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Drug-Evoked Synaptic Plasticity of Excitatory Transmission in the Ventral Tegmental Area

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Cocaine leads to a strong euphoria, which is at the origin of its recreational use. Past the acute effects, the drug leaves traces in the brain that persist long after it has been cleared from the body. These traces eventually shape behavior such that drug use may become compulsive, and addiction develops. Here, we discuss cocaine-evoked synaptic plasticity of glutamatergic transmission onto dopamine (DA) neurons of the ventral tegmental area (VTA) as one of the earliest traces after a first injection of cocaine. We review the literature that has examined the induction requirements, as well as the expression mechanism of this form of plasticity, and ask the question about its functional significance.

Addiction is a disease that starts with recreational drug consumption but evolves to compulsive use in some people (Hyman 2005).[3] Relapse can occur even after prolonged periods of abstinence, long after the substance has been eliminated from the body. This clinical reality points to the existence of persistent traces left by the addictive drug in the brain. Over the last decade, drug-evoked synaptic plasticity has emerged as one of the cellular correlates of these drug traces (Lüscher and Malenka 2011; Lüscher 2016). Much research has now characterized drug-evoked plasticity at various synapses throughout the brain, suggesting a temporal sequence of the synapses involved. The earliest changes are observed on excitatory afferents onto dopamine (DA) neurons in the ventral tegmental area (VTA) within hours of a first drug exposure (Ungless et al. 2001). A single injection of cocaine to a mouse or a rat will affect excitatory transmission for days and, although one dose of cocaine is not sufficient to get the rodent "addicted," these initial traces are likely to represent building blocks toward more permanent alterations in response to repetitive exposure. In support of this idea, all addictive drugs tested so far, but not other psychoactive substances such as fluoxetine or carbamazepine, trigger common synaptic adaptations in the VTA. A single dose of morphine, benzodiazepines, ethanol, amphetamines, or nicotine leads to the same synaptic changes as a single dose of cocaine (Saal et al. 2003; Brown et al. 2010; Mao et al. 2011). Therefore, studying the molecular mechanisms of these early drug-evoked traces in the VTA may lead to a better

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understanding of how addictive behavior is established.

INDUCTION MECHANISM: MESOLIMBIC DA

A systemic injection of a single dose of cocaine followed 24 hours later by the preparation of midbrain slices increases the ratio between the amplitude of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR)- and *N*-methyl-D-aspartate receptor (NMDAR)-mediated excitatory postsynaptic currents (EPSCs) onto DA neurons in the VTA (Ungless et al. 2001). The ex vivo AMPA/*N*-methyl-D-aspartate (NMDA) ratio was used (rather than measuring absolute amplitudes), because in the acute slice preparation the number of synapses recruited by the extracellular stimulation electrode cannot be controlled. Regardless of the mechanistic interpretation (see below), the increase of the AMPA/NMDA ratio at glutamatergic synapses can be considered a bona fide trace of the drug exposure. After a single injection to a mouse or a rat, the increase of the AMPA/NMDA ratio persists for 5 days but is reversed after 10 days (Ungless et al. 2001; Borgland et al. 2004; Kauer and Malenka 2007). In contrast, when an animal is trained to self-inject cocaine (which obviously results in many repetitive doses), the AMPA/NMDA ratio remains increased for more than a month (Chen et al. 2008). Although not strictly comparable (it takes rats several days to learn to self-administer), these experiments suggest that the context of the injection may influence its persistence. Because all addictive drugs cause an increase in the AMPA/NMDA ratio of DA neurons in the VTA, is there a common effect of these substances that drives the induction of this plasticity? It is well established that all addictive drugs strongly increase mesolimbic DA concentrations (Fig. 1) (Di Chiara et al. 2004; Nestler 2005; Lüscher and Ungless 2006). Psychostimulants, for example, interfere with the reuptake of DA in target regions as well as in the VTA itself, where the DA neurons are capable of dendritic release (Sulzer 2011). Nicotine can directly depolarize DA neurons (Maskos et al. 2005), whereas opioids, benzodiazepines (Tan et al. 2010), cannabinoids (Melis et al. 2004), and γ-hydroxybutyrate (GHB) (Cruz et al. 2004) preferentially inhibit γ-aminobutyric acid (GABA) interneurons, thus causing a disinhibition. In the case of opioids, DA neurons in the medioventral VTA are preferentially activated (Corre et al. 2018), in line with the demonstration of circuit-specific disinhibitory motives (Lammel et al. 2012). Indeed, laterodorsal tegmentum neurons preferentially synapse on DA neurons projecting to the nucleus accumbens (NAc) lateral shell to convey positive valence. It remains to be shown that this is the population activated by opioids and undergoes plasticity of afferent glutamate synapses, but the demonstration of occlusion between optogenetic VTA GABA neuron self-inhibition and injected intraperitonealy heroin is in line with this scenario.

Through three distinct cellular mechanisms (interference with reuptake, direct excitation, and disinhibition of DA neurons), addictive drugs therefore converge to cause an increase of mesolimbic DA (Lüscher and Ungless 2006). Direct evidence that increased mesolimbic DA is sufficient to cause a synaptic trace is provided by the experiments in which DA neurons are driven with optogenetic effectors. Such in vivo stimulation mimics drug-evoked synaptic plasticity (Brown et al. 2010; Bariselli et al. 2018). The induction process is confined to the VTA because it can be reproduced in the slice preparation (Schilström et al. 2006) and because a local infusion of the D1 (DA receptor) antagonist SCH23390 is sufficient to block the plasticity in response to optogenetic stimulation (Brown et al. 2010). This is in line with earlier reports that a systemic D1 antagonist blocks cocaine-evoked plasticity (Ungless et al. 2001). An additional induction requirement is the activation of NMDARs on the DA neurons. In inducible conditional GluN1-knockout (KO) mice in which NMDARs are selectively abolished in DA neurons, cocaine fails to elicit the synaptic trace (Engblom et al. 2008). The locus of the D1/5R required for the induction is not identified and may be a D1R expressed on glutamatergic afferents or alternatively D5Rs found on the DA neurons themselves (Khan et al. 2000). This

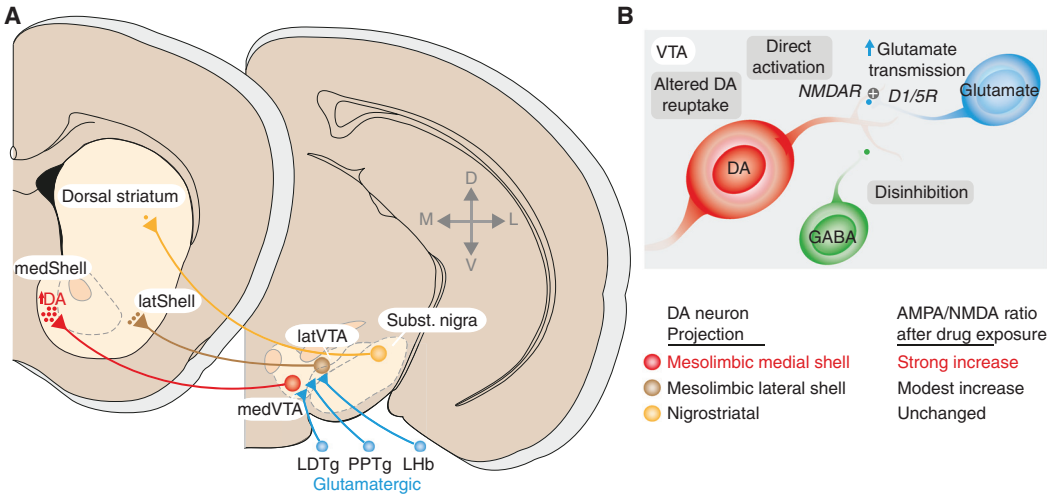

Figure 1. Circuit diagram and proposed induction mechanisms of drug-evoked synaptic plasticity. (*A*) Schematic showing the midbrain dopamine (DA) system with projection neurons originating in the lateral and ventral tegmental area (VTA) or in the substantia nigra. A common effect of addictive drugs is an increase in DA concentration in the nucleus accumbens (NAc), particularly in the shell. Major afferents include glutamatergic projection from the brainstem (laterodorsal tegmental [LDTg]; peduncolupontine tegmentum [PPTg]) and the lateral habenula (LHb). (*B*) Addictive drugs use distinct cellular mechanisms to cause a surge in DA. Although psychostimulants such as cocaine, ecstasy, and amphetamine interfere with DA reuptake, nicotine can directly depolarize DA neurons. Drugs including opioids, cannabinoids, γ-hydroxybutyrate (GHB), and benzodiazepines have a disinhibitory effect, which is a consequence of the hyperpolarization of VTA γ-aminobutyric acid (GABA) neurons. Drug-evoked synaptic plasticity is induced by the activation of presynaptic D1-like receptors, in conjunction with *N*-methyl-D-aspartate receptor (NMDAR).

D1 or D5 receptor activation would prime NMDAR function (Schilström et al. 2006; Argilli et al. 2008). Another interesting observation is that orexin or corticotropin-releasing factor (CRF) antagonists abolish the appearance of cocaine-evoked synaptic plasticity in the VTA (Bonci and Borgland 2009). Orexin neurons are located in the hypothalamus and project among several targets to the VTA, where they make synapses onto DA neurons, particularly in the caudomedial region (Vittoz et al. 2008). According to these studies, the orexin-1 receptor expressed on the surface of the DA neurons drives (via protein kinase C [PKC]) an increase in NMDAR function, a prerequisite for the long-lasting cocaine-evoked plasticity described above. In addition, orexin signaling has been shown critical for morphine-induced synaptic plasticity in the VTA (Baimel and Borgland 2015) and a study monitoring c-fos as a marker of neuronal activity suggests that there is indeed an increased activity of orexin afferents during drug-related behavior (Aston-Jones et al. 2010). Taken together, these pieces of data suggest that orexin plays a role as a gate keeper of the cocaine-evoked effects on glutamatergic transmission. However, more studies with genetic manipulations and selective control of the activity of the orexin projections will be needed to achieve a comprehensive understanding. CRF also potentiates NMDARs (via CRF2 receptors and similar to orexin byway of PKC) that again prime the synapse for the effects of cocaine (Ungless et al. 2003). Interestingly, it has been shown that CRF act in concert with α1 adrenergic receptor to enhance plasticity of NMDAR-mediated plasticity in the VTA to promote cocaine conditioning (Tovar-Díaz et al. 2018). However, the presence of a large Ih current, which was used to identify DA neurons in this study (Ungless et al. 2003), has subsequently been shown not to apply to all DA neurons

(see also below) (Lammel et al. 2008; Brown et al. 2009). In addition, although it has been shown that CRF2R are expressed presynaptically and postsynaptically in the VTA (Slater et al. 2016), it will have to be established from where the CRF afferents arrive and under which circumstances they are activated. Although orexin's and CRF's effect on the VTA share many commonalities, it remains to be shown whether the changes of the NMDAR function are mechanistically identical. The signaling pathway of cocaine-evoked synaptic plasticity relies on PKC activation, but is only incompletely known. A study also implicated protein kinase Mζ (PKMζ) (Ho et al. 2012), based on pharmacological interventions, which abolished the appearance of cocaine-evoked plasticity and subsequent activity-dependent potentiation.

EXPRESSION MECHANISM

The concept that drugs of abuse simply strengthen excitatory afferences onto VTA neurons has been challenged by the investigation of the contribution of both AMPARs and NMDARs on the increase AMPA/NMDA ratio. For AMPARs, the current-voltage curve of the EPSCs is indeed also affected. Linear at baseline or after saline control injections, a change in slope is observed after a single injection of cocaine (Bellone and Lüscher 2006; Argilli et al. 2008) and other additive drugs (Heikkinen et al. 2009; Wiltgen et al. 2010), indicating that AMPAR-EPSCs in drug-treated animals are inwardly rectifying. Inward rectification is a property conferred by endogenous polyamines that inhibit AMPARs at positive potentials (Nishida and MacKinnon 2002). Rectification can be quantified by calculating the ratio of the chord conductance measured at negative over positive potentials. After drug treatment, this rectification index typically has a value of two compared with a rectification index of one in cells from naive or saline-injected mice. Such partial rectification suggests that the drug treatment causes the insertion of AMPARs that lack the subunit GluA2 into synapses of DA neurons (Bellone and Lüscher 2006; Mameli et al. 2007). Most AMPARs in the central nervous system (CNS) contain at least one GluA2 subunit, which through posttranslational editing has an arginine residue in the pore region. This arginine modification regulates the single-channel conductance for monovalent ions such as Na^+ and K^+, makes the channel impermeable for Ca^{++}, and blocks the polyamine-binding site (Seeburg and Hartner 2003). Because this editing mechanism is also crucial for masking an endoplasmic reticulum (ER) retention signal, only edited GluA2-containing receptors reach the cell surface. This is in contrast with GluA1 homomeric or GluA1/3 heteromeric channels, which are made of subunits that are not subject to editing. The crucial residues in the pore regions of these subunits are glutamine. As a consequence, GluA1 homomeric or GluA1/3 heteromeric channels have a larger single-channel conductance for sodium, are calcium permeable, and are inhibited by polyamines at positive potentials. Data from expression systems (for review, see Liu and Zukin 2007) and the analysis of the constitutive KO mice in DA neurons (Engblom et al. 2008) indicate that pure GluA2-lacking receptors are fully rectifying (i.e., no current flows at positive potentials). In native receptors, this may not always be the case as rectification is also determined by auxiliary subunits, such as the Tarps (Kelly et al. 2009). Regardless of the exact molecular mechanism, the appearance of rectifying AMPAR-EPSCs after drug exposure suggests that some synapses contain a fraction of AMPARs, which lack the GluA2 subunit. In line with this interpretation, drug treatment significantly increases the mean single-channel conductance of AMPARs (Mameli et al. 2007). These changes occur without affecting receptor number, however, as revealed by statistical analysis of the trial-to-trial fluctuation of EPSC amplitude (nonstationary fluctuation analysis). This model is further confirmed by electron microscopy, in which immunogold labeling shows a significant redistribution of the GluA2 subunit. After cocaine treatment, its cytoplasmic pool strongly grows at the expense of the pool of receptors at the surface (Mameli et al. 2007; Brown et al. 2010). Interestingly, it has been shown that a single cocaine exposure decreases GluA2-containing AMPARs at several excita-

tory inputs, although exposure to tetrahydrocannabinol (THC) selectively alters glutamate inputs from pedunculopontine tegmentum ([PPTg], sometimes also referred to as pedunculopontine [PPN] tegmentum) (Good and Lupica 2010), suggesting that drugs of abuse may recruit different glutamatergic circuits. Interestingly, although afferents to the substantia nigra pars compacta (SNc) do not undergo drug-evoked synaptic plasticity, the PPTg inputs may constitute an exception (Beaudoin et al. 2018).

Remarkably, it has been shown that although cocaine exposure potentiates AMPAR-EPSCs, it also reduces the amplitude of NMDAR-EPSCs and drives the insertion of GluN3A/GluN2B subunits. Changes in NMDAR subunit composition not only affects calcium permeability but are necessary for the expression of cocaine-evoked AMPAR changes (Yuan et al. 2013) and enhances the activity of DA neurons in the VTA (Creed et al. 2016). This finding explains why the AMPA/NMDA ratio actually increases (particularly when both components are typically measured at +40 mV). Initially, studies suggested that the NMDA component remained unchanged (Ungless et al. 2001). This was based on the observation that bath application of NMDA elicited currents of similar magnitude in slices of mice regardless of whether they were exposed to cocaine or saline. However, this approach has the disadvantage of activating synaptic as well as extrasynaptic receptors. A direct quantification of the amplitude of NMDA-EPSCs using standard extracellular stimulation techniques also remains problematic because across different slices the number of synapses stimulated (a parameter that crucially determines the amplitude) cannot be controlled. A technique that circumvents these problems is to elicit unitary responses at single synapses by generating a small artificial source of glutamate. This can be achieved using a two-photon laser to photolyze caged glutamate in proximity of a synapse. Although this technique is well established for several brain regions (Bloodgood et al. 2009) and typically relies on the presence of a dendritic spine to identify the locus of the synapse, its validation is more difficult in the aspiny synapses of DA neurons of the VTA. Nevertheless, an iterative placement of the laser focus while optimizing kinetics and light power can reveal "hot spots" that fulfill the criteria of unitary EPSCs and allows one to compare absolute EPSC amplitudes in slices from saline versus cocaine-treated animals (Mameli et al. 2011). This approach not only confirmed the existence of rectifying AMPAR-EPSCs but also showed that NMDAR-EPSCs are significantly smaller after the drug treatment. These smaller NMDA currents occur from a redistribution of NMDAR subunit composition (Schilström et al. 2006; Bellone and Lüscher 2012) and the insertion of GluN2B/GluN3A-containing NMDARs (Yuan et al. 2013). GluN3A-containing NMDARs have low calcium conductance and low Mg sensitivity and their expression may provide an explanation for the inability to induce long-term potentiation (LTP).

In summary, increase of the AMPA/NMDA ratio after drug exposure is owing to both a decrease of the NMDAR component and an increase in AMPA-EPSCs because of insertion of Ca^{2+} permeable AMPARs and Ca^{2+}-impermeable NMDARs. Interestingly, both GluA2-lacking AMPARs and GluN3A-containing NMDARs are expressed during early postnatal development at several synapses (Henson et al. 2010; Bellone et al. 2011), suggesting that drugs of abuse may reopen a critical period of postnatal synaptic development (Bellone et al. 2011).

CONSEQUENCES FOR SYNAPTIC FUNCTION

Although several elements of the induction and expression mechanisms are well described, we know very little about the consequences of drug-evoked synaptic plasticity in the VTA. How does the redistribution of glutamate receptors affect synaptic transmission and activity-dependent plasticity? Is the drug-evoked plasticity encoding specific elements of the drug-associated experience? Or is it rather a form of metaplasticity permissive for additional adaptive changes? A first element that still requires clarification is the identification of the actual anatomical projection that is affected.

One thing is clear: the early form of cocaine-evoked synaptic plasticity in DA neurons is limited to the cells in the VTA because neighboring DA neurons of the substantia nigra or GABA neurons in the VTA remain unaffected by a single injection (Ungless et al. 2001). The selectivity for VTA DA neurons along with the requirement for the activation of NMDARs expressed on these cells suggest that activity of afferent glutamatergic projections during the drug exposure is crucial. Although VTA DA neurons receive converging inputs from many upstream nuclei (e.g., prefrontal cortex [PFC], brain stem, amygdala), it is likely that not all undergo drug-evoked synaptic plasticity. In fact, when the plasticity is probed with single synapse resolution there are synapses that remain unaffected (Mameli et al. 2011). However, thanks to the advent of systematic projection targeting with optogenetic effectors, the identification of the relevant afferents should only be a matter of time.

The idea that a portion of DA neurons are part of a specific reward circuit that can undergo drug-evoked changes is shown by the observation that DA cells projecting to the NAc, but not those that project to the PFC, express drug-evoked plasticity. Indeed, recent work selecting the neurons based on the presence of a fluorescent marker that was previously injected in various projection areas (so-called retrobeads) has found that mesocortical DA neurons show an increase in the AMPAR/NMDAR ratio following an injection of formalin to the hind paw (Lammel et al. 2011). Whether this observation can be generalized to any salient but aversive stimulus will require additional work. In particular, it will have to be investigated whether these experiments using a painful stimulus engage a similar mechanism as stress, which also leads to an increase in AMPAR/NMDAR ratio (e.g., forced swim test) (Saal et al. 2003). Regardless of these questions, it is clear that DA neurons do not constitute a homogenous population. An electrical foot shock inhibits most DA neurons (and excites a few), presumably indirectly after activation of upstream GABA neurons (Tan et al. 2012; Van Zessen et al. 2012). However, when monitoring firing properties of DA neurons in vivo, it was observed that a small subpopulation, typically located in the lateral parts of the VTA, is excited by aversive stimuli (Brischoux et al. 2009; Bromberg-Martin et al. 2010). If such excitation is also driven in part by excitatory inputs that activate NMDARs, then this may help to understand the differential induction criteria observed in classes of DA neurons defined by their projection target (Lammel et al. 2011). In the last years, an emergent hypothesis suggests that different DA neurons within the VTA may encode different signals and engage in different circuits (Morales and Margolis 2017). Interestingly, it has been recently shown that DA terminals to the ventral medial NAc shell are excited, although the terminals to other NAc subregions are inhibited by aversive stimuli (de Jong et al. 2019), strongly suggesting that drug-evoked plasticity induced at excitatory synapses onto specific DA neurons, may convey opposite signals to different regions of the NAc.

Another line of research, which may help to understand the functional consequences of drug-evoked plasticity in the VTA is the observation that the changes induced by cocaine in adult mice bring about many features of an immature synapse (Bellone et al. 2011). A systematic characterization of excitatory afferents onto DA neurons of the VTA for the first month revealed that AMPA-EPSCs are rectifying during the first week of life and linear by the third week. In parallel, the amplitudes of NMDA-EPSCs grow larger during the same period (as can be inferred by the change in AMPA/NMDA ratio). The NMDAR-EPSC changes are in part owing to a switch in subunit composition; although GluN2B is the predominate partner of the obligatory subunit GluN1 in the early neonatal period, GluN2A makes up the majority of receptors in adolescent and adult mice. Interestingly, neonatal NMDA receptors flux very little calcium. This is explained by the insertion of GluN3-containing NMDARs as described above.

Interestingly, in synapses of neonatal rodents, the major source for synaptic calcium is also provided by GluA2-lacking AMPARs, whereas this switches to an NMDAR-dependent calcium source in adult mice. Given this developmental profile, the idea has been put forward

that cocaine exposure in adult mice can reopen a critical period similar to that during development and thus change the rules for the induction of subsequent activity-dependent synaptic plasticity (Mameli et al. 2011). In naive animals, coordinated activity involving glutamate release onto depolarized NMDARs leads to efficient calcium influx and synaptic potentiation. After cocaine treatment, this protocol is no longer efficient, presumably because of the reduced calcium permeability of NMDARs.

However, glutamate release onto a hyperpolarized cell now becomes an efficient induction protocol because of the occurrence of calcium-permeable AMPARs. The consequences on the network function have not been worked out in detail. In any case, these observations strongly suggest that this early form of drug-evoked synaptic plasticity is actually a metaplasticity permissive for further adaptive changes with time and repetitive drug exposure. In line with this idea, there is a hierarchical organization between plasticity in the VTA and the NAc. If GluA2-lacking AMPARs are quickly removed in the VTA DA neurons after each injection (Mameli et al. 2009; see also below), no changes of excitatory transmission onto medium spiny neurons of the NAc are observed even after several injections of cocaine. Conversely, synaptic adaptations in the NAc are observed more than a month after a single injection of cocaine, provided that the reversal of the adaptive changes in the VTA in inhibited.

REVERSING DRUG-EVOKED SYNAPTIC PLASTICITY

After a single passive injection of cocaine, calcium-permeable AMPARs and reduced NMDAR EPSCs can be observed for ~5 days (Ungless et al. 2001; Borgland et al. 2004; Bellone and Lüscher 2006). The persistence is prolonged if the injection is self-controlled. For example, after 2 weeks of self-administration of cocaine, the synaptic changes persist for several months (Chen et al. 2008). In all cases, synaptic transmission eventually returns to baseline, which requires the activation of mGluR1 receptors (Bellone and Lüscher 2006). The involvement of mGluR1 is shown by the observation that pharmacological enhancement of mGluR1 causes a reversal within minutes, whereas conversely the disruption of the mGluR1 interaction with Homer1C makes the synaptic plasticity persistent (Mameli et al. 2009). Moreover, constitutive mGluR1 KO mice have rectifying AMPAR-EPSCs throughout their lives (Bellone et al. 2011). Besides the VTA, studies from both the amygdala (Clem and Huganir 2010) and the NAc (McCutcheon et al. 2011) provide evidence that mGluR1-LTD serves as a general mechanism (Lüscher and Huber 2010; Loweth et al. 2013) by which GluA2-lacking AMPARs are removed from the synapse. The mGluR1-induced reversal of cocaine-evoked synaptic plasticity (sometimes also termed mGluR1-LTD) depends on protein synthesis. In fact, mGluR1 through mTOR signaling triggers the translation of the GluA2 subunit, most likely from preexisting messenger RNA (mRNA) (Fig. 2; Mameli et al. 2007). This conclusion was reached by RNA interference, simply by including the small interfering RNA (siRNA) against GluA2 into the patch pipette. This shuts down the synthesis machinery within minutes and thus prevents de novo synthesis, an approach that has also been successfully used to show the involvement of Arc in other forms of protein-synthesis-dependent synaptic plasticity (Mameli et al. 2007; Lüscher and Huber 2010).

ALTERED CIRCUIT FUNCTION

Although the initial demonstration of cocaine-evoked synaptic plasticity was discovered using an ex vivo slice preparation that eludes the identification of the afferents, more recent work points toward afferents from the brain stem. In fact, optogenetic isolation of the laterodorsal tegmental (LDT) to VTA may be one of the major inputs that undergoes plasticity, but a systematic investigation remains elusive. As for the outputs, the DA neurons that undergo the plasticity have been identified (Lammel et al. 2011). Cocaine exposure preferentially increased the efficacy of the excitatory afferents onto DA neurons projecting to the medial and

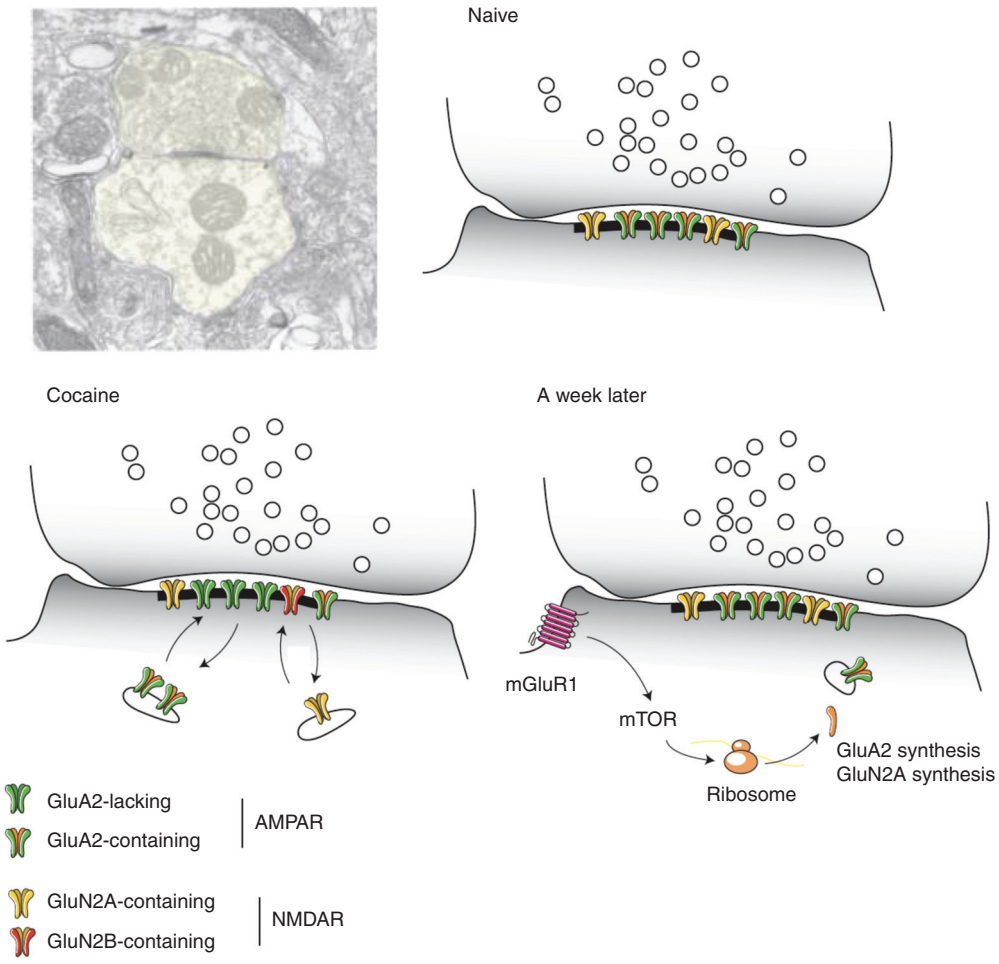

Figure 2. Expression and reversal of drug-evoked synaptic plasticity in dopamine (DA) neurons. Synapses between glutamatergic afferents and dendrites of ventral tegmental area (VTA) DA neurons are aspiny (*top left* panel, electron microscopy picture courtesy of Rafael Lujan). Baseline transmission is mediated by GluA2-containing α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) and GluN2A-dominated *N*-methyl-D-aspartate receptors (NMDARs). After cocaine exposure, GluA2-lacking AMPARs are inserted and NMDARs have a high content of GluN2B (exchange of receptors for GluN3-containing ones). With strong activity, perisynaptic mGluR1s are activated, which through mammalian target of rapamycin (mTOR) synthetize new GluA2 subunits.

lateral shell, but not onto those neurons projecting to the medial prefrontal cortex (mPFC).

Taken together, a picture is emerging whereby specific afferents are subject to plasticity, which then drive the activity of VTA DA neurons projecting primarily to NAc. The molecular determinants (e.g., selective expression of presynaptic DA receptors) and the activity patterns required for induction remain to be determined.

BEHAVIORAL REPERCUSSIONS OF DRUG-EVOKED PLASTICITY IN THE VTA

Linking cocaine-evoked synaptic plasticity in DA neurons of the VTA to a specific behavior has been difficult and the initial attempts looking into various forms of drug-adaptive behavior were not successful. For example, self-administration, behavioral sensitization, and unbiased conditioned place preference, all

behaviors that occur with a time course similar to the time course of drug-evoked synaptic plasticity in the VTA, remained unaffected when plasticity was inhibited (e.g., conditional, inducible GluN1 KO) or quickly reversed (mGluR1 positive modulator) (Engblom et al. 2008; Zweifel et al. 2008). It was only when much later forms of adaptive behavior such as drug-triggered reinstatement or cue-induced cocaine seeking were examined that there was a significant effect of inhibiting plasticity. This was shown by a reduction of these behaviors in mice that lack NMDARs in VTA DA neurons and thus do not show cocaine-evoked synaptic plasticity. This is surprising because these behaviors develop with a delayed time course compared with the plasticity in the VTA, which can be observed within hours after drug injection. The most likely explanation is that the changes in the VTA are permissive for additional adaptations at other synapses, for example in the NAc, which then eventually will have a behavioral impact. Given the identification of the circuits involved and the demonstration of altered rules for activity-dependent plasticity in drug-exposed animals in line with this interpretation, direct pharmacological or optogenetic manipulations of plasticity in the NAc have a more direct impact on behavior. The observation that VTA DA self-stimulation is sufficient to drive adaptive plasticity also in the NAc along with adaptive behavior including compulsive reward consumption (Pascoli et al. 2015, 2018) and seeking (Harada et al. 2019) is in line with DA being the initial trigger of the entire cascade of events.

CONCLUSIONS

Drug-evoked synaptic plasticity is one of the earliest traces that becomes apparent soon after addictive drugs have been eliminated from the body. It is induced by DA transient within the VTA activating presynaptic D1/5Rs and by activity of glutamatergic synapses sufficient to activate NMDARs on the DA neurons. The expression mechanism involves a coordinated exchange of both AMPARs as well as NMDARs. In the case of AMPARs, the initially GluA2-containing receptors are exchanged for GluA2-lacking ones while canonical NMDARs are replaced with GluN3 continuing ones. As a consequence, excitatory transmission onto DA neurons is potentiated (GluA2-lacking AMPARs have a high single-channel conductance) and the conditions for efficient synaptic calcium entry altered. Hyperpolarizing the postsynaptic neurons now facilitates calcium flux and can lead to further activity-dependent potentiation by an anti-Hebbian mechanism (Mameli et al. 2011). Such a metaplasticity has a permissive role for adaptive mechanisms engaged by subsequent drug exposure, gradually remodeling the circuitry and eventually changing behavior toward compulsive drug use without control in addiction.

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