrombolysis have a role to play? J Neurol 2013;260:55–61. http://dx.doi.org/10.1007/s00415-012-6583-6.
[88] Tsirka SE, Gualandris A, Amaral DG, Strickland S. Excitotoxin-induces neuronal degeneration and seizure are mediated by tissue plasminogen activator. Nature 1995;377:340–4.
[89] Tisserand M, Malherbe C, Turc G, Legrand L, Edjlali M, Labeyrie M-A, et al. Is white matter more prone to diffusion lesion reversal after thrombolysis? Stroke 2014;45:1167–9. http://dx.doi.org/10.1161/STROKEAHA.113.004000.
[90] Romanic AM, White RF, Arleth AJ, Ohlstein EH, Barone FC. Matrix metalloproteinase expression increases after cerebral focal ischemia in rats inhibition of matrix metalloproteinase-9 reduces infarct size. Stroke 1998;29:1020–30.
[91] Cheng T, Petraglia AL, Li Z, Thiyagarajan M, Zhong Z, Wu Z, et al. Activated protein C inhibits tissue plasminogen activator-induced brain hemorrhage. Nat Med 2006;12:1278–85. http://dx.doi.org/10.1038/nm1498.
[92] Cuadrado E, Ortega L, Hernández-Guillamon M, Penalba A, Fernández-Cadenas I, Rosell A, et al. Tissue plasminogen activator (t-PA) promotes neutrophil degranulation and MMP-9 release. J Leukoc Biol 2008;84:207–14. http://dx.doi.org/10.1189/jlb.0907606.
[93] Vivien D, Gauberti M, Montagne A, Defer G, Touzé E. Impact of tissue plasminogen activator on the neurovascular unit: from clinical data to experimental evidence. J Cereb Blood Flow Metab 2011;31:2119–34. http://dx.doi.org/10.1038/jcbfm.2011.127.
[94] Rieckmann P. Imatinib buys time for brain after stroke. Nat Med 2008;14:712–3. http://dx.doi.org/10.1038/nm0708-712.
[95] Wahlgren N, Ahmed N, Dávalos A, Ford GA, Grond M, Hacke W, et al. Thrombolysis with alteplase for acute ischaemic stroke in the Safe Implementation of Thrombolysis in Stroke-Monitoring Study (SITS-MOST): an observational study. Lancet 2007;369:275–82.
[96] Hacke W, Kaste M, Bluhmki E, Brozman M, Davalos A, Guidetti D, et al. Thrombolysis with alteplase 3 to 4.5 hours after acute ischemic stroke. N Engl J Med 2008;359:1317–29.
[97] Zhang RL, Zhang ZG, Chopp M, Zivin JA. Thrombolysis with tissue plasminogen activator alters adhesion molecule expression in the ischemic rat brain. Stroke 1999;30:624–9.
[98] Zhang L, Zhang ZG, Ding GL, Jiang Q, Liu X, Meng H, et al. Multitargeted effects of statin-enhanced thrombolytic therapy for stroke with recombinant human tissue-type plasminogen activator in the rat. Circulation 2005;112:3486–94. http://dx.doi.org/10.1161/CIRCULATIONAHA.104.516757.
[99] Zhang C, An J, Haile WB, Echeverry R, Strickland DK, Yepes M. Microglial low-density lipoprotein receptor-related protein 1 mediates the effect of tissue-type plasminogen activator on matrix metalloproteinase-9 activity in the ischemic brain. J Cereb Blood Flow Metab 2009;29:1946–54. http://dx.doi.org/10.1038/jcbfm.2009.174.
[100] Ling C, Zou T, Hsiao Y, Tao X, Chen Z-L, Strickland S, et al. Disruption of tissue plasminogen activator gene reduces macrophage migration. Biochem Biophys Res Commun 2006;349:906–12. http://dx.doi.org/10.1016/j.bbrc.2006.08.111.
[101] Iadecola C, Anrather J. The immunology of stroke: from mechanisms to translation. Nat Med 2011;17:796–808. http://dx.doi.org/10.1038/nm.2399.
[102] Akassoglou K, Kombrinck KW, Degen JL, Strickland S. Tissue plasminogen activator-mediated fibrinolysis protects against axonal degeneration and demyelination after sciatic nerve injury. J Cell Biol 2000;149:1157–66.
[103] Gveric D, Hanemaaijer R, Newcombe J, van Lent NA, Sier CF, Cuzner ML. Plasminogen activators in multiple sclerosis lesions: implications for the inflammatory response and axonal damage. Brain 2001;124:1978–88.
[104] Gveric D, Herrera B, Petzold A, Lawrence DA, Cuzner ML. Impaired fibrinolysis in multiple sclerosis: a role for tissue plasminogen activator inhibitors. Brain 2003;126:1590–8. http://dx.doi.org/10.1093/brain/awg167.
[105] East E, Baker D, Pryce G, Lijnen HR, Cuzner ML, Gverić D. A role for the plasminogen activator system in inflammation and neurodegeneration in the central nervous system during experimental allergic encephalomyelitis. Am J Pathol 2005;167:545–54. http://dx.doi.org/10.1016/S0002-9440(10)62996-3.
[106] Lu W, Bhasin M, Tsirka SE. Involvement of tissue plasminogen activator in onset and effector phases of experimental allergic encephalomyelitis. J Neurosci 2002;22:10781–9.
[107] East E, Gverić D, Baker D, Pryce G, Lijnen HR, Cuzner ML. Chronic relapsing experimental allergic encephalomyelitis (CREAE) in plasminogen activator inhibitor-1 knockout mice: the effect of fibrinolysis during neuroinflammation. Neuropathol Appl Neurobiol 2008;34:216–30. http://dx.doi.org/10.1111/j.1365-2990.2007.00889.x.
[108] Bukhari N, Torres L, Robinson JK, Tsirka SE. Axonal regrowth after spinal cord injury via chondroitinase and

the tissue plasminogen activator (tPA)/plasmin system. J Neurosci 2011;31:14931–43. http://dx.doi.org/10.1523/JNEUROSCI. 3339-11.2011.

[109] Lemarchant S, Pruvost M, Hébert M, Gauberti M, Hommet Y, Briens A, et al. tPA promotes ADAMTS-4-induced CSPG degradation, thereby enhancing neuroplasticity following spinal cord injury. Neurobiol Dis 2014;66C:28–42. http://dx.doi.org/10.1016/j.nbd.2014.02.005.

[110] Nolin WB, Emmetsberger J, Bukhari N, Zhang Y, Levine JM, Tsirka SE. tPA-mediated generation of plasmin is catalyzed by the proteoglycan NG2. Glia 2008;56:177–89. http://dx.doi.org/10.1002/glia.20603.

[111] Cacquevel M, Launay S, Castel H, Benchenane K, Chéenne S, Buée L, et al. Ageing and amyloid-beta peptide deposition contribute to an impaired brain tissue plasminogen activator activity by different mechanisms. Neurobiol Dis 2007;27:164–73. http://dx.doi.org/10.1016/j.nbd.2007.04.004.

[112] Roussel BD, Macrez R, Jullienne A, Agin V, Maubert E, Dauphinot L, et al. Age and albumin D site-binding protein control tissue plasminogen activator levels: neurotoxic impact. Brain 2009;132:2219–30. http://dx.doi.org/10.1093/brain/awp162.

[113] Obiang P, Maubert E, Bardou I, Nicole O, Launay S, Bezin L, et al. Enriched housing reverses age-associated impairment of cognitive functions and tPA-dependent maturation of BDNF. Neurobiol Learn Mem 2011;96:121–9. http://dx.doi.org/10.1016/j.nlm.2011.03.004.

[114] Ledesma MD, Santos J, Silva D, Crassaerts K, Delacourte A, De Strooper B, et al. Brain plasmin enhances APP a - cleavage and A b degradation and is reduced in Alzheimer's disease brains. EMBO Rep 2000;1:530–5.

[115] Tucker HM, Kihiko M, Caldwell JN, Wright S, Kawarabayashi T, Price D, et al. The plasmin system is induced by and degrades amyloid-N_L aggregates. J Neurosci 2000;20:3937–46.

[116] Van Nostrand WE, Porter M. Plasmin cleavage of the amyloid beta-protein: alteration of secondary structure and stimulation of tissue plasminogen activator activity. Biochemistry 1999;38:11570–6. http://dx.doi.org/10.1021/bi990610f.

[117] Melchor JP, Pawlak R, Strickland S. The tissue plasminogen activator-plasminogen proteolytic cascade accelerates amyloid-beta (Abeta) degradation and inhibits Abeta-induced neurodegeneration. J Neurosci 2003;23:8867–71.

[118] Liu R-M, van Groen T, Katre A, Cao D, Kadisha I, Ballinger C, et al. Knockout of plasminogen activator inhibitor 1 gene reduces amyloid beta burden in a mouse model of Alzheimer's disease. Neurobiol Aging 2012;32:1079–89. http://dx.doi.org/10.1016/j.neurobiolaging.2009.06.003.Knockout.

[119] Macrez R, Obiang P, Gauberti M, Roussel B, Baron A, Parcq J, et al. Antibodies preventing the interaction of tissue-type plasminogen activator with N-methyl-D-aspartate receptors reduce stroke damages and extend the therapeutic window of thrombolysis. Stroke 2011;42:2315–22. http://dx.doi.org/10.1161/STROKEAHA.110.606293.

[120] Berkhemer OA, Fransen PSS, Beumer D, van den Berg LA, Lingsma HF, Yoo AJ, et al. A randomized trial of intraarterial treatment for acute ischemic stroke. N Engl J Med 2015;372:11–20. http://dx.doi.org/10.1056/NEJMoa1411587.