

Viewpoints

Drug-Induced Alterations of Endocannabinoid-Mediated Plasticity in Brain Reward Regions

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The endocannabinoid (eCB) system has emerged as one of the most important mediators of physiological and pathological reward-related synaptic plasticity. eCBs are retrograde messengers that provide feedback inhibition, resulting in the suppression of neurotransmitter release at both excitatory and inhibitory synapses, and they serve a critical role in the spatiotemporal regulation of both short- and long-term synaptic plasticity that supports adaptive learning of reward-motivated behaviors. However, mechanisms of eCB-mediated synaptic plasticity in reward areas of the brain are impaired following exposure to drugs of abuse. Because of this, it is theorized that maladaptive eCB signaling may contribute to the development and maintenance of addiction-related behavior. Here we review various forms of eCB-mediated synaptic plasticity present in regions of the brain involved in reward and reinforcement and explore the potential physiological relevance of maladaptive eCB signaling to addiction vulnerability.

Key words: addiction; cocaine; drugs of abuse; endocannabinoid; nucleus accumbens; plasticity; reward; THC; ventral tegmental nucleus

Introduction

Emerging work has identified endocannabinoid (eCB) signaling as an important mediator of synaptic plasticity in mesocorticolimbic and corticostriatal pathways involved in the control of motivated behavior (Melis et al., 2014; Parsons and Hurd, 2015). The eCB system exploits a retrograde signaling mechanism that results in the suppression of neurotransmitter release at both excitatory and inhibitory synapses with both short- and long-lasting effects. Unlike other neuromodulators, eCBs integrate chemical signals from diverse neurotransmitter systems (e.g., GABA, glutamate, dopamine, acetylcholine) with changes in neuronal excitability, and their signaling represents a fundamental mechanism whereby a cell can control the gain of input from its own afferents (Alger, 2002). Through such neuromodulatory functions, eCBs play a vital role in the spatiotemporal regulation of synaptic plasticity that supports adaptive learning of reward-motivated behaviors and maintenance of affective homeostasis. However, the integrity of the eCB system can be compromised by repeated exposure to exogenous cannabinoids and other drugs of abuse, and abnormal eCB signaling has been identified throughout brain reward regions in the pathogenesis of addiction-related behavior (Gerdeman et al., 2003; Sidhpura and Parsons, 2011; Melis et al., 2014; Covey et al., 2015; Parsons and Hurd, 2015). Here we review various forms of eCB-mediated synaptic plasticity in regions of the brain involved in reward and reinforcement and explore the functional significance of maladaptive eCB signaling in drug-motivated behavior.

Overview of the eCB system

The eCB system encompasses several G-protein-coupled receptors (GPCRs) and lipid signaling molecules as well as their biosynthetic and metabolic machinery. There are two classical cannabinoid receptors: the cannabinoid type 1 (CB1) (Devane et al., 1988) and cannabinoid type 2 (CB2) (Munro et al., 1993) receptors. The CB1 receptor is found predominantly in the pre-synaptic compartment of neurons throughout the CNS (Herkenham et al., 1990) and is the most abundant GPCR in the brain (Mechoulam and Parker, 2013). Consistent with its role in reward and cognition, its regions of highest density include the hippocampus, amygdala, PFC, NAc, and caudate-putamen (Fig. 1) (Herkenham et al., 1990). Although the CB2 receptor is expressed primarily by immune cells in the periphery, recent evidence demonstrates that it is also present in neurons and glia (Van Sickle et al., 2005; Atwood and Mackie, 2010) and may functionally modulate neurotransmission in brain reward regions (Zhang et al., 2014, 2016). Sharing 48% sequence homology, both cannabinoid receptors couple to inhibitory G_{i/o} proteins and have the ability to activate several signal transduction mechanisms to inhibit adenylate cyclase activity and calcium influx through N-, P/Q-, and L-type calcium channels (Mackie and Hille, 1992; Twitchell et al., 1997; Gebremedhin et al., 1999) as well as stimulate inward rectifying potassium channels and the MAP kinase pathway (Mackie et al., 1995; Howlett, 2005). Through activation of these intracellular signaling cascades, pre-synaptic CB1 receptors directly reduce the probability of neurotransmitter release at both excitatory and inhibitory synapses and influence synaptic plasticity mechanisms throughout the brain.

Unlike GPCRs of other neurotransmitter systems, cannabinoid receptors have more than one endogenous agonist. The best-characterized eCB ligands are arachidonylethanolamide (AEA) or anandamide (from the Sanskrit word *ananda*, meaning

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CB1R Density

Low		High
Ventral Pallidum Central Amygdala Ventral Tegmental Area	Prefrontal Cortex Basolateral Amygdala Nucleus Accumbens	Globus Pallidus Hippocampus Dorsal Striatum

Figure 1. Density of CB1 receptor distribution across brain reward areas. Presynaptic CB1 receptors are $G_{i/o}$ -coupled metabotropic receptors that are located throughout reward regions of the brain with varying levels of expression. Distribution of other components of the eCB system, such as the eCB synthetic enzymes N-arachidonoyl-phosphatidylethanolamine (NPLD) (Egertová et al., 2008) and DAGL (Suárez et al., 2011), follow a similar pattern, and mechanisms of CB1 receptor-mediated synaptic plasticity have been measured in mesocorticolimbic and corticostriatal pathways crucially involved in the pathophysiology of addiction.

“bliss”) (Devane et al., 1992) and 2-arachidonoylglycerol (2-AG) (Fig. 2) (Mechoulam et al., 1995; Sugiura et al., 1995). Both AEA and 2-AG exert agonist activity at CB1 and CB2 receptors (Pertwee, 2010). AEA binds the CB1 receptor with higher affinity than the CB2 receptor but exhibits low efficacy at both receptors. However, 2-AG binds the CB1 and CB2 receptors with similar affinity and demonstrates greater potency and efficacy than AEA at both.

Recent evidence also suggests that AEA and 2-AG are ligands of several other receptors that may be considered part of an “expanded” eCB system. Both molecules are functional agonists of the orphan GPCRs, GPR55 and GPR119 (Overton et al., 2006; Lauckner et al., 2008; Godlewski et al., 2009; Pertwee, 2010), and AEA in particular is an effective activator of the transient receptor potential vanilloid type 1 receptors (Zygmunt et al., 1999; Di Marzo and De Petrocellis, 2010), through which it may stimulate the presynaptic release of neurotransmitter (Musella et al., 2009). Therefore, eCBs may have significant impact on synaptic plasticity through additional mechanisms that are distinct from cannabinoid receptor-mediated signaling, and future work will be required to further characterize the influence of eCBs on these other signaling pathways during reward processing and motivated behavior.

In most instances, eCB ligands are generated and released on an *ad hoc* basis upon physiological or pathological neural activation of the postsynaptic neuron (Di Marzo et al., 1994; Cadas et al., 1996). As eCBs are membrane-diffusible lipid molecules, their physicochemical properties prohibit intracellular vesicle storage; therefore, they are synthesized “on demand” from cleavage of membrane phospholipids for immediate release. Activation of eCB biosynthetic machinery is generally calcium-dependent and occurs in response to sustained levels of neural stimulation (Freund et al., 2003), including depolarization as well as activation of ionotropic (Stella and Piomelli, 2001; Piomelli, 2003) or metabotropic (Giuffrida et al., 1999; Varma et al., 2001; Kim et al., 2002) receptors. Although several synthetic routes have been proposed (Sugiura et al., 1995; Leung et al., 2006; Liu et al., 2006; Simon and Cravatt, 2006), AEA is thought to primarily derive from the phospholipid precursor N-arachidonoyl-phosphatidylethanolamine (NAPE) that is released from the membrane by N-acyl-phosphatidylethanolamine-specific phospholipase D (NAPE-PLD) (Fig. 2) (Liu et al., 2006; but see Leung et al., 2006; Tsuboi et al., 2011). 2-AG, on the other hand, is synthesized from the hydrolytic metabolism of 1,2-diacylglycerol (DAG) by the sn-1-selective DAG lipases (DAGLs), DAGL α and DAGL β (Bisogno et al., 2003; Jung et al., 2007; Murataeva et al., 2014; Shonesy et al., 2015).

eCB signaling is inactivated by cellular reuptake into both neurons and glia where eCBs are enzymatically degraded (Beltramo et al., 1997; Hillard and Jarrhian, 2000). While both AEA

and 2-AG are synthesized and released from the postsynaptic compartment, there is spatial segregation of their catabolic enzymes (Fig. 2). AEA is hydrolyzed ultimately via fatty acid amide hydrolase located in the postsynaptic cell. In contrast, 2-AG undergoes predominant hydrolysis by monoacylglycerol lipase (Blankman et al., 2007; Nomura et al., 2011; Chandra et al., 2013) located in the presynaptic cell (Gulyas et al., 2004; Ludányi et al., 2011), in addition to minor postsynaptic hydrolysis by α - β -hydrolase 6 (Marrs et al., 2010). This opposing organization of catabolic machinery is likely to underlie the differential physiological roles of AEA and 2-AG in eCB-mediated signaling (Gulyas et al., 2004; Kim and Alger, 2010; Sidhpura and Parsons, 2011). Although AEA can be released in an activity-dependent manner (Giuffrida et al., 1999), it is theorized to play a more general role in tonic eCB signaling at the CB1 receptor (Kim and Alger, 2010). The slow time course of AEA production (Giuffrida et al., 1999; Hohmann et al., 2005) and the location of its degradation machinery in the postsynaptic cell may help regulate interstitial levels of AEA (Sidhpura and Parsons, 2011). Furthermore, consistent with the presence of catabolic enzymes close to its site of action, 2-AG shapes phasic modulation of neurotransmission (Kim and Alger, 2010; Jung et al., 2012; Piomelli, 2014). Independent findings demonstrate a functional interaction between AEA and 2-AG (Maccarrone et al., 2008), and both eCB species can be recruited differentially from the same postsynaptic neuron with specific patterns of presynaptic activity (Puente et al., 2011; Lerner and Kreitzer, 2012). It is possible that each ligand participates in separate but overlapping forms of eCB-mediated synaptic plasticity. However, much is still unknown regarding the precise mechanisms underlying the production, transport, and degradation of eCBs, and additional work is essential for understanding their individual contributions to synaptic physiology.

Mechanisms of eCB-mediated synaptic plasticity

Short-term plasticity

Perhaps the best-studied forms of eCB-mediated plasticity are the transient or short-term mechanisms that result in brief (<1 min), stimulation-induced attenuation of neurotransmitter release during ongoing neurotransmission. These mechanisms have been termed depolarization-induced suppression of inhibition (DSI) or depolarization-induced suppression of excitation (DSE) at inhibitory (i.e., GABAergic) (Llano et al., 1991; Pitler and Alger, 1994; Ohno-Shosaku et al., 2001; Wilson and Nicoll, 2001) or excitatory (i.e., glutamatergic) (Kreitzer and Regehr, 2001) synapses, respectively. These forms of eCB neuromodulation have been observed throughout brain regions critical to reward processing and the development of drug addiction, including the VTA (Melis et al., 2004; Riegel and Lupica, 2004), NAc (Hoffman and Lupica, 2001; Robbe et al., 2001), basolateral amygdala (Zhu and Lovinger, 2005), hippocampus (Isokawa and Alger, 2005), neocortex (Trettel and Levine, 2003; Bodor et al., 2005), and substantia nigra (Yanovsky et al., 2003). Induction of DSI/DSE requires specific patterns of afferent stimulation to depolarize the postsynaptic cell and activate voltage-gated calcium channels to elevate intracellular calcium levels and mobilize eCBs. Subsequently, eCBs activate CB1 receptors on the presynaptic cell and trigger intracellular signaling cascades that result in a decrement

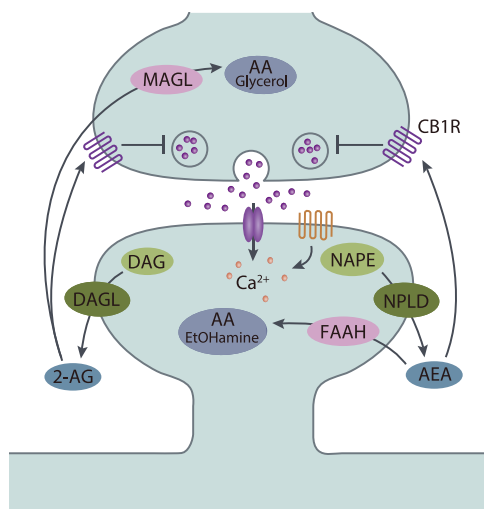


Figure 2. General mechanism of retrograde eCB signaling. Upon release of neurotransmitter (e.g., glutamate, GABA), postsynaptic depolarization results in elevations in intracellular calcium levels through activation of ionotropic receptors, G_q -coupled metabotropic receptors (e.g., Group I mGluRs, M1/M3 mAChRs, D2Rs), and/or voltage-gated calcium channels. The eCBs AEA and 2-AG are not stored in vesicles but instead are synthesized *de novo* from phospholipid precursors through calcium-dependent and -independent mechanisms. NAPE is hydrolyzed by N-arachidonoyl-phosphatidylethanolamine-specific phospholipase D (NPLD) to yield AEA, and DAG is converted to 2-AG by DAGL. Both eCB species traverse the synaptic cleft and activate presynaptic $G_{i/o}$ -coupled CB1 receptors, thereby inhibiting adenylyl cyclase, regulating ion channels, and ultimately suppressing neurotransmitter release. eCB signaling is terminated following degradation by hydrolytic enzymes in the presynaptic and postsynaptic compartments. Primarily, AEA is converted to arachidonic acid (AA) and ethanolamine (EtOHamine) by fatty acid amide hydrolase (FAAH) localized to the postsynaptic cell, whereas 2-AG is hydrolyzed presynaptically into AA and glycerol by monoacylglycerol lipase (MAGL).

in neurotransmitter release for the length of CB1 receptor stimulation (Chevalyere et al., 2006). Most evidence implicates 2-AG as the eCB species mediating DSI/DSE, as DSI is greatly reduced in DAGL knock-out mice (Gao et al., 2010), and inhibition of 2-AG clearance (Makara et al., 2005; Szabo et al., 2006; Pan et al., 2009), but not AEA clearance (Kim and Alger, 2004; Pan et al., 2009), prolongs both DSI and DSE.

An additional form of short-term eCB-mediated plasticity is driven by postsynaptic activation of G_q -coupled GPCRs (e.g., Group I mGluRs, M1/M3 mAChRs, and dopamine D2Rs) (Martin and Alger, 1999; Varma et al., 2001; Kim et al., 2002; Melis et al., 2004; Edwards et al., 2006; Uchigashima et al., 2007). Downstream of metabotropic receptor stimulation, 2-AG mobilization relies on phospholipase C and DAGL activation (Galante and Diana, 2004; Melis et al., 2004; Edwards et al., 2006) and, unlike DSI/DSE, is not calcium-dependent but can be potently augmented by coincident increases in intracellular calcium levels (Maejima et al., 2005; Hashimoto et al., 2007; Kano et al., 2009). Through these short-term plasticity mechanisms, eCBs permit reversible and bidirectional modulation of synaptic strength at both excitatory and inhibitory synapses. This enables critical synaptic scaling and dynamic filtering of specific input frequencies during ongoing neural computation (Abbott et al., 1997; Abbott and Regehr, 2004; Klyachko and Stevens, 2006). Further, by markedly affecting the excitability of the postsynaptic cell (Wagner and Alger, 1996), eCB-mediated short-term synaptic transmission can influence the induction of long-term postsynaptic plasticity mechanisms (Carlson et al., 2002; Chevalyere and Castillo, 2003, 2004; Zhu and Lovinger, 2007). Therefore, although transient, DSI/DSE and metabotropic receptor-mediated short-term plasticity can influence diverse neurotransmit-

ter systems and represent important means by which eCBs make substantial contributions to network function.

Long-term plasticity

Transient eCB-mediated synaptic adaptations, such as DSI/DSE, persist for the duration of CB1 receptor activation, but eCBs can also establish changes in synaptic strength that are maintained beyond initial CB1 receptor stimulation (Chevalyere et al., 2006). Sustained increases and decreases in synaptic strength that last for hours to weeks are referred to as LTP and LTD, respectively. eCB-mediated LTD (eCB-LTD) is the most well-characterized form of long-term presynaptic plasticity affected by eCBs. Although methods of induction at excitatory (eCB-LTDe) and inhibitory (eCB-LTDi) synapses differ somewhat by brain region, the predominant mechanism involves activity-dependent release of eCBs from the postsynaptic cell. eCB mobilization required for eCB-LTD is stimulated by neuronal depolarization or by activation of postsynaptic metabotropic receptors (Freund et al., 2003; Kano et al., 2009). Both high- and low-frequency patterns of neuronal stimulation elicit eCB release through calcium-dependent processes that do not rely on GPCRs (Calabresi et al., 2007; Heifets and Castillo, 2009; Lovinger, 2010). Instead, calcium influx through NMDA receptors and L-type and T-type voltage-gated calcium channels as well as calcium release from intracellular stores trigger postsynaptic eCB release (Beierlein and Regehr, 2006; Isokawa and Alger, 2006; Adermark and Lovinger, 2007; Ohno-Shosaku et al., 2007). In contrast, metabotropic receptor-mediated eCB mobilization is not calcium-dependent and elicits “on-demand” eCB mobilization through G_q (via Group I mGluRs, M1/M3 mAChRs, 5-HT₂Rs, orexin receptors, and cholecystokinin receptors) or $G_{i/o}$ (via D2Rs) protein signaling cascades (Chevalyere et al., 2006; Heifets and Castillo, 2009).

In addition to activity-dependent release of eCBs, induction of eCB-LTD involves coincident presynaptic activity and prolonged CB1 receptor activation (Heifets and Castillo, 2009). The requirement for CB1 receptor binding at active synapses for generation of eCB-LTD imparts some degree of afferent specificity but also allows for both homosynaptic (i.e., target afferent) and heterosynaptic (i.e., nearby afferent) eCB-LTD. Furthermore, evidence suggests that the determining factor for the induction of DSI/DSE versus eCB-LTD may be the nominal time of CB1 receptor activation and subsequent differential recruitment of downstream effectors (Chevalyere et al., 2006; Heifets and Castillo, 2009). Unlike DSI/DSE, eCB-LTD involves downregulation of the cAMP/PKA pathway, and this generates long-term interference with neurotransmitter release machinery (Lonart et al., 2003; Chevalyere et al., 2007) and calcium influx via voltage-gated calcium channels (Robbe et al., 2002; Mato et al., 2005, 2008). Therefore, the extent of neural activity and eCB signaling within the local circuit may crucially determine whether CB1 receptor-mediated signaling results in brief or long-lasting depression of neurotransmission at the synapse. Long-term plasticity induced by eCB mechanisms has been measured in many brain regions, including those involved in addiction, such as the VTA, NAc, and others (Sidhpura and Parsons, 2011; Melis et al., 2014).

Effects of drugs of abuse on eCB-mediated synaptic plasticity in brain reward regions

VTA

The mesolimbic dopamine system is comprised of dopamine cells originating in the VTA of the midbrain that project rostrally to the NAc in the ventral forebrain and to the prefrontal cortex (Wise and Bozarth, 1985). A wide body of evidence supports a

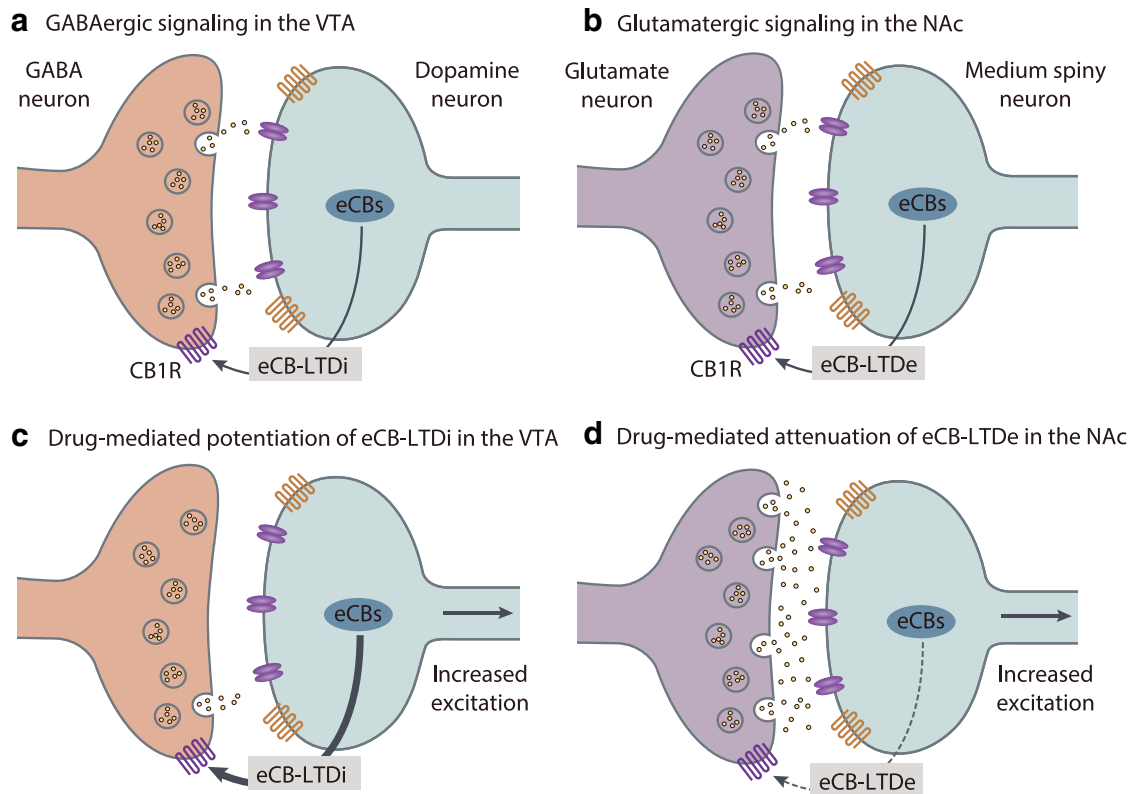


Figure 3. Summary of drug-induced disruptions of eCB-mediated long-term plasticity in the VTA and NAc. **a, b**, Although the specific mechanism differs by brain region, eCB-LTD induction under normal conditions is primarily governed by activity-dependent release of eCBs from the postsynaptic cell and CB1 receptor stimulation on active afferents. **a**, Within the VTA, eCB-LTDi at GABAergic synapses permits adaptive dopamine cell firing, and induction involves additional coordination between presynaptic (e.g., D2 dopamine receptors) and postsynaptic (e.g., Group I mGluRs) metabotropic receptors. **b**, Control over accumbal glutamate release from cortical and limbic afferents through the induction of eCB-LTDe relies on activation of postsynaptic metabotropic receptors (e.g., mGluR5) and release of calcium from intracellular stores. **c, d**, Following exposure to drugs of abuse, such as THC or cocaine, parallel disturbances in eCB-LTD mechanisms that normally provide inhibitory control over VTA dopamine neuron activity and curb excitation of NAc MSNs instead promote activation of reward circuitry. **c**, Drug exposure facilitates the induction of eCB-LTDi in the VTA, removing GABA-mediated inhibition of dopamine neurons and enhancing their excitability. **d**, Drug-induced loss of eCB-LTDe at glutamatergic synapses in the NAc prevents control over excitation of MSNs.

role for mesolimbic DA in reinforcement learning and motivation for incentive stimuli, such as reward-predictive cues (Wise and Rompre, 1989; Ikemoto, 2007; Berridge, 2009; Taber et al., 2012). Additionally, the rewarding properties of all known abused drugs are thought to arise, in part, from dopamine release in the NAc (Roberts et al., 1977; Wise and Bozarth, 1985; Di Chiara and Imperato, 1988; Volkow and Morales, 2015), and synaptic plasticity in the mesolimbic dopamine system is a critical target for drugs of abuse (Kauer, 2004). Importantly, administration of THC or cannabinoid agonists (French et al., 1997; Diana et al., 1998; Cheer et al., 2003) or cocaine (Sombers et al., 2009) increases the burst firing of VTA dopamine neurons as well as stimulates phasic dopamine release in the NAc in a CB1-dependent manner (Cheer et al., 2004, 2007; Wang et al., 2015). However, dopamine neurons do not express CB1 receptors (Herkenham et al., 1991; Julian et al., 2003), and current data support a CB1 receptor-mediated increase in dopamine neuron activity due to induction of local disinhibitory mechanisms, such as DSI (Lupica and Riegel, 2005) and eCB-LTDi (Pan et al., 2008a, b). During periods of burst firing, VTA dopamine neurons synthesize and release 2-AG predominantly onto presynaptic CB1-expressing GABAergic terminals originating from the globus pallidus, rostromedial tegmental nucleus, and local interneurons (Lecca et al., 2012). CB1 receptor binding transiently suppresses GABA-mediated inhibition and initiates a disinhibitory feedback loop that enhances dopamine cell firing (Melis et

al., 2004; Riegel and Lupica, 2004; Lupica and Riegel, 2005; Alger and Kim, 2011). The long-term reduction in GABAergic inhibitory input to dopamine neurons via eCB-LTDi (Fig. 3a) also involves the coordination between D2 receptors and Group I metabotropic glutamate receptors (Pan et al., 2008a, b). Activation of postsynaptic mGluRs enhances the synthesis and release of eCBs and CB1 receptor activation, and presynaptic D2 receptor stimulation facilitates eCB-LTDi by augmenting the downstream effects of CB1 receptor signaling, such as inhibition of the cAMP/PKA pathway.

Consistent with its role in promoting dopaminergic transmission (Cheer et al., 2007; Wang et al., 2015), cocaine interferes with both short-term (Lupica and Riegel, 2005; Wang et al., 2015) and long-term (Fig. 3a, c) (Liu et al., 2005; Pan et al., 2008a, b) eCB-mediated plasticity mechanisms in the VTA. Acute cocaine attenuates inhibition of dopamine neurons through facilitation of DSI at GABAergic synapses in the VTA (Wang et al., 2015). Specifically, cocaine mobilizes 2-AG via local inhibition of norepinephrine uptake and promotion of α_1 -adrenergic receptor stimulation of dopamine neurons. This results in a cocaine-mediated enhancement dopamine cell firing and dopamine release in the NAc.

Moreover, cocaine reduces inhibition of VTA dopamine neurons through facilitated induction of eCB-LTDi at GABAergic synapses (Liu et al., 2005; Pan et al., 2008a, b). *In vivo* cocaine exposure for 5–7 d occludes further induction of eCB-LTDi (Liu et al., 2005; Pan et al., 2008a, b), and cocaine's effects are blocked

by pretreatment with D2, mGluR5, and CB1 receptor antagonists (Fourgeaud et al., 2004; Liu et al., 2005). Under many conditions, GABAergic inhibition suppresses LTP induction at excitatory synapses (Wigström and Gustafsson, 1983; Meredith et al., 2003). Accordingly, evidence suggests that cocaine-induced attenuation of LTD in GABAergic neurons in the VTA facilitates the induction of LTP at excitatory synapses (Liu et al., 2005). Therefore, through enhancement of eCB-LTDi induction, cocaine has the ability to increase the excitability of VTA dopamine neurons and may ultimately influence maladaptive drug-seeking behaviors. Other endogenous factors within the VTA, such as norepinephrine (Wang et al., 2015), brain-derived neurotrophic factor (Zhong et al., 2015), neurotensin-1 (Kortleven et al., 2012), and insulin (Labouèbe et al., 2013), also modulate eCB signaling and, in some cases, are involved in the effects of cocaine on dopamine neuron excitability (Wang et al., 2015; Zhong et al., 2015). Future work will determine the role these factors play in drug-mediated long-term plasticity mechanisms within the VTA.

NAc

The NAc is one of the major target regions of dopaminergic projections from the VTA as well as glutamatergic projections from cortical and limbic areas that are vital to the regulation of motivated behavior (Meredith et al., 2008; Sesack and Grace, 2010). Through convergent inputs from the VTA, PFC, amygdala, and hippocampus and output projections to motor regions, such as the ventral pallidum, the NAc enables adaptive behavioral responding to rewards and reward-predictive cues. Extensive work has identified long-term dysfunctional synaptic alterations in the NAc that promote vulnerability to relapse-related behavior (Gipson et al., 2014; Wolf, 2016). The majority of neurons (90%–95%) in the NAc are GABAergic medium spiny neurons (MSNs) that express either D1 or D2 dopamine receptors (Bock et al., 2013; MacAskill et al., 2014; Kupchik et al., 2015). Unlike projections of MSNs of the dorsal striatum, projections of D1- and D2-positive MSNs of the NAc do not segregate strictly via direct and indirect pathways to output nuclei (Kupchik et al., 2015). Instead, D1-positive MSNs project to the VTA and substantia nigra, whereas both D1- and D2-positive MSNs send projections to the ventral pallidum. Under many circumstances, these individual MSN populations are differentially impacted by exposure to drugs of abuse, and imbalance among their activity contributes to drug-induced behavior (Bock et al., 2013; MacAskill et al., 2014).

Like dopamine neurons of the VTA, MSNs participate in both short-term (Robbe et al., 2001) and long-term (Robbe et al., 2002) eCB-mediated synaptic plasticity (Fig. 3*b*). Although accumbal MSNs do not express CB1 receptors (Winters et al., 2012), CB1 receptors provide inhibitory control over local glutamate and GABA release in the NAc through their expression on cortical and limbic afferents as well as fast-spiking interneurons (Hoffman and Lupica, 2000, 2001; Robbe et al., 2001; Pistis et al., 2002; Winters et al., 2012). eCB-LTDe at glutamatergic synapses in the NAc is facilitated by postsynaptic activation of the Group I metabotropic receptor mGluR5 (Fig. 3*b,d*) (Robbe et al., 2001; Grueter et al., 2010) and release of calcium from intracellular ryanodine-sensitive stores (Robbe et al., 2001). Results suggest AEA as the eCB species mediating eCB-LTDe in the NAc, as preventing its degradation enhances LTD induction (Grueter et al., 2010). Moreover, accumbal eCB-LTDe manifests preferentially in D2-positive MSNs (Grueter et al., 2010). D2 receptor activation, however, is not necessary for eCB-LTDe induction in the accumbens (as opposed to the dorsal striatum) (Gerdeman et

al., 2002) but can stimulate AEA production (Giuffrida et al., 1999). Thus, striatal dopamine and D2 receptors likely modulate fundamental eCB-LTDe induction mechanisms by facilitating eCB mobilization (Kreitzer and Malenka, 2005; Lerner and Kreitzer, 2012).

Studies from several laboratories have demonstrated disruption of eCB-LTDe in the NAc following exposure to drugs of abuse. Both single (Mato et al., 2004) and repeated (Hoffman et al., 2003; but see Mato et al., 2005) exposure to THC prevented eCB-LTDe in the NAc and reduced sensitivity to CB1 receptor agonists at the synapse (Hoffman et al., 2003), supporting pronounced cannabinoid-induced downregulation of the CB1 receptor. Additionally, accumbal eCB-LTDe was abolished 24 h after a single exposure to cocaine (Fourgeaud et al., 2004) and as long as 45 d after prior long-term cocaine self-administration (McCutcheon et al., 2011). However, in contrast to the effects of THC exposure, signaling at the CB1 receptor is not impaired following exposure to cocaine (Fourgeaud et al., 2004; McCutcheon et al., 2011). Instead, during extended withdrawal from cocaine self-administration, there is enhanced sensitivity to CB1 receptor agonists and an apparent uncoupling of mGluR5 with eCB synthetic machinery, suggesting a persistent attenuation of eCB tone (McCutcheon et al., 2011). Disabling of mGluR5-associated eCB-LTDe during protracted cocaine withdrawal is part of series of synaptic adaptations in the NAc that increase excitability of MSNs and promote susceptibility to relapse to drug-seeking behavior (McCutcheon et al., 2011; Wolf and Tseng, 2012; Wolf, 2016). More work will be needed to understand the full significance of drug-induced disruptions in accumbal eCB-LTD and motivated behavior, but present findings demonstrate significant abnormalities in eCB-mediated plasticity following exposure to drugs of abuse.

In conclusion, eCBs are key modulators of synaptic function and mediate mechanisms of both short- and long-term presynaptic plasticity in regions of the brain crucially involved in reward processing, such as the VTA and NAc. Accumulating evidence demonstrates perturbations in normal eCB-mediated synaptic plasticity in these brain areas following experience with drugs of abuse. Specifically, drug-induced disruptions in eCB plasticity result in concurrent loss of inhibitory control over dopamine neurons in the VTA and impairment in the regulation of excitatory signaling in the NAc. Together, these maladaptive synaptic alterations have the ability to potentiate reactivity of the mesocorticolimbic and corticostriatal pathways that are critical to drug-motivated behavior. Indeed, loss of accumbal eCB-LTD and related enhancement in excitability of MSNs are associated with heightened drug-seeking behavior (McCutcheon et al., 2011; Wolf, 2016). These findings suggest that therapeutic approaches aimed at restoring normal eCB-mediated synaptic plasticity might have significant impact on the treatment of addiction. Therefore, additional work is necessary to further characterize mechanisms of eCB-mediated plasticity throughout reward circuitry and determine their contribution to the pathophysiology and treatment of drug addiction.

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