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Hippocampus-specific deletion of tissue plasminogen activator "tPA" in adult mice impairs depression- and anxiety-like behaviors

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tPA

Abstract

Anxiety and depression are multifactorial disorders that have become prominent health problems all over the world. Neurotrophic factors have emerged underlying pathogenesis of these diseases. Although a number of studies indicate that the hippocampus-brain-derived neurotrophic factor (BDNF) may be involved in these psychiatric illnesses, little is known about the molecular mediators of these disorders. In this study we further investigate the role of tissue plasminogen activator (tPA), a serine protease involved in pro-BDNF cleavage to BDNF, in depression and anxiety-like behaviors in adult mice. To address this issue, we investigated the effect of hippocampus tPA manipulation, using viral vectors, on anxiety- and depression-like behaviors, including the marble burying test (MBT), elevated plus maze (EPM), tail suspension test (TST), novelty suppressed feeding (NSF) and forced swim test (FST). Our results showed that tPA knock-down – using lentiviral vectors expressing specific short hairpin RNAs (LV-shRNA) – increased the number of buried marbles together with the digging time in the MBT and decreased the time spent in open the arms of an EPM. In addition, tPA-knock down in the hippocampus increased immobility in the FST and TST, and increased time to feed in the NSF test. These effects were reversed when tPA-over-expressing vectors (LV-tPA) were injected in the hippocampus. We also found that BDNF protein levels were elevated in the hippocampus of mice receiving tPA-expressing vectors. Together, our results imply that

Abbreviations: BDNF, brain-derived neurotrophic factor; CA, closed Arms; EPM, elevated plus maze; FST, forced swim test; HEK293T, human embryonic kidney 293T; LV, lentivirus vector; MBT, marble burying test; NSF, novelty suppressed feeding; OA, open Arms; PLC, phospholipase C; shRNA, short hairpin RNA; tPA, tissue plasminogen activator; TST, tail suspension test; uPA, urokinase plasminogen activator.

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1. Introduction

Anxiety and depression affect number of people throughout the world and are leading cause of disability. In fact the World Health Organization estimates that 2–3% in general populations of countries across the world tend to be affected by severe mental disorders (World Mental Health Survey 2000 data). However, the cellular and molecular basis for the development of these psychiatric disorders is still largely unknown. There is a substantial body of evidence suggesting a strong relation between neurotrophic factors and depression and anxiety pathogenesis (Li et al., 2008; Matrisciano et al., 2009; Strohle et al., 2010).

Brain-derived neurotrophic factor (BDNF), a member of the neurotrophic family known to regulate neuronal plasticity and survival (D'Sa and Duman, 2002; Noble et al., 2011; Numakawa et al., 2010), may play an important role in the pathophysiology of depression and anxiety. In fact, it has been reported that serum BDNF levels of the patients with psychological job stress or panic disorders were significantly lower than those in control patients (Kobayashi et al., 2005; Mitoma et al., 2008). Different studies indicated that bipolar patients who were exposed to traumatic events have anxiety comorbidity and lower levels of serum BDNF compared to those without a history of traumatic events (Kauer-Sant'Anna et al., 2007). Also baseline serum BDNF levels were significantly lower in depressed patients compared to controls (Kim et al., 2007; Lee et al., 2007; Matrisciano et al., 2009). Very recently Schmidt and Duman have shown that when administered peripherally, BDNF has an antidepressant and anxiolytic effect in mice. These effects were associated with an enhanced survival rate of newborn neurons and increases BDNF-mediated signaling in the hippocampus (Schmidt and Duman, 2010). Furthermore, BDNF-deficient mice developed aggressiveness and anxiety as well as hyperactive response to stressors (Lyons et al., 1999; Rios et al., 2001), which suggest that BDNF is implicated in the regulation of anxiety-related behaviors. In addition, a variant in the human BDNF gene, which leads to a valine-to-methionine change in the proBDNF protein, was associated with lower activity-dependent secretion of BDNF (Enoch et al., 2008) and has been implicated with increased susceptibility to neuropsychiatric disorders including depression, anxiety-related dysfunction, and bipolar disorder (Lang et al., 2005; Minelli et al., 2011; Montag et al., 2010). More importantly, mutation of the PLCγ-docking site downstream of TrkB affects experimentally induced long-term potentiation and associative learning in behaving mice (Gruart et al., 2007). Taken together, all these studies suggest that BDNF is likely to be an important etiological factor in memory, depression and anxiety disorders.

BDNF arises from a precursor, proBDNF, which is cleaved to produce the mature protein through the tissue plasminogen activator (tPA) pathway, and represents one mechanism that can regulate the action of BDNF (Sartori et al., 2011; Tsai, 2006). tPA is a serine protease that catalyzes the conversion of plasminogen to plasmin and plays a role in fibrinolysis (Zorio et al., 2008). In addition, tPA is expressed by many types of neurons in the developing and adult brain (for review see Melchor and Strickland, 2005; Teesalu et al., 2002). tPA is highly expressed in the adult rodent brain in regions involved in fear and anxiety (amygdala) (Pawlak et al., 2003; Qian et al., 1993; Zhou et al., 2010), motor learning (cerebellum) (Seeds et al., 1999), learning and memory (hippocampus/amygdala) (Lochner et al., 2006; Pothakos et al., 2010), and addiction (Bahi and Dreyer, 2008, 2011; Nagai et al., 2008; Noel et al., 2011).

More importantly, the tPA-plasmin proteolytic pathway was highly associated to major depressive disorders (MDD) (for review see Hou et al., 2009). In addition and after acute restraint stress, tPA activity was up-regulated in the central and medial amygdala promoting stress-related synaptic plasticity and anxiety-like behavior in mice an effect that was inhibited by plasminogen activator inhibitor-1 (PAI-1) (Pawlak et al., 2003). The same groups have shown later that the amygdala corticotropin-releasing factor (CRF) mediated tPA activity up-regulation through the CRF type-1 receptors (Matys et al., 2004). These findings suggest that tPA is involved in the regulation of numerous aspects of neuronal remodeling and psychiatric disorders.

In the present study we performed a series of experiments to examine the effects of stress on tPA expression and enzymatic activity in the hippocampus. Using lentiviral-mediated gene transfer and shRNA expression, we investigated how tPA manipulation in the hippocampus may affect anxiety- and depression-like behaviors in adult mice.

2. Experimental procedures

2.1. Subjects

Male C57BL/6 mice weighing 25–30 g were group-housed (five per cage) with a 12-h light/dark cycle (lights on at 7 am) and provided ad libitum access to food and water until the age of 10–14 weeks. Age- and body weight-matched male mice were used for behavioral experiments. The experiments were conducted in an isolated behavioral testing room in the animal facility to avoid external distractions. In order to facilitate adaptation to the experimental environment, mice were housed in the testing room for at least 1 week before the experiments started. All animal care and use were in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All experimental procedures were approved by the local Research Ethic Committee. All efforts were made to minimize both the suffering and the number of animals used.

2.2. Drugs

Imipramine chlorhydrate (10 mg/kg, i.p.) and diazepam (1 mg/kg, i.p.) (Sigma, USA) were dissolved in isotonic saline solution (NaCl

0.9%) and the volume administered to each animal was 10 mL/kg body weight. Drugs were injected 30 min before testing the mice.

2.3. Construction and production of lentiviral vectors

These vectors were prepared as described previously (Bahi and Dreyer, 2008, 2011; Bahi et al., 2008b). Briefly, for tPA-expressing lentiviral vectors (LV-tPA), tPA was amplified from total brain cDNA using specific primers and ligated into pTK431. For tPA-specific shRNA expressing lentiviral vectors (LV-shRNA), using PCR amplification shRNA oligos were added to the mouse U6 promoter using pSilencer 1.0-U6 (Ambion, UK) as a template and the PCR product was digested with *Bam HI* and *Xho I* and cloned into similar sites in pTK431. After cloning and sequencing, all plasmids were $CsCl_2$-purified and lentiviruses were produced by the transient calcium phosphate co-transfection of human embryonic kidney 293T (HEK293T) cells with pTK vectors together with pMDG-VSV-G and pΔNRF as described previously (Bahi et al., 2004a, 2004b, 2005a, 2005b, 2006, 2008a, 2008b, 2008c; Bahi and Dreyer, 2008, 2011; Ortiz et al., 2010). The control mice were injected with LV-Mock, representing the empty cloning vector.

2.4. Stereotaxic microinjection of lentiviral vectors

Stereotaxic injections were performed as described previously (Bahi and Dreyer, 2011; Bahi et al., 2009). Mice were anesthetized with ketamine/xylazine (100 mg/kg, 10 mg/kg, i.p.) and placed in a stereotaxic apparatus. Lentiviral vectors were stereotaxically injected bilaterally into the hippocampus using following coordinates (Franklin and Paxinos, 1996): +2.92 mm antero-posterior, ±1.4 mm lateral from the bregma, and 2.0 mm ventral from the skull (striatum coordinates: AP +0.62 mm, Lateral ±2.0 mm and DV 3.0 mm). Viruses were infused at a rate of 0.2 μL/min for 5 min (final volume 1 μL/site). Following recovery from the operation, mice were used for behavioral experiments as depicted in Table 1.

2.5. Measurement of tPA and uPA activities and BDNF protein content

To test tPA (and uPA) enzymatic activities, brains were removed after rapid decapitation 60 min following 6 min forced swim stress. The tissue was punched from 2-mm sections with an 18-gauge syringe and placed immediately in lysis buffer. Fifty micrograms of protein from each sample were incubated with a specific substrate as described previously (Bahi and Dreyer, 2011; Parolini et al., 1996). Reactions were stopped and colorimetric intensity was measured in a micro-plate spectrophotometer. BDNF protein content was measured using ELISA BDNF Emax immunoassay system (Promega, USA). The protein concentration in each sample was measured using a BCA protein assay kit (Bio-Rad, Switzerland).

2.6. Behavioral studies

2.6.1. Locomotor activity

Mice were habituated to the testing room for 1 h before testing. Locomotor activity was recorded by individually placing the mice into activity chambers and monitored using EthoVision tracking system (Noldus, Netherlands) (Bahi and Dreyer, 2011). Activity was recorded for 90 min and measures were collected in 5 min bins. Data were expressed as distance traveled (cm).

Table 1 Time line of the behavioral experiments.

Days	1	14	15	17	19	21	23
Test	Surgery	Locomotion	MBT	EPM	TST	NSF	FST

2.6.2. Marble burying test (MBT)

Before testing mice were habituated to the testing room for 1 h. Mice were individually placed in Plexiglace cages with 6 cm of fine sawdust, for a 45-min habituation period. Subsequently, animals were briefly removed and 20 marbles (1 cm diameter) were placed in each cage, on top of the sawdust. Mice were then returned to the cages, and their behavior was recorded for the following 30 min. Measures included the number of buried marbles (when at least two-thirds of its surface area was covered in sawdust), and the total duration of digging bouts as measured by time spent burying marbles (Abe et al., 1998; Njung'e and Handley, 1991; Popova et al., 2011; Umathe et al., 2011; Uz et al., 2002).

2.6.3. Elevated plus maze test (EPM)

The EPM test is an anxiety paradigm based on the mice's natural aversion to a novel environment represented by the open and elevated spaces (Bahi et al., 2009). The elevated plus maze apparatus is a plus (+) shaped structure constructed from Plexiglas, elevated 40 cm above the ground, with two open arms (40 cm × 6 cm × 0.2 cm) and two enclosed arms (40 cm × 6 cm × 17 cm) separated by a central platform (6 cm × 6 cm). One hour before testing, mice were habituated to the testing room. Mice were then placed individually into the center of the maze facing an open arm and allowed to explore the maze freely for a 5 min testing period. The maze was cleaned between runs with a wet paper towel. The traditional elevated plus maze measures time spent in open and closed arms and the number of arm entries that were collected.

2.6.4. Novelty suppressed feeding test (NSF)

Twenty-four hours before testing, food was removed from the cage. At the day of testing, mice were transferred to the testing room and after 1-hour habituation period, they were placed in a clear plastic enclosure (40 cm x 40 cm x 20 cm). The floor was covered with 2 cm of bedding. A small pre weighed food pellet chow was placed in the center of the arena on a piece of white circular filter paper. At the start of the experiment, each mouse was placed in the corner of the testing arena, and the time to the first feeding event was recorded during 10 min. The subject was then placed alone for 5 min in its home cage with the pre weighed piece of food chow. At the end of the 5-min period, the amount of food consumed was also measured. For this experiment latency to begin chewing food was measured as well as amount of food consumed (Guilloux et al., 2011; Liu et al., 2011; Olivier et al., 2008).

2.6.5. Tail suspension test (TST)

The procedure is similar to the forced swim test in that it measures "behavioral despair" in mice (i.e. mice stop struggling in the face of an inescapable stressor). After 1-hour habituation to testing room, each mouse was suspended individually by its tail from a metal rod. The rod was fixed 50 cm above the surface of a table covered with soft cloth in a sound-attenuated room. The tip of the mouse's tail was fixed on the rod using adhesive Scotch tape. The duration of the test was 6 min and the immobility time (s) of the tail-suspended mice was measured as previously described (Bahi et al., 2009).

2.6.6. Forced swim test (FST)

The procedure is based on that described previously (Bahi et al., 2009). Mice were habituated to the testing room for 2 h before the experiment. The forced swim test was conducted by individually placing the mice into cylindrical plastic transparent containers (height, 25 cm; diameter, 18 cm) containing 15 cm of water at ~25 °C. Immobility was analyzed during 6 min and mice were removed from the container and left to dry in a heated enclosure before they were returned to their home cages. Test-swims were subsequently assessed for immobility time (s). Immobility behavior was described as when the animal remains floating passively in the water without any vertical or horizontal movements.

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Both TST and FST experimental procedures are based on the fact that when mice are forced into an aversive situation from which they cannot escape they will become immobile. Antidepressants, like imipramine, reduce the immobility time in these tests (Gadotti et al., 2012; Liang et al., 2008; Sales et al., 2011; Shieh et al., 2008) indicating that these methods can be used to test for depression-related behavior in mice.

2.7. Statistical analysis

For statistical comparisons, the software package SPSS (version 19.0) was used. Mean values and SEM were calculated for each group and all experiments were subjected to a between-subjects design with virus as the independent variable. Dependent variables for each behavioral model were analyzed using a one-way ANOVA. For the experiment testing the effect of imipramine in TST or Diazepam in EPM, the data were analyzed using a two-way ANOVA where the main factors under consideration were virus (Mock, LV-shRNA or LV-tPA mice) and treatment (vehicle or imipramine/Diazepam). For the locomotor activity study, repeated measures were collected from a single animal at different time points and data were analyzed using a one-way repeated measure ANOVA. $p<0.05$ denotes a statistically significant difference.

3. Results

3.1. Stress decreases tPA activity in the hippocampus

In this experiment we examined tPA and uPA activities in the hippocampus protein extracts after a forced swim stress using an enzymatic assay. The striatum was used as a control region. Mice were stressed for 6 min and killed 60 min later. As shown in Fig. 1A, stress decreased tPA activity [$F(3,18)=3.647$; $p<0.05$, $n=5–6$]. Post-hoc analysis revealed that forced-swim mice displayed lower tPA enzymatic activity in the hippocampus compared to naïve littermates ($p<0.05$). In contrast, when tPA enzymatic activity was tested in the striatum there was no difference between naive and stressed mice ($p>0.05$). In the same experiment we decided to test the enzymatic activity of the urokinase plasminogen activator (uPA), another neuronal serine protease involved in the plasminogen-plasmin degradation pathway, but not able to cleave proBDNF to BDNF. Results showed that uPA enzymatic activity was not affected upon forced swimming [$F(3,18)=0.117$; $p>0.05$] (Fig. 1B). These results suggest that acute stress induces a down-regulation of tPA activity in the hippocampus but not in the striatum.

Based on these findings we decided to manipulate tPA expression in the hippocampus and in the striatum to determine whether tPA knock down, using shRNA-expressing lentiviral vectors, or over-expression can affect depression- and anxiety-like behaviors in adult mice. The injection sites are summarized in (Fig. 1C, D).

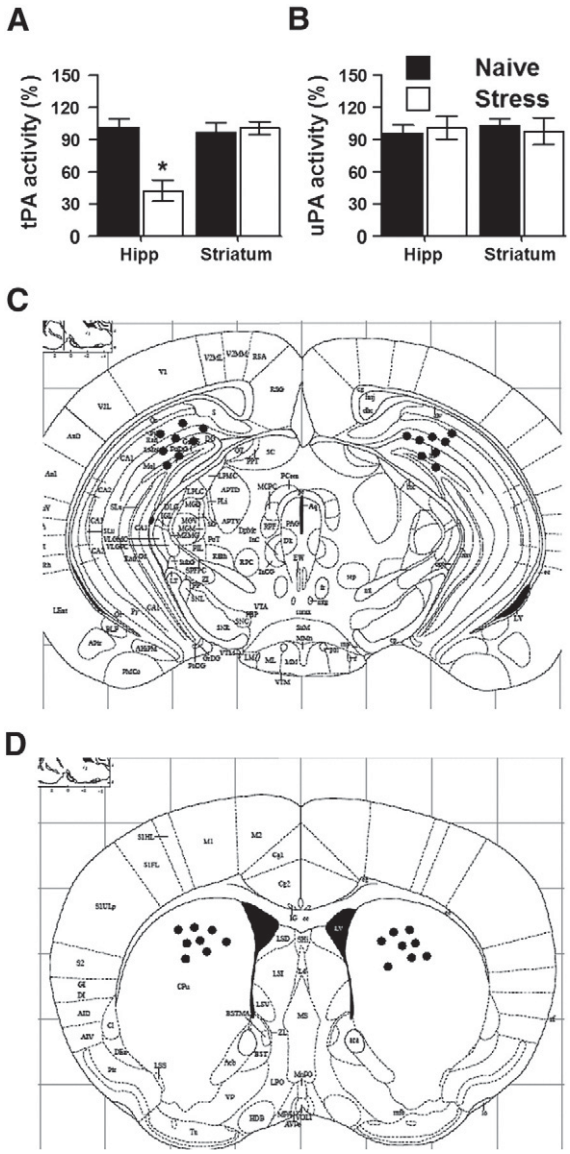

Figure 1 tPA and uPA enzymatic activities in the hippocampus. A) tPA and B) uPA enzymatic activities in mice hippocampal and striatal protein extracts following force swim test. *$p<0.05$ compared to "Naive". In both panels, data represent the Mean ± SEM. $n=5–6$ mice per group. Schematic representation of the sites of lentiviral vector injection into the C) hippocampus and D) dorsal striatum.

3.2. Locomotor activity

Spontaneous locomotor activity study revealed no significant virus effect. Total distance moved during the 90 min testing was similar in all groups [$F(2,67)=0.013$; $p>0.05$, $n=9–11$] (Fig. 2A). Also there was no effect of the brain region tested [$F(1,67)=0.071$; $p>0.05$]. Consequently no virus×region interaction was detected [$F(2,67)=0.482$; $p>0.05$]. There were no differences in distance moved, when the data were analyzed at different time points or intervals using a one-way repeated measures analysis (e.g. 0–30 min, 31–60 min, 61–90 min) (Fig. 2B). There was a significant effect of time [$F(2,66)=13.358$; $p<0.05$], but no significant effect of virus [$F(2,66)=0.224$; $p>0.05$] or time×virus interaction [$F(4,66)=0.429$; $p>0.05$], suggesting a normal habituation profile in locomotion when tested in a novel environment (Fig. 2B).

3.3. Elevated anxiety-like behaviors in tPA-deficient mice

As depicted in Fig. 3A tPA manipulation using stereotaxic injection of viral vectors had a significant effect on marble-burying

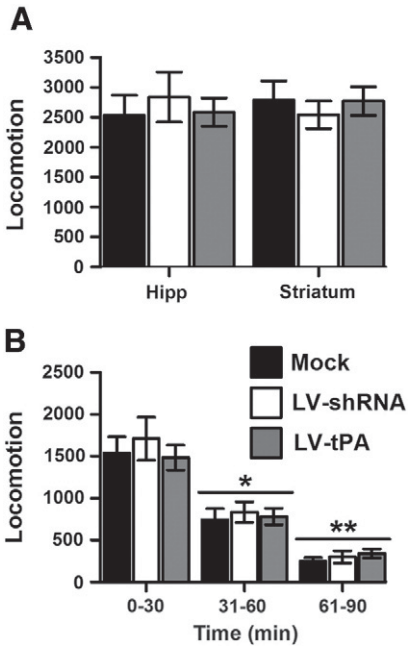

Figure 2 Lentiviral-mediated locomotor activity behavior of mice. A) The total distance during 90 min in mice injected with viral vectors either in the hippocampus or in the striatum. B) The total distance in 30 min blocks times from mice injected with viral vectors in the hippocampus only. There were no significant differences between the three groups. *$p<0.05$ and **$p<0.01$ compared to 0–30 min. In both panels, data represent the mean ± SEM. n = 9–11 mice per group. Mock: Empty vector, LV-shRNA: shRNA expressing- and, LV-tPA: tPA expressing-lentiviral vectors.

indicated by ANOVA analysis [$F(2,30)=10.623$; $p<0.01$, n = 9–11]. LV-tPA decreased the number of marbles buried but LV-shRNA increased it (Fig. 3A). This effect was absent when viral vectors were injected into the striatum [$F(2,31)=0.103$; $p>0.05$]. Also, the time spent digging was similarly affected by tPA manipulation in the hippocampus [$F(2,30)=11.694$; $p<0.01$] but not in the striatum [$F(2,31)=0.945$; $p>0.05$] (Fig. 3B).

Next, we examined the differences in behavior of Mock, LV-shRNA, and LV-tPA-injected mice in the EPM, which is widely used in laboratories to evaluate anxiety-like behaviors in mice (Bahi et al., 2009; Lister, 1987; Moser, 1989; Pellow et al., 1985). This technique is based on the innate aversion of rodents to open areas and on the spontaneous exploratory behavior of the animals. It is thought that the time spent in the open arms of the maze is inversely correlated to their level of anxiety-related proneness. As shown in Fig. 3C, LV-shRNA mice spent less time in open arms as compared to LV-tPA or Mock-injected mice [$F(2,30)=15.811$; $p<0.001$, n = 9–11]. In contrast, when viral vectors were injected into the striatum, tPA knock down or overexpression did not affect the time spent in open arms [$F(2,31)=0.171$; $p=0.843$]. Also, as shown in Fig. 3D the number of entries in open arms was affected when tPA expression was manipulated into the hippocampus [$F(2,30)=28.742$; $p<0.001$]. Post hoc analysis showed that LV-shRNA-injected mice displayed less open arm entries compared to Mock control animals ($p=0.002$) but tPA over expressing mice displayed greater open arms entries ($p=0.001$). Also, tPA manipulation in the striatum did not affect the open arm entries in this test [$F(2,31)=0.134$; $p>0.05$]. The overall activity was estimated by the number of entries performed into the closed arms during a 5-min observation period. Interestingly, as depicted in Fig. 3E this parameter was not affected by lentiviral injection in the hippocampus [$F(2,30)=0.278$; $p>0.05$] neither in the striatum [$F(2,31)=0.052$; $p>0.05$] indicating no change in overall ambulatory behavior.

As the results of the elevated plus maze experiments suggested that tPA knockdown leads to an increase in anxiety-like behaviors, we decided to confirm these observations using an anxiolytic drug primarily used to reverse anxiogenic behaviors (Corbett et al., 1991; Dunn et al., 1989; Golda and Petr, 1989; Karakas et al., 2011; Nomikos and Spyraki, 1988). As shown in Fig. 3F, treatment of mice with Diazepam (1 mg/kg, i.p.) significantly increased the time spent in open arms in both LV-shRNA and control mice but not in tPA overexpression mice [$F(2,34)=0.200$; $p>0.05$, n = 9–11]. An overall analysis of the data with a two-way ANOVA indicated a significant effect interaction virus × treatment [$F(2,67)=4.143$; $p<0.05$].

3.4. Elevated depression-like behaviors in tPA-deficient mice

The novelty suppressed feeding (NSF), the tail-suspension test (TST) and the forced-swimming test (FST) were employed in this study to investigate the tendency towards depression-like behavior in the hippocampal tPA knock down mice groups. We, therefore, quantified the behavioral-despair with one more standard paradigm: the novelty suppressed feeding test (NSF). In this paradigm, tPA manipulation in the hippocampus impairs the time to feed in mice [$F(2,31)=12.505$; $p<0.05$, n = 9–11] (Fig. 4A). Post-hoc evaluation has revealed that LV-shRNA significantly increases the time to approach the food ($p<0.05$) whereas LV-tPA overexpression has the opposite effect compared to the Mock-injected mice ($p<0.01$). When lentiviral vectors were injected in the striatum, the time to feed was not affected [$F(2,31)=0.249$; $p>0.05$] (Fig. 4A). The impaired response of LV-shRNA mice in the NSF test suggested that they had an increase in depression levels. We also measured the total amount of food consumed in the 3 groups but no statistical difference was found (data not shown). In the tail-suspension test, as shown in Fig. 4B, tPA manipulation in the hippocampus influenced immobility time in mice [$F(2,33)=11.245$; $p<0.05$]. Thus when tPA expression was blocked in the hippocampus using LV-shRNA, immobility time for mice was significantly higher than that of mock control animals ($p<0.05$). In contrast, overexpressing tPA level in the same brain region reduced immobility time compared to control ($p<0.05$). However, when lentiviral vectors were injected into the striatum, there was no difference in immobility time of mice between LV-shRNA, LV-tPA and mock groups [$F(2,33)=0.095$; $p>0.05$]. This phenomenon was also observed in the forced-swim test [$F(2,31)=17.726$; $p<0.05$] (Fig. 4C), which showed significant increased immobility time in LV-shRNA mice compared to controls ($p<0.01$). In contrast, tPA overexpression in the hippocampus reduced immobility time in mice compared to Mock-injected mice ($p<0.01$). Treatment of mice with the tricyclic uptake inhibitor imipramine significantly reduced immobility time in both LV-shRNA and control mice but not in tPA overexpression mice [$F(2,34)=1.620$; $p>0.05$] (Fig. 4C). An overall analysis of the data

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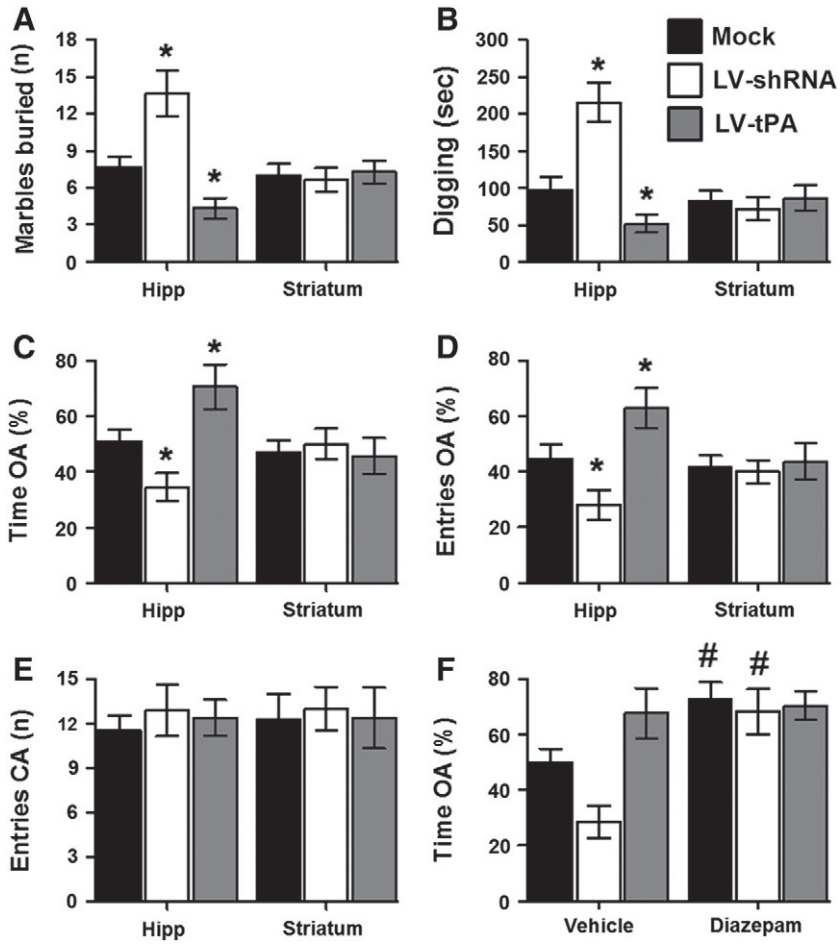

Figure 3 Effect of tPA manipulation in the hippocampus on anxiety like-behavior in mice. A) The number of buried marbles and, B) the digging time in marbles burying test. C) The percentage of time in open arms (OA) and, D) the percentage of entries in the OA and, E) the number of entries in closed arms (CA) of an elevated plus maze (EPM). F) The percentage of entries in OA of the EPM following diazepam administration. *$p<0.05$ compared to "Mock"; #$p<0.05$ compared to "Vehicle". In all panels, data represent the mean± SEM. n=9–11 mice per group.

with a two-way ANOVA indicated a significant effect interaction virus×treatment [$F(2,65)=9.111$; $p<0.05$]. These results demonstrate that targeted viral expression of tPA in the hippocampus of non-stressed mice produces profound depressive-like responses similar to the effect of forced-swim induced stress.

3.5. tPA enzymatic activity and BDNF protein expression

The spatial distribution of tPA gene transfer after injection via the stereotaxic injection described in Experimental procedures has been previously published (Bahi and Dreyer, 2008; Bahi et al., 2008b). 60 min after completion of the last behavioral experiment (FST), we quantified tPA enzymatic activity in the hippocampus. Results revealed that when mice were injected with LV-shRNA, tPA activity was reduced approximately 50% [$F(2,16)=12.613$; $p<0.05$, n=5–7] (Fig. 5A). In contrast, LV-tPA increased activity levels in the hippocampus by approx. 60 to 70% from baseline. Overall, these results indicate that anxiety- and depression-like behavioral changes depend on tPA activity in the hippocampus. More importantly, ELISA evaluation revealed that BDNF protein was reduced approximately 30% after treatment with AAV-shRNA (Fig. 5B) and increased by approx. 40 to 50% from baseline when mice were injected with LV-tPA [$F(2,17)=11.032$; $p<0.05$]. Thus, lentiviral-mediated brain delivery of tPA is effective at increasing its mRNA and enzymatic activity in the adult mouse hippocampus and consequently increased BDNF protein in the same brain region.

4. Discussion

In this study we examined the effects of tissue plasminogen activator "tPA" manipulation in the hippocampus in depression- and anxiety-like behaviors in mice. The main findings of the present study were: first, hippocampus tPA, but not uPA, activity was decreased after forced swim stress. Second, lentiviral tPA over-expression in the hippocampus shows robust antidepressant and anxiolytic-like effect. In contrast, blocking tPA expression using shRNA-expressing lentiviral vectors shows depressant and anxiogenic-like effect. Finally, tPA over-expression protective effect was associated to higher levels of BDNF proteins in the hippocampus. Taken together, these findings demonstrate that tPA plays an important role

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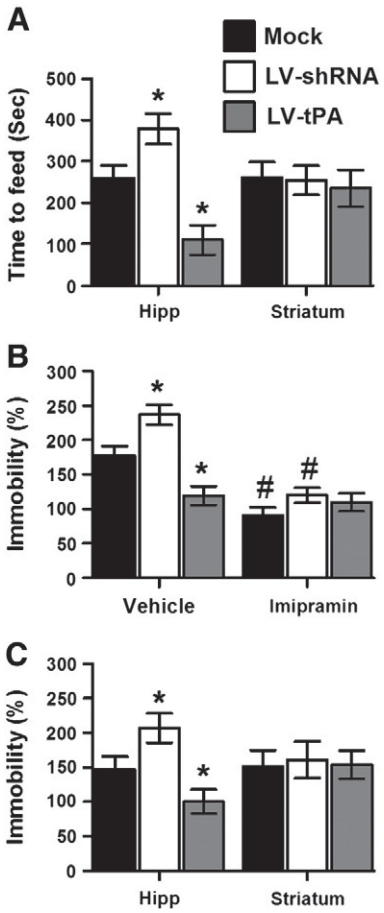

Figure 4 Effect of tPA manipulation in the hippocampus on depression like-behavior in mice. A) The time to feed in the novelty suppressed feeding. B) The immobility time in the tail suspension test following Imipramin administration. C) The immobility time in the forced swim test. *$p<0.05$ compared to "Mock"; #$p<0.05$ compared to "Vehicle", . In all panels, data represent the mean ± SEM. n = 9–11 mice per group.

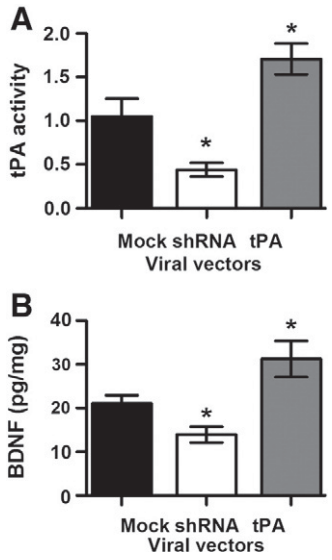

Figure 5 Effect of tPA manipulation in the hippocampus on tPA enzymatic activity and BDNF protein expression. A) The tPA activity. B) The BDNF protein levels in mice hippocampal extracts following the last forced swim test.*$p<0.05$ compared to "Mock". In both panels, data represent the mean ± SEM. n = 5–7 mice per group.

in psychiatric disorders most probably trough a BDNF mechanism.

The extracellular serine protease tPA was found to be highly expressed in mood disorders-associated brain nuclei including the hippocampus, amygdala, prefrontal cortex and infra-limbic regions (Lohman et al., 2008; Wu et al., 2000; Zhou et al., 2010). In the first experiment we found a decrease of tPA enzymatic activity in the hippocampus of mice from the stress group when compared to mice from the control group. More importantly, this effect was tPA-specific as uPA, a different serine protease, was not affected after stress in mice. Changes in stress-induced tPA expression and activity were reported both in vitro and in vivo. In fact, it has been shown that tPA mRNA levels were higher in primary cultures endothelial cells subjected to shear stress or cyclic strain than in controls (Diamond et al., 1990; Iba and Sumpio, 1992; Nollert et al., 1991). This effect was generalized to several cell lines (Sjogren et al., 2000; Ueba et al., 1997; Yang et al., 2006). In addition, the signaling cascade involved in stress-induced tPA expression is most probably PKC-independent (Levin et al., 1993) suggesting that environmental stressors may regulate tPA gene expression. More interestingly, tPA expression changes were found in humans. In fact, when assessing the effects of acute laboratory stressors on tPA in a sample of cardiac patients, participants subjected to mental stress condition revealed a statistically significant decrease in tPA levels in blood samples (Hevey et al., 2000). In addition, a significant decrease of plasma tPA concentration was observed in patients with depression (Pietraszek et al., 1991). Interestingly, tPA expression was enhanced in the amygdala following acute restraint stress (Pawlak et al., 2003). Thus, we can hypothesize that the enhanced ability of stress to induce tPA enzymatic activity in the hippocampus may affect stress-related mood disorders like anxiety and depression.

To further investigate the physiological significance of stress-induced decrease in tPA enzymatic activity in the hippocampus, we explored the effect of tPA manipulation on anxiety- and depression-like behaviors in mice. To this end, we over-express tPA or knock-down its mRNA expression by mean of shRNA-expressing lentiviral vectors. These tools have been successfully used to investigate gene function in the central nervous system (Bahi et al., 2004a, 2006, 2008b, 2008c; Bahi and Dreyer, 2008, 2011). Using marble burying and elevated plus maze tests we found that anxiety-like behavior was reduced in tPA over-expressing mice but significantly exacerbated when tPA transcripts were inhibited by mean of specific shRNA-expressing lentiviral vectors in the hippocampus. More importantly, these effects were region specific as tPA manipulation in the dorsal striatum had no effect. These findings are not in the line with previously published studies in which tPA deficient mice did not show restraint-induced anxiety (Bennur et al., 2007; Matys et al., 2004; Pawlak et al., 2003). These findings seem to be in contrast to our current observations, but it is likely that the lack of stress-induced anxiety in tPA knockout mice involves

unknown compensatory mechanisms characterizing conventional gene knockout. Also, developmental neuronal alterations may explain the apparent conflicting results. In fact, these same KO mice displayed attenuated neuronal remodeling, impaired hormonal response and attenuated stress-induced spine retraction in the medial amygdala (Bennur et al., 2007; Matys et al., 2004) which makes it difficult to compare those results with our findings. In the elevated plus maze test, no changes in the number of entries onto the closed arms were found. The finding that this last index did not change allowed us to discard some effect of tPA manipulation on motor activity. Besides, the time spent on the open arms increased after tPA over-expression. Because this parameter is poorly influenced by locomotion, it appeared as a clear index of anxiety, suggesting that tPA exerted an anxiolytic effect. Indeed, normal exploratory behavior is directed to the closed arms, and this tendency can be enhanced by anxiogenic drugs, which increment the natural aversion towards open arms. In contrast, anxiolytic agents reduce this aversion and promote exploration (Britton et al., 1991; Fernandez-Guasti et al., 2005; Rex et al., 1993; Zangrossi et al., 1992). This was confirmed in the current study as we have shown that tPA knock-down-induced anxiety-like behavior was reversed when diazepam was applied before the test. Taken together, it is likely that the tPA is crucial in regulating anxiety-like behavior in mice.

We also investigated whether tPA manipulation is involved in depression-like behavior using novelty suppressed feeding, tail suspension and forced swim tests. Our findings suggest that depression-like behavior was enhanced in shRNA-expressing mice but tPA over-expression leads to an attenuated response. In fact, latency to feed and immobility time were significantly enhanced when tPA mRNA was blocked by means of shRNA expressing viral vectors compared with control mice (Mock vector). In contrast, those parameters were attenuated when tPA was over-expressed in the hippocampus. Importantly, higher BDNF levels were found in tPA over-expressing hippocampal lysates. These findings suggest that tPA/BDNF complex activity in the hippocampus plays a crucial role in depression-like behavior in mice.

A possible role of tPA in Major depressive disorders was reviewed (Hou et al., 2009; Tsai, 2006, 2007a,b). More interestingly, when plasma BDNF and tPA were measured in late-onset geriatric depression (LGD) before treatment, it has been found that, compared to controls, these levels were significantly lower in LGD patients (Shi et al., 2010). In addition, BDNF immune-reactivity was higher after antidepressant treatment. Also, post-stroke depression (PSD) patients displayed significantly lower plasma BDNF than non-PSD patients (Yang et al., 2011). Very recently, it has been reported that voluntary physical activity induced a robust increase in hippocampal BDNF levels, as well as in tPA mRNA expression; these changes were associated to anti-depressive effects as compared with sedentary mice (Sartori et al., 2011). In addition, it has been shown that the BDNF receptor TrkB, modulate fear learning and amygdala synaptic plasticity through two main phosphorylation docking sites [Y515/Shc and Y816/PLCγ] (Musumeci et al., 2009). Taken together, the antidepressive effect of tPA over-expression may depend, at least in part, on changes in BDNF post-translational modification. In fact, it is well established that Pro-BDNF cleavage depends on tPA activity (Barnes and Thomas, 2008; Pang et al., 2004). Although the cellular and molecular mechanisms remain to be further clarified, it is suggested that the effects of tPA might be due to BDNF processing changes in stress-induced depression- and anxiety-like behaviors.

It is possible that mood regulation involves the tPA/BDNF system also in other brain areas. tPA is also expressed in the amygdala and infra-limbic regions, which may participate in the regulation of stress-related psychiatric illness. In any case, our data show that tPA plays an important role in mood regulation, thus providing a potential molecular link between extracellular proteases and mood regulation. Targeting the tPA/BDNF system may be a new avenue for the treatment of mood disorders.

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Contributors

The work presented here was carried out in collaboration between both authors. AB and JLD defined the research theme. AB designed methods and experiments, carried out the laboratory experiments, analyzed the data, interpreted the results and drafted the manuscript. JLD co-worked on data interpretation and improved manuscript writing. Both authors have approved the manuscript.

Conflict of interest

The authors have no financial interests that might be perceived to influence the results or the discussion reported in this article.

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References

Abe, M., Nakai, H., Tabata, R., Saito, K., Egawa, M., 1998. Effect of 5-[3-[((2S)-1,4-benzodioxan-2-ylmethyl) amino]propoxy]-1,3-benzodioxole HCl (MKC-242), a novel 5-HT1A-receptor agonist, on aggressive behavior and marble burying behavior in mice. Jpn. J. Pharmacol. 76, 297–304.

Bahi, A., Dreyer, J.L., 2008. Overexpression of plasminogen activators in the nucleus accumbens enhances cocaine-, amphetamine- and morphine-induced reward and behavioral sensitization. Genes Brain Behav. 7, 244–256.

Bahi, A., Dreyer, J.L., 2011. Involvement of tissue plasminogen activator "tPA" in ethanol-induced locomotor sensitization and conditioned-place preference. Behav. Brain Res. 226, 250–258.

Bahi, A., Boyer, F., Gumy, C., Kafri, T., Dreyer, J.L., 2004a. In vivo gene delivery of urokinase-type plasminogen activator with regulatable lentivirus induces behavioural changes in chronic cocaine administration. Eur. J. Neurosci. 20, 3473–3488.

http://doc.rero.ch

http://doc.rero.ch

Bahi, A., Boyer, F., Kafri, T., Dreyer, J.L., 2004b. CD81-induced behavioural changes during chronic cocaine administration: in vivo gene delivery with regulatable lentivirus. Eur. J. Neurosci. 19, 1621–1633.

Bahi, A., Boyer, F., Bussard, G., Dreyer, J.L., 2005a. Silencing dopamine D3-receptors in the nucleus accumbens shell in vivo induces changes in cocaine-induced hyperlocomotion. Eur. J. Neurosci. 21, 3415–3426.

Bahi, A., Boyer, F., Kolira, M., Dreyer, J.L., 2005b. In vivo gene silencing of CD81 by lentiviral expression of small interference RNAs suppresses cocaine-induced behaviour. J. Neurochem. 92, 1243–1255.

Bahi, A., Boyer, F., Kafri, T., Dreyer, J.L., 2006. Silencing urokinase in the ventral tegmental area in vivo induces changes in cocaine-induced hyperlocomotion. J. Neurochem. 98, 1619–1631.

Bahi, A., Boyer, F., Chandrasekar, V., Dreyer, J.L., 2008a. Role of accumbens BDNF and TrkB in cocaine-induced psychomotor sensitization, conditioned-place preference, and reinstatement in rats. Psychopharmacology (Berl) 199, 169–182.

Bahi, A., Kusnecov, A., Dreyer, J.L., 2008b. The role of tissue-type plasminogen activator system in amphetamine-induced conditional place preference extinction and reinstatement. Neuropsychopharmacology 33, 2726–2734.

Bahi, A., Kusnecov, A.W., Dreyer, J.L., 2008c. Effects of urokinase-type plasminogen activator in the acquisition, expression and reinstatement of cocaine-induced conditioned-place preference. Behav. Brain Res. 191, 17–25.

Bahi, A., Mineur, Y.S., Picciotto, M.R., 2009. Blockade of protein phosphatase 2B activity in the amygdala increases anxiety- and depression-like behaviors in mice. Biol. Psychiatry 66, 1139–1146.

Barnes, P., Thomas, K.L., 2008. Proteolysis of proBDNF is a key regulator in the formation of memory. PLoS One 3, e3248.

Bennur, S., Shankaranarayana Rao, B.S., Pawlak, R., Strickland, S., McEwen, B.S., Chattarji, S., 2007. Stress-induced spine loss in the medial amygdala is mediated by tissue-plasminogen activator. Neuroscience 144, 8–16.

Britton, K.T., Page, M., Baldwin, H., Koob, G.F., 1991. Anxiolytic activity of steroid anesthetic alphaxalone. J. Pharmacol. Exp. Ther. 258, 124–129.

Corbett, R., Fielding, S., Cornfeldt, M., Dunn, R.W., 1991. GABAmimetic agents display anxiolytic-like effects in the social interaction and elevated plus maze procedures. Psychopharmacology (Berl) 104, 312–316.

Diamond, S.L., Sharefkin, J.B., Dieffenbach, C., Frasier-Scott, K., McIntire, L.V., Eskin, S.G., 1990. Tissue plasminogen activator messenger RNA levels increase in cultured human endothelial cells exposed to laminar shear stress. J. Cell. Physiol. 143, 364–371.

D'Sa, C., Duman, R.S., 2002. Antidepressants and neuroplasticity. Bipolar Disord. 4, 183–194.

Dunn, R.W., Corbett, R., Fielding, S., 1989. Effects of 5-HT1A receptor agonists and NMDA receptor antagonists in the social interaction test and the elevated plus maze. Eur. J. Pharmacol. 169, 1–10.

Enoch, M.A., White, K.V., Waheed, J., Goldman, D., 2008. Neurophysiological and genetic distinctions between pure and comorbid anxiety disorders. Depress Anxiety 25, 383–392.

Fernandez-Guasti, A., Reyes, R., Martinez-Mota, L., Lopez-Munoz, F.J., 2005. Influence of inflammatory nociception on the anxiolytic-like effect of diazepam and buspirone in rats. Psychopharmacology (Berl) 180, 399–407.

Franklin, K.B.J., Paxinos, G., 1996. The mouse brain in stereotaxic coordinates. New York .

Gadotti, V.M., Bonfield, S.P., Zamponi, G.W., 2012. Depressive-like behaviour of mice lacking cellular prion protein. Behav. Brain Res. 227, 319–323.

Golda, V., Petr, R., 1989. Animal model of anxiety: effect of acute diazepam treatment in the older adult genetically hypertensive rats of Koletsky type and in the older adult rats of Wistar strain. Sb. Ved. Pr. Lek. Fak. Karlovy Univerzity Hradci Kralove 32, 407–423.

Gruart, A., Sciarretta, C., Valenzuela-Harrington, M., Delgado-Garcia, J.M., Minichiello, L., 2007. Mutation at the TrkB PLC {gamma}-docking site affects hippocampal LTP and associative learning in conscious mice. Learn. Mem. 14, 54–62.

Guilloux, J.P., David, D.J., Xia, L., Nguyen, H.T., Rainer, Q., Guiard, B.P., Reperant, C., Deltheil, T., Toth, M., Hen, R., Gardier, A.M., 2011. Characterization of 5-HT (1A/1B)−/− mice: an animal model sensitive to anxiolytic treatments. Neuropharmacology 61, 478–488.

Hevey, D., McGee, H.M., Fitzgerald, D., Horgan, J.H., 2000. Acute psychological stress decreases plasma tissue plasminogen activator (tPA) and tissue plasminogen activator/plasminogen activator inhibitor-1 (tPA/PAI-1) complexes in cardiac patients. Eur. J. Appl. Physiol. 83, 344–348.

Hou, S.J., Yen, F.C., Tsai, S.J., 2009. Is dysfunction of the tissue plasminogen activator (tPA)-plasmin pathway a link between major depression and cardiovascular disease? Med. Hypotheses 72, 166–168.

Iba, T., Sumpio, B.E., 1992. Tissue plasminogen activator expression in endothelial cells exposed to cyclic strain in vitro. Cell Transplant. 1, 43–50.

Karakas, A., Coskun, H., Kaya, A., Kucuk, A., Gunduz, B., 2011. The effects of the intraamygdalar melatonin injections on the anxiety like behavior and the spatial memory performance in male Wistar rats. Behav. Brain Res. 222, 141–150.

Kauer-Sant'Anna, M., Tramontina, J., Andreazza, A.C., Cereser, K., da Costa, S., Santin, A., Yatham, L.N., Kapczinski, F., 2007. Traumatic life events in bipolar disorder: impact on BDNF levels and psychopathology. Bipolar Disord. 9 (Suppl. 1), 128–135.

Kim, Y.K., Lee, H.P., Won, S.D., Park, E.Y., Lee, H.Y., Lee, B.H., Lee, S.W., Yoon, D., Han, C., Kim, D.J., Choi, S.H., 2007. Low plasma BDNF is associated with suicidal behavior in major depression. Prog. Neuropsychopharmacol. Biol. Psychiatry 31, 78–85.

Kobayashi, K., Shimizu, E., Hashimoto, K., Mitsumori, M., Koike, K., Okamura, N., Koizumi, H., Ohgake, S., Matsuzawa, D., Zhang, L., Nakazato, M., Iyo, M., 2005. Serum brain-derived neurotrophic factor (BDNF) levels in patients with panic disorder: as a biological predictor of response to group cognitive behavioral therapy. Prog. Neuropsychopharmacol. Biol. Psychiatry 29, 658–663.

Lang, U.E., Hellweg, R., Kalus, P., Bajbouj, M., Lenzen, K.P., Sander, T., Kunz, D., Gallinat, J., 2005. Association of a functional BDNF polymorphism and anxiety-related personality traits. Psychopharmacology (Berl) 180, 95–99.

Lee, B.H., Kim, H., Park, S.H., Kim, Y.K., 2007. Decreased plasma BDNF level in depressive patients. J. Affect. Disord. 101, 239–244.

Levin, E.G., Santell, L., Saljooque, F., 1993. Hyperosmotic stress stimulates tissue plasminogen activator expression by a PKC-independent pathway. Am. J. Physiol. 265, C387–C396.

Li, Y., Luikart, B.W., Birnbaum, S., Chen, J., Kwon, C.H., Kernie, S.G., Bassel-Duby, R., Parada, L.F., 2008. TrkB regulates hippocampal neurogenesis and governs sensitivity to antidepressive treatment. Neuron 59, 399–412.

Liang, Y., Shaw, A.M., Boules, M., Briody, S., Robinson, J., Oliveros, A., Blazar, E., Williams, K., Zhang, Y., Carlier, P.R., Richelson, E., 2008. Antidepressant-like pharmacological profile of a novel triple reuptake inhibitor, (1S,2S)-3-(methylamino)-2-(naphthalen-2-yl)-1-phenylpropan-1-ol (PRC200-SS). J. Pharmacol. Exp. Ther. 327, 573–583.

Lister, R.G., 1987. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology (Berl) 92, 180–185.

Liu, J., Perez, S.M., Zhang, W., Lodge, D.J., Lu, X.Y., 2011. Selective deletion of the leptin receptor in dopamine neurons produces anxiogenic-like behavior and increases dopaminergic activity in amygdala. Mol. Psychiatry 16, 1024–1038.

http://doc.rero.ch

Lochner, J.E., Honigman, L.S., Grant, W.F., Gessford, S.K., Hansen, A.B., Silverman, M.A., Scalettar, B.A., 2006. Activity-dependent release of tissue plasminogen activator from the dendritic spines of hippocampal neurons revealed by live-cell imaging. J. Neurobiol. 66, 564–577.

Lohman, R.J., O'Brien, T.J., Cocks, T.M., 2008. Protease-activated receptor-2 regulates trypsin expression in the brain and protects against seizures and epileptogenesis. Neurobiol. Dis. 30, 84–93.

Lyons, W.E., Mamounas, L.A., Ricaurte, G.A., Coppola, V., Reid, S.W., Bora, S.H., Wihler, C., Koliatsos, V.E., Tessarollo, L., 1999. Brain-derived neurotrophic factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. Proc. Natl. Acad. Sci. U. S. A. 96, 15239–15244.

Matrisciano, F., Bonaccorso, S., Ricciardi, A., Scaccianoce, S., Panaccione, I., Wang, L., Ruberto, A., Tatarelli, R., Nicoletti, F., Girardi, P., Shelton, R.C., 2009. Changes in BDNF serum levels in patients with major depression disorder (MDD) after 6 months treatment with sertraline, escitalopram, or venlafaxine. J. Psychiatr. Res. 43, 247–254.

Matys, T., Pawlak, R., Matys, E., Pavlides, C., McEwen, B.S., Strickland, S., 2004. Tissue plasminogen activator promotes the effects of corticotropin-releasing factor on the amygdala and anxiety-like behavior. Proc. Natl. Acad. Sci. U. S. A. 101, 16345–16350.

Melchor, J.P., Strickland, S., 2005. Tissue plasminogen activator in central nervous system physiology and pathology. Thromb. Haemost. 93, 655–660.

Minelli, A., Zanardini, R., Bonvicini, C., Sartori, R., Pedrini, L., Gennarelli, M., Bocchio-Chiavetto, L., 2011. BDNF serum levels, but not BDNF Val66Met genotype, are correlated with personality traits in healthy subjects. Eur. Arch. Psychiatry Clin. Neurosci. 261, 323–329.

Mitoma, M., Yoshimura, R., Sugita, A., Umene, W., Hori, H., Nakano, H., Ueda, N., Nakamura, J., 2008. Stress at work alters serum brain-derived neurotrophic factor (BDNF) levels and plasma 3-methoxy-4-hydroxyphenylglycol (MHPG) levels in healthy volunteers: BDNF and MHPG as possible biological markers of mental stress? 'acol. Biol. Psychiatry 32, 679–685.

Montag, C., Basten, U., Stelzel, C., Fiebach, C.J., Reuter, M., 2010. The BDNF Val66Met polymorphism and anxiety: support for animal knock-in studies from a genetic association study in humans. Psychiatry Res. 179, 86–90.

Moser, P.C., 1989. An evaluation of the elevated plus-maze test using the novel anxiolytic buspirone. Psychopharmacology (Berl) 99, 48–53.

Musumeci, G., Sciarretta, C., Rodriguez-Moreno, A., Al Banchaabouchi, M., Negrete-Diaz, V., Costanzi, M., Berno, V., Egorov, A.V., von Bohlen Und Halbach, O., Cestari, V., Delgado-Garcia, J.M., Minichiello, L., 2009. TrkB modulates fear learning and amygdalar synaptic plasticity by specific docking sites. J. Neurosci. 29, 10131–10143.

Nagai, T., Nabeshima, T., Yamada, K., 2008. Basic and translational research on proteinase-activated receptors: regulation of nicotine reward by the tissue plasminogen activator (tPA)—plasmin system via proteinase-activated receptor 1. J. Pharmacol. Sci. 108, 408–414.

Njung'e, K., Handley, S.L., 1991. Effects of 5-HT uptake inhibitors, agonists and antagonists on the burying of harmless objects by mice; a putative test for anxiolytic agents. Br. J. Pharmacol. 104, 105–112.

Noble, E.E., Billington, C.J., Kotz, C.M., Wang, C., 2011. The lighter side of BDNF. Am. J. Physiol. Regul. Integr. Comp. Physiol. 300, R1053–R1069.

Noel, M., Norris, E.H., Strickland, S., 2011. Tissue plasminogen activator is required for the development of fetal alcohol syndrome in mice. Proc. Natl. Acad. Sci. U. S. A. 108, 5069–5074.

Nollert, M.U., Diamond, S.L., McIntire, L.V., 1991. Hydrodynamic shear stress and mass transport modulation of endothelial cell metabolism. Biotechnol. Bioeng. 38, 588–602.

Nomikos, G.G., Spyraki, C., 1988. Influence of oestrogen on spontaneous and diazepam-induced exploration of rats in an elevated plus maze. Neuropharmacology 27, 691–696.

Numakawa, T., Suzuki, S., Kumamaru, E., Adachi, N., Richards, M., Kunugi, H., 2010. BDNF function and intracellular signaling in neurons. Histol. Histopathol. 25, 237–258.

Olivier, J.D., Van Der Hart, M.G., Van Swelm, R.P., Dederen, P.J., Homberg, J.R., Cremers, T., Deen, P.M., Cuppen, E., Cools, A.R., Ellenbroek, B.A., 2008. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. Neuroscience 152, 573–584.

Ortiz, O., Delgado-Garcia, J.M., Espadas, I., Bahi, A., Trullas, R., Dreyer, J.L., Gruart, A., Moratalla, R., 2010. Associative learning and CA3-CA1 synaptic plasticity are impaired in D1R null, Drd1a−/− mice and in hippocampal siRNA silenced Drd1a mice. J. Neurosci. 30, 12288–12300.

Pang, P.T., Teng, H.K., Zaitsev, E., Woo, N.T., Sakata, K., Zhen, S., Teng, K.K., Yung, W.H., Hempstead, B.L., Lu, B., 2004. Cleavage of proBDNF by tPA/plasmin is essential for long-term hippocampal plasticity. Science 306, 487–491.

Parolini, S., Flagiello, D., Cinquetti, A., Gozzi, R., Cristini, S., Cappiello, J., Nicolai, P., Rusnati, M., Presta, M., Tosatti, M.M., 1996. Up-regulation of urokinase-type plasminogen activator in squamous cell carcinoma of human larynx. Br. J. Cancer 74, 1168–1174.

Pawlak, R., Magarinos, A.M., Melchor, J., McEwen, B., Strickland, S., 2003. Tissue plasminogen activator in the amygdala is critical for stress-induced anxiety-like behavior. Nat. Neurosci. 6, 168–174.

Pellow, S., Chopin, P., File, S.E., Briley, M., 1985. Validation of open:closed arm entries in an elevated plus-maze as a measure of anxiety in the rat. J. Neurosci. Methods 14, 149–167.

Pietraszek, M.H., Takahashi, S., Takada, Y., Ohara, K., Inatomi, H., Kondo, N., Ohara, K., Takada, A., 1991. Diurnal patterns of serotonin, 5-hydroxyindoleacetic acid, tryptophan and fibrinolytic activity in blood of depressive patients and healthy volunteers. Thromb. Res. 64, 243–252.

Popova, N.K., Morozova, M.V., Naumenko, V.S., 2011. Ameliorative effect of BDNF on prenatal ethanol and stress exposure-induced behavioral disorders. Neurosci. Lett. 505, 82–86.

Pothakos, K., Robinson, J.K., Gravanis, I., Marsteller, D.A., Dewey, S.L., Tsirka, S.E., 2010. Decreased serotonin levels associated with behavioral disinhibition in tissue plasminogen activator deficient (tPA−/−) mice. Brain Res. 1326, 135–142.

Qian, Z., Gilbert, M.E., Colicos, M.A., Kandel, E.R., Kuhl, D., 1993. Tissue-plasminogen activator is induced as an immediate-early gene during seizure, kindling and long-term potentiation. Nature 361, 453–457.

Rex, A., Marsden, C.A., Fink, H., 1993. Effect of diazepam on cortical 5-HT release and behaviour in the guinea-pig on exposure to the elevated plus maze. Psychopharmacology (Berl) 110, 490–496.

Rios, M., Fan, G., Fekete, C., Kelly, J., Bates, B., Kuehn, R., Lechan, R.M., Jaenisch, R., 2001. Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. Mol. Endocrinol. 15, 1748–1757.

Sales, A.J., Biojone, C., Terceti, M.S., Guimaraes, F.S., Gomes, M.V., Joca, S.R., 2011. Antidepressant-like effect induced by systemic and intra-hippocampal administration of DNA methylation inhibitors. Br. J. Pharmacol. 164, 1711–1721.

Sartori, C.R., Vieira, A.S., Ferrari, E.M., Langone, F., Tongiorgi, E., Parada, C.A., 2011. The antidepressive effect of the physical exercise correlates with increased levels of mature BDNF, and proBDNF proteolytic cleavage-related genes, p11 and tPA. Neuroscience 180, 9–18.

Schmidt, H.D., Duman, R.S., 2010. Peripheral BDNF produces antidepressant-like effects in cellular and behavioral models. Neuropsychopharmacology 35, 2378–2391.
Seeds, N.W., Basham, M.E., Haffke, S.P., 1999. Neuronal migration is retarded in mice lacking the tissue plasminogen activator gene. Proc. Natl. Acad. Sci. U. S. A. 96, 14118–14123.
Shi, Y., You, J., Yuan, Y., Zhang, X., Li, H., Hou, G., 2010. Plasma BDNF and tPA are associated with late-onset geriatric depression. Psychiatry Clin Neurosci 64, 249–254.
Shieh, C.H., Hong, C.J., Huang, Y.H., Tsai, S.J., 2008. Potential antidepressant properties of cysteamine on hippocampal BDNF levels and behavioral despair in mice. Prog. Neuropsychopharmacol. Biol. Psychiatry 32, 1590–1594.
Sjogren, L.S., Gan, L., Doroudi, R., Jern, C., Jungersten, L., Jern, S., 2000. Fluid shear stress increases the intra-cellular storage pool of tissue-type plasminogen activator in intact human conduit vessels. Thromb. Haemost. 84, 291–298.
Strohle, A., Stoy, M., Graetz, B., Scheel, M., Wittmann, A., Gallinat, J., Lang, U.E., Dimeo, F., Hellweg, R., 2010. Acute exercise ameliorates reduced brain-derived neurotrophic factor in patients with panic disorder. Psychoneuroendocrinology 35, 364–368.
Teesalu, T., Kulla, A., Asser, T., Koskiniemi, M., Vaheri, A., 2002. Tissue plasminogen activator as a key effector in neurobiology and neuropathology. Biochem. Soc. Trans. 30, 183–189.
Tsai, S.J., 2006. The possible role of tissue-type plasminogen activator and the plasminogen system in the pathogenesis of major depression. Med. Hypotheses 66, 319–322.
Tsai, S.J., 2007a. The P11, tPA/plasminogen system and brain-derived neurotrophic factor: Implications for the pathogenesis of major depression and the therapeutic mechanism of antidepressants. Med. Hypotheses 68, 180–183.
Tsai, S.J., 2007b. Statins may enhance the proteolytic cleavage of proBDNF: implications for the treatment of depression. Med. Hypotheses 68, 1296–1299.
Ueba, H., Kawakami, M., Yaginuma, T., 1997. Shear stress as an inhibitor of vascular smooth muscle cell proliferation. Role of transforming growth factor-beta 1 and tissue-type plasminogen activator. Arterioscler. Thromb. Vasc. Biol. 17, 1512–1516.
Umathe, S.N., Manna, S.S., Jain, N.S., 2011. Involvement of endocannabinoids in antidepressant and anti-compulsive effect of fluoxetine in mice. Behav. Brain Res. 223, 125–134.
Uz, T., Dimitrijevic, N., Tueting, P., Manev, H., 2002. 5-Lipoxygenase (5LOX)-deficient mice express reduced anxiety-like behavior. Restor. Neurol. Neurosci. 20, 15–20.
Wu, Y.P., Siao, C.J., Lu, W., Sung, T.C., Frohman, M.A., Milev, P., Bugge, T.H., Degen, J.L., Levine, J.M., Margolis, R.U., Tsirka, S.E., 2000. The tissue plasminogen activator (tPA)/plasmin extracellular proteolytic system regulates seizure-induced hippocampal mossy fiber outgrowth through a proteoglycan substrate. J. Cell Biol. 148, 1295–1304.
Yang, Z., Tao, J., Wang, J.M., Tu, C., Xu, M.G., Wang, Y., Pan, S.R., 2006. Shear stress contributes to t-PA mRNA expression in human endothelial progenitor cells and nonthrombogenic potential of small diameter artificial vessels. Biochem. Biophys. Res. Commun. 342, 577–584.
Yang, L., Zhang, Z., Sun, D., Xu, Z., Yuan, Y., Zhang, X., Li, L., 2011. Low serum BDNF may indicate the development of PSD in patients with acute ischemic stroke. Int. J. Geriatr. Psychiatry 26, 495–502.
Zangrossi Jr., H., Leite, J.R., Graeff, F.G., 1992. Anxiolytic effect of carbamazepine in the elevated plus-maze: possible role of adenosine. Psychopharmacology (Berl) 106, 85–89.
Zhou, Y., Maiya, R., Norris, E.H., Kreek, M.J., Strickland, S., 2010. Involvement of tissue plasminogen activator in stress responsivity during acute cocaine withdrawal in mice. Stress 13, 481–490.
Zorio, E., Gilabert-Estelles, J., Espana, F., Ramon, L.A., Cosin, R., Estelles, A., 2008. Fibrinolysis: the key to new pathogenetic mechanisms. Curr. Med. Chem. 15, 923–929.

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