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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Heshmati M. Cocaine-induced LTP in the ventral tegmental area: new insights into mechanism and time course illuminate the cellular substrates of addiction. J Neurophysiol 101: 2735–2737, 2009. First published March 18, 2009; doi:10.1152/jn.00127.2009. 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. A recent study has provided new insights into cocaine-induced LTP, showing that it begins 3–5 h after exposure, requires activation of a dopamine D$_3$/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 (Fig. 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-aminobutyric 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, repack 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 role of N-methyl-D-aspartate receptor (NMDAR) 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 long-term potentiation (LTP), which is expressed by increases in postsynaptic α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid receptor (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 that, in turn, 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 that examine cocaine’s effects on excitatory synaptic transmission have made measurements in brain slices ≥24 h 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 ≥5 but <10 days. Another study reported that cocaine activates dopamine D$_1$/D$_2$ receptors, which in turn activate the cyclic adenosine monophosphate (cAMP)/protein kinase A pathway, leading to an increase in NMDAR currents 20 min after exposure (Schilström et al. 2006). Cocaine was also shown to trigger the insertion of glutamate receptor 1 (GluR1)–containing AMPARs into the postsynaptic membrane (Bellone and Lüscher 2006). However, the precise mechanism and duration of these changes following cocaine exposure were not clarified.

But what happens in the crucial period between 20 min and 24 h 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 h after cocaine exposure. Rats were given intraperitoneal (ip) injections of cocaine or an equivalent amount of saline and horizontal slices containing the VTA were prepared 2 or 23 h later for subsequent whole cell voltage-clamp recordings. GABA currents were eliminated with picrotoxin, a GABA receptor 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.
By 3–5 h after in vivo cocaine exposure, an increased AMPAR/NMDAR ratio was observed in VTA dopamine cells compared with saline-injected controls (Fig. 1, B and C). The magnitude of this increase was similar to that of recordings made 24 h after cocaine exposure (Borgland et al. 2004; Ungless et al. 2001). Saline-injected controls also showed a slightly higher AMPAR/NMDAR ratio than that of naïve rats, an effect that likely resulted from the stress induced by ip 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 h. At this time point, cocaine preexposure significantly increased the AMPAR/NMDAR ratio compared with that of the vehicle controls and this increase was blocked if the slice was pretreated with the NMDAR antagonist d-2-amino-phosphonovaleric acid (d-APV) (Fig. 1B). Interestingly, when AMPAR/NMDAR ratios were measured during cocaine exposure or 10 min 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 D3 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 LTP of glutamatergic synapses. D1-like dopamine receptors are the Gs-coupled D1 and D3 receptors. Application of the D1/D3 antagonist SCH-23390 5 min before and during cocaine exposure blocked the typical increase in AMPAR/NMDAR ratio measured 3–5 h later. This blockade was not observed when using eticlopride, an antagonist of the Gi-coupled D2 receptor. The D1/D3 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 with SKF-81297 alone. This indicates a requirement for coactivation of NMDARs and dopamine receptors. Next, in an effort to distinguish between D1 and D3 dopamine receptors, 3- to 4-wk-old D2 receptor knockout mice were used. The AMPAR/NMDAR ratio recorded following in vivo cocaine exposure in these mice was reduced compared with that of wild-type littermates. Together, these experiments make a strong case for activation of D3 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 that requires NMDAR activation. Indeed, STD LTP measured in the slice was reduced 3–5 h following a single ip injection of cocaine. STD LTP was also occluded 24 h after cocaine injection or 24 h after the 5th day of five consecutive cocaine injections. STD LTP was also tested after in vitro cocaine exposure. STD LTP could be induced 10 min after cocaine washout but was occluded 3–5 h 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 (Fig. 1C).
The authors conclude that cocaine-induced LTP uses mechanisms for potentiating glutamatergic synapses similar to those of STD LTP and this LTP is dependent on the local rapid activation of NMDARs in the VTA, leading to the slower up-regulation 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 ratio when applied 20 min before and during in vitro cocaine exposure. When the translation inhibitors were applied either alone or 1 h 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 LTP by providing evidence of D3 receptor activation and new protein synthesis, along with AMPAR and NMDAR activation. They found that the time course of cocaine-induced LTP develops with a delay of a few hours and begins with an early induction phase that requires dopamine D3 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 up-regulation 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 whether 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 five consecutive cocaine injections. At first glance, their result conflicts with a finding that STD LTP increases following daily ip injections of cocaine (Liu et al. 2005). However, the enhanced STD LTP observed by Liu and colleagues is due to decreased GABAergic inhibition, a mechanism that would not have been at play in the experiments performed by Argilli et al. (2008) 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 those 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? Metabotropic GluR–dependent long-term depression (LTD) 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 presented by Argilli et al. (2008), 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.

REFERENCES