Effects of urokinase-type plasminogen activator in the acquisition, expression and reinstatement of cocaine-induced conditioned-place preference

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Abstract

Cocaine and many other psychostimulants strongly induce urokinase-type plasminogen activator (uPA) expression in the mesolimbic dopaminergic pathway, which plays a major role in drug-mediated behavioral plasticity [Bahi A, Boyer F, Gumy C, Kafri T, Dreyer JL. In vivo gene delivery of urokinase-type plasminogen activator with regulatable lentivirus induces behavioral changes in chronic cocaine administration. Eur J Neurosci 2004;20:3473–88; Bahi A, Boyer F, Kafri T, Dreyer JL. Silencing urokinase in the ventral tegmental area in vivo induces changes in cocaine-induced hyperlocomotion. J Neurochem 2006;98:1619–31; Bahi A, Dreyer JL. Overexpression of plasminogen activators in the nucleus accumbens enhances cocaine-, amphetamine- and morphine-induced reward and behavioral sensitization. Genes Brain Behav 2007]. In this study, the role of mesolimbic dopamine (DA) pathways in the development of cocaine reward was examined by conditioned-place preference in rats with bilateral intra-accumbens injections of uPA-expressing lentiviral vectors. We show that overexpression of uPA in the Nac significantly augments cocaine-induced place preference. Furthermore, while this did not affect the ability of preference to be extinguished, reinstatement with a low dose of cocaine produced significantly greater preference to the cocaine-associated context. Once CPP had been established, and the preference extinguished, reinstatement induced by a priming dose of cocaine was facilitated by uPA. Inhibition of this serine protease expression using doxycycline abolished the augmented acquisition produced by overexpression of uPA but not the expression of the cocaine-induced CPP. When uPA is inhibited during the acquisition phase, animals no longer demonstrate place preference for the environment previously paired with cocaine. B428, a specific uPA inhibitor does not affect drug reinstatement after extinction if uPA has been activated during acquisition, a clear indication that uPA is involved in the acquisition phase of conditioned-place preference. Our results suggest that that increased uPA expression with repeated drug exposure produces conditions for enhanced acquisition of cocaine-induced CPP, indicating that cocaine-induced CPP and reinstatement may be dependent on active extracellular uPA.

1. Introduction

Plasminogen activators are important mediators of extracellular metabolism, involved in remodeling events during development and regeneration in the nervous system. The generation of plasmin from its inactive precursor plasminogen, is mediated by serine enzymes known as tissue-type plasminogen activator (tPA) and urokinase (uPA), and contributes to the turnover of the extracellular matrix in the central nervous system [1]. Urokinase-type plasminogen activator (uPA) exerts a variety of functions during development, and is involved in learning and memory. Expression of uPA and its receptor, uPAR, may play an important role for synaptogenesis, remodeling, and reactive processes other than for cell migration in developing mouse brain [2]. Activity-dependent synaptic plasticity and remodeling of the mesolimbic dopaminergic system play a crucial role in the development of drug dependence [3–6]. It is well established that drugs of abuse, including the stimulants cocaine and metamphetamine, as well as morphine, acutely modulate the activity of mesolimbic dopaminergic neurons, projecting from the ventral tegmental area (VTA) of the midbrain to the nucleus accumbens (NAc) [7–11]. Consistent with its hypo-
esized role in plasticity, uPA expression is strongly induced in the mesolimbic dopaminergic pathway in response to psychostimu-
lant treatment [12]. In turn, uPA induces strong behavioral changes associated with drug delivery. For example, metamphetamine-
induced dopamine release in the nucleus accumbens recruits the plasminogen activator–plasmin system [13], inducing long-term
synaptic plasticity and remodeling, and increasing drug-induced reward [14,15]. The plasmin system also participates in the reward-
ning effects of morphine by acutely regulating morphine-induced dopamine release in the nucleus accumbens (NAc) [13,16]. A sin-
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dopamine release in the nucleus accumbens (NAc) [16,17].

We have previously shown that genetic manipulation of uPA expression in rats’ results in altered reactivity to cocaine adminis-
tration. Lentiviral-mediated overexpression of uPA in the VTA of rats increases doxycycline-dependent expression of its receptor, uPAR, but not its inhibitor, plasminogen activator inhibitor-1 (PAI-1) [18]. The expression of uPAR in the VTA is repressed upon silencing of uPA with lentiviruses expressing siRNAs [18]. Associated with the over-
expression of uPA in the VTA is a 10-fold increase in locomotor activ-
ity following injection of cocaine [12]. However, animals expressing the dominant-negative form of uPA failed to display such hyper-
locomotor activity [12]. These cocaine-induced behavioral changes, associated with uPA expression, could be suppressed in the pres-
ence of doxycycline or of siRNA targeted against uPA [18,19]. Collectively, these and other data [13] strongly support a major role for urokinase in psychostimulant-mediated plasticity changes.

In the present study, we extended our previous observations [12,18,19] by assessing the role of accumal uPA overexpression on the reinforcing properties of cocaine in the conditioned-place preference (CPP) paradigm. Animals were injected into the nucleus accumbens with lentiviruses expressing uPA, and various param-
ters of place preference conditioning were assessed. We show that uPA expression was important for the acquisition, but not expres-
sion of CPP. In addition, when uPA was inhibited during acquisition, animals displayed a memory deficit and no longer associated the environement with cocaine. After extinction, priming with low doses of cocaine reinstated place preference, but reinstatement of place preference was strongly facilitated upon uPA expression. These data confirm that the plasmin system plays an important function in the expression of drug dependence, mainly through activation of extracellular proteases, e.g. uPA.

2. Materials and methods

2.1. Animals

Animals used in this experiment were male Wistar rats weighing 220–250g. All animal experiments were carried out in accordance with the guidelines and regulations for Animal Experimentation, BAG, Bern, Switzerland. The animals were housed in trios in clear plastic cages with wire grid lids. Access to food and water was unrestricted. The animals were kept in the animal facility maintained on a 12-h light:12-h dark cycle (light off at 7 a.m.).

2.2. Lentivirus construction of LV-uPA and LV-GFP

Briefly, the rat uPA cDNA was amplified by reverse transcription using super-
script II reverse transcriptase (Invitrogen, Switzerland) following the manufacturer’s instructions. The cDNA was then PCR amplified, 6 His-tagged, digested with BamHI and XhoI and cloned into similar sites in pTK431 [12,18,19]. A control vector con-
struct, pTK433 in which green fluorescent protein (GFP) expression is regulated by a tetracycline inducible promoter, was generated by cloning a BamHI/BglII DNA fragment containing the GFP gene into a BamHI site in pTK431 [12,18,19–22]. All plasmids were CsCl2 purified.

Vesicular stomatitis virus G pseudo-typed lentiviruses were produced by the transient calcium phosphate co-transfection of HEK293T cells with pTK vectors together with pMDG-YSV-G and pNRF as previously described [12,18–23]. Lentivi-
ral vector quantifications were performed according to the p24 ELISA (KPI, USA) in accordance with the manufacturer’s instructions.

2.3. Surgery

All surgical procedures were performed as previously described [12,18–22]. Briefly, rats were anesthetized with a mix of ketamine/xylazine (100 mg/kg/ 10 mg/kg, i.p.). Using a 5-μl Hamilton syringe, 2 μl of concentrated lentiviral solu-
tions mixed (ca. 200,000 ng of p24 antigen/ml) per site were bilaterally injected into the NAc or the dorsal striatum−, at the corresponding coordinates (NAc: anterior, +1.4; lateral, ±1.6; ventral, −6.8; dorsal striatum: anterior, +0.7; lateral, ±3.2; ven-
tral, −4.5 [24]), with a rate of 1 μl/min, in a stereotaxic frame. The needle was then left in place for an additional 5 min and gently withdrawn. After surgery, ani-
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Fig. 1. LV-uPA enhances cocaine-induced place preference. Animals were injected with either LV-GFP or LV-uPA \((n=9/\text{group})\) and then manipulated as follows. Panels A–C: rats were habituated to the CPP cages for 3 days and tested for baseline preference (pre-conditioning). Panels A–C: after the pre-conditioning phase, rats were trained for conditioned-place preference (CPP) using 20 mg/kg cocaine or 0.9% saline 1 ml/kg for 6 days then tested for place preference (post-conditioning). The first set of animals received 5% sucrose drinking solution throughout the preconditioning, conditioning and test phases (Panels A and A); the second group received 5% sucrose solution with 0.02% doxycycline during only the conditioning phase (CPP acquisition) (Panels B and B); the third group received 5% sucrose solution and 0.02% doxycycline only during the post-conditioning phase (i.e. CPP expression) (Panels C and C). Values indicate means ± S.E.M. \(*p<0.05\) compared to LV-uPA injected animals; \#p<0.05 compared to saline-conditioned animals.

3. Results

3.1. Lentivirus-mediated uPA expression in the NAc affects place preference

To determine the effects of uPA expression on cocaine-mediated place preference, animals were injected with either LV-uPA or LV-GFP into the NAc. Gene expression by both vectors is regulated by doxycycline, which was administered to separate groups of animals to determine whether uPA expression was necessary during acquisition or expression of CPP. Therefore, two separate groups of animals were fed doxycycline either during CPP acquisition (days 3–10) or during the expression phase of CPP (at day 10). A third group was given only sucrose-flavored water without doxycycline throughout all phases of the experiment. Data are summarized in Fig. 1. Before conditioning (at day 3) all animals were pretested for evidence of bias towards either compartment in the conditioning apparatus (Fig. 1 A–C; \(F_{5,60}=1.1\); \(p>0.1\)). No evidence of bias was found, since during pre-conditioning, rats spent the same amount of time in both chambers (\(F_{11,120}=0.26; p>0.990\) (see Fig. 1 A–C). Animals were then conditioned for place preference and tested on day 10. Saline-conditioned animals displayed no behavioral changes, whether they were fed water or doxycycline, and their preference scores were unchanged after the conditioning phase (\(F_{5,60}=1.105; p>0.379\)). However, between subjects comparisons of saline versus cocaine-treated animals showed that GAP-treated animals displayed cocaine-induced place preference (\(F_{5,60}=48.81; p<0.005\)), irrespective of whether they were fed doxycycline or not (see LV-GFP groups in Fig. 1B and 1C; \(F_{5,60}=41.781; p<0.00001\)). Alternatively, uPA-treated animals displayed a much higher preference for the cocaine-paired chamber (\(F_{5,60}=178.94; p<0.0001\) (Fig. 1A’ and spent approximately 90% of the time in this drug-associated chamber. However, when uPA was not expressed during the conditioning phase (i.e. if uPA-expression was inhibited by doxycycline during the acquisition period), cocaine-conditioned LV-uPA animals did not display a greater preference than the cocaine-conditioned GFP-controls (\(p>0.377\) compared to GFP; \(p<0.001\) compared to preconditioning; see Fig. 1B’). These uPA-treated animals, nevertheless, which have been fed doxycycline during conditioning, displayed significantly lower scores than the corresponding group that has not been fed doxycycline (Fig. 1B’ and 1A’; \(F_{11,60}=41.781; p<0.0005\)). However, if uPA expression was suppressed only during CPP expression (i.e. if animals were fed doxycycline only at the end of the learning period (after day 9)), cocaine-conditioned LV-uPA animals again showed approximately 90% preference for the drug-paired chamber (Fig. 1C’; \(F_{11,60}=41.78; p<0.0001\)). These data suggest that the enhancing effects of LV-uPA on cocaine-induced CPP are dependent on the expression of uPA during the acquisition phase of conditioning, rather than during the expression phase of CPP, when animals are tested for place preference.
3.2. Effect of LV-uPA on extinction of CPP

Animals injected with either LV-GFP or LV-uPA, were trained to conditioned-place preference, tested for CPP on day 10, and then subjected to a series of extinction trials in which they were re-exposed to the conditioning apparatus without any injections, but were allowed free-access to both chambers. As shown in Fig. 2, animals which had been conditioned with saline displayed no significant behavioral change during this extinction period, and kept spending approximately 50% of the time in both compartments, irrespective of whether they had been fed doxycycline or not during either the conditioning (acquisition) or post-conditioning (expression) phase (Fig. 2A–C: within subject factor \(F_{(5,60)} = 0.17; p > 0.971\)). In contrast, animals which had been conditioned with cocaine displayed progressive extinction over the 10-day period (within subject factor \(F_{(5,60)} = 38.547; p < 0.0001\)). The rate of extinction among GFP-treated animals was not altered by doxycycline treatment. Between subject analysis of day 5 versus day 6 revealed no significant difference (\(F_{(2,30)} = 0.223; p > 0.803\) and \(F_{(2,30)} = 1.234; p > 0.319\) for days 5 and 6, respectively), indicating that 5–6 days after the CPP test, these GFP-treated animals were unable to distinguish between the two compartments. uPA-treated animals displayed a longer extinction latency, which lasted 7–8 days, but this was likely due to the fact that place preference was initially much higher (Fig. 2A; between subject analysis for days 7 and 8, respectively \(F_{(2,30)} = 25.57; p < 0.001\) and \(F_{(2,30)} = 23.64; p < 0.001\)). This latter effect in uPA-treated animals was observed both in groups fed doxycycline during CPP expression and in animals not fed doxycycline (Fig. 2A and C; within subject analysis of doxycycline in expression vs. no doxycycline \(p > 0.06\)). In contrast, animals fed doxycycline during the acquisition period already displayed lower place preference at the initiation of extinction, similar to GFP-treated animals, and showed an overlapping extinction rate to that observed in GFP-treated animals (see Fig. 2B; \(p > 0.531\)). Alternatively, uPA-treated animals fed doxycycline only during the expression phase displayed similar extinction rate compared to normal (no doxy) regimen (compare Fig. 2C vs. A; \(p > 0.183\)). Finally, at the end of the extinction process (day 10) there were no differences in preference among all the groups (between subject factor \(F_{(11,120)} = 0.487; p > 0.904\)). This observation prompted us to proceed towards an assessment of the effects of cocaine priming on reinstatement of CPP.

3.3. Reinstatement of cocaine-induced place preference following extinction

After a 10-day extinction period, animals were given one single injection of saline or cocaine and tested for CPP. Injection of saline had no effect on reinstatement of place preference (see Fig. 3, upper panels A–C; \(F_{(11,120)} = 1.044; p > 0.421\)), irrespective of whether they had been fed doxycycline during either the acquisition phase or the expression phase of place preference conditioning (between subject factor compared to regimen: \(F_{(11,60)} = 41.78; p > 0.993\)). No change was observed neither in cocaine (between subject factor \(F_{(5,60)} = 1.06; p > 0.401\) nor saline-conditioned animals (between subject factor \(F_{(5,60)} = 1.203; p > 0.332\)). However, if animals were given a single injection of a very low dose of cocaine (2 mg/kg, i.p.), drug-mediated place preference was immediately re-established (\(F_{(11,120)} = 121.48; p < 0.0001\) (Fig. 3 lower panels A; B’ and C’). This was evident in the GFP-treated animals, who displayed a modest preference for the drug-paired compartment and spent 60% of the time in the drug compartment (\(F_{(5,60)} = 56.303; p < 0.005\), which was similar to the behavior observed before extinction (\(F_{(11,120)} = 1.198; p > 0.308\)). In contrast, uPA-treated animals displayed a much stronger reinstatement of place preference, which once more showed up to 90% exploration time in the drug-paired compartment, as observed prior to extinction (Fig. 3A’ and C’; \(F_{(5,60)} = 175.52; p < 0.0001\)). Nevertheless, the substantial magnitude of this reinstatement was not observed if animals had been fed doxycycline during the initial acquisition phase of conditioning; under these conditions animals showed reinstatement of CPP to the degree observed in GFP-treated animals given the priming dose of cocaine (Fig. 3B’; between subject factor compared to LV-GFP \(p > 0.243\); between subject factor compared to regimen without doxycycline \(p < 0.005\); and between subject factor compared to groups fed doxycycline in acquisition \(p > 0.003\), respectively). These data once more indicate that uPA expression during acquisition exerts effects that not only enhance the development of CPP (see Fig. 1A’), but also endow animals with heightened responsiveness to cocaine, such that prior evidence of learning returns in a more robust form.

To further test this hypothesis, an uPA-specific inhibitor, B428 [35–37] was injected into the animals after establishment of CPP. Animals treated with either LV-GFP or LV-uPA, were condi-

![Fig. 2. Rate of extinction for cocaine-induced place preference in LV-uPA and LV-GFP-treated animals. Animals (n = 9/group) were injected with LV-GFP or LV-uPA and pretested, trained and tested for CPP as in Fig. 1 (Panels A–C and A’–C). After CPP monitoring on day 10, rats were placed into the apparatus for 20 min daily for 10 days. No injections were given during this extinction period. Graphs A’–C represent post-training data during the 10 days of drug withdrawal period of cocaine-conditioned animals and start at higher levels than graphs A–C that represent post-training data from saline-conditioned control animals. Values indicate means ± S.E.M.](http://doc.rero.ch)
Fig. 3. Greater reinstatement of cocaine-induced place preference with a priming dose of cocaine in conditioned LV-uPA animals subjected to extinction. Animals (n = 9/group) were injected with LV-GFP or LV-uPA and trained for CPP as in Fig. 1. After CPP monitoring on day 10, extinction was instituted as in Fig. 2. Following extinction, animals were given one single injection of 0.9% saline (1 ml/kg, i.p.) (upper panels) or cocaine (2 mg/kg) (lower panels) and tested for CPP for 20 min. Values indicate means ± S.E.M. *p < 0.05 compared to LV-uPA injected animals; #p < 0.05 compared to saline-conditioned animals.

Fig. 4. Inhibition of uPA by B428 failed to alter conditioned-place preference reinstatement with low dose cocaine priming. Animals (n = 9/group) were injected with LV-GFP or LV-uPA and trained for CPP as in Fig. 1. After CPP monitoring on day 10, rats were placed daily into the apparatus for 20 min for 10 days. During that period, injection with B428 (30 mg/kg, i.p.) was given 30 min prior to injection with cocaine (2 mg/kg, i.p.), and, immediately after cocaine injection, animals were exposed to the CPP chamber for 20 min preference testing. Values indicate means ± S.E.M. *p < 0.05 compared to LV-uPA injected animals; #p < 0.05 compared to saline-conditioned animals.

Fig. 5. (A) Effects of the uPA inhibitor, B428, on CPP expression. Animals (n = 9/group) were injected with LV-GFP or LV-uPA and trained for CPP as in Fig. 1 but during conditioning, each virus group was injected with B428, to inhibit uPA expression, or the vehicle. Half an hour prior to conditioning, animals have been injected 30 mg/kg B428 in their home cage, thereafter animals have been injected 15 mg/kg cocaine (or saline) for conditioning. On test days no B428 injection is performed. *p < 0.05 compared to LV-injected animals after pre-conditioning; #p < 0.05 compared to saline-conditioned animals; ∅p < 0.05 compared to saline-conditioned GFP control animals. (B) Effects of uPA overexpression in the dorsal striatum on acquisition of CPP. Two groups of animals (n = 9/group) were injected with LV-GFP or LV-uPA in the dorsal striatum at following coordinates: anterior, +0.7; lateral, ±3.2; ventral, −4.5 [24], and subsequently pretested, conditioned and tested for CPP as in Fig. 1. *p < 0.05 compared to LV-injected animals after pre-conditioning.
it was no longer expressed at later stages (between subject factor \( p < 0.001 \) vs. doxycycline fed animals). The inhibition of uPA by B428 occurs for both endogenous (in LV-GFP-treated animals) as well as ectopic uPA (in LV-uPA-treated animals). Therefore, as an alternative to using doxycycline, and in order to determine the role of endogenous uPA, the effect of B428 was assessed in animals subjected to cocaine conditioning (Fig. 5A). The results showed that B428 administration during acquisition suppresses CPP to below that of preconditioning. Blockade of endogenous uPA (GFP-treated animals) and exogenous uPA (in LV-uPA-treated group) during acquisition altered expression of cocaine-induced CPP (\( F_{(5,60)} = 50.76; p < 0.001 \)). CPP is in fact augmented in LV-uPA-injected animals when vehicle is given, but there is no CPP in both GFP and uPA groups when B428 was injected. This indicates that suppressing endogenous uPA activity during acquisition alters CPP expression, which agrees with the finding that doxycycline inhibition of ectopic uPA during acquisition does alter CPP expression.

Furthermore, another group of animals was treated in the dorsal striatum, to better assess the specificity of the observed behavioral effects with respect to the NAc (Fig. 5B). Clearly overexpression of uPA in the dorsal striatum does not augment CPP upon cocaine conditioning, compared to controls (\( F_{(5,60)} = 1.22; p > 0.521 \)). This indicates that the behavioral changes observed upon overexpression of uPA are probably very related to the NAc. Further studies should be necessary to test this hypothesis.

In order to assess whether cocaine-induced place preference could be retained after a prolonged period of withdrawal from drug and drug-associated contexts, animals treated either LV-GFP or LV-uPA were trained for conditioned-place preference, then maintained in their home cages for 5 weeks, without further training and without drug injections. After this withdrawal period, animals were placed into the CPP set-up and place preference was measured. As shown in Fig. 6, animals displayed enhanced CPP scores (\( F_{(11,120)} = 119.79; p < 0.0001 \)), while saline-conditioned animals displayed no preference (\( F_{(5,60)} = 0.27; p > 0.93 \)). This indicates that the behavioral changes observed upon overexpression of uPA are probably very related to the NAc. Further studies should be necessary to test this hypothesis.

As shown in Fig. 7, the observed behavioral changes appear to be related to the expression of uPA in the injected sites. In the presence of doxycycline (either in acquisition or in expression of CPP), uPA expression in the NAc is very low, corresponding to cocaine-induced endogenous uPA expression (Fig. 7A). Quantitative RT-PCR in these regions showed no significant changes in uPA mRNA expression in GFP-treated animals on the doxycycline regimen, compared to a normal non-doxycycline drinking water regimen. In LV-uPA-treated animals, uPA expression was 2.9-fold higher under the normal drinking regimen, compared to the doxycycline regimen (Fig. 7B). These changes are reflected in protein changes observed in immunohistochemistry (Fig. 7A).

It is also very likely that behavioral changes are associated with the active enzyme, as shown by zymography (Fig. 7C). Zymograms show two bands, a major bright one (at ca. 46 kDa), corresponding to the high-MW form of uPA, and minor bands (ca. 35 kDa) from a lower MW form of uPA. In the presence of doxycycline either in acquisition or in expression of CPP, but also in presence of B428, all groups displayed no active uPA. However, in the absence of doxycycline, but also during cocaine priming, LV-GFP-treated animals display no significant enzyme activity, compared to LV-uPA-treated animals where enzyme activity was very strong, in good correlation with observed behavioral data. This is also found upon reinstatement. These data strongly indicate that the active form of uPA is involved in observed behavioral changes.

4. Discussion

The goal of the current work was to determine the impact of uPA overexpression on CPP acquisition, extinction and/or reinstatement. The results showed that uPA overexpression in the NAc exerted a significant impact on cocaine-induced CPP and augmented CPP, and led to a more robust re-enlistment of CPP following extinction. The effect was abolished if overexpression was turned off with doxycycline. These effects have been generated by ectopic increases of uPA, and would have been more conclusive about the role of endogenous uPA if silencing of naturally produced uPA was produced, which is a question for future investigations. Nevertheless results are clearly indicate that excess uPA localized to the NAc promotes the development of cocaine-induced CPP. In contrast, when endogenous uPA was inhibited during acquisition, using B428, animals displayed no CPP. Therefore, given that animals with LV-uPA lentivirus showed effects above and beyond the control virus manipulation (GFP), it is clear that increased uPA expression with repeated drug exposure produces conditions for enhanced acquisition of cocaine-induced CPP. Moreover, this overexpression appears to have a localized effect, since infusion of LV-uPA into the dorsal striatum was without significant effect on the development of CPP. Therefore, the present findings suggest that the impact of uPA in the NAc is critical to uPA-induced augmentation of cocaine-induced CPP.

![Image](http://doc.rero.ch)
It is noteworthy that the LV-GFP groups conditioned with cocaine showed only weak, but statistically significant, development of place preference. Therefore, it is possible that the enhancing effects of uPA overexpression operate in the context of suboptimal development of CPP. Needless to say, uPA overexpression shifted preference to 90% to that of the saline-associated context. Therefore, there is little room for further augmentation in the CPP paradigm, and consequently, other procedures, such as self-administration may be better approaches for determining whether suboptimal circumstances for drug-reinforced behavior are enhanced by uPA. Furthermore, the fact that priming in uPA animals led to stronger reinstatement of preference speaks either to greater retention or greater rewarding effects of cocaine.

When uPA expression was inhibited by doxycycline during acquisition of CPP, animals no longer demonstrated evidence of having shown a greater or stronger association of the environment with cocaine. Doxycycline has no effects on endogenous uPA expression, since endogenous uPA is not doxycycline regulatable and in our previous work we have shown that within approximately 8 h after the introduction of doxycycline in the drinking fluid, more than 90% of the LV gene-suppressing doxycycline effects are reached, which has also been confirmed at the protein level by Bahi et al. [12,20], and has been repeatedly observed in other experiments in our laboratory (unpublished data).

The rate of extinction was the same for all cocaine groups no matter what uPA treatment they received. In all cases it took approximately 6 days of extinction trials for a 50% reduction in CPP. The greater number of sessions needed to abolish CPP in uPA overexpressing rats was merely due to a higher starting point, but not to a delay in extinction, implying that uPA overexpression probably does not cause any alteration in extinction. However, after extinction, priming with low doses of cocaine reinstated place preference. Several studies have shown that reinstatement of cocaine-seeking behavior occurs after drug priming [38,39], and the drug itself triggers craving in human addicts [40,41]. Infusion of FN-439, a broad-spectrum MMP inhibitor, prevented cocaine-mediated CPP acquisition, blocked reinstatement of cocaine CPP and suppressed subsequent cocaine-primed reinstatement [42]. This is in full agreement with our finding using B428, a specific uPA inhibitor. Indeed, one goal of the present work was to test whether an inhibitor of uPA activity would also impair the acquisition of cocaine-induced CPP behavior. B428, a potent and specific uPA inhibitor that blocks both endogenous (in LV-GFP-treated animals) and ectopic uPA (in LV-uPA-treated animals) prevented the development of aug-
mented CPP in LV-uPA animals, but did not affect drug-induced CPP reinstatement after extinction if LV-uPA had been active during acquisition. Together these data clearly indicate that uPA plays an important role in the acquisition but not expression of cocaine-induced conditioned-place preference. By virtue of augmenting the preference to a cocaine-associated context, the overexpression of uPA prolongs, but does not maintain, the behavioral inclination for exploring such a context in the absence of further cocaine treatment (as demonstrated by normal extinction). That is, after 5 weeks of withdrawal, CPP was still evident to a greater degree in LV-uPA animals. Therefore, while the presence of uPA overexpression may not produce resistance to extinction, animals appeared to show retention of the learning experience, as well as increased susceptibility to the reinstating effects of cocaine, as already mentioned earlier. It would be of interest to determine whether other potential influences such as stress might have a similar effect in reinstating CPP in LV-uPA animals previously conditioned with cocaine.

Urokinase plaminogen activator is an extracellular serine protease, which is expressed in most brain regions in the CNS [1,43–45] and exerts its main enzymatic actions by converting inactive pre-protease, which is expressed in most brain regions in the CNS [1,43–45] in LV-uPA animals previously conditioned with cocaine. The present study firmly consolidates these data and points to an important role of the plasmin system in chronic cocaine. Other studies have shown that the plasmin system releases dopamine in the NAc upon psycho-stimulants, which activates long-term synaptic plasticity and remodeling, and acutely participates in the rewarding effects of drugs such as methamphetamine or morphine [13,14,16,17,69]. It has been shown that tPA regulates nicotine-induced reward and dopamine release through protease activated receptor-1 [70]. Using the morphine self-administration procedure, the same group described very recently that tPA deficient mice display more morphine intake in a dose-dependent manner as compared to their wild type littermates [71]. However, under a progressive ratio schedule of morphine reinforcement, tPA null mice display a lower breaking point than wild-type mice [71]. These results and those reported here clearly support the hypothesis that plasminogen activators (tPA and uPA) play a central role in synaptic plasticity and drug-associated learning and memory phenomena.

There is some possibility that the dose of cocaine used in the present study could be aversive to some extent, as one would expect from sympathomimetic agents such as cocaine (a perplexing issue that Berridge, for example, has addressed). However, many other authors have used this same dose of cocaine in similar contexts ([25,27–32]; Leri et al. [33,34]). In any case, while we did not have a robust effect on CPP in the LV-GFP animals, it was nevertheless in the predicted direction, as opposed to 'place aversion.' Whatever the neurobiological reasons for why highly arousing substances like cocaine result in dependent behavior, the dose that we used was nevertheless effective as an unconditioned stimulus. It is nevertheless possible that uPA rendered the “aversive” effects of cocaine less intense or even more tolerable. This question might be better addressed through self-administration studies where the operant behavior of the animal will provide an indication of whether uPA overexpression is altering the CNS response to cocaine.

A question that needs to be further addressed in future studies is whether there are neuroanatomical restrictions on the ability of uPA overexpression to augment cocaine-induced place preference. The current study was mainly restricted to overexpression in the NAc, based on previous studies that showed very strong locomotor changes when uPA was overexpressed in that brain region Bahl et al. [12,20]. However, it is possible that increased expression of uPA outside the NAcc might exert similar effects. This needs to be determined to confirm whether unique interactions between uPA and the neurons of the NAc are necessary and sufficient to produce an increase in place preference. Nevertheless, as already mentioned earlier, a control experiment in the dorsal striatum showed no significant behavioral changes, implying that unique interactions between uPA and the neurons of the NAc are necessary and sufficient to produce an increase in place preference. This however requires further detailed studies.

In conclusion, the present study suggests that uPA in the NAc facilitates and strengthens learning for a cocaine-associated context, even after extinction, and while animals are under the influence of a small priming dose of cocaine during reinstatement testing. Although not directly tested, these findings suggest that endogenous uPA may mediate cocaine-associated contextual learning.

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