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Cortical consequences of HIV-1 Tat exposure in rats are enhanced by chronic cocaine

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Abstract

The life span of individuals that are sero-positive for human immunodeficiency virus (HIV) has greatly improved; however, complications involving the central nervous system (CNS) remain a concern. While HIV does not directly infect neurons, the proteins produced by the virus, including HIV transactivator of transcription (Tat), are released from infected glia; these proteins can be neurotoxic. This neurotoxicity is thought to mediate the pathology underlying HIV-associated neurological impairments. Cocaine abuse is common among HIV infected individuals, and this abuse augments HIV-associated neurological deficits. The brain regions and pathophysiological mechanisms that are dysregulated by both chronic cocaine and Tat are the focus of the current review.

Keywords

addiction; calcium channels; Cav1.2; neuropathogenesis; prefrontal cortex

Introduction

Since the beginning of the acquired immunodeficiency syndrome (AIDS) epidemic, there has been a strong link between human immunodeficiency virus (HIV) and drug abuse [1]. Recreational use of psychostimulants, including cocaine, increases the likelihood that the user will engage in impulsive and unsafe behaviors such as risky sexual practices. These risky behaviors increase the chance of exposure to HIV and other diseases [2]. Cocaine is also used to self-medicate for mental health stressors [3], and recent users (i.e., self-reported to have used cocaine within 6 months) and former users (i.e., self-reported to have ever used

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Conflict of Interest

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cocaine) report more depressive symptoms that non-users [4]. Upon learning that they are HIV$^+$, many individuals experience depression-like symptoms including fear, anguish, and thoughts of suicide [5], and these stressors may serve to promote cocaine abuse. Regardless of the original motive for abusing drugs, chronic exposure to these drugs can exacerbate HIV-related disease progression. For example, cocaine increases viral replication [6] and accelerates loss of CD4$^+$ T-cells [7] in the HIV$^+$ individual. This review focuses on the brain, and mechanisms that may contribute to enhanced vulnerability and/or exacerbated neuropathology that can occur during HIV/AIDS and cocaine abuse co-morbidity.

HIV and cocaine in the brain

HIV-associated neurocognitive disorders (HAND) include impaired concentration, memory deficits, and motor impairment [8,9]. These symptoms reflect neuronal damage subsequent to HIV-infection; however, the virus does not directly infect neurons. Damage is inflicted by HIV-infected monocytes, which readily cross the blood-brain barrier (BBB), [10] and subsequently release toxic viral proteins within the central nervous system (CNS) [11,12]. CNS damage occurs early during infection; HIV-1 RNA can be detected in cerebral spinal fluid (CSF) as early as 8 days after the estimated introduction of the virus into the human host [13]. Combination antiretroviral therapies (cART) do not readily cross the BBB, which reduces efficacy for suppressing HIV infection within the CNS [14]. Accordingly, HAND are reported in up to 50% of HIV$^+$ patients, even with cART-controlled viral replication [8,15–17]. The neurocognitive deficiencies associated with HIV are augmented in those that are cocaine abusers [4,18], as cocaine use disrupts BBB integrity and facilitates HIV penetration into the CNS [19,20]. Thus, in spite of cART-controlled infection, the brain of cocaine-abusing HIV$^+$ individuals may be particularly vulnerable to the destructive features of virus.

Regions of the brain that show an accelerated pathology in the co-morbid condition include the hippocampus, striatum, and frontal cortex (including the prefrontal cortex, PFC) [21]. Cocaine addicts (that were not screened for HIV infection) exhibit hypo-activation of the PFC [22]; atrophy and tissue thinning also occurs in the PFC of HIV$^+$ individuals [23,24]. HIV$^+$ men exhibit deficits in attention and executive function during tasks that involve the PFC [25]. Another study conducted with women revealed that blood oxygen level-dependent (BOLD) functional magnetic resonance imaging (fMRI) during verbal learning tasks correlates with severe cognitive impairment and both current and former cocaine using HIV$^+$ women demonstrate reduced PFC activation compared to HIV$^+$ non-users [26]. These outcomes likely reflect a convergence of neuronal dysregulation within the co-morbid brain. Understanding the common sites of action for, and/or maladaptations to, HIV-1 infection and cocaine abuse within the PFC would help decipher the underpinnings of these co-morbid consequences.

Neurological consequences of Tat

Following entry into the CSF/CNS, HIV-1 spreads from monocytes and macrophages to the astrocytes and the resident immune cells of the brain, microglia [10,27]. Microglia and astrocytes play key roles in brain innate immunity and mediate the neuroinflammatory

response to HIV [28,29]. The turnover rate for these cells is relatively slow, which provides a potential resevoir for HIV-1 in the brain [30]. HIV-1 expresses at least nine toxic proteins that can be released from infected immune cells, and some of these proteins impair the biochemical and physiological mechanisms of neurons, resulting in dysregulation [11]. Of these proteins, one that is known to be neurotoxic and has been extensively studied is a transactivator of transcription (Tat). Tat is a small, intrinsically flexible and highly basic protein of ~80–103 amino acids (14–16kD) that can bind with high affinity to a large number of diverse partners (proteins or lipids), to form complexes with a wide range of action [31]. Tat drives replication of the HIV-1 *via* transactivation of the promoter region of the viral genome [32]. Tat also can inhibit proteolysis [33–35], and even a transient exposure to Tat is sufficient to dysregulate neurons [36]. Moreover, the pathological consequences of Tat can persist long after the protein itself is degraded [37], reflecting the ability of Tat to induce the expression of a large variety of cellular genes, interact with cellular proteins and to promote production of pro-inflammatory cytokines and chemokines [38,39]. These characteristics also underlie the ability of Tat to exert its effects far from its site of release [40]. Extracellular Tat can be transported into neurons *via* the low density lipoprotein receptor (LRP)-mediated endocytotic pathway [41]. Intracellular Tat can disrupt cytoplasmic Ca^{2+} concentrations either by inducing release from intracellular stores (i.e., disrupting function of 1,4,5-triphosphate receptors) or by enhancing influx of extracellular Ca^{2+} *via* voltage- and ligand-gated channels (i.e., voltage-gated Ca^{2+} channels and N-methyl-D-aspartate receptors (NMDAR)) on the plasma membrane [42,43]. Thus, Tat-induced consequences (including neurotoxicity) are dependent on extracellular Ca^{2+} influx [44]. The Tat-induced rise in cytoplasmic Ca^{2+} is followed by mitochondrial Ca^{2+} uptake and generation of mitochondrial reactive oxygen species (ROS) [45]. Accumulation of ROS can lead to oxidative stress and induce signaling cascades for neuronal apoptosis [45,46]. In addition to direct effects on neurons, Tat also alters the function of glial cells that then contribute to neuronal pathology. HIV-1 Tat promotes reactive gliosis and secretion of pro-inflammatory cytokines. This action reduces neuronal synaptic densities to simplify synaptic connections, leading to neuronal injury [40,47–54]. Tat concentrations in the CSF of HIV^+ patients range from 10–1000nM [55–57]. These concentrations of Tat *in vitro* can be neurotoxic [36]. Accordingly, Tat-induced neurotoxicity is thought to be a major contributor to HIV-related neuropathogenesis.

Neurological consequences of chronic cocaine

Cocaine binds to monoamine, (i.e., serotonin, dopamine and norepinephrine) transporters on nerve terminals and prevents the re-uptake of the respective neurotransmitters from the synapse. As a result, synaptic concentrations of neurotransmitters are dramatically increased, enhancing the activation of synaptic and peri-synaptic receptors. The chronic presence of cocaine decreases the sensitivity of postsynaptic neurons to monoamines (i.e. dopamine in the nucleus accumbens (NAc)) and increases the sensitivity of dopamine auto-receptors, resulting in a functional loss of dopamine in the synapse [58,59]. During periods of repeated drug exposure, reduced neurotransmitter production, receptor internalization, and synaptic simplification can occur in order to compensate for the exaggerated activation of the

mesocorticolimbic system. These maladaptations endure long after terminating cocaine exposure.

The mesocorticolimbic system is critically involved in the motivational aspects of drug abuse including reward, and salience attribution to drug-associated cues. Neurons in the medial prefrontal cortex (mPFC) provide excitatory input to the NAc [60], ventral pallidum (VP) [60,61], and ventral tegmental area (VTA) [60,62], key brain regions involved in addictions. Chronic cocaine increases the excitatory drive from the mPFC and enhances activity of dopaminergic cells, which are linked with reward-associated, cue-elicited behaviors [63–65]. Specifically, projections from the infralimbic (IL) and prelimbic (PrL) subregions of the mPFC terminate within the NAc shell and NAc core, respectively [60]. Activation of the PrL to NAc core projections promotes drug-seeking, whereas activation of IL to NAc shell projections inhibits drug-seeking behaviors [65,66]. Cocaine, but not food, engages projections originating in the PrL mPFC and increases glutamate levels in the NAc core, to stimulate reinstatement of responding in rats [66]. These findings suggest that the cocaine-induced rise in NAc core glutamate levels is abnormal rather than physiological. Rats that self-administer cocaine in the presence of discrete cues show drug-seeking in the presence of these cues during periods of withdrawal [67,68] likely reflecting persistent molecular neuroadaptations in the mPFC [69,70]. Functional imaging performed on abstinent cocaine addicts reveal hyper-reactivity of the mPFC that is strongly correlated with self-reports of craving for cocaine during the presentation of cocaine-associated cues [22,71]. Therefore, mPFC-associated circuitry is thought to underpin the abnormally enhanced reactivity to drug-associated cues experienced by addicted individuals during periods of drug abstinence.

Common pathways for cocaine and Tat effects in mPFC

The high prevalence of cocaine abuse in HIV^+ individuals and the ability of cocaine addiction to exacerbate HAND indicates that chronic cocaine and HIV-1 toxic proteins may present overlapping neuropathological events in brain regions that govern HAND and reward-motivated behaviors. Using Tat as a prototype, we overview here current evidence for such an overlap, and explain the mechanisms that contribute to the neuropathological convergence.

Indirect effects of Tat and cocaine on neurons

In a healthy CNS, astrocytes outnumber neurons five-fold. Astrocytes envelope neuronal synapses to aid many essential neuronal functions that are critical for normal synaptic transmission [72], including the maintenance of fluid, ion, pH, and transmitter homeostasis of the synaptic interstitial fluid [73,74]. Insults to the CNS induce reactive astrogliosis, a process which has become a pathological hallmark for numerous neurological diseases [73]. Activated astrocytes lose the capacity to maintain synaptic homeostasis of glutamate, resulting in excessive glutamate-induced calcium influx which when excessive can be neurotoxic [74].

Tat exerts a powerful and persistent influence on astrocytes. *In vitro* studies revealed that astrogliosis can occur within 7 days of exposure to Tat [50]. Extending these studies to the

mammalian brain, we assessed astrogliosis in the mPFC after Tat administration [75]. Glial fibrillary acidic protein (GFAP) is a structural protein in astrocytes, and increased GFAP expression signifies astrogliosis. GFAP expression was increased in the mPFC 14 days after a single intracerebroventricular (i.c.v.) injection of Tat [75]. Tat up-regulates GFAP expression through direct interaction with the enhancer elements activator protein 1 (AP1) and specificity protein 1 (SP1). The transcription factor early growth response-1 (Egr-1) regulates p300 transcription, and deletion of the Egr-1 *cis*-transacting element within the p300 promoter abolishes Tat-induced GFAP expression [76]. Though the mechanisms are less clear for cocaine, the psychostimulant is known to increase GFAP immunoreactivity in the PFC (and nucleus accumbens) following repeated administration and 3 weeks of withdrawal [77]. Astrogliosis caused by cocaine would diminish the capacity of astrocytes to support neuronal health and in so doing, enhance neuron vulnerability to the detrimental consequences of Tat.

Direct effects of Tat and cocaine on neurons

The ability of cocaine to bind to and block the dopamine transporter (DAT) is well established. Recent studies have revealed that Tat also can bind directly to DAT [78,79]. Tat binding to DAT inhibits the reuptake of dopamine released from activated neurons [78] and promotes DAT internalization [80]. Tat-induced conformational changes in DAT also increase the affinity of cocaine for the transporter protein [78]. Thus, Tat has the capacity to significantly enhance extracellular concentrations of dopamine through multiple mechanisms [81]. Excessive extracellular dopamine over-actives dopaminergic receptors and drives the reward-motivation known to underlie cocaine-mediated behaviors. Recent studies indicate that a similar outcome occurs following exposure to Tat, which can potentiate behaviors mediated by cocaine reward [82].

In the frontal cortex and striatum of rats, acute or repeated administration of cocaine alters mitochondrial complex I subunits (i.e. nicotinamide adenine dinucleotide-4) to decrease oxidative phosphorylation and increase ROS production [83]. These cocaine-mediated effects occur without the loss of neurons. The absence of neuronal death may reflect ROS buffering as cocaine causes a concomitant increase in the production of antioxidant enzymes which prevents the activation of cellular apoptotic signaling cascades [83]. In contrast, Tat induces ROS production that can result in death of cultured neurons; cocaine enhances this effect [84]. These reports indicate that cocaine may enhance the Tat-induced rise in cytoplasmic Ca^{2+} and mitochondrial Ca^{2+} uptake leading to the generation of mitochondrial ROS, and that excessive intracellular Ca^{2+} may be a common mechanism by which cocaine abuse accelerates HIV^{+} associated neuropathology.

Evaluations of Ca^{2+} function in neurons indicate that this mechanism is a site of convergence for chronic cocaine and Tat. Removal of extracellular Ca^{2+} decreases Tat-induced Ca^{2+} influx and toxicity of cultured rat cortical neurons [44,85]. Enhanced Ca^{2+} influx is regulated, at least in part, by NMDAR [42,86,87] and voltage-gated Ca^{2+} channels (likely the L-type Ca^{2+} channels) [88]. Tat (1–500 nM) dose-dependently induces a fast, transient increase of Ca^{2+} influx in cultured rat cortical neurons, which is not blocked by inhibition of NMDAR [85]. In contrast, blockade of the L-channels significantly reduces

Tat-induced Ca^{2+} influx and neurotoxicity in cultured human fetal microglia and monocytes [89,90]. Tat increases neuronal excitability in mPFC pyramidal neurons, and this increase can be inhibited by diltiazem, an L-type Ca^{2+} channel blocker (Fig 2). Physiological low nanomolar concentrations (~15–20nM) of Tat delivered *via* i.c.v. injection enhances L-channel expression in the mPFC of rats as compared to vehicle injected controls (Fig. 3). The major advantage of using the i.c.v. injection is that a known concentration of Tat can be introduced into the brain, bypassing the BBB. The resulting CSF concentrations in these rats are within the range of the Tat CSF concentrations detected in humans [55–57]. Tat-induced upregulation of these channels could abnormally increase Ca^{2+} influx and intracellular Ca^{2+} levels, enhancing reactivity of mPFC pyramidal cells to membrane depolarization. This phenomenon is present for up to 14 days after Tat administration, and it overlaps with the time-course of L-channel plasticity during cocaine withdrawal [91]. After a prolonged (2–3 weeks) withdrawal from repeated, experimenter administered cocaine, surface expression of the L-type Ca^{2+} channels has been shown to be significantly increased in the mPFC [91].

We recently reported that *in vitro* application of Tat, while recording mPFC pyramidal neurons from rat brain slices, dramatically increases firing and NMDAR-independent Ca^{2+} influx through L-type Ca^{2+} channels [88]. Tat enhances Ca^{2+} influx at concentrations between 10–40nM, the magnitude of which correlates with Tat concentration. Elevated intracellular Ca^{2+}, under normal conditions, will bind to calmodulin to inactivate L-type Ca^{2+} channels. Recovery from inactivation occurs as the membrane becomes re-polarized or hyperpolarized [92]. Tat can bind calmodulin [93] and inhibit Ca^{2+}-dependent inactivation of the channel, which would allow for excessive influx of Ca^{2+} into the neuron. A history of repeated, non-contingent cocaine treatment (i.e., cocaine administered by the experimenter) increases neuronal firing that is augmented by 40nM Tat (Fig.4A). Additionally, cocaine treatment enhances Ca^{2+} influx, and acute exposure to 10nM Tat enhances this effect (Fig. 4B). Rats that self-administer or self-titrate their dose of cocaine (similar to the human scenario) exhibit pyramidal neuron firing that is greater than that recorded from saline-yoked (SAL-Yoked) controls [94]. Pyramidal neurons from rats that self-administered cocaine (COC-SA) exhibit Tat-induced increases in firing at much lower concentrations of Tat (e.g., 5nM) [94], and these neurons are more easily driven to over-excitation, wherein firing neurons become inhibited with low Tat concentrations (Fig 5). The neurons recorded in these studies [94] were in cortical layers 5/6, had a pyramidal morphological profile, prominent apical dendrite, and exhibited regular firing patterns. These characteristics are consistent with glutamatergic pyramidal neurons [95]. GABAergic interneurons typically feature multiple small apical dendrites and exhibit irregular firing patterns [96]. While literature on L-type Ca^{2+} channel expression in prefrontal GABAergic interneurons is limited, hippocampal interneurons have been shown to express L-type Ca^{2+} channels [97]. However, it is important to note that any effect of Tat-induced upregulation of L-type Ca^{2+} channels on mPFC interneurons is overridden by the increased excitability of the pyramidal neurons. Thus, L–type Ca^{2+} channels may be one of the common pathways for cocaine- and Tat-induced neuroadaptations in mPFC pyramidal neurons, and this convergence may explain how cocaine can accelerate the onset and increase the severity of neurocognitive impairment in HIV^{+} individuals.

The consequences of cocaine and Tat on L-type Ca^{2+} channels in the mPFC appear to be synergistic. When both cocaine and Tat are present, the effects are greater than additive and this is confirmed by interaction statistics [88]. Cocaine itself is not lethal to neurons, but cocaine does alter neuronal function and responses from glia. The consequences from exposure to low nanomolar concentrations of Tat also occurred in the absence of a significant loss of neurons [75], suggesting that Tat has not yet produced the neurotoxicity that is observed in other studies. However, these same concentrations of Tat (that normally would be subthreshold for evoking neuronal pathology of pathophysiology) may become toxic in the presence of cocaine.

Conclusion

In summary, we extend *in vitro* work with Tat and cocaine to *in vivo* work and demonstrate that the changes may be long lasting even if Tat is no longer present. These changes reflect alterations to glial cells, which may set the stage for a hostile environment and increase the vulnerability of neurons to become damaged or dysfunction. We identify that alterations to L-channel expression and function as common mechanism between the pathophysiology arising from exposure to the Tat protein and cocaine. The neuroadaptations that occur in pyramidal neurons within the mPFC as a result of repeated cocaine exposure may potentiate the acute effects of Tat, including upregulated L-channel function and increased neuron excitability. In addition, rats that self-administer cocaine appear to be more sensitive to the enhanced excitability induced by cocaine and the acute effects of Tat. Taken together L-channels are a mechanism that may contribute to an enhanced vulnerability and/or exacerbated neuropathology during HIV/AIDS and cocaine abuse co-morbidity.

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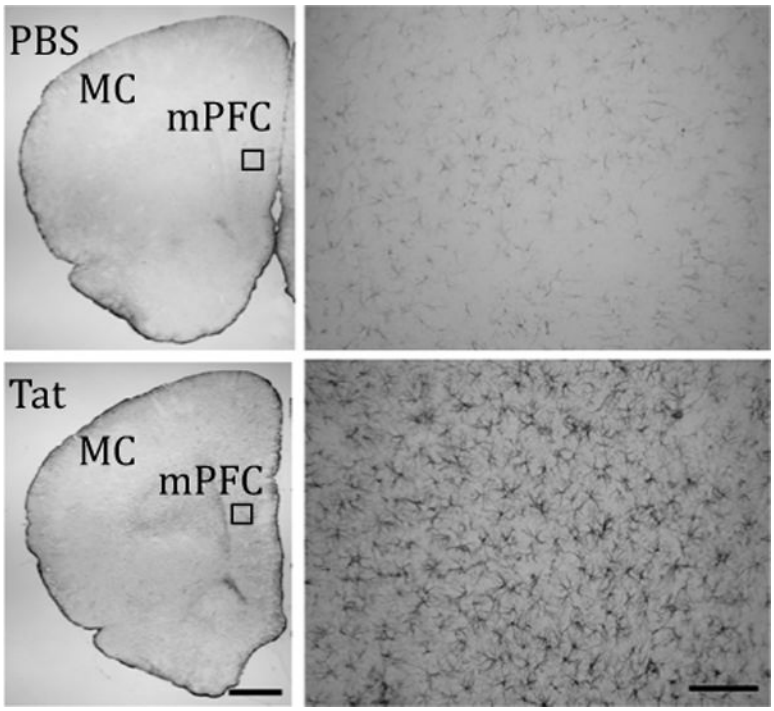

Fig. 1.

Tat up-regulates GFAP expression in the rat mPFC. Shown is GFAP staining from representative brain sections taken 14 days after a single intracerebroventricular injection of Tat (80μg/20μl) (bottom) or the PBS vehicle (20μl) (top). Left are brain sections containing the medial prefrontal cortex (mPFC) and motor cortex (MC) photographed at 1.25× (scale bar=1mm). Right are representative 20× magnification images of the mPFC and the location for which are indicated with a box on the 1.25× images (scale bar=100μm). Reprinted with permission from Wayman *et al. Neuroreport.* 3 October 2012-Vol 23-Issue 14-p825–829. Wolters Kluwer Health Lippincott Williams & Wilkins©.

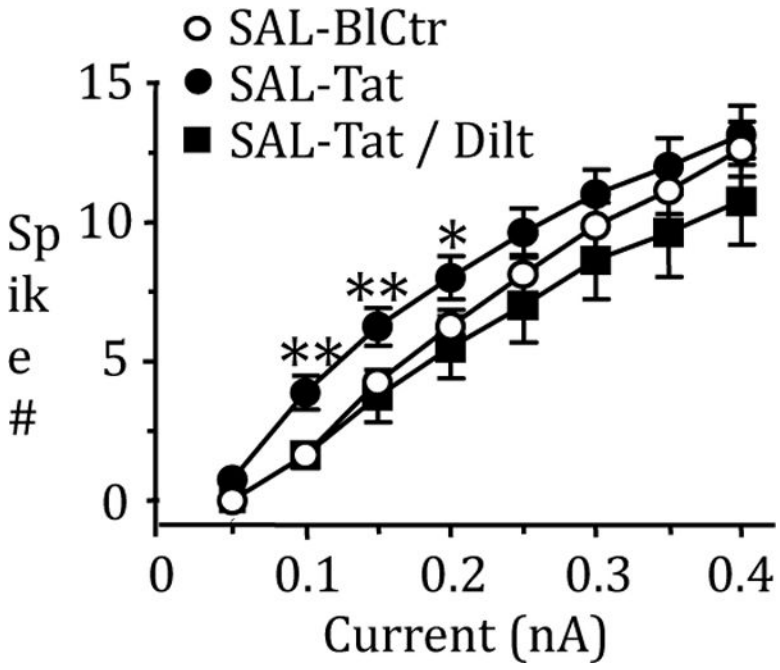

Fig. 2.

Tat increases evoked firing of rat mPFC pyramidal neurons in a Ca^{2+}-dependent manner. Curves illustrate the number of action potentials (spikes) evoked by depolarizing current pulses at pretreatment baseline (SAL-BlCtr; open circles) is significantly increased following exposure to 40nM of recombinant Tat (SAL-Tat; filled circles) and this effect was blocked by co-perfusion with an antagonist of open state L-Type Ca^{2+} channels, diltiazem (40nM; SAL-Tat/Dilt; filled squares). Two-way rmANOVA; $p<0.01$ for treatment history effect, current effect, and the interaction with *post hoc* Newman-Keuls *$p<0.025$, **$p<0.01$. SAL refers to the ip injections of saline that these rats received prior to killing and harvesting the brain slices. Reprinted with permission from Napier et al. *JNIP*. 1 June 2014-Vol 9-Issue 3-p354–368. Springer US©.

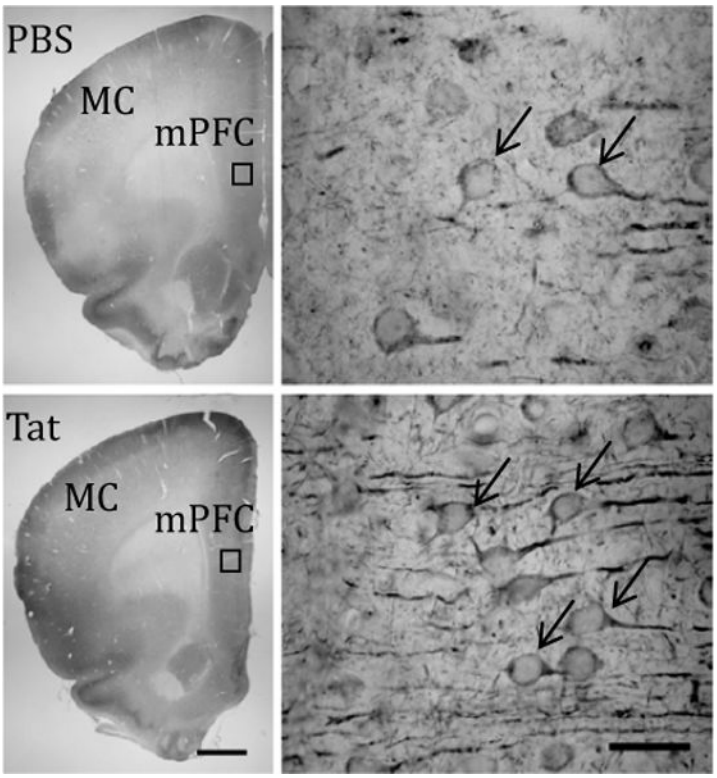

Fig. 3.

Tat increases $Ca_v1.2$-α1c immuno-reactive cells in the rat mPFC. Shown is staining from representative brain sections taken 14 days after a single intracerebroventricular injection of Tat (80μg/20μl; bottom) or PBS (20μl; top). Left are brain sections containing the mPFC and MC photographed at 1.25× (scale bar=1mm). Right are representative 20× magnification images of the mPFC and the location for which are indicated with a box on the 1.25× images (scale bar=100μm). Examples of $Ca_v1.2$-α1c immuno-reactive cells are indicated by the arrows. Reprinted with permission from Wayman *et al. Neuroreport.* 3 October 2012-Vol 23-Issue 14-p825–829. Wolters Kluwer Health Lippincott Williams & Wilkins©.

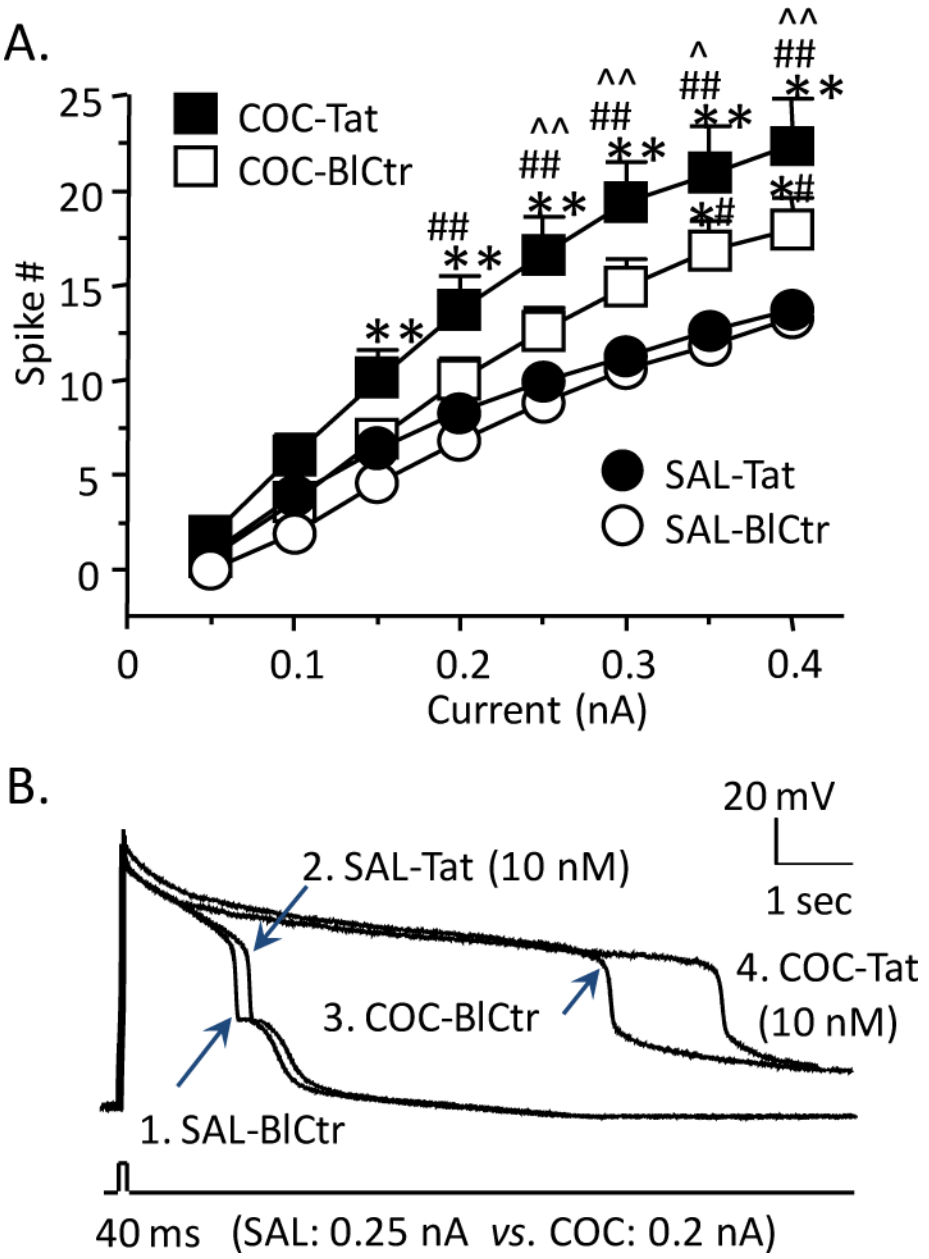

Fig. 4.

Repeated non-contingent administration of cocaine to rats enhanced Tat-induced firing and Ca^{2+} potentials recorded from mPFC pyramidal neurons *ex vivo*. (A.) Curves illustrate that the number of action potentials (spikes) evoked by depolarizing currents is markedly greater in a mPFC pyramidal neurons from cocaine (COC)-treated rats (open squares) than that recorded from saline (SAL)-treated rats (open circles). BlCtr, baseline control. Bath-applied Tat (40nM) facilitated the evoked firing in both neurons from SAL-treated rats (filled circles) and COC-treated rats (open squares). Data are presented as mean ± S.E.M. Two-way rmANOVA, $p<0.05$ for treatment histry effect, current effect, and the interaction. *Post hoc* Newman-Keuls illustrated as follows: *p < 0.025 and **p < 0.01, compared to SAL-BlCtr; #p < 0.025 and ##p < 0.01, compared with SAL-Tat; ^p < 0.025, compared to COC-BlCtr.

(B.) At baseline, the duration of Ca^{2+} potentials were remarkably prolonged in an mPFC pyramidal neuron from a COC-treated rat (COC-BlCtr) as compared to a neuron from a SAL-treated rat (SAL-BlCtr). Bath-applied Tat (10nM) enhanced the Ca^{2+} potential duration in a neuron from a SAL-treated rat (SAL-Tat) and exaggerated the enhanced Ca^{2+} potential observed in the neuron from a cocaine-treated rat (COC-Tat). Reprinted with permission from Napier et al. *JNIP*. 1 June 2014-Vol 9-Issue 3-p354–368. Springer US©.

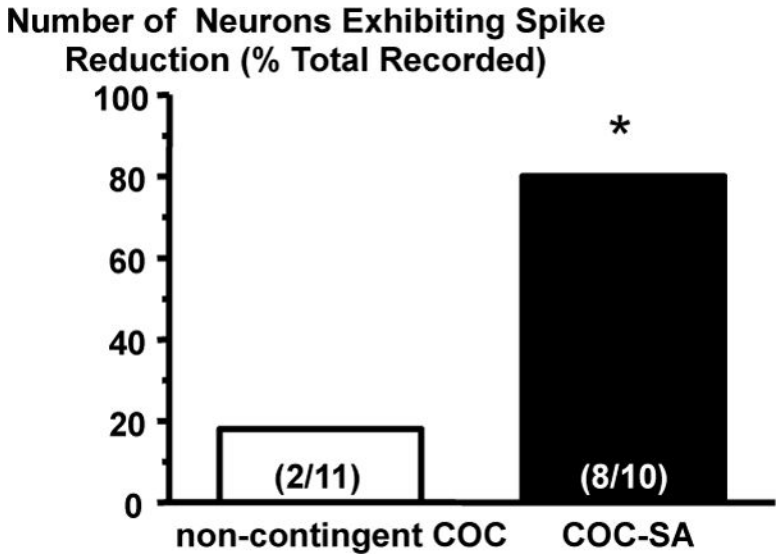

Fig. 5.

The ability of Tat to excessively activate neurons is greater in rats that self-administer cocaine. Shown is the number of pyramidal neurons that exhibited a Tat-induced "excitotoxicity" as defined by an abnormal action potential profile associated with a reduction in spike number during membrane depolarization. Two cocaine administration profiles were compared, non-contingent and self-administration (SA). Chi-square test revealed that the pyramidal neurons from adolescent rats that received experimenter delivered cocaine were less sensitive to 40nM Tat than adult COC-SA rats were when exposed to 10nM Tat ($\chi^2_{(1)}=8.03$, $p<0.005$). This indicates that SA (and/or the adult brain state) makes mPFC pyramidal neurons more sensitive to the toxic effects of Tat.