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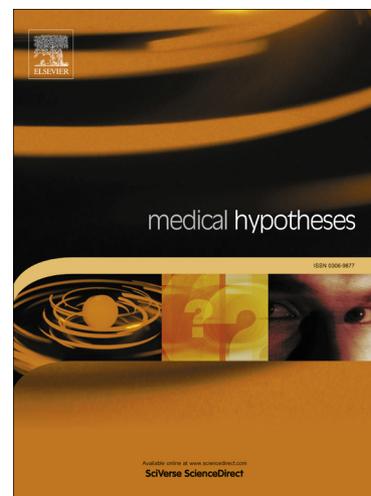
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**The Fibrinolytic System: a New Target for Treatment of Depression with Psychedelics**

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## Introduction

Major depression is among the most common mental health disorders in the United States and carries the heaviest burden of disability among mental and behavioral disorders [1]. Over one third of patients will not achieve remission after initiation of an antidepressant treatment and the likelihood of achieving remission diminishes with subsequent medication trials [2]. These considerations demand exploration of alternative treatment strategies and identification of novel mechanisms that can be exploited to improve outcomes. The finding that glutamate modulators such as ketamine have rapid acting antidepressant effects has expanded the current model of depression beyond the traditional monoamine theory involving serotonin, dopamine and norepinephrine. Recent research has focused on the role of cytokine-mediated inflammation, neurogenesis, and the role of the glutamate in the pathogenesis of depression [3].

The involvement of inflammation in the development of depression has been extensively studied, while that of the fibrinolytic system, which is down-regulated by inflammation in a range of disorders including lung and pleural injury, cardiovascular disease, glomerulonephritis, and cirrhosis [4–6] has been comparatively ignored, even though depression is considered to be an independent risk factor for coronary artery disease that predicts increased morbidity and mortality [7]. Brain derived neurotrophic factor (BDNF), a neurotrophic factor regulated by tissue type plasminogen activator (tPA), has been posited as a key link between stress, cardiovascular disease, and depression as well as its treatment, mainly via tPA-mediated cleavage of BDNF by plasmin [8–10]. Animal models with BDNF Val66Met polymorphisms are associated with decreased BDNF activity and demonstrate increased activation of platelets, alterations in coagulation pathways, changes in vessel wall protein composition and increased depressive phenotype [11]. Here, we review these prior hypotheses and extend them in light of more recent understanding of the role of impaired fibrinolysis, aberrant extravascular fibrin

deposition and tissue remodeling that has been the longstanding focus of our group. We also provide new supporting evidence, some from our own laboratory, that newly recognized mechanisms underlying impaired fibrinolysis in the lung and pleural space could occur within the brain parenchyma to promote architectural alterations that lead to the development of depressive symptoms. While psychological explanations for depression are certainly important, our focus in this manuscript is on biologic mechanisms that specifically involve the fibrinolytic system, that could likewise contribute to the pathogenesis of depression and that may be amenable to novel pharmacologic approaches including administration of psychedelic agents.

tPA, urokinase (uPA), plasminogen activator inhibitor 1 (PAI-1), neuroserpin, and urokinase receptor (uPAR) are expressed in the brain in neurons, microglia and astrocytes [12,13] while soluble uPAR (suPAR) is a proteolytic cleavage product that can be detected within the systemic circulation. Interestingly, increased suPAR is a biomarker of depression [12] and potentially suicidality [13]. This supports the concept that abnormalities of the fibrinolytic system occur in depression and could potentially contribute to its pathogenesis (**Figure 1**). We posit that plasminogen activators, uPAR, and the inhibitors, PAI-1 and neuroserpin, are integral to the pathogenesis of depression, in part by regulating the production of mature BDNF (mBDNF) as well as remodeling of brain architecture, neurotransmission and synaptic plasticity. We also propose that psilocybin, a 5-HT<sub>2A</sub> serotonin receptor agonist and classic psychedelic can exert anti-depressant effects that are mediated by salutary changes in the fibrinolytic system. The classic psychedelic 5-HT<sub>2A</sub> receptor agonists, which include diethylamide (LSD), mescaline, and N,N-dimethyltryptamine (DMT) are capable of inducing robust changes in affect, perception, and cognition. Psilocybin in particular may induce positive psychological experiences of high personal significance leading to enduring beneficial changes in mood, thinking, and behavior [14].

Our overarching hypothesis is that exploration of fibrinolytic system in the brain will provide an avenue for the development of new treatments for depression including administration of psychedelic agents to patients with refractory depression [15]. We specifically propose that serotonergic classic psychedelics such as psilocybin will demonstrate therapeutic effects on the brain including normalization of changes that occur as a result of chronic stress and disruption of the fibrinolytic system through interactions between neuroserpin, tPA and PAI-1 (**Figure 2**). We infer that psilocybin may decrease PAI-1 activity via reduction of TNF- $\alpha$  levels, leading to disinhibition of tPA, increased mBDNF production, normalized synaptic plasticity, and resolution of depression (**Figure 3**). This hypothesis relies in part on the established link between peripheral and central inflammation in the pathogenesis of depression, which is described in the next section.

### **The Linkage Between Peripheral or Cerebral Inflammation and Suppression of the Fibrinolysis in the Brain**

Evidence from human studies suggests that increased peripheral cytokine levels are associated with depression and suicidality [16]. A recent meta-analysis indicates that levels of interleukin-6 (IL-6) and TNF- $\alpha$  are significantly higher in depressed patients [17]. Aberrant cytokine elevation profiles have been shown to be potential biomarkers of treatment responses, as a recent study showed that subjects with non-remitted Major Depressive Disorder (MDD) have high baseline levels of TNF- $\alpha$ , which falls with exercise as opposed to antidepressant medications [18]. This study underscores the potential utility of alternative depression strategies or treatments, such as treatment with psilocybin for resistant depression.

Peripheral proinflammatory cytokines may access or directly affect the central nervous system (CNS) through several mechanisms such as activating the vagus nerve, disrupting the blood brain barrier

(BBB), or accessing the brain directly via the circumventricular organs or saturable transport systems [19]. The BBB is regulated by several mechanisms involving tPA, which opens the BBB and PAI-1, which tightens the BBB [20]. Overexpression of proinflammatory cytokines in the CNS or in the circulation with induction of a relatively more open BBB, may contribute to neuroinflammation. These and other proinflammatory mediators activate microglia and astrocytes, leading to an enhanced local inflammation and the potential for glial scarification. These processes may suppress normal hippocampal neurogenesis which is associated with depression [21].

Disruption of hippocampal neurogenesis may occur throughout the life cycle as a result of chronic peripheral inflammation due to medical illness [22] or chronic stress [23]. In humans, numerous medical comorbidities including inflammatory bowel disease, diabetes mellitus, and obesity have all been linked to depression and cognitive impairment [24], likely mediated by disruption of hippocampal neurogenesis. These reports suggest a link between chronic inflammation due to co-morbidities with suppression of the fibrinolytic system in the brain.

### **Synaptic Plasticity, Depression, And The Fibrinolytic System In The Brain**

Inflammation, coagulation, and fibrinolysis are intricately interwoven systems that are interactive [4,25]. While inflammation has been intensely studied as a risk factor for depression, the role of the fibrinolytic system in the pathogenesis of this disorder remains poorly understood. Several key elements of the fibrinolytic system linked to synaptic plasticity appear to be altered during stress. For example, uPA and uPAR have been shown to increase cellular viability in the stressed lung epithelium [26] and in lung fibroblasts [27] and we infer that they may similarly do so in the brain. uPA may also promote synaptic plasticity in the brain when upregulated by proinflammatory cytokines involved in depression. tPA and uPA convert plasminogen to its active form plasmin. In the vasculature, plasmin is

known to degrade fibrin clots. In the brain, fibrin has been demonstrated to activate microglia, increasing neuroinflammation and potentially depression [28]. While it is currently unclear whether or not transitional fibrin occurs with low grade inflammation in the brain in depression, fibrin possesses high affinity for both tPA [29] and plasmin [30,31]. Its presence in the brain parenchyma could adversely affect neuroplasticity via inhibition of proBDNF activation, thus promoting depression. Moreover, PAI-1, which is upregulated in alveolar epithelial cells during lung inflammation, has been shown to inhibit neutrophil apoptosis and subsequent phagocytosis thereby leading to prolonged inflammation within the injured lung [32]. PAI-1 is primarily located in astrocytes and has been suggested to be anti-apoptotic for neurons in the brain [33]. This suggests that both tPA and PAI-1 may be neurotoxic or neurotrophic depending on their relative concentrations. PAI-1 is a biomarker and mediator of poor outcomes in lung and pleural diseases [4,34] and we infer that elevated PAI-1 could likewise be a biomarker of depression (Figures 1 and 2).

While the fibrinolytic system plays a key role in the pathogenesis of renal [35,36], hepatic [37] and acute and chronic lung [4] injuries, this system may be of particular importance in the pathogenesis of depression in patients with lung disease. A recent study has shown that about one in four patients with chronic obstructive pulmonary disease (COPD) have persistent depressive symptoms which are associated with risk of COPD exacerbation and decreased exercise tolerance [38]. Additionally, patients with cystic fibrosis (CF) have been shown to have increased incidence of depression and anxiety [39]. The link between lung illness and depression is becoming better characterized by new research showing the influence of mood on other organ systems and vice versa. Inflammation induced epithelial-mesenchymal transition (EMT) in the lungs is associated with increased expression of epithelial PAI-1 and suppressed uPA and loss of lung function[40].

A process analogous to that which occurs in pulmonary remodeling may occur in the brain as a result of stress-induced inflammation. While fibrinogen, the precursor to fibrin, is not present at extravascular sites within the healthy brain it is elevated in the brains of individuals with schizophrenia [41], multiple sclerosis [42], Alzheimer's disease [43] and normal aging [44], all of which are disorders characterized by neuroinflammation and transient or long lasting BBB disruption[20]. These findings suggest that increased inflammation, decreased fibrinolysis and fibrosis may be involved in the pathogenesis of depression. Transitional fibrin deposition may perpetuate microglial activation, in addition to stress-induced, cytokine mediated microglial activation associated with decreased fibrinolysis, increased neuroinflammation, and depression. uPAR is involved in the recruitment of immune cells and cellular adhesion [45] and is a very sensitive marker of low grade inflammation typically seen in depression [12,13]. Further studies are necessary to characterize the roles of fibrin, tPA, uPA, uPAR, PAI-1 and neuroserpin in regulating stress, neural inflammation and mood (Figure 1 and 2).

In the CNS, tPA has been shown to exert effects that extend beyond traditional fibrinolysis and bridge to the regulation of neural cell functionality and behavior. For example, tPA has been demonstrated to influence synaptic plasticity through several mechanisms including degradation of the extracellular matrix through activation of matrix metalloproteases and interaction with the N-methyl-D-aspartate (NMDA) receptor in addition to activation of proBDNF [46,47]. In the brain, tPA has been shown to have a role in memory, learning, and stress dependent behavioral responses [48]. In addition to its role in learning, tPA through NMDA receptor mediated activity, has been shown to be involved in the development of the fear response to acute stress through regulation of plasticity in the hippocampus [49]. While the administration of tPA itself for treatment of depression carries the risk of intracerebral bleeding and potentially neurotoxicity in the ischemic brain [50], the up-regulation of

endogenous tPA activity is in principal an approach that could be pursued for the treatment of depression.

The literature also provides a link between the fibrinolytic system in the brain, BDNF and depression. BDNF is synthesized in cell bodies of neurons and glia and is transported to terminals where it is released [51]. It has been demonstrated to play a pivotal role in neurogenesis and depression. proBDNF and mBDNF have been shown to have opposite effects on neurogenesis and accordingly patients with major depression demonstrate elevated levels of proBDNF and decreased levels of mBDNF [52]. proBDNF induces dendritic retraction and apoptosis and long-term depression, while mBDNF promotes dendritic growth, cell survival, and long term potentiation indicating the importance of appropriate levels of functional tPA or perhaps uPA. A recent study demonstrated that an enriched environment increases tPA-dependent plasmin cleavage of proBDNF in mice [46]. Exercise has also been shown to increase mBDNF and tPA levels in humans which correlates with euthymia [53]. In rats, electro convulsive therapy, a rapid acting neuromodulatory treatment that is the gold standard treatment for resistant depression, has been shown to increase mBDNF and tPA levels [54], unlike imipramine, suggesting a novel tPA-mediated regulatory mechanism of action.

In the normal brain with no infiltrating microglia, PAI-1 is minimally expressed, and biochemical evidence showing strong inhibition of tPA suggests that the primary inhibitor of tPA is likely neuroserpin [55], particularly as overexpression of neuroserpin decreases activity levels of tPA [56]. Neuroserpin and tPA are expressed in neurons throughout the developing and adult nervous system [47]. Both overexpression and knockout of neuroserpin lead to increased anxiety like responses in mice, further supporting the role of the fibrinolytic system in regulating mood [57].

The literature suggests that tPA is a gliotransmitter mediating cross talk between neurons and astrocytes [58]. Astrocytes regulate the effective concentration of extracellular tPA and glutamate,

influencing NMDA receptor signaling involved in regulation of mood. During chronic stress, excess hypothalamic–pituitary–adrenal (HPA) activation leads to activation of microglia and impaired astrocyte reuptake of glutamate leading to excess extracellular NMDA receptor stimulation, neuro-inflammation and subsequent depression [59]. The disruption of the homeostatic balance of available extrasynaptic tPA, which is regulated by neuroserpin and astrocyte reuptake of glutamate, appears to be critical to cell survival and limitation of neuroinflammation.

uPA may be involved in the regulation of mood as well. Both tPA and uPA activate plasminogen to plasmin leading to degradation of extracellular matrix, which is critical to axonal and synaptic plasticity. Mice deficient in uPAR exhibit increased anxiety and reduced social behavior [60] and cognitive impairment [61], a finding that lends strength to our postulate that this receptor, through localization of uPA within brain cells, also contributes to regulation of cleavage of BDNF and alleviation of depressive symptoms. Notably, uPA<sup>-/-</sup> mice have been demonstrated to exhibit a clear reduction in exploratory activity, an enhanced fear response to tone [62] and increased neuroinflammation [63].

### **Antidepressants, Ketamine and Psilocybin: Established and Hypothetical Effects on the Brain Fibrinolytic System and Depression**

Selective serotonin reuptake inhibitors (SSRIs) such as fluoxetine, sertraline, and citalopram inhibit the serotonin transporter (SERT, *SLC6A4*), a plasma membrane integral glycoprotein found in neurons and platelets that regulates reuptake of serotonin across the plasma membrane. Inhibition of SERT leaves more serotonin available in the synaptic cleft to increase serotonergic neurotransmission, one of the putative mechanisms of SSRIs. SSRIs have been shown to have significant efficacy in severe depression [64]. However, these drugs exert various side effects such as akathisia, sexual dysfunction

and weight gain that may lead to discontinuation [65]. Furthermore, SSRIs may take up to 3-4 weeks to take effect, during which time the medication may be discontinued prior to titration to the optimal dose.

Neural circuits involved in emotional processing and reward seeking have been demonstrated to be dysfunctional in major depressive disorder. Individuals with major depressive disorder have demonstrated decreased resting state functional connectivity (RSFC) among specific circuits in the affective network involved in emotional processing and increased RSFC among default mode network neural circuits involved in self referential processing [66]. These imaging studies suggest electrophysiologic circuits that could be interrogated to see if these areas are functionally altered by disruptions of fibrinolysis or transient fibrin deposition in the depressed brain (Figure 1 and 2). Interestingly, psilocybin and mindfulness meditation has been shown to decrease activity in the default mode network [15,67] which could be therapeutic in depression by decreasing rumination or negative self-referencing contributing to the stress response, neuroinflammation and decreased fibrinolysis associated with depression.

Depressive derangements of the hippocampus have also been associated with altered functionality of the fibrinolytic system in the brain (Figure 2). In rats, sertraline (Zoloft), a selective SSRI, has been shown to increase the expression of tPA in the hippocampus and reverse depressive behavioral change in rats exposed to five weeks of chronic unpredictable mild stress [68]. In the same study, sertraline increased BDNF levels in the prefrontal cortex and hippocampus.

There is a growing body of additional clinical evidence supporting the hypothesis that the activity of the fibrinolytic system is decreased in depression. A study of women with MDD on anti-depressant therapy showed elevated plasma PAI-1 associated with increased abdominal fat compared with controls [69]. Variants of the SERPINE1 gene that encodes PAI-1 have been demonstrated to predict response to SSRI's in adults with MDD [70] and in individuals with Alzheimer's disease (AD) and depression [71].

These findings indicate that AD and MDD may show overlapping pathophysiology regulated by derangements in fibrinolysis, potentially alleviated by psilocybin.

Blockade of serotonin uptake in platelets by SSRIs could decrease associated PAI-1 release and its subsequent activity in the blood and brain, in effect elevating Plasminogen Activator (PA) activity leading to higher plasmin levels with cleavage of proBDNF to mBDNF (Figures 2 and 3). Increased mBDNF promotes hippocampal neurogenesis and resolution of depressive symptoms associated with chronic stress induced hypersecretion of cortisol and adrenaline. Whether SSRIs regulate PAI-1 in the systemic circulation in patients receiving these drugs and how cardiovascular risk is affected with chronic use of these drugs is currently unclear.

Ketamine, an NMDA receptor antagonist and fast acting antidepressant has been shown to modulate the inflammation mediated kynurenine pathway with treatment response predicted by baseline levels of IL-6 [72]. To our knowledge, the effect of ketamine on PAI-1 expression within the brain has not been evaluated. Because PAI-1 can revert to an irreversibly latent form or can be cleaved with loss of inhibitory activity, the processing of PAI-1 in response to exposure to ketamine and other drugs and how it is processed in depression itself are issues that are germane to the better understanding of the pathogenesis of this condition. It is possible that by decreasing TNF- $\alpha$  and other pro-inflammatory cytokine activity, ketamine may indirectly affect PAI-1 levels, fibrin deposition, and increases of PA activity involved in hippocampal neurogenesis and the pathogenesis of depression.

Psilocybin binds with high affinity to the 5-HT<sub>1A</sub>, 5-HT<sub>2A/C</sub>, 5-HT<sub>6</sub> and 5-HT<sub>7</sub> subtypes of the serotonin receptors [73]. Psilocybin and other serotonergic classic psychedelics have been shown to exert their effects through agonism at the 5-HT<sub>2A</sub> subtype of the serotonin receptor [74], which is associated not only with psilocybin's psychedelic effects, but also with vascular smooth muscle contraction, platelet aggregation, thrombus formation, and coronary artery spasm [75]. The psychedelic

effects of psilocybin include perceptual changes, labile moods vacillating from joy to anxiety, and cognitive changes including sense of meaning, insight or ideas of reference. The mystical experience, as defined by Roland Griffiths at Johns Hopkins, induced by psilocybin, is defined by abstract traits such as transcendence of time/space, unitary being, sense of sacredness, and ineffability. Researchers at Johns Hopkins have demonstrated that these traits of psilocybin experiences can be quantified by questionnaires that assess the occurrence of the mystical experience after psilocybin administration, the presence of which can be correlated with enduring positive attitudes, mood and behavior after psilocybin administration [14].

Recent studies suggest that 5-HT<sub>2A</sub> receptor modulation may have therapeutic potential in treating depression. Downregulation of 5-HT<sub>2A</sub> receptors, which may occur in response to 5-HT<sub>2A</sub> receptor stimulation, mediates antidepressant and anti-anxiety effects of antidepressants and atypical antipsychotic drugs [76]. Studies have shown that cortical 5-HT<sub>2A</sub> receptor expression is increased in postmortem samples of depressed and suicidal patients [77,78]. It is possible that the region-specific upregulation of 5-HT<sub>2A</sub> receptors associated with mood disorders and borderline personality disorder [79] may occur in response to stress-related decreases in serotonin transmission, and that these receptors may subsequently be regulated to salutary levels in response to increased 5-HT<sub>2A</sub> stimulation associated with psychedelic use.

It appears that the 5-HT<sub>2A</sub> receptor upregulation is linked to the response to inflammation and fibrinolysis as well. C57BL/6J mice have varying degrees of psychological resiliency when exposed to chronic stress, with some mice manifesting behavioral symptoms of depression such as floating in the forced swim test and hyperactivity under stressful lighting conditions. Mice that manifest depressive symptoms demonstrate elevated TNF- $\alpha$  and SERT in the pre-frontal area, while all chronically stressed animals, resilient or susceptible, showed enhanced expression of 5-HT<sub>2A</sub> and Cox-1 in the pre-frontal

area. The study suggests a unique, direct correlation between TNF- $\alpha$  levels, neuroinflammation and expression of 5-HT<sub>2A</sub> receptors. Previous studies in rabbits demonstrated that 5-HT<sub>2A</sub> agonists such as LSD can significantly reduce cortical 5-HT<sub>2A</sub> expression by 33-66% within days [80]. Of note, it has already been established that TNF- $\alpha$  and PAI-1 levels are directly correlated, suggesting that psilocybin could restore the 5-HT<sub>2A</sub> receptor to pre-stress levels with concomitant and potentially critical salutary downregulation of PAI-1 or perhaps neuroserpin.

R-DOI, a potent psychedelic and 5-HT<sub>2A</sub> agonist has been demonstrated to have potent anti-inflammatory effects. R-DOI is noteworthy for its specificity to the 5-HT<sub>2A</sub> receptor which differentiates it from psilocybin. Studies show that R-DOI can block progression of a mouse model of asthma [81], possibly via blockade of TNF- $\alpha$  signaling, which could lead to reduction in PAI-1 levels and alleviation of depressive symptoms. There is also evidence that demonstrates that the microglial 5-HT<sub>2A</sub> receptor may regulate the release of microglial vesicles called exosomes that may contain cytokines involved in neuroinflammation and growth factors involved in neurogenesis [82]. It is plausible that 5-HT<sub>2A</sub> agonism may regulate the stress response at the level of the microglia via regulation of exosomal cytokines and growth factors necessary for neuronal growth and survival.

Emerging evidence supports the idea that psilocybin may exert effects through the limbic system including the hippocampus and amygdala that are involved in emotional processing. Psilocybin has been shown to normalize limbic hyperactivity in individuals with depressed mood by attenuating amygdala hyperactivity and increasing positive affect [83]. In mice, low dose psilocybin has been demonstrated to rapidly extinguish cued fear conditioning and induce a slight non-significant trend toward increased hippocampal neurogenesis, suggesting a potential role in the treatment of Post-Traumatic Stress Disorder (PTSD) [84] and possibly depression. Psilocybin has also been shown to be well-tolerated in humans in a controlled setting with proper supervision, screening and preparation [14] and may lead to

increases in the personality domain of openness in healthy adults [85]. Small pilot studies in humans have shown efficacy of psilocybin in the treatment of anxiety in advanced stage cancer [86], as well as tobacco [87] and alcohol addiction [88]. Analysis of data from the National Survey of Drug Use and Health pooled across 2008-2012 by Hendricks et al. [89] demonstrated that psilocybin may be protective in patients with psychological distress and suicidality. Recently, a clinical study has demonstrated that psilocybin may reduce high sensitivity to social rejection that is characteristic of depression, suggesting that this drug is useful in reducing predisposing factors to depression [90]. Another small recent pilot study suggests that psilocybin administration with psychological support is a safe, efficacious treatment for refractory depression [91].

The role of ketamine and psilocybin in the regulation of the fibrinolytic system in the brain has not yet to our knowledge been studied. However, previous studies of the 5-HT<sub>2A</sub> receptor suggest a possible therapeutic role for psilocybin in depression involving the fibrinolytic system. Hypertrophic adipocytes have been shown to upregulate expression of the 5-HT<sub>2A</sub> receptor and PAI-1, while suppression of 5-HT<sub>2A</sub> gene expression enhances adiponectin expression, which is involved in regulation of glucose levels as well as fatty acid breakdown and is normally downregulated in obesity [92]. This finding demonstrates that downregulation of the 5-HT<sub>2A</sub> receptor by psilocybin could be associated with reduced PAI-1 expression and potentially help with weight loss and depression simultaneously. PAI-1 is deleteriously up-regulated in both conditions. Whether 5-HT<sub>2A</sub> receptor modulation and PAI-1 upregulation predispose to depression through interactions with components of the fibrinolytic system remains to be determined.

The role of psilocybin in the treatment of depression remains to be delineated. However, we posit that its administration is beneficial in reducing neuroinflammation, and restoring the fibrinolytic system to baseline, a postulate that can be tested in wild type mice with depressive behavior. If we are

correct that effective therapy for depression is unified around a restoration of tPA leading to plasmin mediated activation of BDNF, preservation of neurogenesis and synaptic plasticity and that psilocybin reverses downregulation of tPA activity, the postulate can be confirmed. Additionally, the importance of changes in key components of the fibrinolytic system; tPA, uPA, uPAR or PAI-1 to depressive phenotype can be tested in knockout mice or in animals deficient in plasminogen and exposed to depressive stimuli. Transgenic mice that have upregulation of PAI-1 are also available to determine how its overexpression affects depressive symptoms.

Through *in vitro* studies with astrocytes involving the assessment of the effect of inflammatory cytokines such as TGF- $\beta$  and TNF- $\alpha$  on the fibrinolytic system, we can assess the potential of psilocybin to alleviate pro- inflammatory, anti-fibrinolytic changes induced in glial cells. Clinically, we are assessing the role of mindfulness mediation on inflammatory and fibrinolytic biomarkers in depression. These studies may add support to pursuing further clinical trials using psilocybin for treatment resistant depression.

In conclusion, the preponderance of literature suggests that the pathogenesis of depression involves dysregulation of the fibrinolytic system and that antidepressants in common use and treatment candidates could be involved in their regulation. We specifically hypothesize that the fibrinolytic system exerts potentially critical effects in the pathogenesis of depression and that psychedelics such as psilocybin may regulate the fibrinolytic system in a salutary manner. We further posit that the therapeutic effects of psilocybin and perhaps commonly used antidepressants act as anti-inflammatory, profibrinolytic treatments that reduces neuroinflammation engendered by chronic stress. Increased inflammation through TNF- $\alpha$  and likely other proinflammatory mediators expressed in the brain is associated with elevated PAI-1 levels, increased 5-HT<sub>2A</sub> cortical expression, and decreased tPA activity in the CNS. These alterations disrupt synaptic plasticity in structures that mediate functional brain circuits

involved in mood. We hypothesize that psilocybin exerts an antidepressant effect by reducing PAI-1 levels, decreases neuro-inflammation and fibrin deposition in the brain and restores the 5-HT<sub>2A</sub> receptor to pre-stress levels leading to normalized serotonergic tone associated with euthymic mood. Psychedelics have been historically stigmatized, and are currently schedule 1 substances in the U.S. making initiation of new research into the treatment of resistant depression with psilocybin a challenging endeavor. Despite these challenges, we believe that it is imperative to leave no stone unturned when alleviation of chronic suffering from depression is a potential outcome.

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## Captions

**Figure 1. Hypothesis: The contribution of the fibrinolytic system to processing of BDNF as a link to depression.** Serpins (PAI-1 and NSP) are elevated in depression and inhibit both tissue (tPA) and urokinase (uPA) plasminogen activators (including uPA bound to its receptor uPAR), suppress activation of plasminogen, and block activation of proBDNF. Inhibition of the fibrinolytic activity promotes transient extravascular fibrin deposition in the brain. An increase in PA activity also results in cleavage of plasminogen producing plasmin, which then activates proBDNF to the activated cleavage product; mature (m)BDNF. However, transient fibrin, due to its high affinity to plasmin and tPA competes with proBDNF for plasmin, and thus affects proBDNF processing.

**Figure 2. Proposed effects of dysregulated fibrinolysis on the pathogenesis of depression.**

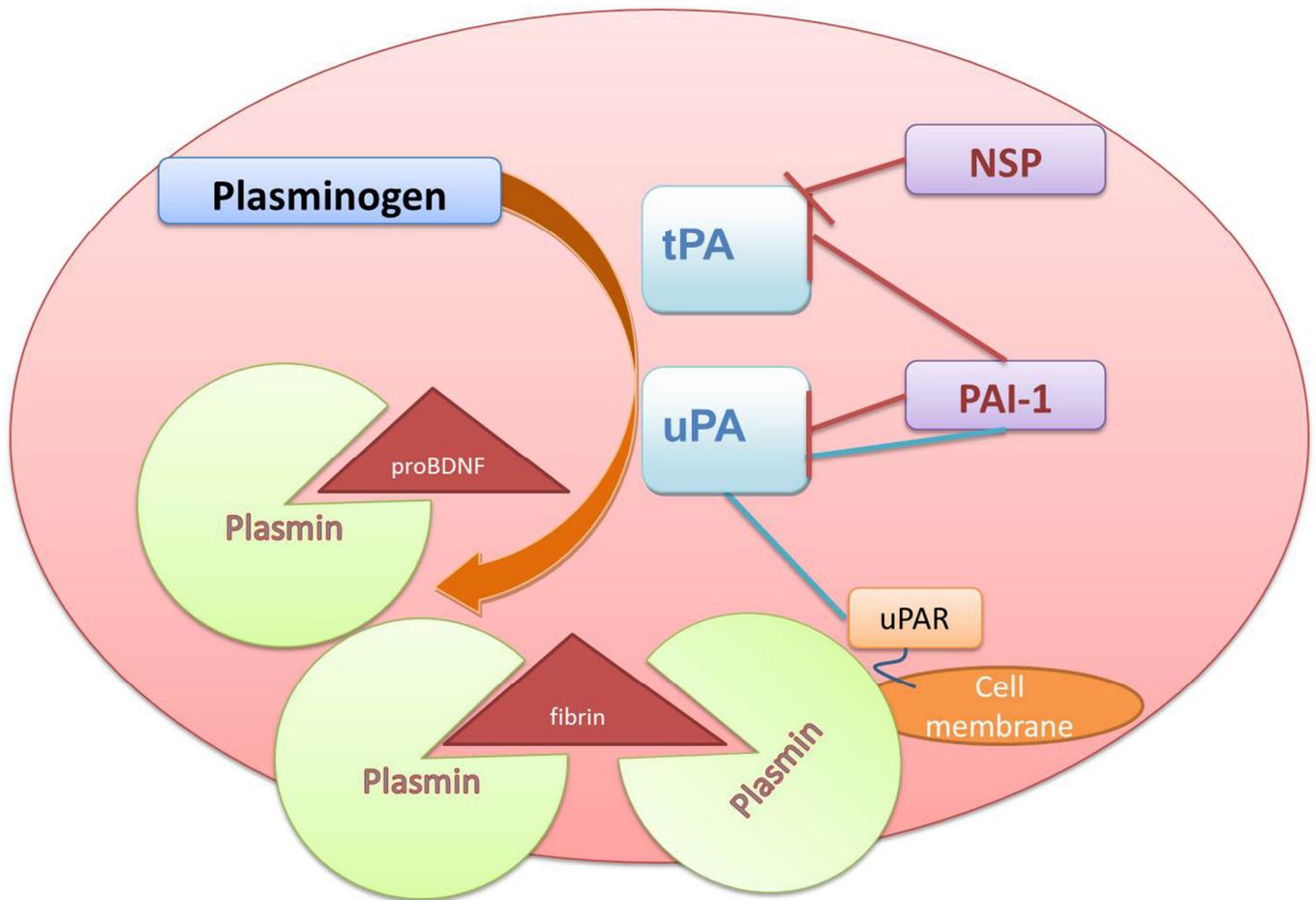
Derangements of the fibrinolytic system are integral to the pathogenesis of depression: Stress initiates a cascade in the brain wherein proinflammatory cytokines such as TNF- $\alpha$  increase PAI-1 levels. This inhibits PA activity and blocks plasmin-mediated cleavage of proBDNF to mBDNF. Inhibition of PA activity also promotes transient fibrin deposition. A decrease in mBDNF (and increase in the proBDNF/mBDNF ratio) leads to disruption of hippocampal neurogenesis, synaptic plasticity, and increased hippocampal atrophy resulting in depression.

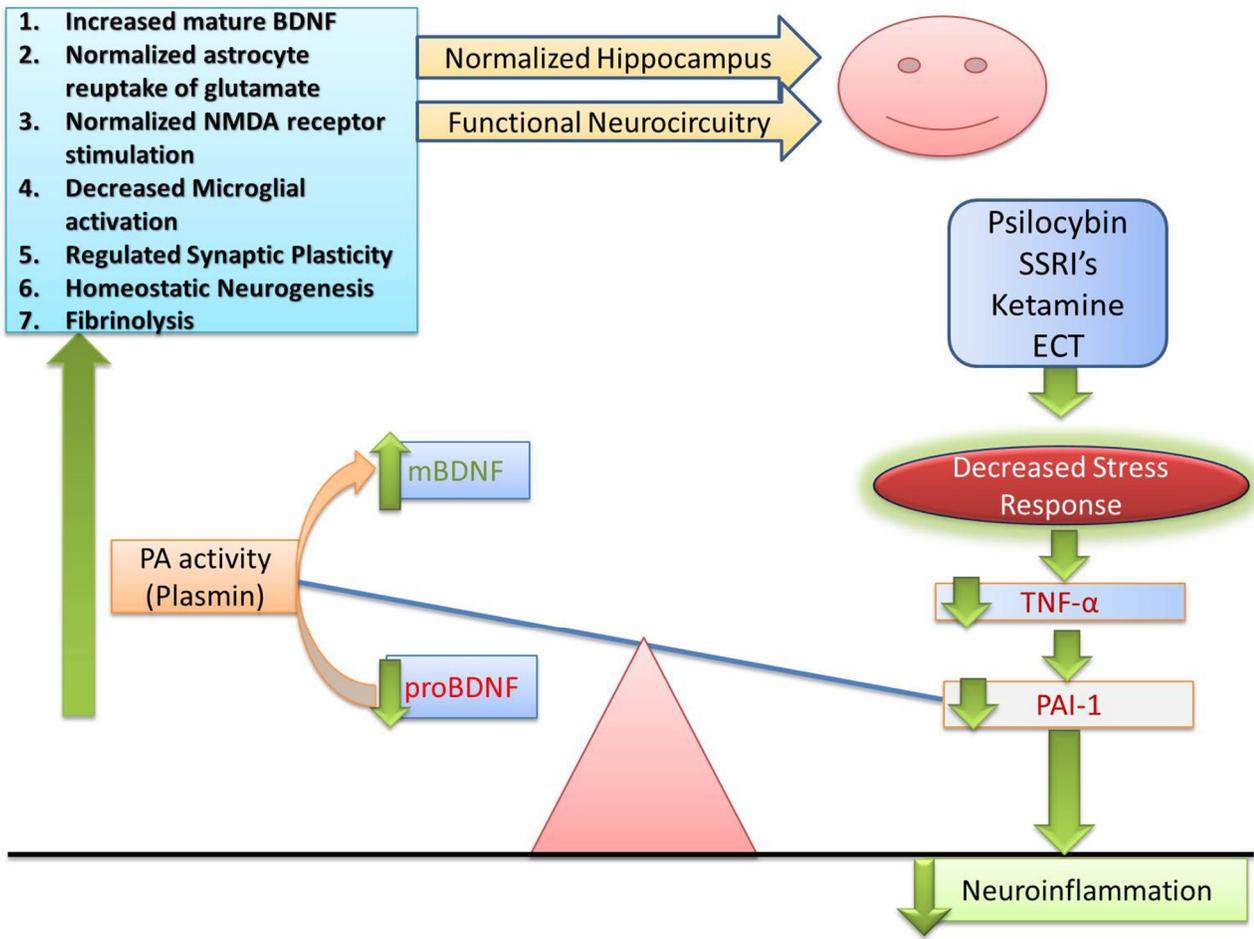
**Figure 3. Proposed contribution of the fibrinolytic system to the mitigation of depression by psilocybin and other treatments.**

Psilocybin, Ketamine, Electroconvulsive therapy and SSRI's may act through antiinflammatory, profibrinolytic mechanisms simultaneously decreasing TNF- $\alpha$  and PAI-1 levels thereby increasing PA

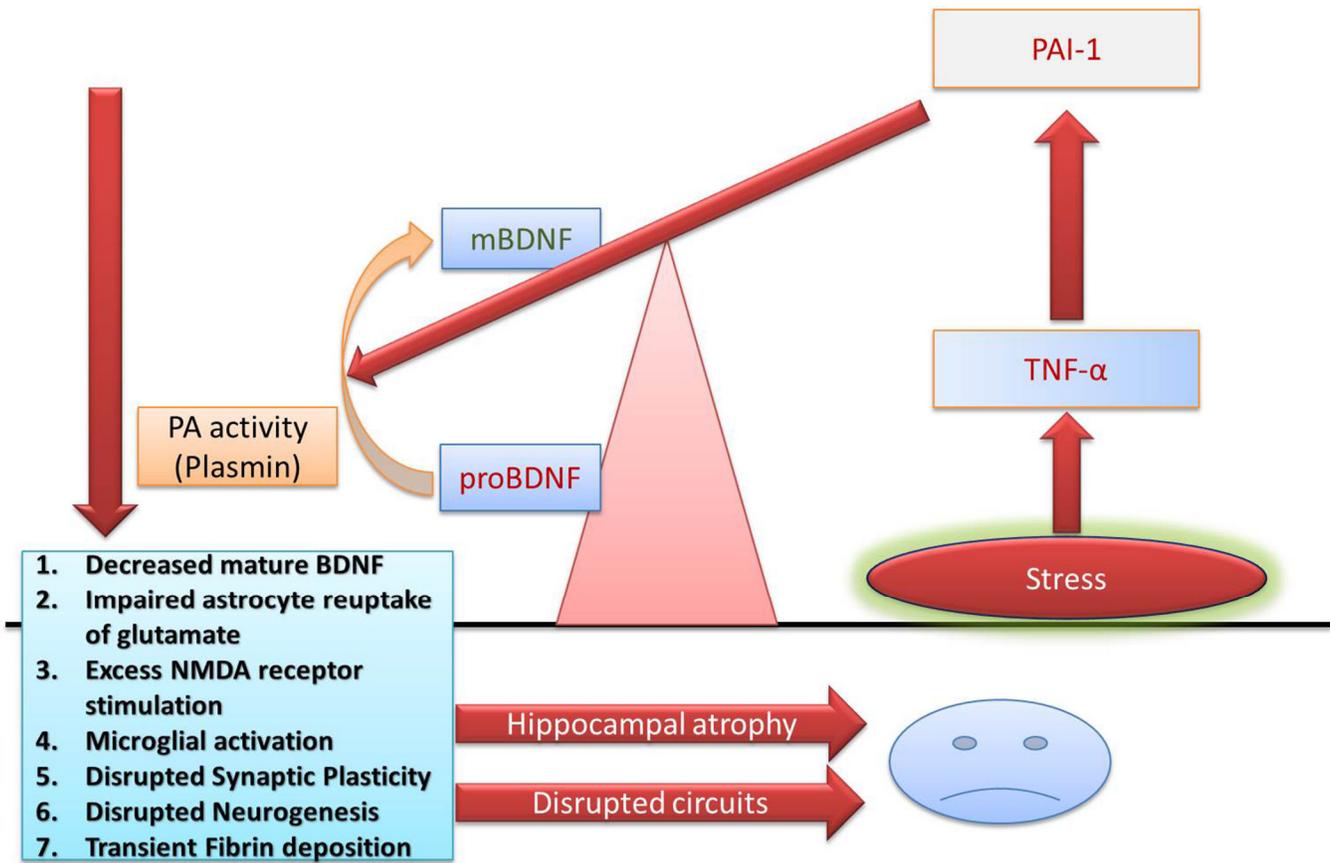
activity to generate plasmin which cleaves pro BDNF to mature; mBDNF. This may lead to restoration of homeostatic functional neurocircuitry, increased hippocampal neurogenesis, and euthymia. Possible delay in activation of proBDNF could occur due to competition for plasmin and tPA with transient extravascular fibrin deposition within the brain parenchyma (Figure 1).

ACCEPTED MANUSCRIPT





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