Glutamate systems in cocaine addiction
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All addictive drugs facilitate dopamine transmission, and determining the role of dopamine has been the predominant focus of biomedical research in addiction for 20 years. Newer data and hypotheses have begun to shift our focus to involvement of cortex and corticofugal glutamate projections. The rationale for shifting focus to glutamate ranges from evidence showing that cortical activity is altered in addicts to data from animal models demonstrating drug-induced changes in the function of proteins that regulate pre- and postsynaptic glutamate neurotransmission. Recent studies have particularly focused on involvement of a circuit that includes glutamate projections from the prefrontal cortex to the nucleus accumbens.

Introduction
The most widely studied neurobiological characteristic of cocaine addiction is the role played by dopamine transmission [1]. It is clear that enhanced dopamine transmission in neurons projecting from the ventral mesencephalon to the limbic forebrain, including the medial prefrontal cortex and nucleus accumbens, is the pharmacological target for cocaine-induced reinforcement and locomotor stimulation [2]. However, persistence of the behavioral characteristics of cocaine addiction, such as paranoia (sensitization) and the propensity to relapse years after the acute rewarding effects of the drug have disappeared, indicates that there must also be neuronal substrates undergoing long-term neuroplastic changes. Although studies have endeavored to identify enduring changes in dopamine transmission that might underlie behavioral sensitization and the reinstatement of drug-seeking (relapse), the results have not been entirely consistent with an obligatory role for dopamine [3]. In contrast to dopamine, glutamate transmission appears to be a primary contributor in the majority of examples of enduring neuroplasticity in the brain, and the development and expression of cocaine addiction is no exception [4]. For example, convincing neuropharmacological evidence for involvement of glutamate transmission in the development and expression of behavioral sensitization to repeated cocaine administration has accumulated over the past decade [5]. In this review, we proceed from this neuropharmacological foundation and explore the involvement of glutamate in the reinstatement model of relapse. Specifically, we examine recent data supporting the hypothesis that altered glutamate transmission in the projection from the prefrontal cortex to the nucleus accumbens mediates behavioral neuroplasticity associated with addiction, including relapse and sensitization to components of the drug experience.

Neurocircuitry in addiction
Figure 1 shows the interconnected circuit amalgamated from a 20-year research effort that is thought to be necessary to develop addiction and to manifest addictive behaviors, such as relapse and sensitization [6,7]. This circuit is derived from studies that employ neuroimaging in addicts [8**], behavioral pharmacology in animal models of addiction [7,9**,10] and, most recently, cell physiology and molecular biology [10,11*,12,13]. The dopamine projection to the prefrontal cortex, nucleus accumbens and amygdala is a primary site of pharmacological action by cocaine, as well as a site where addictive behaviors such as relapse and sensitization can be initiated [1]. The regions of the prefrontal cortex most clearly tied to addiction in both neuroimaging studies in addicts and lesion/pharmacological studies in animal models of addiction are the anterior cingulate/limbic cortex and the ventral orbital cortex [7,8**,14]. Similarly, neuroimaging and animal studies show that the amygdala is a primary component of the circuitry mediating cue-primed relapse [8**,15]. The nucleus accumbens is composed of two compartments termed the core and the shell and, although the shell is more clearly associated with dopamine-dependent reward, the core has been linked to the enduring cellular changes elicited by repeated use of addictive drugs [7,16]. The projections from the amygdala and prefrontal cortex to the nucleus accumbens are glutamatergic, as are the reciprocal connections between the basolateral amygdala and prefrontal cortex. The prefrontal cortex also sends glutamatergic efferents to the dopamine cell body region in the ventral tegmental area. This topographically organized circuit has primary output through co-localized γ-amino butyric acid (GABA)ergic and peptidergic neurons in the nucleus accumbens mediates behavioral neuroplasticity associated with addiction, including relapse and sensitization to components of the drug experience.
acumbens that project to the ventral pallidum and ventral tegmental area [17].

**Focus on glutamatergic projections**

Recent data indicate that activation of glutamatergic efferents from the amygdala and prefrontal cortex is critical in the expression of addictive behaviors. The importance of these projections can be seen superficially in neuroimaging studies where blood flow to the anterior cingulate cortex, ventral orbital cortex and amygdala is increased during craving for a variety of addictive drugs, including alcohol, cocaine, methamphetamine, heroin and nicotine [8**,18]. More invasive and quantitative techniques applicable in animal studies clearly reveal the importance of these cortical and allocortical glutamatergic structures. The most common animal model employed for these studies is the reinstatement model, in which animals are trained to self-administer cocaine and are then provided with a stimulus that will cause the animal to perform an operant task (e.g. lever pressing or nose poke) to obtain only saline [7,9**]. The number of operant responses for saline is used as a measure of drug-seeking behavior (e.g. relapse). This reinstatement behavior is typically engendered by exposing the animal to one of three stimuli: a single administration of cocaine, a cue that was previously associated with cocaine self-administration, or an experimental stressor such as mild footshock. Using the reinstatement model of relapse, it has been shown that, regardless of the stimulus modality, there is a dependence on neurotransmission in the prefrontal cortex [9**,11*,19]. Thus, pharmacological inactivation of the prefrontal cortex with voltage-dependent sodium channel blockers or GABA receptor agonists prevents reinstatement induced by stress, cocaine-associated cues or administration of cocaine itself [19–22]. Moreover, studies using the induction of immediate early genes as an index of neuronal activity demonstrate involvement of the prefrontal cortex in reinstatement behavior [10,14]. The basolateral amygdala appears to be critical selectively for reinstatement elicited by a drug-associated cue [15,23,24]. As shown in Figure 1, both the amygdala and prefrontal cortex send glutamatergic projections to the nucleus accumbens and, through using the reinstatement model of relapse, the nucleus accumbens has also been shown to be critical for cocaine- and stress-primed reinstatement [20,21]. Moreover, the administration of AMPA glutamate receptor antagonists into the accumbens prevents reinstatement elicited by cocaine administered directly into the prefrontal cortex [25]. Using microdialysis, both drug- and stress-primed reinstatement were shown to produce an increase in glutamate release into the core of the accumbens, and inhibition of prefrontal cortical afferents blocked both reinstatement and the increase in accumbens glutamate [11*,20].

**Glutamatergic neuroadaptations in the ventral tegmental area**

A critical role for glutamatergic projections from the prefrontal cortex and amygdala to the nucleus accumbens in the expression of addictive behaviors is consistent with a well-developed literature showing long-term changes in gene expression and protein function in the nucleus accumbens induced by chronic administration of addictive drugs [13]. As with circuitry, the historic focus by neurochemists and molecular biologists has been the sequence of intracellular events precipitated by stimulation of dopamine receptors as a result of repeated use of cocaine. This research effort has identified molecular candidates related to dopamine transmission as important mediators of establishing sensitization and reinstatement behaviors. For example, dopamine D1 receptor stimulation of cAMP-dependent protein kinase (or PKA) and subsequent changes in protein function and gene expression in the nucleus accumbens and ventral tegmental area appear critical to establishing sensitization [13]. The most well-characterized effect of increased cAMP-dependent protein kinase activity is the induction of cAMP response element and the subsequent change in deltaFosB and cyclin-dependent kinase 5 [26,27]. Furthermore, manipulating dopamine D2 receptor signaling by regulating the in vivo level of the regulator-of-G-protein-signaling 9-2 (RGS9-2) was found to alter the rewarding effects of cocaine [28]. In addition to the immediate consequences of dopamine receptor signaling, calcium/calmodulin and ras/mitogen-activated protein kinase activity in the ventral tegmental area are critical to the development of sensitization [29**].
Although these dopamine-dependent changes have been linked to the development of cocaine-induced behavior and neuroplasticity, it is generally thought that the transient molecular adaptations in the ventral tegmental area are most critical to the development of addictive behaviors, and glutamate transmission in the ventral tegmental area has been shown to regulate dopamine-dependent alterations. For example, acute cocaine sensitizes the glutamatergic input from the prefrontal cortex and enhances the induction of long-term potentiation in dopamine cells [30,31,32]. In addition, cocaine induces a transient increase in glutamate receptor-1 (GluR1), which is linked to more enduring cellular changes in the nucleus accumbens and the development of sensitization [33**], and blocking ionotropic glutamate receptors in the ventral tegmental area prevents the development of conditioned place preference to cocaine [34]. Finally, following a cocaine overdose, addicts demonstrate elevated levels of several ionotropic glutamate receptor subunits in the ventral tegmental area [35]. Taken together, these newer studies are consistent with the idea that the ventral tegmental area is a site of action for cocaine, where increasing dopamine release produces a cascade of events that facilitates enduring cellular changes elsewhere in the brain; this cascade includes a transient increase in pre- and postsynaptic glutamate transmission.

**Glutamatergic neuroadaptations in the nucleus accumbens**

Although neuroadaptations related directly to dopamine receptor stimulation appear critical for the development of addiction, once addiction is established a variety of emerging data indicates that changes in proteins regulating glutamate transmission are critical for the expression of behaviors that characterize addiction, such as sensitization and relapse. Thus, a sequence of neuroadaptations produced by repeated cocaine might first involve adaptations in signaling pathways related directly to dopamine transmission that become more permanently manifested by changes in glutamate transmission [36*]. For example, it has been known for some time that acute cocaine administration does not alter glutamate release in the accumbens of naïve animals, but produces marked glutamate release in animals previously treated with repeated cocaine, especially when cocaine is associated with environmental cues [11*,37]. The enhanced release of glutamate occurs against a background of significantly reduced basal levels of glutamate in the extracellular space and inside presynaptic terminals [38*,39]. It has been speculated that the reduced glutamate background may accentuate the synaptic signal delivered by glutamate released in the projection from the prefrontal cortex to nucleus accumbens [11*,38*]. Recently, it was discovered that the reduced basal level of extracellular glutamate results from diminished activity of the cystine-glutamate exchanger, and restoration of cystine-glutamate exchange normalized extracellular glutamate levels and prevented cocaine-primed reinstatement [38*]. In the brain, it appears that the majority of cystine-glutamate exchange occurs in glia [40], and recent studies have identified enduring changes in other glial proteins following repeated cocaine administration [41]. The reduction in cystine-glutamate exchange might be related to the reported reduction in group I metabotropic glutamate receptor (mGluR1/5) regulation of extracellular glutamate, which results in part from a cocaine-induced reduction in Homer proteins that scaffold mGluR1/5 to inositol trisphosphate-sensitive intracellular calcium pools [42,43]. The importance of the reduction of Homer proteins in the accumbens in addiction is indicated by findings that antisense oligonucleotide reductions in Homer1 or deletion of the Homer2 gene produces a behavioral phenotype resembling cocaine addiction, including sensitization of cocaine-induced locomotion and reward [44]. In apparent contradiction to the effects of reduced mGluR1/5 signaling through Homer proteins, which causes enhanced responsiveness to cocaine, deletion of the mGluR5 gene or administration of mGluR5 antagonists inhibits the behavioral response to cocaine [45,46]. This contradiction is readily explained by the fact that enhanced release of glutamate through the cystine-glutamate exchanger, caused by mGluR1/5 stimulation, is mediated by mGluR1, not mGluR5 [42]. Another adaptation in presynaptic glutamate is the apparent desensitization of group II mGluR5 (mGluR2/3) following withdrawal from cocaine. Signaling through mGluR2/3 and the ability of mGluR2/3 to inhibit glutamate release is blunted, and this arises in part from an increased phosphorylation of the receptor, as well as a rise in activator of G protein signaling-3 (AGS3), which selectively binds to Gz [47,48].

The way in which this sequence of adaptations could synergize to dysregulate presynaptic glutamate transmission in cocaine addiction is illustrated in Figure 2. This hypothetical model describes how reduced Homer1bc could account for reduced activity of the cystine-glutamate exchanger and the accompanying reduced basal levels of extracellular glutamate. The reduced levels of glutamate, combined with desensitization of the mGluR2/3 receptor, results in a loss of regulatory feedback on synaptic glutamate release. Thus, lower basal levels of glutamate, combined with increased release of synaptic glutamate in response to activation of prefrontal cortical afferents to the nucleus accumbens, results in an amplified signal and behavioral drive to engage drug-seeking (e.g. to relapse).

In addition to adaptations in presynaptic and possibly glial release of glutamate that regulate the expression of sensitization and/or reinstatement, a variety of changes in postsynaptic glutamate transmission have been documented in the nucleus accumbens. Interestingly, although
presynaptic release of glutamate was augmented by withdrawal from repeated cocaine, most data indicate a reduction in postsynaptic responses to glutamate. Electrophysiological responses to iontophoretic or stimulated glutamate release are blunted [49,50]. This blunting might be associated with changes in ionotropic glutamate receptor subunits, although these data are variable, with the direction of change depending on the laboratory and withdrawal time [27,51–54]. A clear reconciliation of how augmented presynaptic glutamate transmission and reduced postsynaptic glutamate transmission might mediate the expression of addictive behaviors is not yet available. However, one consideration is that reduced electrophysiological estimates of postsynaptic glutamate transmission are suppressed after withdrawal from chronic cocaine. Against this suppressed background, the enhanced release of glutamate from prefrontal glutamatergic afferents (as occurs during reinstatement of drug-seeking; see above) will be more easily detected as a biologically relevant signal. This would especially be true if the reinstatement stimulus promotes postsynaptic as well as presynaptic transmission. For example, if the stimulus (e.g. a cocaine injection or stressor) increases dopamine release simultaneously with glutamate in the nucleus accumbens, it would be expected to rapidly increase surface expression of GluR1 [36].

Glutamatergic neuroadaptations in the prefrontal cortex

Enduring cellular changes in the prefrontal cortex produced by withdrawal from repeated cocaine are not as well characterized as in the nucleus accumbens. However, in vivo intracellular recording of pyramidal cells in the prefrontal cortex projecting to the nucleus accumbens or ventral tegmental area reveals a loss in membrane
The membrane potential of pyramidal cells normally fluctuates between relatively depolarized and hyperpolarized potentials. This fluctuation is regulated by both dopaminergic and glutamatergic afferents, and is thought to reflect tonic activity in cortical circuitry [55,56]. Thus, the loss of membrane bistability following chronic cocaine reflects changes within the pyramidal cells or changes in dopaminergic and/or glutamatergic afferents. There is emerging evidence for all of these cocaine-induced neuroadaptations. For example, after withdrawal from repeated cocaine, signaling through Gz coupled receptors is diminished (including mGluR2/3 and the GABA-B receptor) [57,58], probably as a result of elevated levels of AGS3 [48]. The ability to release dopamine in the prefrontal cortex is also altered [59], and there is evidence of glial proliferation that could affect glutamate transmission through altered glutamate uptake or activity of the cystine-glutamate exchanger [41]. At present, there is not sufficient information to determine the functional significance of these cocaine-induced alterations in prefrontal protein expression. However, the loss of membrane bistability is consistent with an emerging view in the neuroimaging literature that the prefrontal cortex might be hypoactive in cocaine addicts, resulting in decreased cognitive ability to regulate drug-seeking behavior [8**].

Conclusions
Research over the past two or three years has generally confirmed earlier hypotheses that transient neuroadaptations in the ventral tegmental area elicited by repeated cocaine are necessary for more enduring cellular changes elsewhere in the circuit (Figure 1). Importantly, recent studies have confirmed the important role of pre- and postsynaptic glutamate transmission in the ventral tegmental area. Using the reinstatement model of relapse, a strong focus has emerged on the role of glutamate transmission in the projection from the prefrontal cortex to the nucleus accumbens. Enduring alterations in both pre- and postsynaptic glutamate transmission in the accumbens can increase the signal-to-noise ratio of prefrontal excitatory afferents. Primary gaps in our understanding of how cocaine-induced adaptations in prefrontal glutamate projections might mediate relapse include an electrophysiological understanding of how changes in protein expression alter membrane physiology in both cortical pyramidal cells and accumbens spiny cells. In addition, it is clear that activity in both cell populations is state-dependent, and conclusions drawn from examining basal activity and protein expression might not be consistent with dynamic cellular responses produced by a stimulus capable of eliciting reinstatement (relapse). This latter lacuna in our knowledge will prove the most difficult to remedy, as it requires measurement of cellular function in behaviorally responding animals. At present, the technical capability to make these measurements is extremely limited.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:


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A provocative review summarizing this group’s work on the role of glutamate transmission in addiction, and providing balanced coverage of the literature regarding evidence supporting glutamate in the ventral tegmental area as one primary determinant of addiction.

34. Harris GC, Aston-Jones G: Critical role for ventral tegmental glutamate in prefrontal cortex as a gateway for a cocaine-conditioned environment. Neuropsychopharmacology 2003, 28:73-76.


A important paper providing a molecular mechanism whereby dopamine transmission could quickly alter glutamate transmission. This paper offers an important insight into how ongoing changes in dopamine transmission, as would occur in behaving animals, might translate into increased efficacy of postsynaptic glutamate signaling.


The paper outlines a novel mechanism underlying cocaine relapse that involves changes in glutamatergic tone in the nucleus accumbens mediated by the cystine-glutamate exchanger.


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