Cocaine-induced LTP in the ventral tegmental area: new insights into mechanism and time-course illuminate the cellular substrates of addiction

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Running head: Cocaine-induced LTP in the VTA

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Previous work has shown that a single dose of cocaine can produce long-term potentiation (LTP) of the glutamatergic synapses received by dopamine neurons in the ventral tegmental area (VTA). This and other plastic changes in the brain’s reward circuitry have been suggested to underlie addiction. Argilli et al. (2008) have provided new insights into cocaine-induced LTP, showing that it begins 3-5 hours after exposure, requires activation of a dopamine D5 / NMDA receptor cascade, and can be evoked by cocaine application directly to the VTA.
After many drug-free months, why does the sight of a white powder line still excite a cocaine addict? Cocaine causes long-lasting changes in the brain’s reward circuitry that prompt the drug-seeking behaviors exhibited by addicts. The mesolimbic dopamine system is the brain’s reward and motivational center, consisting of the ventral tegmental area (VTA), nucleus accumbens, striatum, prefrontal cortex, and associated limbic structures (Figure 1A). The motivation for natural rewards, such as food, water and sex, stems from the mesolimbic dopamine system. Drugs of abuse are thought to hijack the same pathways as natural rewards when causing euphoria (Kauer and Malenka 2007).

The VTA plays an important role in goal-directed behavior, including drug-seeking behavior. It is driven by excitatory, glutamatergic inputs from the prefrontal cortex, lateral hypothalamus, bed nucleus of the stria terminalis, pedunculopontine nuclei, and the superior colliculus, among other brain regions (Fields et al. 2007). The VTA contains populations of γ-amino butyric acid (GABA)–containing and dopamine-containing cells, as well as glutamatergic cells that are neither GABAergic nor dopaminergic. The substantia nigra pars compacta and the VTA are the only sources of dopaminergic drive for the striatum and limbic forebrain (Fields et al. 2007). Dopaminergic efferents from the VTA project to targets that include the nucleus accumbens, a region that appears to have a central role in the reinforcement of drug associated cues (Kauer and Malenka 2007).

Cocaine works by inhibiting plasma membrane dopamine transporters that take up dopamine from the synaptic cleft into the presynaptic cytoplasm (where separate vesicular transporters, the vesicular monoamine transporters repackaged it into vesicles for
later release). As a result, excess dopamine is available to bind to dopamine receptors in the nucleus accumbens and other VTA targets, creating a characteristic euphoria. The NMDA receptor’s role in cocaine addiction came to light after in vivo rodent studies revealed that the NMDAR antagonist MK-801 blocks the behavioral sensitization to cocaine that develops following repeated exposure (Karler et al. 1989). A more recent study also argued for the role of NMDARs in drug-seeking behavior by selectively knocking out the functional NR1 subunit of NMDARs in rat dopamine cells and observing a lack of conditioned place preference for a cage compartment in which cocaine was previously given (Zweifel et. al., 2008). NMDAR-dependent LTP, which is expressed by increases in postsynaptic AMPAR current, has been suggested as the mechanism for cocaine-induced synaptic plasticity in the VTA (Ungless et. al. 2001). This LTP is similar to that seen in other brain regions, like CA1 pyramidal cells of the hippocampus (Malenka and Bear 2004). Potentiation of excitatory synapses onto dopaminergic cells in the VTA increases the dopamine release to VTA targets. This triggers downstream changes in the reward circuitry that may account for the persistent drug-seeking behavior seen in addicts (Kauer and Malenka 2007).

Most studies to examine cocaine’s effects on excitatory synaptic transmission have made measurements in brain slices 24 or more hours after exposure to cocaine in vivo. Using recordings from midbrain slices containing the VTA, Ungless et al. (2001) found that a single in vivo exposure to cocaine causes a potentiation that lasts at least 5 but less than 10 days. Another study reported that cocaine activates dopamine D1/D5 receptors, which in turn activate the cAMP/PKA pathway, leading to an increase in NMDAR currents 20 minutes after exposure (Schilström et al. 2006). Cocaine was also
shown to trigger the insertion of GluR1-containing AMPARs into the postsynaptic membrane (Bellone and Lüscher 2006). However, the precise mechanism and duration of these changes following cocaine exposure was not clear.

But what happens in the crucial period between 20 minutes and 24 hours post-cocaine? Argilli et al. (2008) now provide a more detailed description of the time-course and intervening signaling events of cocaine-induced LTP by measuring changes that occur 3-5 hours after cocaine exposure. Rats were given intraperitoneal (i.p.) injections of cocaine or an equivalent amount of saline and horizontal slices containing the VTA were prepared 2 hours or 23 hours later for subsequent whole-cell voltage-clamp recordings. GABA currents were eliminated with picrotoxin, a GABAR antagonist, and dopamine neurons in the VTA were identified by a characteristic large, hyperpolarizing potassium current that is not seen in GABAergic cells. The AMPAR/NMDAR ratio was measured with voltage clamp recordings at +40 mV to relieve the Mg$^{2+}$ block of the NMDAR. Unlike measurements of the AMPA current amplitude alone, measurement of the AMPAR/NMDAR ratio enables comparisons of basal synaptic strength by normalizing small differences in slice recordings, like the positioning of electrodes or the number of activated synapses.

3-5 hours after in vivo cocaine exposure, an increased AMPAR/NMDAR ratio was observed in VTA dopamine cells compared to saline-injected controls (Figure 1B and C). The magnitude of this increase was similar to recordings made 24 hours after cocaine exposure (Borgland et al. 2004; Ungless et al. 2001). Saline-injected controls also showed a slightly higher AMPAR/NMDAR ratio than naïve rats, an effect that likely resulted from the stress induced by i.p. injection (Saal et al. 2003).
Is systemically-applied cocaine acting locally, within the VTA and its afferents, to evoke LTP or is it producing a broader network effect that is simply expressed in the VTA? Normal slices were incubated for 10 min with cocaine or vehicle, washed, and then allowed to recover for 3 - 5 hours. At this time point, cocaine pre-exposure significantly increased the AMPAR/NMDAR ratio compared with the vehicle controls and this increase was blocked if the slice was pretreated with the NMDAR antagonist APV (Figure 1B). Interestingly, when AMPAR/NMDAR ratios were measured during cocaine exposure or 10 minutes after, the ratios were reduced. This last result is consistent with a previous study, which found that acute cocaine perfusion causes NMDAR but not AMPAR potentiation via D₅ receptor activation of NMDARs (Schilstrom et al. 2006). Taken together, these results suggest that cocaine acts by a local, delayed mechanism to potentiate AMPARs. In this model, application of cocaine leads to activation of D₁-like dopamine receptors, an increase in cAMP, and a subsequent potentiation of NMDARs. The increase in NMDAR currents is required for potentiation of AMPARs via LTP-like mechanisms of AMPAR insertion in the postsynaptic density.

A series of in vitro experiments were performed to test an aspect of this model: that increased activation of D₁-like dopamine receptors on VTA dopamine cells leads to long-term potentiation of glutamatergic synapses. D₁-like dopamine receptors are the Gₛ-coupled D₁ and D₅ receptors. Application of the D₁/D₅ antagonist SCH-23390 five minutes before and during cocaine exposure blocked the typical increase in AMPAR/NMDAR ratio measured 3-5 hours later. This blockade was not observed when using eticlopride, an antagonist of the Gₛ-coupled D₂ receptor. The D₁/D₅ receptor agonist SKF-81297 also mimicked cocaine-induced LTP, causing an increased
AMPAR/NMDAR ratio. When the NMDAR antagonist D-APV was used along with SKF-81297, the AMPAR/NMDAR ratio was significantly reduced compared to SKF-81297 alone. This indicates a requirement for co-activation of NMDARs and dopamine receptors. Next, in an effort to distinguish between D1 and D5 dopamine receptors, 3-4 week old D5 receptor knockout mice were used. The AMPAR/NMDAR ratio recorded following in vivo cocaine exposure in these mice was reduced compared to wild-type littermates. Together, these experiments make a strong case for activation of D5 receptors and NMDARs as necessary steps in the induction phase of cocaine-induced LTP.

If the effects of cocaine on glutamatergic synapses received by VTA dopamine neurons are indeed similar to LTP induced by physiological patterns of activity, then prior cocaine exposure should occlude subsequent LTP. To perform this test, the authors made use of spike-timing-dependent LTP (STD LTP), a form thought to be physiologically relevant and which requires NMDAR activation. Indeed, STD LTP measured in the slice was reduced 3-5 hours following a single i.p. injection of cocaine. STD LTP was also occluded 24 hours after cocaine injection, or 24 hours after the 5th day of 5 consecutive cocaine injections. STD LTP was also tested after in vitro cocaine exposure. STD LTP could be induced 10 minutes after cocaine washout but was occluded 3-5 hours later, at the presumed onset of cocaine-induced LTP. The addition of D-APV, an NMDAR antagonist, before and during cocaine exposure rescued STD LTP induction (Figure 1C). The authors conclude that cocaine-induced LTP uses similar mechanisms for potentiating glutamatergic synapses as STD LTP and this LTP is dependent on the local rapid activation of NMDARs in the VTA leading to the slower upregulation of
AMPARs. An alternative explanation is that cocaine exposure blocks subsequent STD LTP through other mechanisms that are independent of occlusion.

If cocaine-induced LTP is comparable to synaptically-evoked LTP in other brain regions like the hippocampus, the delayed AMPAR potentiation should require the synthesis of a new protein. Anisomycin or cycloheximide, mRNA translation inhibitors, blocked an increase in AMPAR/NMDAR when applied 20 min before and during in vitro cocaine exposure. When the translation inhibitors were applied either alone or 1 hour following washout of cocaine, there was no significant change in the expected AMPAR/NMDAR ratio. These findings indicate that new protein synthesis must occur in the induction phase of cocaine-induced LTP triggered by drug exposure. The requirement for new protein synthesis suggests that cocaine-induced changes in the VTA are similar to other long-term memory processes in CA1 of the hippocampus (Malenka and Bear, 2004). Even a single exposure to cocaine causes the nuclear machinery to actively produce new proteins, which might include new AMPARs that will be inserted into the postsynaptic membrane.

Argilli et al. (2008) offer new insight into cocaine-induced long-term potentiation by providing evidence of D5 receptor activation and new protein synthesis, along with AMPAR and NMDAR activation. They have found that the time course of cocaine-induced long-term potentiation develops with a delay of a few hours, and begins with an early induction phase that requires dopamine D5 receptor activation. This leads to NMDAR activation together with new protein synthesis. A later phase involves the insertion of GluR1 subunit-containing AMPARs and the consequent upregulation of synaptic AMPAR currents.
In interpreting these results it should be noted that the VTA receives glutamatergic synapses from a variety of structures, including the bed nucleus of the stria terminalis, the lateral hypothalamus, and the prefrontal cortex (Fields et al. 2007). It remains unclear if these different populations of glutamatergic synapses show similar changes following cocaine exposure. In the future, testing these synaptic populations separately may provide insight into cocaine’s effect on information flow from specific cortical regions.

The authors suggest that since STD LTP is occluded after both in vivo and in vitro cocaine exposure, cocaine-induced LTP must be similar to other activity-dependent LTP in the VTA and may share the same molecular substrates. They also found that STD LTP is reduced after 5 consecutive cocaine injections. At first glance, their result conflicts with a finding that STD LTP increases following daily i.p. injections of cocaine (Liu et al. 2005). However, the enhanced STD LTP observed by Liu et al. is due to decreased GABAergic inhibition, a mechanism that would not have been at play in the experiments performed by Argilli et al., where GABAergic currents were pharmacologically blocked to focus on the effects of excitatory glutamatergic transmission. The differential effects of single versus multiple cocaine injections warrant further exploration, particularly since repeated cocaine exposure will better model the human addict.

How might a greater understanding of cocaine-induced LTP lead to treatments for cocaine addiction and other drug addictions? Since dopamine release from the VTA to the nucleus accumbens is implicated in the motivation for natural rewards as well as drugs, a better understanding of LTP in the VTA could lead to therapies for a number of
addictive behaviors. Coupling purely electrophysiological experiments, like the ones described here, to behavioral studies examining cocaine self-administration could provide insight into the mechanisms underlying addiction. Cocaine self-administration produces a potentiation of VTA glutamatergic synapses that persists longer than that evoked by exogenous cocaine (Chen et al. 2008). The use of self-administration paradigms will better model the human addict by showing the effects of repeated, voluntary drug use.

Is it possible to reverse cocaine-induced LTP? mGluR-dependent long-term depression reverses the cocaine-induced change in AMPAR distribution (Bellone and Lüscher 2006). A better understanding of the underlying mechanisms may enable the development of compounds to attenuate LTP or evoke LTD in the VTA. These compounds could then be tested using cocaine self-administration protocols in rats or mice as an assay of reward-seeking behavior. An understanding of the immediate mechanisms and time course of cocaine exposure, as Argilli et al. have presented, is an important first step in detailing the changes in VTA circuitry that could be manipulated by targeting the specific receptors involved at each stage of addiction. If the craving for cocaine is eliminated pharmacologically, the compulsive, cocaine-seeking behavior displayed by human addicts may be treated.


Figure Legend

Figure 1. Cocaine induced synaptic potentiation in glutamatergic synapses received by dopamine neurons in the ventral tegmental area (VTA).

(A) A simplified sketch of mesolimbic dopamine system circuitry (adapted from Kauer and Malenka, 2007). Glutamatergic drive is shown in blue, dopaminergic in red, and GABAergic in green. Dopamine-using cells in the ventral tegmental area (VTA) receive excitatory synapses from the prefrontal cortex (PFC). The VTA dopamine cells project to the nucleus accumbens (NAc) and the prefrontal cortex (PFC). The amygdala (AMG) sends excitatory, glutamatergic axons to the NAc, while the NAc inhibits the VTA via GABAergic synapses.

B) Population bar graph illustrating AMPAR/NMDAR ratios measured in VTA dopamine cells in brains slices. The left portion of the graph shows groups of rats which received no treatment, i.p. injection of saline, or cocaine. The right portion of the graph shows these ratio measurements 3-5 hours after a 10 min exposure to cocaine (or cocaine + APV) in vitro. This and following panel adapted from Argilli et al., 2008.

C) VTA slices were briefly exposed to cocaine or cocaine + APV. 3 – 5 hours later, a stimulation protocol designed to evoked spike-timing-dependent LTP (STP LTP) was delivered and a time course of evoked EPSPs was recorded.
A Mesolimbic Dopamine System

B

AMPAR/NMDAR Ratio

In vivo cocaine In vitro cocaine

C

EPSP amplitude (%)

STD LTP

0 10 20 30 40 50

Time (min)