

Synaptic plasticity and addiction

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Abstract | Addiction is caused, in part, by powerful and long-lasting memories of the drug experience. Relapse caused by exposure to cues associated with the drug experience is a major clinical problem that contributes to the persistence of addiction. Here we present the accumulated evidence that drugs of abuse can hijack synaptic plasticity mechanisms in key brain circuits, most importantly in the mesolimbic dopamine system, which is central to reward processing in the brain. Reversing or preventing these drug-induced synaptic modifications may prove beneficial in the treatment of one of society's most intractable health problems.

Long-term potentiation (LTP). Activity-dependent strengthening of synaptic transmission that lasts at least one hour.

Long-term depression (LTD). Activity-dependent weakening of synaptic transmission that lasts at least one hour.

More than a century ago, Ramon y Cajal speculated that information storage in the brain results from alterations in synaptic connections between neurons¹. The discovery in 1973 of long-term potentiation (LTP) of glutamate synapses in the hippocampus² launched an exciting exploration into the molecular basis and behavioural correlates of synaptic plasticity. Partly because LTP was first described at synapses in the hippocampus, a brain region necessary for declarative memory formation, there was an early assumption that synaptic plasticity represents a cellular building block used exclusively for learning and memory. However, it has since become clear that LTP and its counterpart, long-term depression (LTD), are basic properties of most excitatory synapses throughout the CNS, and are used for multiple brain functions in addition to learning and memory³. For example, LTP and LTD appear to be essential in the stabilization and elimination of synapses during the developmental fine-tuning of neural circuits in many areas of primary sensory cortex⁴.

It therefore may not be surprising that evidence accumulated over the last decade demonstrates that drugs of abuse can co-opt synaptic plasticity mechanisms in brain circuits involved in reinforcement and reward processing. Indeed, an influential hypothesis is that addiction represents a pathological, yet powerful, form of learning and memory^{5–10}. Although the brain circuitry underlying addiction is complex, it is unequivocal that the mesolimbic dopamine system, consisting of the ventral tegmental area (VTA) and nucleus accumbens (NAc), as well as associated limbic structures (FIG. 1), are critical substrates for the neural adaptations that underlie addiction. It is also clear that the interactions between addictive drugs and synaptic plasticity in different brain regions will contribute to specific aspects of addiction, such as craving, withdrawal and, perhaps most importantly, relapse.

Addiction is not triggered instantaneously upon exposure to drugs of abuse. It involves multiple, complex neural adaptations that develop with different time courses ranging from hours to days to months (BOX 1). Work to date suggests an essential role for synaptic plasticity in the VTA in the early behavioural responses following initial drug exposures, as well as in triggering long-term adaptations in regions innervated by dopamine (DA) neurons of the VTA⁹. By contrast, downstream synaptic changes in the NAc and other brain regions, are likely to represent the formation of powerful and persistent links between the reinforcing aspects of the drug experience and the multiple cues (both internal and external) associated with that experience^{5–10}. Here we review emerging evidence that addictive drugs elicit or modify synaptic plasticity in many of the key brain regions involved in addiction, and that these synaptic modifications have important behavioural consequences. A major motivation for this research is the assumption that addictions to the different classes of abused substances share important underlying brain mechanisms. Identifying these mechanisms will advance our ability to treat and prevent these often devastating disorders, as well as other related behaviours, such as gambling.

Of course, the brain adaptations that underlie addiction are complex and involve drug-induced changes in essentially every parameter that has been studied including gene transcription, membrane excitability and neuronal morphology. Moreover, because of advances in our understanding, and the societal importance, of the neurobiology of addiction, this topic has been the subject of numerous reviews in both the basic science and clinical literatures. Thus, we will intentionally limit our discussion primarily to those studies that most directly demonstrate drug-induced modulation of

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Conditioned place preference

A behavioural task during which a subject learns to associate the drug experience with a specific physical environment. A subject will choose to spend more time in an environment in which it previously had a 'rewarding' experience and less time in an environment in which it had an aversive experience.

synaptic plasticity mechanisms and try to construct a coherent picture from an often confusing and, as yet, incomplete literature.

Addiction and learning

Synaptic plasticity is required for neuroadaptations that result from a wide range of environmental stimuli. It was therefore attractive to hypothesize that drugs of abuse cause long-term changes on behaviour by altering synaptic function and plasticity in relevant brain circuits. Moreover, data from diverse behavioural experiments with drugs of abuse has implicated specific signalling molecules already identified as key players in LTP and LTD at other synapses¹⁰. Indeed, accumulating evidence links various behavioural models of key features of addiction with synaptic plasticity in brain areas that process reinforcement and reward.

Studies demonstrating that blocking N-methyl-D-aspartate receptors (NMDARs) could short-circuit the development of drug-induced behavioural adaptations in certain addiction models were among the first clues that addictive drugs might access the same processes that are used to store learned information. For example, NMDAR blockade, known to prevent many forms of LTP and LTD in other brain regions³, also prevents conditioned place preference, behavioural sensitization and self-administration of drugs of abuse^{14–21} (BOX 2). Furthermore, NMDAR blockade specifically within the VTA (but not the NAc) effectively prevents both behavioural sensitization and conditioned place preference, supporting the idea that NMDAR-dependent processes in the VTA might have a pivotal role in the development of addiction^{15–17}. Importantly, NMDAR blockade does not prevent the acute locomotor response to psychostimulant drugs, only the sensitization that occurs with

repeated exposure. Given the critical role that NMDAR-dependent synaptic plasticity is thought to have in normal learning and memory¹³, these findings immediately suggested that processes akin to associative learning are essential in the early development of addiction.

Abundant additional evidence^{25–27} supports the notion that excitatory synaptic function within mesolimbic dopamine circuits is crucial for the behavioural responses to drugs of abuse. Furthermore, human imaging studies in addicted subjects demonstrate the powerful cognitive and emotional effects of cues that were previously associated with the drug experience²⁸. Preventing relapse is the major clinical problem in the treatment of addiction, suggesting the need to understand the cellular nature of the powerful 'memories' caused by prior drug experiences. Thus, experimental work in animal models, as well as clinical studies, provides compelling support for the importance of learning and memory mechanisms in addiction.

LTP and LTD mechanisms

A ubiquitous property of all synapses is their ability to undergo activity-dependent changes in synaptic strength, that is, synaptic plasticity. Much of the mechanistic work on long-term synaptic plasticity in the mammalian brain over the last few decades has focused on the forms of LTP and LTD observed at excitatory synapses, although it is now clear that inhibitory synapses can exhibit LTP and LTD as well. Synaptic plasticity can be studied most effectively using electrophysiological methods in brain slices that are viable for several hours, and therefore, the cellular mechanisms underlying the first few hours of LTP and LTD are the best understood. Before discussing the interactions between drugs of abuse and long-term synaptic plasticity, it is useful to review our mechanistic understanding of the most common forms of LTP and LTD (FIG. 2). Only by understanding these core synaptic mechanisms can we hope to understand how drugs of abuse usurp or modify them.

NMDAR-dependent LTP. NMDAR-dependent LTP, first observed in the hippocampus, has been intensively examined for over three decades and remains the best understood form of long-lasting synaptic plasticity in the mammalian brain^{2,3} (FIG. 2a). It requires the activation of NMDARs by presynaptically released glutamate when the postsynaptic membrane is significantly depolarized. This relieves the voltage-dependent block of the NMDAR by Mg²⁺, allowing Ca²⁺ to enter postsynaptic dendritic spines. The rise in postsynaptic Ca²⁺ concentration, the crucial trigger for LTP, activates complex intracellular signalling cascades that include several protein kinases, most notably CaMKII²⁹. The primary mechanism underlying the increase in synaptic strength during LTP is a change in α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA) trafficking that results in an increased number of AMPARs in the postsynaptic plasma membrane with no effect on NMDARs^{3,29}. Within a few hours, the maintenance of LTP requires protein synthesis³⁰, and there is

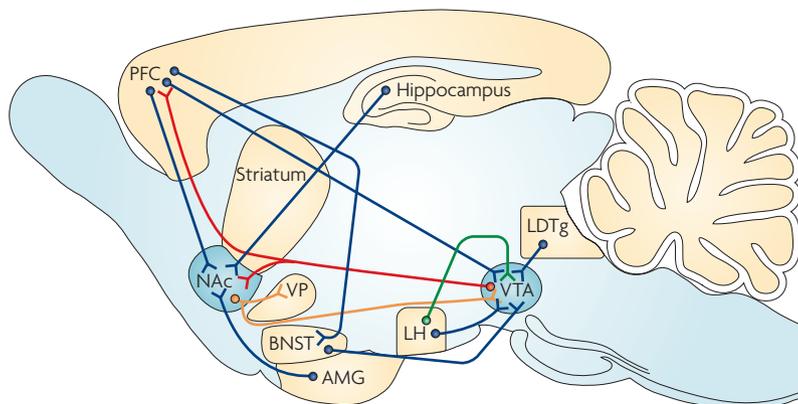


Figure 1 | Mesolimbic dopamine system circuitry. Simplified schematic of the circuitry of the mesolimbic dopamine system in the rat brain highlighting the major inputs to the nucleus accumbens (NAc) and ventral tegmental area (VTA) (glutamatergic projections, blue; dopaminergic projections, red; GABAergic projections, orange; orexinergic projections, green). Glutamatergic synapses excite postsynaptic neurons and GABAergic synapses inhibit postsynaptic neurons. Dopamine release exerts more complex modulatory effects. The release of dopamine from VTA neurons increases in response to administration of all drugs of abuse^{5–10,50}. These neurons also fire in response to novelty and their firing patterns may encode a prediction signalling the reward value of a stimulus relative to its expected value¹⁴³. AMG, amygdala; BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamus; PFC, prefrontal cortex; VP, ventral pallidum.

Induction of synaptic plasticity

Refers to the cellular mechanisms required for the events initiating or triggering LTP or LTD.

growing evidence that LTP is accompanied by observable enlargements of dendritic spines and associated postsynaptic densities^{3,31,32}. These structural changes may be essential to cement the information-storage process initiated at synapses upon LTP induction. It should also be noted that an alteration in the trafficking or numbers of NMDARs at synapses could potentially alter the threshold for induction of NMDAR-dependent LTP (and LTD; see below). A subunit switch between particular NMDAR subunits can also up- or downregulate NMDAR-mediated synaptic currents so that more or less Ca²⁺ will enter the postsynaptic neuron during receptor activation, thereby altering the induction of synaptic plasticity³³.

Presynaptic LTP. A distinct form of LTP was first described at synapses between the mossy fibres of the dentate granule cells and area CA3 hippocampal pyramidal neurons, but similar examples have been found in the neocortex and cerebellum^{3,34}. This form of LTP does not require NMDARs and postsynaptic factors may not be required (although this remains controversial^{15,36}). Instead, presynaptic LTP appears to be initiated by an activity-dependent rise in intracellular Ca²⁺ within the presynaptic terminals (FIG. 2b). The Ca²⁺ rise activates adenyl cyclases to produce cyclic AMP, with subsequent activation of protein kinase A (PKA)^{3,34}. This in turn leads to a persistent increase in the amount of glutamate released each time an action potential reaches the nerve terminal. Rab3A and RIM1 α , proteins that act to coordinate synaptic vesicle interactions with the presynaptic active zone, have an essential role in the increased glutamate release^{37,38}.

NMDAR-dependent LTD. NMDAR-dependent LTD is induced by weak activation of NMDARs (for example, due to modest membrane depolarization or low stimulation frequencies) and is thought to result from a smaller rise in postsynaptic Ca²⁺ than is required for LTP³. This triggers a different subset of Ca²⁺-dependent intracellular signalling molecules than those required

for LTP, including serine/threonine phosphatases, which dephosphorylate critical synaptic substrates, including the AMPARs themselves³ (FIG. 2c). The depression of synaptic strength during NMDAR-dependent LTD is due to the removal of synaptic AMPARs via dynamin- and clathrin-dependent endocytosis^{3,39}. An intriguing feature of NMDAR-dependent LTD is that NMDAR-mediated synaptic responses are also depressed by mechanisms that are distinct from those responsible for the LTD of AMPAR-mediated responses^{40,41}. This observation suggests that after this form of LTD is induced, further NMDAR-dependent synaptic plasticity will be limited, at least temporarily.

Metabotropic glutamate receptor-dependent LTD.

Activation of metabotropic glutamate receptors (mGluRs) can also lead to a postsynaptically induced and expressed LTD; this was first described at parallel fibre synapses on cerebellar Purkinje cells⁴² (FIG. 2d). Other forms of mGluR-dependent LTD using somewhat overlapping cellular mechanisms have subsequently been described in the hippocampus and the neocortex³. At the parallel fibre synapse, LTD is associative, requiring both postsynaptic Ca²⁺ influx through voltage-gated ion channels and postsynaptic group I mGluR activation, whereas at other synapses, activation of postsynaptic mGluRs alone appears to be sufficient. In most cases, however, this form of LTD is mediated by clathrin-dependent endocytosis of synaptic AMPARs. Interestingly, at certain developmental stages, rapid protein synthesis is required for both mGluR-triggered AMPAR endocytosis and LTD⁴³.

Endocannabinoid-mediated LTD. At many CNS glutamatergic and γ -aminobutyric acid (GABA)-releasing synapses, a brief, strong postsynaptic Ca²⁺ influx (and in some cases activation of mGluRs or muscarinic receptors alone) triggers the synthesis of endocannabinoids (eCBs), lipophilic molecules that travel retrogradely across the synapse to bind to presynaptic CB1 receptors and transiently depress neurotransmitter release for a period of many seconds⁴⁴ (FIG. 2e). At some synapses, however,

Box 1 | Drug administration protocols

There are many different ways to administer drugs of abuse. A drug may be administered by the investigator (passive administration) or an animal can be trained to self-administer the drug in response to cues. The time course over which drugs are administered and the time point at which assays are performed, during drug administration or following drug withdrawal, are also parameters that are under experimental control. These details are important because neuroadaptations to drugs of abuse occur over varying timescales and can be greatly influenced by the mode and duration of drug administration. An additional complexity is that distinct behavioural and neurobiological results can ensue depending on the novelty or other features of the environment in which the animal receives the drug. For example, behavioural sensitization is much more robust when the investigator-administered drug is given in a novel cage compared with the home cage¹¹. Recent studies also have found differences between groups of self-administering animals depending on the length of time the drug is available each day¹².

By varying drug administration protocols, investigators may selectively highlight particular aspects of the drug experience and the processes contributing to addiction. Assays at relatively early time points can detect changes related to tolerance or the symptoms of acute withdrawal, as well as changes underlying the development of craving. Adaptations related to craving and relapse may be most apparent when time points of days or weeks after drug withdrawal are examined. In some studies, after a period of withdrawal, a subsequent drug dose is given (for example, for the assessment of a behavioural response) and this re-exposure to the drug itself can have significant effects on synaptic and circuit properties. It is important to note carefully the administration protocol and the assay time points as contradictory results can be obtained from minor differences in these variables.

Box 2 | Behavioural sensitization

Sensitization is the gradually escalating behavioural and motivational response to a fixed drug dose. Two features of sensitization are intriguing in the context of drug addiction: multiple different addictive drugs produce sensitization, and after cessation of drug exposure, sensitization routinely lasts for weeks or months. Behavioural sensitization is most commonly assayed as drug-induced increases in locomotor activity²² and this has been associated with enhancement of the rewarding properties of drugs of abuse²³. Results from the behavioural sensitization model were some of the first to hint that NMDAR-dependent synaptic plasticity might be an essential contributor to the neural adaptations leading to drug addiction^{24,25}. There is strong evidence that the ventral tegmental area is involved in the triggering of behavioural sensitization, whereas the nucleus accumbens is crucial for its expression, but not its triggering.

prolonged eCB release instead causes LTD, which is mediated by a long-lasting depression of transmitter release (eCB-LTD)⁴⁵. Why eCB release produces only a transient synaptic depression at some synapses while at other synapses persistent LTD is elicited is not fully understood. Recent work suggests that the presynaptic mechanisms underlying the transient depression due to eCBs versus eCB-LTD differ, with PKA and RIM1 α dependent signalling being necessary only for eCB-LTD⁴⁶.

Homeostatic synaptic scaling. In addition to LTP and LTD, which usually are synapse specific, synaptic strength can be modified when activity levels are changed for prolonged periods (hours to days). Specifically, prolonged decreases in activity globally increase synaptic strength whereas prolonged increases in activity decrease synaptic strength⁴⁷. These widespread changes in synaptic strength are thought to be homeostatic responses that maintain the activity of individual cells within some finite range while keeping constant the relative differences in strength between synapses, caused by LTP and LTD, constant. Most evidence suggests that synaptic scaling is caused by changes in synaptic AMPAR content together with presynaptic changes in transmitter release⁴⁸. Mechanisms for this form of synaptic plasticity are currently under investigation and might include changes in local protein synthesis, nerve growth factors or diffusible factors, such as tumour necrosis factor α (TNF α)⁴⁹.

Drug exposure triggers LTP in the VTA

Different classes of drugs of abuse all increase the release of DA in the NAc⁵⁰ and this convergence, along with compelling evidence from behavioural studies, indicates that the mesolimbic DA system is required for drug addiction^{5-10,22,23,28}. The major cell type in the VTA is the dopaminergic neuron, which receives excitatory inputs from the prefrontal cortex (PFC), laterodorsal tegmental nucleus and lateral hypothalamus⁵¹. Dopaminergic neurons are inhibited by local interneurons, which generate GABA_A receptor-mediated responses, as well as by GABAergic projections from the NAc and ventral pallidum. VTA DA neurons themselves provide major projections to the NAc and PFC. As many as 35% of VTA neurons are GABAergic, and in addition to providing local inhibition, these neurons also project to the NAc and PFC (FIG. 1). Precise anatomical relationships exist between neurons in the VTA and projection targets. For

example, excitatory inputs from the PFC selectively form synapses onto VTA dopaminergic neurons that project back to the PFC but not onto neighbouring dopaminergic cells in the VTA that project to the NAc⁵².

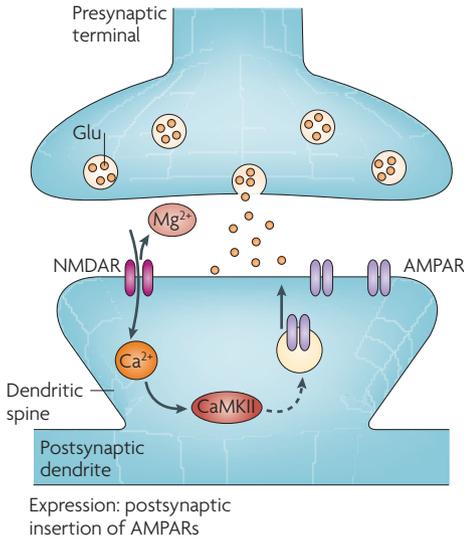
Before addressing the question of whether drugs of abuse can trigger synaptic plasticity in the VTA, it was important to establish that phenomena such as LTP and LTD do in fact occur at VTA synapses. Indeed, excitatory synapses on VTA DA cells express a form of NMDAR-dependent LTP⁵³⁻⁵⁶, as well as LTD that, surprisingly, requires voltage-dependent Ca²⁺ channels, not NMDARs^{57,58}. Furthermore, an mGluR-dependent LTD has also been reported at VTA synapses⁵⁹, and is described in the next section. These findings set the stage for a study that directly tested whether *in vivo* administration of an addictive drug produced long-term changes at excitatory synapses on VTA DA neurons⁶⁰. To monitor changes in excitatory synaptic strength, the investigators measured the ratio of AMPAR-mediated excitatory postsynaptic currents (EPSCs) to NMDAR-mediated EPSCs (the AMPAR/NMDAR ratio) (FIG. 3), and found that a single exposure to cocaine caused a large increase in this ratio in VTA DA cells when measured 24 hours later in brain slices. Additional assays indicated that this cocaine-induced change, like NMDAR-dependent LTP, was due to an upregulation of AMPARs and potentially shared mechanisms with the synaptically evoked LTP elicited in VTA slices. Furthermore, this drug-induced LTP was prevented when animals were pre-treated with an NMDAR antagonist.

These findings support the hypothesis that *in vivo* cocaine exposure elicits LTP at excitatory synapses on VTA DA neurons. An obvious yet critical question is whether other drugs of abuse cause the same synaptic modification. The finding that application of nicotine could evoke LTP at VTA DA excitatory synapses⁵⁵ is consistent with this idea. Further experiments show a similar potentiation of the AMPAR/NMDAR ratio 24 hours after *in vivo* administration of a number of diverse addictive drugs, including amphetamine, morphine, ethanol and nicotine⁶¹ (FIG. 4). The increased ratio could be detected within two hours of amphetamine exposure *in vivo*, as expected if LTP is the underlying mechanism⁶². Importantly, administration of widely used non-addictive drugs (fluoxetine and carbamazepine), did not change the AMPAR/NMDAR ratio⁶¹. Furthermore, no change in the AMPAR/NMDAR ratio was observed at hippocampal synapses or at excitatory synapses on VTA GABAergic cells, indicating that the effect of cocaine at VTA DA cell synapses was specific⁶⁰. The finding of an increased AMPAR/NMDAR ratio after administration of multiple different classes of drugs of abuse with distinct molecular targets and differing behavioural profiles suggests that this synaptic adaptation (that is, LTP at excitatory synapses on VTA DA neurons) might be directly related to the addictive properties of these compounds.

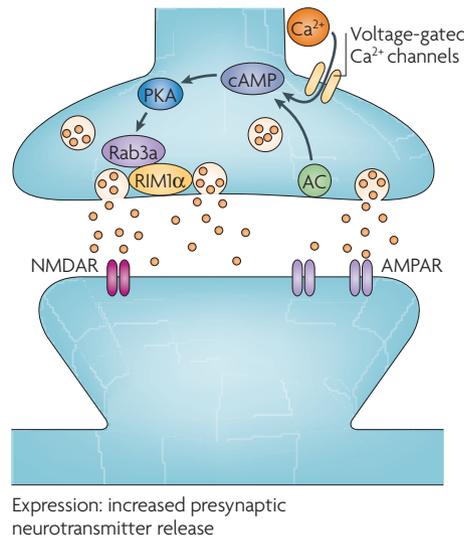
Stress is a potent trigger of relapse in humans and many animal addiction models⁶³⁻⁶⁶. This observation provided the motivation to test whether acute stress also increased the AMPAR/NMDAR ratio in DA neurons.

Excitatory postsynaptic currents
(EPSCs). Currents measured using electrophysiological recordings from a single neuron while electrically stimulating axons to release neurotransmitter. For the purposes of this Review, EPSCs are glutamate-mediated.

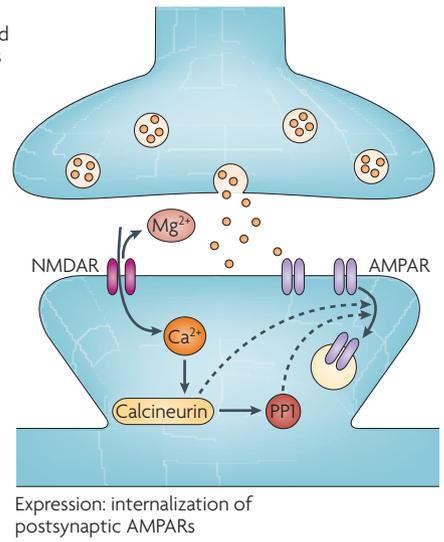
a NMDAR-dependent LTP



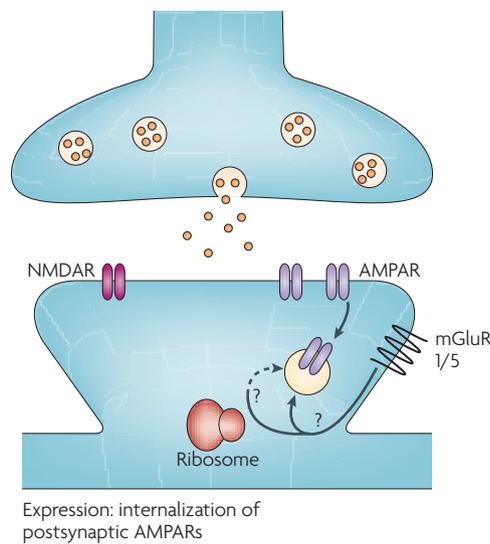
b Presynaptic LTP



c NMDAR-dependent LTD



d mGluR-dependent LTD



e eCB-LTD

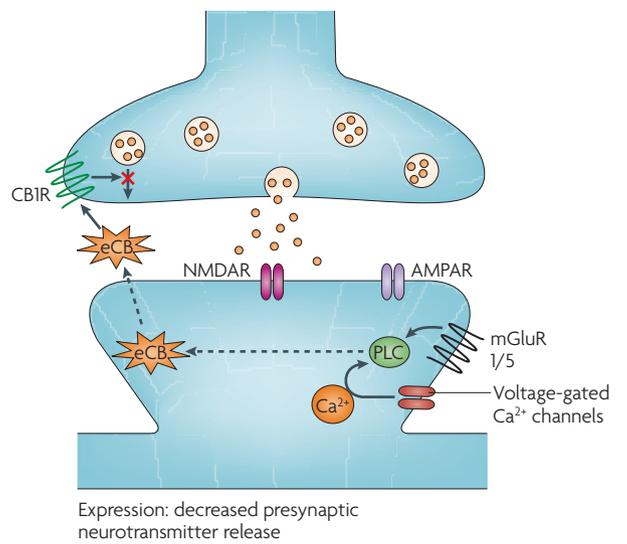


Figure 2 | Well-described forms of LTP and LTD. Highly simplified diagrams of the induction and expression of synaptic plasticity observed in the rodent brain. **a** | N-methyl-D-aspartate receptor (NMDAR)-dependent long-term potentiation (LTP) has been observed in many different brain regions and is dependent on postsynaptic NMDAR activation and calcium/calmodulin-dependent protein-kinase II (CaMKII) for its initiation³. The voltage-dependent relief of the magnesium block of the NMDAR channel allows the synapse to detect coincident presynaptic release of glutamate (Glu) and postsynaptic depolarization. α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA) insertion into the postsynaptic membrane is a major mechanism underlying LTP expression. **b** | Presynaptic LTP has been best characterized at mossy fibre–CA3 hippocampal synapses as well as at parallel fibre–Purkinje cell cerebellar synapses^{3,34}. Repetitive synaptic activity leads to the entry of presynaptic Ca^{2+} , which activates a Ca^{2+} -sensitive adenylylate cyclase (AC) leading to a rise in cAMP and the activation of cyclic AMP-dependent protein kinase A (PKA). This in turn modifies the functions of Rab3a and RIM1 α leading to a long-lasting increase in glutamate release^{34,37,38}. Involvement of postsynaptic signalling molecules (not shown) has also been reported^{35,36}. **c** | NMDAR-dependent long-term depression (LTD) is triggered by Ca^{2+} entry through postsynaptic NMDAR channels, leading to increases in the activity of the protein phosphatases calcineurin and protein phosphatase 1 (PP1). The primary expression mechanism involves internalization of postsynaptic AMPARs and a downregulation of NMDARs by an unknown mechanism^{3,41}. **d** | Metabotropic glutamate receptor (mGluR)-dependent LTD has been best characterized at cerebellar parallel fibre–Purkinje cell synapses and hippocampal synapses. Activation of postsynaptic mGluR1/5 triggers the internalization of postsynaptic AMPARs, a process that under some conditions appears to require protein synthesis⁴³. **e** | Endocannabinoid-LTD is the most recently discovered form of LTD, and has been observed in many brain regions. Either mGluR1/5 activation, leading to activation of phospholipase C (PLC) or an increase of intracellular Ca^{2+} (or both), in the postsynaptic neuron initiates the synthesis of an endocannabinoid (eCB). The eCB is subsequently released from the postsynaptic neuron, travels retrogradely to bind to presynaptic cannabinoid 1 receptors (CB1R) and this prolonged activation of CB1Rs depresses neurotransmitter release via unknown mechanisms⁴⁵.

Expression of synaptic plasticity
Refers to the cellular mechanisms responsible for maintaining a change in synaptic strength, for example, an increase in neurotransmitter release.

Indeed, 24 hours after a cold water swim (a manipulation commonly used to elicit stress in rodents), the ratio was increased and, like the response to cocaine, the increase was blocked by a preceding dose of an NMDAR antagonist⁶¹. This result raised the possibility that administration of drugs of abuse elicited a stress response that was responsible for the observed drug-induced LTP. However, a glucocorticoid receptor antagonist, which blocked the potentiation caused by stress, did not block the potentiation by cocaine⁶¹. Conversely, a D1 dopamine receptor antagonist blocked the increase in the AMPAR/NMDAR ratio elicited by cocaine but not the increase caused by stress⁶⁷. These results demonstrate that even though stress and cocaine elicit the same synaptic adaptation in VTA DA neurons, they do so through distinct mechanisms.

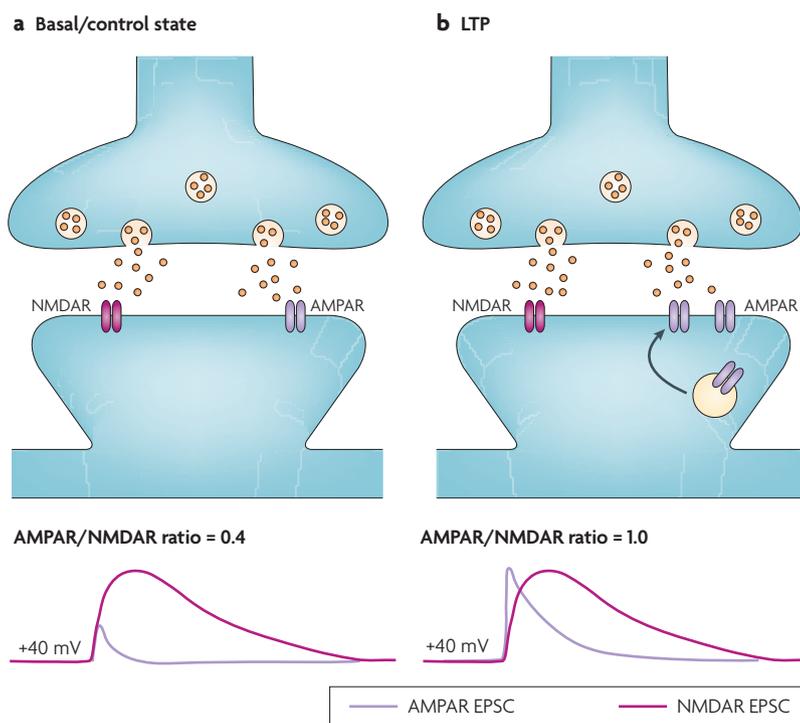


Figure 3 | Synaptic strength measured using the AMPAR/NMDAR ratio. The basal strength of excitatory synapses is difficult to compare between different cells and preparations. Calculating the ratio of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA)-mediated synaptic currents to N-methyl-D-aspartate receptor (NMDAR)-mediated synaptic currents of a population of stimulated synapses is a normalization procedure that facilitates such comparisons because it is independent of parameters such as the positioning of electrodes or the number of synapses that are activated. It is commonly calculated by holding the membrane potential of cells at positive potentials (for example, +40 mV), to completely relieve the block of NMDARs by magnesium, and measuring the amplitude of AMPAR excitatory postsynaptic currents (EPSCs) and NMDAR EPSCs. A typical procedure is to record a dual component EPSC (mediated by both AMPARs and NMDARs; not shown) and then apply the NMDAR antagonist D-APV to isolate the AMPAR EPSC. The NMDAR EPSC is obtained by digital subtraction of the AMPAR EPSC from the dual component EPSC. **a** | A synapse is illustrated in the basal or control state. The isolated AMPAR and NMDAR components of the EPSC are shown below the synapse diagram. **b** | After LTP induction, if AMPARs are inserted into the postsynaptic membrane, the AMPAR component of the EPSC is enhanced, whereas the NMDAR component remains unchanged, increasing the ratio. This method assumes that there are no significant changes in the proportion of rectifying (Ca^{2+} -permeable) AMPARs that would confound measurements made at +40 mV.

The studies reviewed thus far provide strong evidence that drugs of abuse interact with synaptic plasticity mechanisms in VTA DA neurons. In fact, *in vivo* administration of amphetamine not only elicits LTP at excitatory VTA synapses, but also blocks LTD at the same synapses when applied to slices, an effect that may contribute to its potentiating effects⁵⁷. Do these synaptic adaptations have any behaviourally relevant consequences? A standard way of beginning to address this issue is to examine the behaviour of mutant mice that lack relevant forms of synaptic plasticity in the VTA and examine them for behavioural deficits. Current models of NMDAR-dependent LTP support the idea that insertion of glutamate receptor 1 (GluR1)-containing AMPARs is an early necessary step in LTP expression⁶⁸. Furthermore, overexpression of GluR1 subunits in the VTA produced sensitized behavioural responses to morphine⁶⁹. Thus, the electrophysiological and behavioural effects of cocaine exposure in mutant mice lacking GluR1 were explored⁶⁷. Administration of cocaine or an acute stress to GluR1 knock-out mice did not increase the AMPAR/NMDAR ratio measured 24 hours later, demonstrating a critical role for this AMPAR subunit in VTA LTP. Although the mutant animals still developed locomotor sensitization to cocaine (BOX 2), conditioned place preference to cocaine was absent, as was their conditioned increase in locomotor activity when placed in the activity box in which they had previously experienced cocaine⁶⁷. These results are consistent with the idea that drug-induced LTP of excitatory synapses on VTA DA neurons might be necessary for attributing motivational significance to the drug experience or for the learned association between context and drug experience. Of course, in these studies GluR1 was absent throughout the brain and thus, more work will be needed to prove that the absence of LTP in the VTA, rather than other brain structures, caused the observed behavioural impairments. It is somewhat puzzling that GluR1 overexpression in the VTA produces behavioural sensitization on its own, suggesting that LTP-like changes in the VTA are sufficient to drive behavioural responses to morphine toward a sensitized phenotype⁶⁹, whereas GluR1 knock-out animals exhibit normal behavioural sensitization to cocaine⁶⁷. Further work will be necessary to determine whether these differences reflect important mechanistic differences in the actions of cocaine and morphine, or rather are due to the different methodologies that were used to study the role of GluR1.

What happens to excitatory synaptic function in VTA DA neurons after repeated exposure to cocaine — do the AMPAR/NMDAR ratios become even bigger, or is there a ceiling effect? Surprisingly, after seven daily cocaine injections, the AMPAR/NMDAR ratios remained at the same level seen 24 hours after a single injection⁷⁰. The persistence of the potentiation was also similar in both groups: ratios remained elevated five days after the last cocaine injection but were near control levels after ten days. Moreover, immediately following the first cocaine administration the precise AMPAR/NMDAR ratio from a given animal correlated well with its drug-induced locomotor behaviour, but after this time point

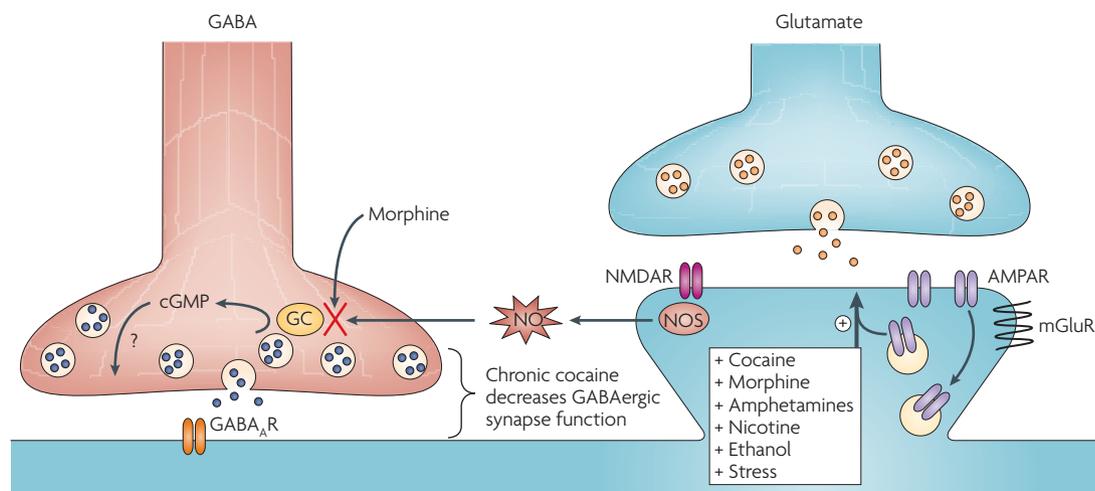


Figure 4 | Drugs of abuse modulate synaptic function and plasticity in the ventral tegmental area (VTA). Diagram showing the major effects of drugs of abuse on synaptic plasticity in the VTA. Several classes of drugs of abuse (indicated in the white box), as well as acute stress, elicit long-term potentiation (LTP) possibly by increasing postsynaptic α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) at glutamatergic synapses^{60–62,67,70}, metabotropic glutamate receptor-dependent long term depression (LTD). N-methyl-D-aspartate receptor (NMDAR) activation also activates nitric oxide synthase (NOS), which leads to the production of nitric oxide (NO). NO is a diffusible messenger that is released from postsynaptic neurons and activates guanylate cyclase (GC) in neighbouring presynaptic inhibitory terminals. A rise in cyclic GMP (cGMP) elicits a long-lasting increase in γ -aminobutyric acid (GABA) release at GABA_A receptor-containing synapses (LTP_{GABA})⁹⁶. Morphine prevents LTP_{GABA} by inhibiting the signalling of NO to guanylate cyclase⁹⁶. Chronic cocaine decreases inhibitory synaptic transmission via unknown mechanisms⁵⁶.

the locomotor response and AMPAR/NMDAR ratio became uncorrelated⁷⁰. In a related study, AMPAR/NMDAR ratios were examined in mice that received both an acute stress and cocaine, and these ratios also did not increase above the level seen with either stimulus alone⁶⁷. These results are consistent with the idea that although potentiation of excitatory synapses on VTA DA neurons may initially contribute to the incentive value attributed to the drug or stress experience, adaptations in downstream circuitry are likely to be more important for the longer-lasting behavioural changes associated with addiction (BOX 2).

Another reported effect of chronic (5–7 day) cocaine administration is to enhance a form of LTP elicited by a spike-timing protocol⁵⁶. However, we (P. Luu and R. C. Malenka, unpublished observations) and others (E. Argilli and A. Bonci, personal communication) have found that following cocaine administration (1 day or 5 days), spike-timing dependent LTP, like pairing-induced LTP⁶⁰, was absent presumably because of occlusion by the LTP that had already occurred *in vivo*.

Synaptic adaptations that influence LTP in the VTA may also occur during drug withdrawal. For example, expression of *c-FOS* (a marker for neuronal activation) increased sharply in rats re-exposed to an environment associated with withdrawal⁷¹. In a related finding, brain-derived neurotrophic factor (BDNF) levels in the VTA increased during prolonged (10–15 day) drug withdrawal; this has been suggested to enhance the ability to elicit LTP using a weak induction protocol that normally does not elicit LTP⁷². This effect might be related to the ability of BDNF when injected into the VTA immediately after a regimen of cocaine self-administration to enhance

drug-seeking behaviour, even after several weeks of cocaine withdrawal⁷³. Further work is required to test the idea that growth factors like BDNF may be regulatory molecules linking rapid changes at synapses caused by drug exposure with longer-lasting modifications of circuit activity.

The results summarized in this section provide strong evidence that drugs of abuse or stress cause potentiation of excitatory synapses on VTA DA neurons. It is clear, however, that this one synaptic adaptation alone does not predict that addiction will follow. Addiction rarely occurs after, for example, a single exposure to nicotine or alcohol, yet one exposure to either drug potentiates synapses on VTA DA neurons⁶¹. Furthermore, a single acute stress does not lead to drug addiction despite the fact that this experience also potentiates VTA synapses⁶¹. Instead, these experiments suggest that the LTP at excitatory synapses on VTA DA neurons elicited by a single drug experience or stress contributes to the early neural adaptations that are required for the subsequent development of addiction. An important question for future work is whether this potentiation of VTA synapses also contributes significantly to relapse, which is commonly triggered by either of these experiences. This might occur because stronger excitatory synapses on VTA DA neurons will change the levels or patterns of DA release in target structures, such as the NAc, and thereby modulate DA-dependent learned associations and behaviours^{5–10}. It will be important to establish stronger links between the synaptic changes in the VTA that are triggered rapidly by addictive drugs and the downstream neural circuit adaptations that ultimately underlie the persistent behavioural changes that define addiction.

Occlusion

The observation that synaptic stimulation produces no further LTP (or LTD) presumably because the underlying cellular mechanisms have been maximally activated by some preceding stimulus. When LTP (or LTD) is absent, it is often difficult to determine whether it has been 'occluded' or blocked by inhibition or inactivation of one or more essential cellular mechanisms.

Ca²⁺-permeable AMPARs in the VTA

Most AMPARs are heteromers of at least two different AMPAR subunits including GluR2 and, therefore, are not very permeable to Ca²⁺ (REF. 74). However, AMPARs lacking GluR2, such as GluR1 homomeric receptors, are Ca²⁺-permeable and, therefore, can initiate Ca²⁺-triggered intracellular signalling cascades in a manner that is analogous to NMDARs. Based on the dramatic behavioural changes elicited by the overexpression of GluR1 in VTA DA neurons, increased numbers of synaptic Ca²⁺-permeable AMPARs have been proposed to have an important role in the development of behavioural sensitization⁷⁵. Recent electrophysiological evidence from cortical and hippocampal preparations suggests that GluR2-lacking AMPARs can in fact be inserted at potentiated synapses that previously expressed GluR2-containing AMPARs^{76–78} (although some of these results are controversial)^{79,80}. Similarly, insertion of GluR2-lacking AMPARs has also recently been suggested to occur at synapses on VTA DA neurons after *in vivo* cocaine administration. Notably, this process can be reversed by mGluR-dependent LTD, which results from the removal of GluR2-lacking synaptic AMPARs and their replacement by GluR2-containing ones^{59,81,82}. This is a potentially important observation as it suggests a strategy for reversing or preventing the drug-induced LTP at VTA DA cell synapses.

A limitation to this hypothesis, however, is that the well-established biophysical properties of GluR2-lacking AMPARs⁷⁴ suggest that following cocaine administration, only a modest fraction of synaptic AMPARs can be GluR2-lacking as such receptors show strong inward rectification and pass little current at positive membrane potentials. If most synaptic AMPARs following cocaine administration were GluR2-lacking, the increase in the AMPAR/NMDAR ratio, which has been measured at +40 mV in all studies to date (FIG. 3), would be minimal or absent. Furthermore, other groups have not observed a change in the rectification of AMPAR EPSCs despite simultaneously observing a clear drug-induced increase in the AMPAR/NMDAR ratio⁶². Technical differences in experimental conditions may contribute to these inconsistencies and, thus, this intriguing hypothesis deserves further study.

Orexin receptors and LTP in the VTA

Orexin A and B (also known as hypocretin-1 and -2) are neuropeptides with behavioural effects on arousal and feeding that are synthesized exclusively in neurons of the lateral hypothalamus^{83,84}. Growing evidence links the orexin system with reward and reinforcement⁸⁵. For example, orexin neurons are activated in response to rewarding stimuli such as food or addictive drugs⁸⁵ and innervate the dendrites of dopaminergic neurons⁸⁶ as well as cells within the NAc⁸⁷. Furthermore, orexin A re-instates cocaine-seeking in rats⁸⁸, whereas an *orexin receptor 1* (OXR1) antagonist blocks acquisition of conditioned place preference when delivered locally to the VTA^{89,90}.

Recently, a synaptic effect of orexin A that might contribute to its behavioural effects has been reported. Brief (5 minute) exposure to orexin A in slices rapidly

and transiently enhanced NMDAR-mediated EPSCs (but not AMPAR-mediated EPSCs) in VTA DA neurons; this effect was blocked by the OXR1 antagonist, SB 334867 (REF.91) (FIG. 5). Interestingly, an increase in AMPAR miniature EPSCs (mEPSCs) and the AMPAR/NMDAR ratio was observed 3–4 hours later. The delayed potentiation of AMPAR mEPSCs did not occur when orexin A was applied with an NMDAR antagonist, suggesting that the increase in NMDAR-mediated responses promotes LTP induction at these synapses within a few hours. Consistent with these observations, when mice were pre-injected with SB 334867 before each of the daily cocaine injections for 5 days, the AMPAR/NMDAR ratio was not significantly increased 24 hours after the last cocaine treatment. Moreover, cocaine-induced locomotor sensitization was prevented by either systemic administration of SB 334867 or local injection of SB 334867 into the VTA⁹¹.

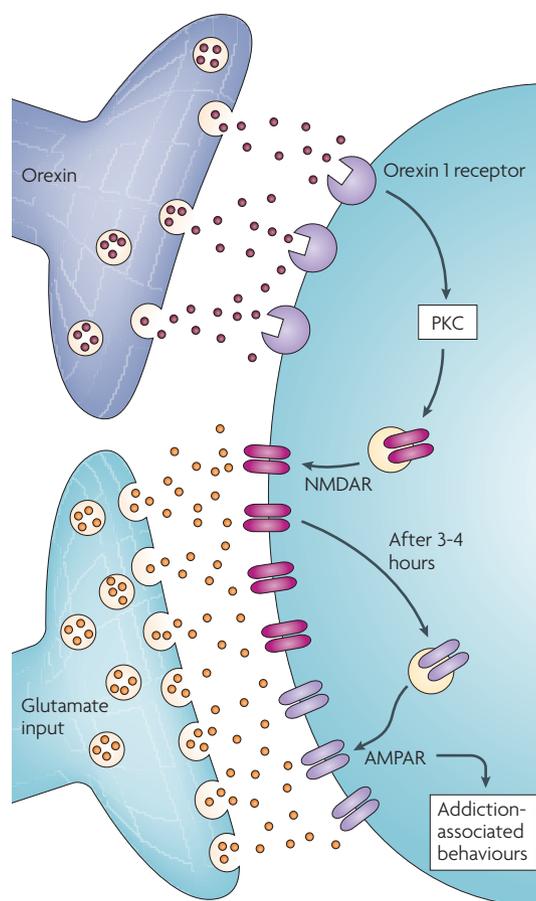


Figure 5 | Orexin A enhances NMDAR EPSCs in VTA dopamine neurons. Orexinergic afferents from the lateral hypothalamus innervate dopamine neurons of the ventral tegmental area (VTA). Over a period of several minutes, activation of orexin 1 receptors by orexin A enhances N-methyl-D-aspartate receptor (NMDAR) excitatory postsynaptic currents (EPSCs) by a protein kinase C (PKC)-dependent insertion of NMDARs at synapses. This process is followed within 3–4 hours by an increase of α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptor (AMPA) EPSCs that results from AMPAR insertion⁹¹.

These data suggest that OXR1 receptors in the VTA are necessary for the development of locomotor sensitization, possibly by increasing NMDAR-mediated currents. Cocaine application to slices, by D5-like receptor activation, has also been found to increase NMDAR-mediated EPSCs in VTA DA cells⁹² suggesting that upregulation of NMDARs through multiple mechanisms could be a critical trigger for the drug-induced LTP of AMPAR-mediated responses. Indeed, previous work has shown that NMDAR activation is a prerequisite for psychostimulants to elicit locomotor sensitization as well as to increase the AMPAR/NMDAR ratio^{15–17,60}. Together, these findings suggest that the orexin pathway to the VTA is a critical player in reward-based learning and memory and, therefore, also in addiction. Important questions for the future include how cocaine promotes the release of orexin A from lateral hypothalamic neurons and whether other drugs of abuse also work through the orexin system.

GABAergic VTA synapses as drug targets

Inhibitory synapses in the VTA also have a critical role in controlling the firing rate of DA neurons. Blockade of GABA_A receptors strongly increases DA cell firing both *in vivo* and in slices^{93,94}, and thus plasticity of GABAergic synapses might have a profound influence on the activity of VTA DA neurons. Indeed, daily injections of cocaine (over 5–7 days) decreased the size of GABA_A receptor-mediated miniature inhibitory postsynaptic currents (IPSCs) on DA neurons as well as their maximal evoked GABA_A currents⁵⁶. Associated with this decrease in inhibition was an increased likelihood of firing to a fixed stimulus. Although the mechanism by which cocaine depresses GABAergic function is unknown, this work suggested that enhancing GABA_A currents might be a way to counteract some of the neuroadaptations caused by cocaine in the VTA. Nicotine also produces a persistent depression of GABA release for at least an hour; longer-term effects have yet to be examined⁹⁵.

GABA_A receptor synapses on VTA dopamine neurons also exhibit robust LTP in slices from naive animals in response to high-frequency stimulation⁹⁶ (FIG. 4). This LTP of IPSCs can be triggered by NMDAR activation, suggesting that NMDAR-dependent LTP at excitatory synapses may ordinarily be accompanied by NMDAR-dependent LTP of inhibitory synapses, thus, helping to keep the firing rate of DA cells relatively constant. Several lines of evidence suggest that this potentiation (termed LTP_{GABA}) is initiated by the postsynaptic release of nitric oxide (NO) from DA cells that feeds back on inhibitory terminals to activate guanylate cyclase, which in turn causes a long-lasting enhancement of GABA release⁹⁶. Importantly, LTP_{GABA} was entirely absent in slices from animals treated with morphine 24 hours earlier. This was due to a disruption of the coupling between NO and guanylate cyclase, as in slices from morphine treated animals, cyclic GMP was still effective at producing LTP_{GABA} but NO itself was not. These data suggest that a single *in vivo* exposure to morphine either causes a loss of guanylate cyclase from presynaptic GABA-releasing terminals or renders guanylate cyclase NO-insensitive⁹⁶. Together with LTP at excitatory synapses, this loss of

LTP_{GABA} is expected to increase the firing of VTA DA neurons following morphine exposure. Restoration of guanylate cyclase function might represent an avenue that could be exploited to rescue or prevent the loss of normal inhibition after opiate exposure. It will be important to determine whether other drugs of abuse target LTP_{GABA} in the same manner as morphine.

Plasticity in the NAc

As is the case for the VTA, it is generally accepted that plasticity in the NAc and its associated circuitry has a key role in many forms of reward-dependent learning and that the powerful reinforcing effects of drugs of abuse can hijack these circuits to produce the pathological behaviours that define addiction^{5–10,26–28,97–100}. Adaptations in the excitatory synaptic inputs onto NAc medium spiny neurons seem to be particularly important for mediating addiction-related changes in behaviour and, thus, the detailed molecular mechanisms by which drugs of abuse modify excitatory synaptic function in the NAc have received increased attention. The major cell type in the NAc is the GABAergic medium spiny neuron, which makes inhibitory connections with cells in the ventral pallidum and VTA, and receives excitatory inputs from limbic structures, specifically the hippocampus and amygdala, as well as the PFC (FIG. 1). The NAc can be further divided into two subregions termed the core and the shell, which differ in their detailed histochemical architecture and, presumably, their function. The NAc core is often considered a functional extension of the dorsal striatum and may be particularly important for instrumental learning, including cue-induced reinstatement of drug seeking behaviour⁹⁸. The shell is a transitional zone between the striatum and extended amygdala and may be preferentially involved in mediating the primary reinforcing effects of drugs of abuse^{99,100}.

LTD and LTP in the NAc. Within the NAc, the phenomena of LTD and LTP have once again served as models for the types of changes that drugs of abuse may cause in this brain region. However, drawing parallels between LTP and LTD in the NAc and drug-induced plasticity is particularly difficult because very little is known about the mechanisms of synaptic plasticity at NAc excitatory synapses. NMDAR-dependent LTP and LTD have been reported to occur at these synapses^{58,101–103} as well as eCB-LTD^{104,105}. Similar to these forms of plasticity at hippocampal CA1 pyramidal cell synapses, LTP in the NAc is enhanced in mice lacking postsynaptic density protein 95 (PSD-95)¹⁰³, whereas NMDAR-dependent LTD appears to involve the endocytosis of AMPARs¹⁰⁶ (FIG. 2c). eCB-LTD involves activation of postsynaptic mGluRs leading to the release of an endogenous cannabinoid that activates presynaptic CB1 receptors to cause a long-lasting decrease in glutamate release^{45,104} (FIG. 2e).

Does *in vivo* administration of a drug of abuse modify excitatory synapses in the NAc by activating or interfering with one or more of these synaptic plasticity mechanisms? Evidence that this may occur came from studies, similar to those performed in the VTA, in which the AMPAR/NMDAR ratio was used as a measure of

Inhibitory postsynaptic currents

(IPSCs). Currents measured using electrophysiological recordings from a single neuron while electrically stimulating axons to release neurotransmitter. For the purposes of this Review, IPSCs are GABA-mediated.

basal synaptic strength¹⁰⁷. Although unlike the VTA, a single *in vivo* dose of cocaine caused no change in this ratio, chronic (5 days) cocaine administration followed by 10–14 days of withdrawal caused a decrease in the AMPAR/NMDAR ratio recorded from medium spiny neurons in the NAc shell. This decrease probably involves a downregulation of AMPARs and shares mechanisms with NMDAR-dependent LTD as this form of plasticity was reduced by the cocaine experience¹⁰⁷. Analogous results were found in subsequent studies examining the synaptic effects of prolonged cocaine self-administration. Evoked excitatory synaptic responses recorded extracellularly in the shell were reduced¹⁰⁸, and in both the core and shell LTD was absent⁹⁷. Importantly, animals that self-administered food or that received cocaine passively in a yoked design still expressed LTD. Furthermore, in the core (but not the shell), LTD could not be elicited in cocaine self-administering animals even after 21 days of abstinence⁹⁷.

These results suggest that *in vivo* cocaine administration promotes depression of excitatory synaptic transmission in the NAc (FIG. 6), although the exact circuits in which this occurs (for example, core versus shell) and how long this synaptic plasticity lasts might depend on additional variables, such as whether the drug was self-administered or not. The approach used in these studies, however, cannot address the behavioural relevance of such synaptic modifications. To directly examine whether LTD in the NAc has a role in amphetamine-induced behavioural sensitization, a membrane permeable peptide that was shown to block clathrin-mediated endocytosis, and thus LTD, was administered into the NAc of sensitized rats immediately before a challenge dose of amphetamine¹⁰⁶. Remarkably, this prevented the increase in locomotor activity normally elicited by the drug and, therefore, the expression of behavioural sensitization, while having no effect on the acute response to amphetamine in non-sensitized animals. Injection of this peptide into the VTA, however, had no behavioural effect.

Although these behavioural results provide support for the functional importance of the drug-induced decreases in excitatory synaptic strength in the NAc reported in earlier studies, an important caveat is that the peptide blocked the acute expression of behavioural sensitization (its effects on the development of sensitization were not tested)¹⁰⁶. This observation suggests that acute amphetamine administration elicits LTD in the NAc rather than LTD induced by the previous drug experience. Indeed, the original observation of a decrease in the AMPAR/NMDAR ratio in the NAc of cocaine-sensitized animals was made following a challenge dose of cocaine¹⁰⁷. To determine whether this single challenge dose of cocaine influenced excitatory synaptic strength in sensitized animals, AMPAR/NMDAR ratios were measured in NAc shell neurons from animals who received a repeated cocaine treatment that was sufficient to cause sensitization but did not receive a single dose of cocaine 10–14 days later¹⁰⁹. Surprisingly, the AMPAR/NMDAR ratio was significantly increased, probably due to an upregulation of AMPARs and, consistent with the previous study,

this increase was reversed by an *in vivo* challenge dose of cocaine. These findings are consistent not only with the behavioural effects of the LTD-blocking peptides but also with reports that chronic *in vivo* administration of psychostimulants increase the density of dendritic spines on NAc medium spiny neurons¹¹⁰, the surface expression of AMPARs in the NAc¹¹¹ and the behavioural responses to infusion of AMPA into the NAc^{112,113}.

All of the work discussed thus far presumably deals with a form of NMDAR-dependent LTD in the NAc that is mainly due to the loss of synaptic AMPARs³. As mentioned above, eCB-LTD also exists in the NAc and can be modified by *in vivo* administration of drugs of abuse. Specifically, a single dose of cocaine or the partial CB1 receptor agonist Δ^9 -tetrahydrocannabinol (Δ^9 -THC), or chronic administration of Δ^9 -THC, abolishes eCB-LTD^{105,114,115}. The effects of cocaine on this form of plasticity might be due to a loss of the surface expression of postsynaptic group I mGluRs (that is, mGluR5), which are required for endocannabinoid production¹¹⁶. Chronic Δ^9 -THC, however, appears to cause a functional downregulation of presynaptic CB1 receptors^{105,116} and a homeostatic upregulation of a different form of presynaptic LTD that is triggered by activation of presynaptic group II mGluRs¹¹⁶.

Compared with the effects of drugs of abuse on LTD, much less is known about their influence on LTP in the NAc. In one study, chronic (5 day) cocaine administration led to an enhancement of LTP in slices of the NAc measured 2–3 days after the last injection¹⁰³. This was associated with a decrease in the NAc levels of the synaptic scaffolding protein PSD-95 and, consistent with this observation, LTP in the NAc was also enhanced in mutant mice lacking fully functional PSD-95 (REF.103). However, LTP in the NAc *in vivo* elicited by tetanization of hippocampal afferents was reported to be blocked, not enhanced, 18–25 days after chronic (6 day) cocaine administration¹¹⁷. The interpretation of the results from these studies is limited by the fact that extracellular recording techniques were used, making it difficult to assess what proportion of any observed changes in the responses was due to synaptic modifications rather than other parameters such as cell excitability.

Other approaches examining NAc synaptic plasticity.

Although examining drug-induced changes in synaptic function, by definition, requires measuring synaptic responses using electrophysiological recording techniques, additional assays have provided useful information about the potential role of synaptic adaptations in the NAc in addition. Extracellular glutamate levels in the NAc decrease during cocaine withdrawal, partly because of a downregulation of the glial cystine-glutamate transporter^{99,118}. Specific isoforms of the synaptic protein Homer, which helps cluster mGluRs and associated signalling proteins, are also decreased by cocaine administration¹¹⁹ as is PSD-95¹⁰³. All of these changes have been shown to affect drug-induced behavioural adaptations but exactly how they influence excitatory synaptic function and plasticity within the NAc needs further investigation. A different approach

Yoked design

Experimental protocol in which a 'yoked' control animal receives a drug administered by the investigator in a non-contingent manner, in the same amount and temporal pattern as an animal that is self-administering the drug.

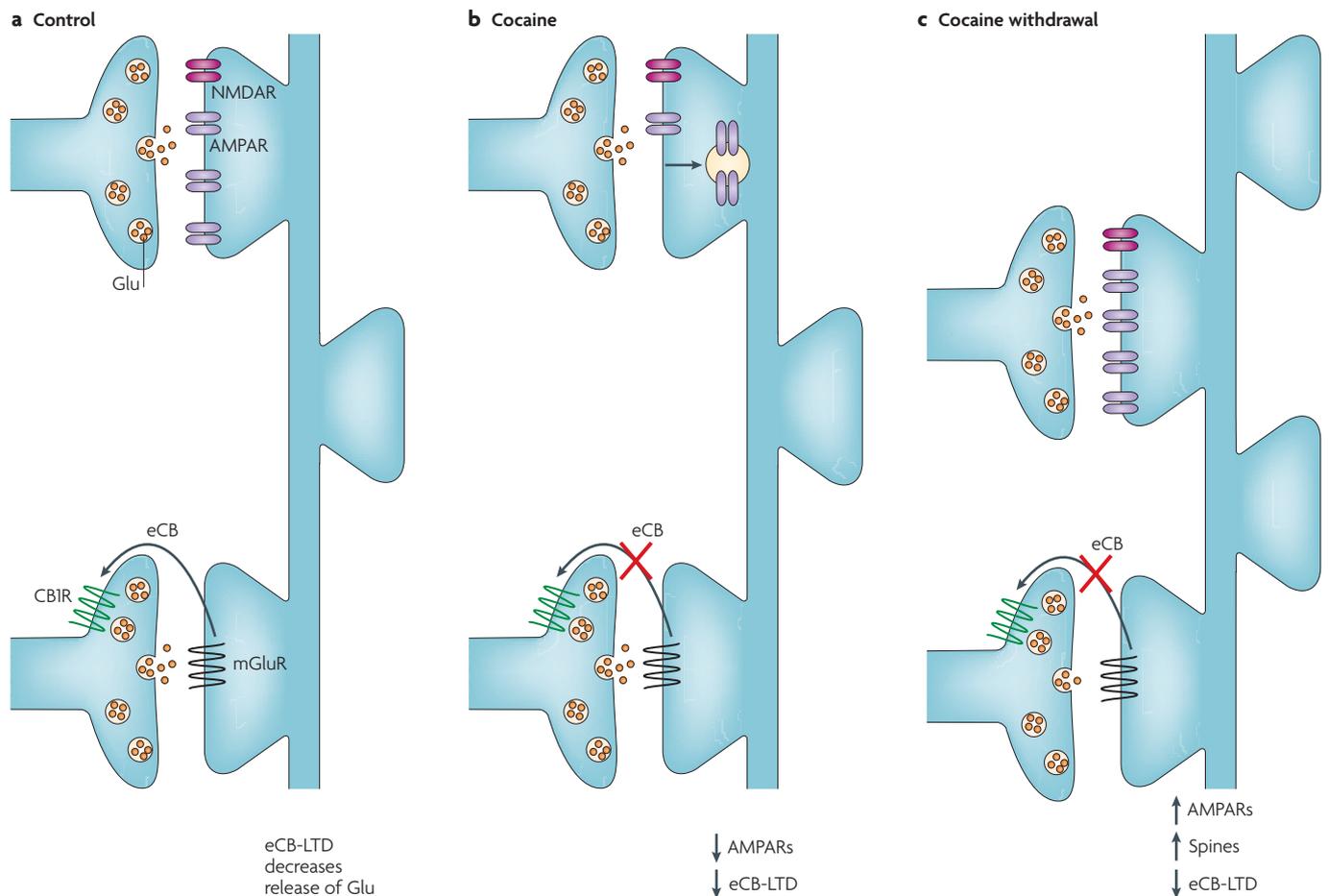


Figure 6 | Drugs of abuse modulate synaptic function and plasticity in the nucleus accumbens (NAc). Highly simplified summary of the effects of cocaine (and perhaps other drugs of abuse) on synaptic function and plasticity in the NAc. **a** | In control conditions, a normal complement of synaptic α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid receptors (AMPA) and N-methyl-D-aspartate receptors (NMDARs) exists, and endocannabinoid-mediated long-term depression (eCB-LTD) can be induced. **b** | Cocaine administration causes a loss of synaptic AMPARs due to their internalization^{106,107} and a concomitant block of eCB-LTD¹¹⁴. **c** | During withdrawal from chronic cocaine administration there is an increase in surface AMPARs and in dendritic spine density^{109–111}, perhaps due to homeostatic synaptic scaling⁴⁷ in response to the decrease in synaptic strength and excitability elicited by cocaine (seen in **b**). The synaptic adaptations during withdrawal may differ between the NAc shell and core (not shown) as LTD is blocked in the core, but not the shell, 21 days after withdrawal from cocaine self-administration⁹⁷. The effects of withdrawal from cocaine administration on LTP are also complex (not shown)^{103,117}. CB1R, cannabinoid receptor 1; Glu, glutamate; mGluR, metabotropic glutamate receptor.

has been to examine the behavioural consequences of overexpression of wild type or dominant negative AMPAR subunits in the NAc. Overexpression of GluR1 in the NAc, which would be expected to increase excitatory synaptic strength, facilitated the extinction of cocaine-seeking responses¹²⁰, attenuated the rewarding effects of cocaine in conditioned place preference¹²¹ and increased brain reward stimulation thresholds¹²². Conversely, overexpression of a pore-dead GluR1, which should decrease synaptic strength, exacerbated cocaine-induced locomotor sensitization and reinstatement of drug seeking (R.K. Bachtell and D.W. Self, personal communication). These results are consistent with the idea that rapid drug-induced depression of excitatory synaptic function in the NAc contributes to the long-lasting sensitized behavioural responses caused by previous drug exposure.

Other cellular adaptations. Although the focus of this review is on synaptic plasticity, it is important to mention that drugs of abuse may cause cellular adaptations that do not directly affect synaptic transmission, yet may profoundly influence neural circuit function. Among the most important of these non-synaptic adaptations are changes in the intrinsic excitability of NAc neurons. Chronic administration of psychostimulants causes a decrease in neuronal excitability in the NAc, due to the modulation of voltage-dependent conductances^{123–125}, and this adaptation alone is sufficient to cause a sensitized behavioural response to cocaine¹²⁵. Another important issue that is just beginning to be explored¹²⁵ is how drug-induced gene transcription and epigenetic regulation¹²⁶ influence synaptic and circuit function in the mesolimbic dopamine system and, thereby, exert their behavioural effects.

BNST and amygdala

Although the VTA–NAc axis has been the most extensively studied circuit in relation to motivation and reward in drug addiction, it is clear that other brain regions are essential components as well. Here we briefly review evidence that synaptic plasticity in two additional regions, the bed nucleus of the stria terminalis (BNST) and amygdala, might also be modified by drugs of abuse. The BNST is considered a component of the extended amygdala and has a role in stress- and reward-related limbic circuitry. It contributes to stress-induced relapse to drug-seeking behaviour and its neurons project to areas involved in feeding and reward processing, including the NAc and VTA^{127,128}. NMDAR-dependent LTP can be triggered in the BNST and acute ethanol impairs this LTP in part by attenuating NMDAR-mediated synaptic currents¹²⁹. In contrast, potentiation of the AMPAR/NMDAR ratio at excitatory synapses in the ventral lateral BNST occurred in rats that self-administered either palatable food pellets or intravenous cocaine daily for eleven days¹³⁰. Intriguingly, neither experimenter-administered food nor drug produced an enhancement of the ratio, suggesting that LTP at this set of excitatory synapses in the BNST occurs only when an operant task is performed to obtain a reward. LTD in the BNST has also been examined and requires activation of group I mGluRs, which elicit LTD via an eCB-independent, extracellular-signal-regulated kinase 1 (ERK1)-dependent mechanism¹³¹. This form of LTD is prevented by chronic (10 day) but not single day administration of cocaine. Further work will be necessary to determine whether this effect of cocaine is due to an occlusion or block of LTD.

The amygdala is known to be involved in forms of memory that involve a strong emotional component and LTP in the lateral nucleus of the amygdala has long been suggested to have an important role in fear conditioning¹³². In human subjects, cocaine-associated cues that induce craving in substance abusers robustly alter neuronal activity in the amygdala¹³³. Recently, LTP at excitatory synapses in the central amygdala was found to increase dramatically two weeks after withdrawal from chronic (14 day) cocaine administration¹³⁴. However, this effect was not seen 24 hours after the last cocaine injection, indicating that it increases with time of withdrawal. Both NMDARs and corticotropin-releasing factor (CRF) are essential for the LTP to be triggered *in vitro*. This latter observation is of particular interest because CRF release in the amygdala is increased during acute withdrawal¹³⁵. Even a brief application of CRF was reported to potentiate excitatory synaptic currents and this effect was also enhanced after 2 weeks withdrawal from cocaine¹³⁶. Enhanced synaptic plasticity in the central amygdala may be one cellular adaptation contributing to the CRF-dependent signalling that appears to cause anxiety and stress responses during withdrawal from cocaine. If the ability to elicit LTP in the amygdala does in fact correlate with the aversiveness of withdrawal symptoms, it would be of interest to see whether LTP returns to control levels after an additional drug exposure that reduces withdrawal symptoms.

Conclusions

We have attempted to review the growing literature on the synaptic modifications elicited by drugs of abuse in the mesolimbic DA system and how these may contribute to the development of addiction. The changes that occur are complex and much work remains to establish which of the myriad reported changes in synaptic function are the most behaviourally relevant. Nonetheless, it can be argued that more than any other commonly studied form of experience-dependent plasticity, we are beginning to understand the potential causal relationships between the neural circuit adaptations elicited by drugs of abuse and the behavioural consequences of that experience. To determine whether these synaptic adaptations are important in the development or maintenance of human addictions, however, will require much further effort. By necessity the study of synaptic plasticity depends on the use of electrophysiological recording techniques, which permit real-time measurements of the functional state of synapses in defined populations of neurons. Because recordings from individual cells are difficult to make in older animals, many of the studies to date have been carried out in young animals. Furthermore, most studies have not used protocols involving drug self-administration (in part because of the necessity of using older animals), which is the most faithful representation of human drug abuse. Thus, in future work, it will be important to balance the pragmatic advantages of using simple *in vivo* procedures involving direct administration of drugs by the experimenter with more difficult, but clinically relevant, protocols that more accurately mirror human drug-seeking and drug-taking behaviour. Whole cell recording from individual cells continues to provide the most sensitive and complete picture of synaptic function. However, combining this technique with fluorescence imaging techniques or with simpler electrophysiological approaches, such as field potential recordings that can more easily be made *in vivo*, may improve our ability to assay synaptic function across larger cell populations.

The cell populations in critical brain regions such as the VTA and NAc are likely to be heterogeneous, differing in their molecular and physiological properties as well as their anatomical connectivity. Therefore, it will be essential to determine whether drugs of abuse influence the different cell types in the same manner. Compounding this issue is the difficulty of knowing which specific sets of synapses (that is, inputs) on a given cell are being assayed and modified by the drug experience. The powerful combination of single cell electrophysiology with animals that are genetically modified, by engineering from birth or by using viral vectors in the postnatal brain, offers an excellent approach to define the precise neuronal populations and physiological parameters modified by exposure to drugs of abuse. A recent exciting advance is the use of genetically modified mice in which fluorescent markers (for example, green fluorescent protein (GFP)) are expressed in specific, molecularly-identified cell populations¹³⁷. Such mice have already facilitated our understanding of the cell type-specific synaptic modifications that occur in

the dorsal striatum following dopamine depletion^{138,139} as well as cocaine-induced changes in spine density in the NAc¹⁴⁰. Additional molecular approaches, such as the restricted expression of light-activated ion channels and pumps that depolarize or hyperpolarize neurons¹⁴¹, should allow activation or inhibition of specific afferent inputs and, thereby, permit a more precise assay of synaptic adaptations in specific mesolimbic DA circuits. This approach could potentially also be exploited in experiments *in vivo* by implanting a fibre optic light source within a relevant brain structure and monitoring

behaviour in unanaesthetized animals during neuronal activation or inactivation¹⁴². With further advances in our understanding of the molecular mechanisms of synaptic plasticity in the key circuits mediating addiction combined with more sophisticated applications of animal and human brain imaging, we anticipate that our knowledge of the neural circuit adaptations that underlie addiction will grow exponentially. These advances will facilitate more informed and effective approaches to the treatment of addiction and related disorders, which remain one of society's most challenging health problems.

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DATABASES

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 BDNF | c-FOS | ERK1 | Orexin A | Orexin receptor 1 | PSD-95

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