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Inhibitory Plasticity of Mesocorticolimbic Circuits in Addiction and Mental Illness

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Abstract

Behavioral adaptations occur through remodeling of brain circuits, as arising for instance from experience-dependent synaptic plasticity. Drugs of abuse and aversive stimuli, such as stress, act on the mesocorticolimbic system, dysregulating adaptive mechanisms and leading to a variety of aberrant behaviors associated with neuropsychiatric disorders. Until recently, research in the field has commonly focused on experience-dependent synaptic plasticity at excitatory synapses. However, there is growing evidence that synaptic plasticity within inhibitory circuits is an important contributor to maladaptive behaviors. We speculate that restoring normal inhibitory synaptic transmission is a promising therapeutic target for correcting some of the circuit abnormalities underlying neuropsychiatric disorders.

Keywords

drugs of abuse; stress; dopamine; GABA; chloride; KCC2; nucleus accumbens; ventral tegmental area; mesolimbic

Neuropsychiatric disorders and synaptic plasticity

Neuropsychiatric disorders are common causes of disability in the United States, leading to a significant rise in the cost of mental health care over the last decade [1, 2]. By 2020, substance abuse, anxiety, depressive and psychotic disorders are expected to surpass all physical diseases as major causes of disability worldwide [3]. Despite traditional nosological boundaries, some neuropsychiatric disorders share broadly comparable impairments in reward processing, motivation, and executive function that reflect an underlying dysregulation in mesocorticolimbic circuitry, including the dopaminergic (DA) projections [4]. Accordingly, animal studies show that drugs of abuse, stress, and aversive stimuli activate and remodel partially overlapping networks within the mesocorticolimbic system [5–7]. At the cellular level, alterations in circuitry can be achieved by inducing long-lasting changes in the synaptic connectivity between neurons, a phenomenon termed experience-

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dependent synaptic plasticity. Likewise, drugs of abuse and aversive stimuli trigger or modulate multiple forms of synaptic plasticity within the DA system. In some cases, researchers have demonstrated that restoring basal synaptic transmission repairs neural circuitry and alleviates aberrant behaviors, indicating that targeting experience-dependent synaptic plasticity within mesocorticolimbic circuitry may be a promising therapeutic tool for neuropsychiatric disorders [8–10].

Although glutamatergic synaptic plasticity is most commonly studied, there is a growing appreciation that inhibitory synapses also undergo plasticity and regulate circuit function and adaptability. For decades, inhibitory synapses were assumed to be relatively inflexible, functioning mainly to ensure stable and reliable control of the neuronal network. Recent progress in fluorescent labeling and in optogenetic and chemogenetic manipulation of neurons has provided evidence that inhibitory circuits can drive behavioral changes through multiple forms of plasticity at inhibitory synapses. In particular, inhibitory synaptic plasticity within the mesocorticolimbic system alters circuit function and mediates various stress and drug-related behavioral adaptations. In this review, we discuss these recent advances, and also describe another type of postsynaptic plasticity characterized in the ventral tegmental area (VTA) that shifts the chloride (Cl^-) gradient across the neuronal membrane in response to stress, pain, and drugs of abuse. We propose that shifts in the Cl^- gradient are an ongoing form of inhibitory synaptic plasticity. Within the mesocorticolimbic system, this form of plasticity contributes to experience-dependent circuit remodeling and to the emergence of maladaptive behaviors.

Presynaptic forms of inhibitory plasticity in mesocorticolimbic circuitry

Plasticity of inhibitory inputs onto VTA DA neurons

The VTA is a key brain region containing subpopulations of DA cell bodies that send distinct projections to various striatal and cortical areas [11]. These heterogeneous DA projections may exhibit specific biases in their input selection (Box 1) thereby forming anatomically separate yet partly overlapping circuits that mediate different behavioral and physiological processes including reward, salience, stress, fear, and aversion [11, 12]. There are various circumstances, however, where this circuit-specific information flow becomes more unified. For example, during a classical conditioning task, almost all VTA afferents provide similar, evenly distributed input to DA neurons (Box 1) [13]. Likewise, an addictive drug administration (e.g., nicotine) elicits a similar response across many DA neurons in a relatively broad area of the VTA [14, 15]. Overall, the response of the VTA can be fairly varied and flexible, with additional heterogeneity of DA neuron subpopulations arising not only from their input-output architecture but also from differences in baseline firing activity, intra- and extracellular modulation, and multi-neurotransmitter release [16–19].

DA neuron activity is powerfully controlled by inhibitory synaptic inputs that undergo plasticity in response to multiple environmental stimuli. These stimuli can trigger enduring increases and decreases in neurotransmitter release, thereby producing long-term potentiation (LTP) and long-term depression (LTD), respectively (Box 2). Early studies described at least three different types of experience-dependent presynaptic GABAergic plasticity onto VTA DA neurons: cAMP/PKA-dependent enhanced GABA release, cAMP/

PKA-dependent reduced GABA release, and cGMP/PKC-dependent enhanced GABA release. Activation of adenylyl cyclase during morphine withdrawal and acute ethanol exposure enhances cAMP/PKA pathway signaling leading to increased GABA release onto VTA DA neurons [20, 21]. This form of plasticity correlates with somatic signs of opiate withdrawal, decreased opioid conditioned place preference, and increased acquisition of ethanol intake [21–23]. In contrast, inhibition of the cAMP/PKA pathway by repeated cocaine exposure attenuates GABA release onto DA neurons and contributes to cocaine-induced conditioned place preference [24]. Another form of synaptic plasticity involves nitric oxide-mediated activation of the cGMP-PKC pathway, which leads to LTP of GABAergic inputs onto DA neurons [25]. Acute stress blocks this form of plasticity, and rescuing LTP at GABAergic synapses prevents stress-induced reinstatement of cocaine seeking [26].

DA neurons receive GABA afferents from multiple sources, but it remains unclear how various forms of inhibitory plasticity are expressed in different circuits. Recent studies provide a proof of concept that different types of plasticity can be expressed by distinct inhibitory inputs onto VTA DA neurons [27, 28]. Specifically, inputs from local VTA GABA neurons and afferents from the nucleus accumbens (NAc) express both adenylyl cyclase and cGMP-dependent forms of plasticity [28]. In contrast, inputs from the rostromedial tegmental nucleus (RMTg) do not express cGMP-dependent plasticity, suggesting that these afferents might be insensitive to acute stress [27, 28]. These results support the idea that different life experiences trigger diverse forms of synaptic plasticity to modulate specific inhibitory inputs differentially onto VTA DA neurons. These inputs can carry distinct behavioral information, and in the next section we will describe the major inhibitory afferents onto VTA DA neurons.

Multiple roles of inhibitory inputs onto DA neurons

VTA neurons receive inputs from multiple brain areas (Box 1), but the densest and most studied inhibitory inputs are projections from the NAc and the RMTg onto VTA DA neurons, as well as local projections from GABAergic neurons (Figure 1). High-frequency optogenetic activation of VTA GABA neurons blocks DA neuron firing, decreases reward consumption, and increases conditioned place aversion [29, 30]. However, the role of VTA GABA neurons is not limited simply to the cessation of motivated behaviors. In fact, increased GABAergic input from local neurons onto DA cells correlates with increased ethanol and nicotine self-administration [21, 31–33]. Furthermore, experimental and modeling studies demonstrate that GABA-mediated persistent decreases in tonic DA levels result in increased stimulus-evoked phasic DA signaling that drives Pavlovian and instrumental learning [34–36]. Combining in vivo electrophysiological recordings during behavior with optogenetic stimulation shows that temporally precise activation of VTA GABA neurons shapes the DA reward prediction error signal, thereby contributing to the formation of cue-reward associations [37]. According to the reward prediction error hypothesis, the behavioral outcome during Pavlovian or instrumental conditioning depends on DA signaling that results from a discrepancy between reward expectation and the actual reward [37–39]. Specifically, if the reward exceeds expectation triggered by a cue, increased DA signaling promotes positive reinforcement and approaching behavior. In contrast,

attenuated DA signaling mediates avoidance when the reward is less than expected. Finally, when the cue reliably predicts the reward, then there is no change in DA signaling. VTA GABA neurons attenuate DA neuron activity during the delivery of predicted reward, contributing to the reward expectation and behavioral reaction to cue presentation [37]. In addition to the multifaceted function of GABA neurons in the VTA, a recent observation indicates that these neurons primarily innervate neighboring cells and do not project widely throughout the VTA [27]. This limited distribution of axons suggests that distinct VTA GABA neuronal subsets control discrete subpopulations of DA neurons, which may serve different behavioral functions.

The RMTg sends dense GABAergic efferents to VTA DA neurons and serves as a major source of aversive signals by relaying information from the lateral habenula (LHb), a brain area implicated in negative emotional states [40, 41]. Activation of RMTg leads to inhibition of VTA DA neurons and promotes various behaviors, such as fear-conditioned freezing, place avoidance, and anxiety-like behavior [40]. In contrast, inhibition of RMTg terminals in the VTA increases reward seeking even in the face of aversive consequences [42, 43]. Despite the RMTg's involvement in aversive behaviors, acute exposure to stressful or painful stimuli does not induce c-Fos expression in the RMTg [44]. Similarly, acute exposure to many drugs of abuse, except psychostimulants, has no effect on immediate early gene expression in the RMTg [45]. In contrast to acute exposures, repeated foot shocks or withdrawal from chronic morphine induces c-Fos expression as well as long-lasting adaptations in RMTg neurons [44, 46, 47]. Interestingly, acute exposure to ethanol or lithium chloride, which causes visceral illness, has no effect on immediate early gene expression. However, c-Fos expression is induced in response to ethanol- and LiCl-induced conditioned taste aversion [48]. These findings suggest that behavioral responses to stimuli, but not the stimuli themselves, trigger plasticity in the RMTg.

NAc GABAergic inputs to the VTA exert multiple influences on motivated behaviors reflecting the complex anatomical organization of connections between NAc subregions and DA neuron subpopulations [19, 49]. The NAc has been divided into three main subregions: the core, the medial shell, and the lateral shell. These subregions contain two subtypes of medium spiny neurons (MSNs), which express either dopamine D1 (D1R) or D2 (D2R) receptors and project to the midbrain and the basal ganglia. A recent study demonstrated that D1-MSNs in the NAc medial shell exert direct inhibitory control over two distinct VTA DA subpopulations by activating different types of GABA receptors (Figure 1) [19]. Specifically, D1-MSNs signal via GABA_A receptors to inhibit DA neurons that project back to NAc medial shell, whereas DA neurons projecting to the lateral shell receive GABA_B receptor-mediated input. Selective optogenetic activation of D1-MSNs that signal via GABA_A receptors produces rewarding behavior expressed as a real-time preference for the compartment paired with stimulation [19]. In contrast to medial shell, D1-MSNs in the NAc lateral shell do not innervate VTA DA neurons directly, but they synapse preferentially onto VTA GABA neurons (Figure 1) [19].

The canonical view postulates that only D1 neurons project to the VTA. Intriguingly, recent studies report that VTA DA neurons receive GABAergic input from NAc medium spiny neurons (MSNs) that express D2 receptors [46, 50]. It remains unclear how this pathway

modifies rewarding or aversive behavior, but generally, D2-expressing MSNs mediate aversion. D2-MSNs have a well-established role in morphine withdrawal symptoms, and it is possible that the D2-MSN input contributes to the increased GABAergic drive onto DA neurons that is induced by morphine withdrawal [20, 51, 52].

Presynaptic inhibitory plasticity in NAc target regions

Given that the NAc is a primary site mediating reward and that its main output neurons are GABAergic, it is reasonable to expect that inhibitory plasticity in MSN target regions may be linked to various motivated behaviors. Activity-dependent inhibitory synaptic plasticity in MSN terminals has been described most extensively in the VTA and in the ventral pallidum (VP), a heterogeneous structure within the basal ganglia that projects to multiple downstream structures including the VTA [53, 54]. High frequency stimulation (HFS) of D1-MSN terminals triggers LTP of inhibitory currents in VTA GABA and VTA-projecting VP neurons (Figure 2) [55]. The induction of this inhibitory LTP does not involve activity of the postsynaptic neurons. Instead, HFS enhances GABA release probability via presynaptic D1 receptor activation and subsequent activation of the cAMP-PKA cascade. In contrast, HFS of D2-MSN terminals leads to LTD of inhibitory inputs in the VP (Figure 2) [56, 57]. It remains unclear how a single stimulation protocol triggers opposite forms of inhibitory plasticity in D1 and D2 terminals, but the plasticity may arise from differential expression of endogenous opioids and their receptors [56, 57].

Recent studies indicate that various cocaine-related behavioral outputs are associated with synaptic plasticity in MSN D1 and D2 terminals. Specifically, repeated cocaine exposure blocks HFS-induced LTP of D1 inputs to VTA GABA neurons. This block of plasticity is accompanied by increased GABA release onto VTA GABA neurons, indicating that cocaine has already produced PKA-dependent potentiation of D1 inputs (i.e., cocaine occludes further LTP) [55]. Similarly, withdrawal from cocaine potentiates output of D1 terminals in the VP and simultaneously decreases output of D2 terminals in the VP, leading to the occlusion of LTP and LTD in D1 and D2 terminals, respectively [56, 57]. To assess the causal link between synaptic neuroadaptations and behavior, recent studies employed optogenetic stimulation protocols designed to either induce or rescue cocaine-induced synaptic plasticity [55, 56]. In behaving animals, the authors showed that inducing LTP at D1 terminals in the VTA and VP underlies locomotor sensitization to cocaine, an effect associated with the reinforcing effects of addictive drugs [55, 56, 58]. In contrast, inhibitory plasticity at D2-MSN terminals mediates the negative symptoms of cocaine withdrawal such as anhedonia and decreased motivation for sucrose [56]. Therefore, divergent forms of plasticity within distinct inhibitory circuits can mediate opposing behavioral effects of addictive drugs. While ex vivo studies of synaptic plasticity in the VP have focused on neurons that project to the VTA, in vivo manipulation of synaptic plasticity could modify multiple VP outputs. Another recent study reports decreased inhibitory input onto LHB-projecting VP neurons after social defeat stress, an effect that contributes to behavioral despair depressive-like phenotypes [59]. Altogether, these studies demonstrate the critical role of inhibitory plasticity in the emergence of aberrant behaviors. From the therapeutic perspective, these findings suggest that restoring normal inhibitory transmission could be

used for combating some of the behavioral abnormalities associated with neuropsychiatric disorders.

Postsynaptic inhibitory plasticity

Plasticity of GABA_A receptors

In addition to presynaptic mechanisms, inhibitory synaptic plasticity may arise from GABA_A receptor regulation on the postsynaptic membrane (Box 2). In mesocorticolimbic pathways, only a few reports have linked postsynaptic forms of experience-dependent inhibitory plasticity to behavioral outputs. Repetitive exposure to addictive drugs modifies GABA_A receptor subunit composition in the NAc leading to long-lasting effects on GABAergic transmission [60, 61]. For example, morphine reduces the expression of various GABA_A receptor subunits in D1 MSN leading to decreased GABAergic transmission in the NAc shell [60]. In contrast, cocaine increases inhibitory tone in the NAc shell by upregulating GABA_A receptor expression [61]. Antagonism of GABA_A receptors in the NAc enhances morphine conditioned place preference and blocks cocaine locomotor sensitization, suggesting that postsynaptic forms of drug-dependent inhibitory plasticity contribute to associative and non-associative reward-related responses to drugs of abuse [60, 61].

Chloride gradient-mediated synaptic plasticity

A form of inhibitory plasticity that has gained increased attention over the last several years involves long-lasting alterations in the transmembrane Cl⁻ gradient, which determines the strength of synaptic inhibition (Box 2 and Box 3). Elevated intracellular Cl⁻ concentration that leads to depolarizing or even excitatory GABA_A receptor signaling was originally described in very young animals and in seizure generation [62]. However, recent studies in adult animals demonstrate a depolarizing transmembrane Cl⁻ gradient and impaired GABAergic inhibition after exposure to multiple environmental and endogenous stimuli.

Within the DA system, long-term changes in the Cl⁻ gradient have been shown in GABA neurons of the VTA after exposure to stress and during withdrawal from drugs of abuse (Figure 3A, Key figure). The Cl⁻ gradient of VTA GABA neurons is maintained, in part, by the K⁺, Cl⁻ transporter, KCC2. A single exposure to restraint stress leads to a glucocorticoid-mediated, post-translational down-regulation of KCC2 in the VTA (Box 2 and Box 3; Figure 3A) [32]. DA neurons express little or no KCC2 [63], but in VTA GABA neurons, down-regulation of KCC2 leads to Cl⁻ accumulation, producing a depolarizing shift in the reversal potential for GABA_A receptor-mediated currents (E_{GABA} ; Box 3). This depolarized reversal potential results in decreased synaptic inhibition or even paradoxical GABAergic excitation of GABA neurons in response to GABA_A receptor activation (Figure 3B). Behavioral studies show that stress leads to increased alcohol self-administration and restoring normal synaptic inhibition in the VTA mitigates this effect [32]. At the circuitry level, a stress-induced shift in E_{GABA} fundamentally alters the pharmacological action of alcohol on the mesolimbic pathway, attenuating alcohol-induced DA signaling in the VTA and medial NAc [32]. Therefore, stress-induced alterations in the Cl⁻ gradient impact reward circuitry and link stressful experiences to the increased intake of drugs of abuse.

An excitatory shift in GABA_A receptor signaling and elevated alcohol consumption have also been observed after nicotine exposure. Importantly, the animal's age determines the duration of the inhibitory plasticity. Rats treated with nicotine during adolescence, but not those treated in adulthood, show excitatory shifts in GABA_A receptor signaling and elevated alcohol self-administration that last at least for several weeks [64]. KCC2 down-regulation and altered inhibitory transmission in the VTA persist for more than 4 weeks after adolescent nicotine exposure but last for less than one week after adult nicotine exposure [31, 64]. More importantly, enhancing KCC2 function in adolescent nicotine-treated rats normalizes both GABAergic signaling and alcohol consumption [64]. These results indicate that adolescent nicotine exposure produces long-lasting alterations in Cl⁻ homeostasis and GABA_A receptor signaling, contributing to elevated ethanol self-administration in adulthood (at least in this rodent model).

Other treatments that shift GABA_A receptor signaling toward excitation in VTA GABA neurons include peripheral nerve injury, which causes chronic pain, and abrupt withdrawal from chronic morphine or ethanol [63, 65, 66]. Like acute stress, chronic pain and drug withdrawal disrupt Cl⁻ homeostasis in VTA GABA neurons via KCC2 downregulation, which leads to decreased synaptic inhibition or even paradoxical excitation in response to GABA_A receptor activation [63, 65–67]. Compromised GABAergic inhibition of VTA GABA neurons attenuates morphine and cocaine-mediated DA signaling in the NAc, linking synaptic plasticity to circuit-wide modifications. At the behavioral level, opioid- or pain-mediated decreases in KCC2 protein expression lead to impaired reward behavior, such as blunted cocaine or morphine place preference [63, 66].

Multiple stimuli (including stress, pain, and withdrawal from nicotine or morphine) trigger KCC2-mediated plasticity that leads to blunted behavioral and neural reward sensitivity, but that blunted reward sensitivity also commonly promotes drug self-administration [32, 63, 64, 66]. In humans, attenuated behavioral and neuronal responses to rewards predict the onset, escalation, and relapse in drug abuse, implying that drug taking may partially alleviate disrupted reward function caused by stress, chronic pain, drug withdrawal, or depressive disorders [68–74]. We hypothesize that KCC2-mediated synaptic plasticity in VTA GABA neurons represents one mechanistic pathway linking attenuated reward sensitivity to increased motivation for drug intake. Given that this single form of synaptic plasticity within the VTA concurrently mediates disrupted reward processing and drug intake, manipulation of Cl⁻ homeostasis is a potential therapeutic target for treating addiction and reward-related dysfunction in neuropsychiatric disorders.

Recent studies suggest that VTA GABA neurons participate in multiple local and long-range inhibitory circuits, and therefore, KCC2-mediated synaptic plasticity may affect behavior differently based on which distinct inhibitory pathways are affected. Stress, chronic pain, and drug withdrawal downregulate KCC2 in VTA GABA neurons that provide local inhibition onto NAc-projecting DA neurons [32, 63, 64, 66]. Future studies should investigate how KCC2-mediated plasticity regulates the activity of DA neurons that project to other brain areas. Furthermore, in addition to local inhibition, KCC2-mediated plasticity can impact VTA GABA neurons that project to other brain regions. Selective viral-genetic labeling and manipulation of VTA GABA neurons indicate that these cells send reciprocal

projections to multiple brain regions, modulating specific learning and motivated behaviors (Figure 3C) [75–78]. For example, NAc-projecting VTA GABA neurons inhibit accumbal cholinergic interneurons enhancing the association between cues and salient outcomes [76, 79]. Inhibition of LHb activity by VTA GABA neurons decreases RMTg firing, disinhibits VTA DA neurons, and thereby promotes reward-related behaviors [77, 78]. In addition to possible projection-specificity, KCC2-mediated plasticity might differentially modulate distinct inhibitory inputs onto VTA GABA neurons. Midbrain GABA neurons receive inhibitory projections from multiple brain areas, and optogenetic activation of some of these inputs leads to DA neuron disinhibition, which supports various reward-related behaviors, such as place preference, social interaction, and investigation of novel objects [19, 80, 81]. In summary, recent studies suggest that VTA GABA neurons consist of distinct subpopulations, which differ anatomically in their input and output organization and participate in distinct behaviors. An important next step is to identify whether these GABA subpopulations are equally susceptible to KCC2-mediated shifts in the Cl^- gradient, as well as other forms of synaptic plasticity.

Growing evidence suggests that addictive drugs, stress, and aversive stimuli alter Cl^- homeostasis not only in the VTA, but also in the NAc, hippocampus, amygdala, and along the HPA axis [82–85]. Accordingly, human and animal studies implicate Cl^- transporter dysfunction in a variety of neuropsychiatric disorders, including anxiety, depression, and substance use disorders [66, 86–88]. Based on the increasing number of studies, we propose that ongoing shifts in neuronal Cl^- gradients are a typical form of experience-dependent synaptic plasticity that contributes to circuit remodeling and the emergence of adaptive behaviors in health and disease.

Concluding remarks and future perspectives

Neuropsychiatric disorders are often characterized by the impairment of reward processing and motivation that arise from experience-dependent dysregulation of mesocorticolimbic circuitry. A majority of studies have focused on dopaminergic and glutamatergic transmission within the mesocorticolimbic system, while inhibitory circuits remain relatively understudied. The recent literature indicates that GABA neurons in various regions of the mesocorticolimbic system form distinct local and long-range inhibitory circuits that participate in a wide array of motivated behaviors. Importantly, behavior adjustments involve synaptic plasticity within these inhibitory circuits that are triggered by addictive drugs and aversive stimuli. Reversing maladaptive plasticity at inhibitory synapses holds potential for treating various neuropsychiatric disorders, including addiction, depression, and anxiety. While the translational relevance of this approach remains to be examined, an increasing number of studies show that pharmacologic targeting of inhibitory synaptic plasticity can prevent or block various aberrant behaviors in animal models of pathology [26, 31, 32, 59, 61, 64, 89]. A form of inhibitory plasticity that has gained increasing attention involves long-lasting alterations in the transmembrane Cl^- gradient. Dysregulation of Cl^- homeostasis mediates stress, pain, and drug-induced reward circuitry dysfunction, suggesting that targeting anion transporters has potential as a new strategy for treating anxiety, depression, and substance use disorders.

Apart from pharmacology, long-term alterations in synaptic strength can be induced by various brain stimulation techniques, such as deep brain stimulation and transcranial magnetic stimulation. Reversing maladaptive excitatory plasticity with brain stimulation is becoming an effective procedure to treat symptoms of addiction and depression [90]. Given that deep brain and transcranial magnetic stimulation can also trigger plasticity at inhibitory synapses, these methods could also be used to target inhibitory circuits [91, 92]. However, development of new pharmacotherapies and brain stimulation techniques will require precise dissection of the critical inhibitory circuits and a better understanding of inhibitory plasticity mechanisms.

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References

1. Murray CJ, et al. The state of US health, 1990-2010: burden of diseases, injuries, and risk factors. *JAMA*. 2013; 310(6):591–608. [PubMed: 23842577]
2. Tucci V, Moukaddam N. We are the hollow men: The worldwide epidemic of mental illness, psychiatric and behavioral emergencies, and its impact on patients and providers. *J Emerg Trauma Shock*. 2017; 10(1):4–6. [PubMed: 28243005]
3. Substance Abuse and Mental Health Services Administration. Prevention of Substance Abuse and Mental Illness. 2017. <https://www.samhsa.gov/prevention>, (accessed 29 Mar 2018)
4. American Psychiatric Association, et al. Diagnostic and statistical manual of mental disorders: DSM-5. American Psychiatric Association; 2013.
5. Luthi A, Lüscher C. Pathological circuit function underlying addiction and anxiety disorders. *Nat Neurosci*. 2014; 17(12):1635–43. [PubMed: 25402855]
6. Taylor AMW. Corticolimbic circuitry in the modulation of chronic pain and substance abuse. *Prog Neuropsychopharmacol Biol Psychiatry*. 2017
7. Ostroumov A, Dani JA. Convergent Neuronal Plasticity and Metaplasticity Mechanisms of Stress, Nicotine, and Alcohol. *Annu Rev Pharmacol Toxicol*. 2018; 58:547–566. [PubMed: 28977763]
8. Creed M, et al. Addiction therapy. Refining deep brain stimulation to emulate optogenetic treatment of synaptic pathology. *Science*. 2015; 347(6222):659–64. [PubMed: 25657248]
9. Duman RS, et al. Synaptic plasticity and depression: new insights from stress and rapid-acting antidepressants. *Nat Med*. 2016; 22(3):238–49. [PubMed: 26937618]
10. Lüscher C. The Emergence of a Circuit Model for Addiction. *Annu Rev Neurosci*. 2016; 39:257–76. [PubMed: 27145911]
11. Lammel S, et al. Reward and aversion in a heterogeneous midbrain dopamine system. *Neuropharmacology*. 2014; 76(Pt B):351–9. [PubMed: 23578393]
12. Beier KT, et al. Circuit Architecture of VTA Dopamine Neurons Revealed by Systematic Input-Output Mapping. *Cell*. 2015; 162(3):622–34. [PubMed: 26232228]
13. Tian J, et al. Distributed and Mixed Information in Monosynaptic Inputs to Dopamine Neurons. *Neuron*. 2016; 91(6):1374–1389. [PubMed: 27618675]
14. Li W, et al. Acute in vivo nicotine administration enhances synchrony among dopamine neurons. *Biochem Pharmacol*. 2011; 82(8):977–83. [PubMed: 21684263]
15. Zhang T, et al. Dopamine signaling differences in the nucleus accumbens and dorsal striatum exploited by nicotine. *J Neurosci*. 2009; 29(13):4035–43. [PubMed: 19339599]
16. Tarfa RA, et al. Enhanced Sensitivity to Hyperpolarizing Inhibition in Mesoaccumbal Relative to Nigrostriatal Dopamine Neuron Subpopulations. *J Neurosci*. 2017; 37(12):3311–3330. [PubMed: 28219982]

17. Barker DJ, et al. Multiplexed neurochemical signaling by neurons of the ventral tegmental area. *J Chem Neuroanat.* 2016; 73:33–42. [PubMed: 26763116]
18. Mejias-Aponte CA, et al. A subpopulation of neurochemically-identified ventral tegmental area dopamine neurons is excited by intravenous cocaine. *J Neurosci.* 2015; 35(5):1965–78. [PubMed: 25653355]
19. Yang H, et al. Nucleus Accumbens Subnuclei Regulate Motivated Behavior via Direct Inhibition and Disinhibition of VTA Dopamine Subpopulations. *Neuron.* 2018; 97(2):434–449 e4. [PubMed: 29307710]
20. Bonci A, Williams JT. Increased probability of GABA release during withdrawal from morphine. *J Neurosci.* 1997; 17(2):796–803. [PubMed: 8987801]
21. Melis M, et al. Long-lasting potentiation of GABAergic synapses in dopamine neurons after a single in vivo ethanol exposure. *J Neurosci.* 2002; 22(6):2074–82. [PubMed: 11896147]
22. Madhavan A, et al. Opioid-Induced GABA potentiation after chronic morphine attenuates the rewarding effects of opioids in the ventral tegmental area. *J Neurosci.* 2010; 30(42):14029–35. [PubMed: 20962224]
23. Madhavan A, et al. micro-Opioid receptor endocytosis prevents adaptations in ventral tegmental area GABA transmission induced during naloxone-precipitated morphine withdrawal. *J Neurosci.* 2010; 30(9):3276–86. [PubMed: 20203187]
24. Pan B, et al. D2 dopamine receptor activation facilitates endocannabinoid-mediated long-term synaptic depression of GABAergic synaptic transmission in midbrain dopamine neurons via cAMP-protein kinase A signaling. *J Neurosci.* 2008; 28(52):14018–30. [PubMed: 19109485]
25. Nugent FS, et al. Opioids block long-term potentiation of inhibitory synapses. *Nature.* 2007; 446(7139):1086–90. [PubMed: 17460674]
26. Polter AM, et al. Poststress block of kappa opioid receptors rescues long-term potentiation of inhibitory synapses and prevents reinstatement of cocaine seeking. *Biol Psychiatry.* 2014; 76(10):785–93. [PubMed: 24957331]
27. Polter AM, et al. Synaptic function and plasticity in identified inhibitory inputs onto VTA dopamine neurons. *Eur J Neurosci.* 2018
28. Simmons DV, et al. Differential expression of long-term potentiation among identified inhibitory inputs to dopamine neurons. *J Neurophysiol.* 2017; 118(4):1998–2008. [PubMed: 28701538]
29. van Zessen R, et al. Activation of VTA GABA neurons disrupts reward consumption. *Neuron.* 2012; 73(6):1184–94. [PubMed: 22445345]
30. Tan KR, et al. GABA neurons of the VTA drive conditioned place aversion. *Neuron.* 2012; 73(6):1173–83. [PubMed: 22445344]
31. Doyon WM, et al. Nicotine decreases ethanol-induced dopamine signaling and increases self-administration via stress hormones. *Neuron.* 2013; 79(3):530–40. [PubMed: 23871233]
32. Ostroumov A, et al. Stress Increases Ethanol Self-Administration via a Shift toward Excitatory GABA Signaling in the Ventral Tegmental Area. *Neuron.* 2016; 92(2):493–504. [PubMed: 27720487]
33. Tolu S, et al. Co-activation of VTA DA and GABA neurons mediates nicotine reinforcement. *Mol Psychiatry.* 2013; 18(3):382–93. [PubMed: 22751493]
34. Morozova EO, et al. Contribution of synchronized GABAergic neurons to dopaminergic neuron firing and bursting. *J Neurophysiol.* 2016; 116(4):1900–1923. [PubMed: 27440240]
35. Schindler AG, et al. Reversal of Alcohol-Induced Dysregulation in Dopamine Network Dynamics May Rescue Maladaptive Decision-making. *J Neurosci.* 2016; 36(13):3698–708. [PubMed: 27030756]
36. Kruse LC, et al. Maladaptive Decision Making in Adults with a History of Adolescent Alcohol use, in a Preclinical Model, Is Attributable to the Compromised Assignment of Incentive Value during Stimulus-Reward Learning. *Front Behav Neurosci.* 2017; 11:134. [PubMed: 28790900]
37. Eshel N, et al. Arithmetic and local circuitry underlying dopamine prediction errors. *Nature.* 2015; 525(7568):243–6. [PubMed: 26322583]
38. Schultz W. Dopamine reward prediction error coding. *Dialogues Clin Neurosci.* 2016; 18(1):23–32. [PubMed: 27069377]

39. Stauffer WR. Systems Neuroscience: Shaping the Reward Prediction Error Signal. *Curr Biol.* 2015; 25(22):R1081–4. [PubMed: 26583899]
40. Barrot M, et al. Braking dopamine systems: a new GABA master structure for mesolimbic and nigrostriatal functions. *J Neurosci.* 2012; 32(41):14094–101. [PubMed: 23055478]
41. Brown PL, et al. Habenula-Induced Inhibition of Midbrain Dopamine Neurons Is Diminished by Lesions of the Rostromedial Tegmental Nucleus. *J Neurosci.* 2017; 37(1):217–225. [PubMed: 28053043]
42. Zhou TC, et al. Cocaine drives aversive conditioning via delayed activation of dopamine-responsive habenular and midbrain pathways. *J Neurosci.* 2013; 33(17):7501–12. [PubMed: 23616555]
43. Vento PJ, et al. Learning From One's Mistakes: A Dual Role for the Rostromedial Tegmental Nucleus in the Encoding and Expression of Punished Reward Seeking. *Biol Psychiatry.* 2017; 81(12):1041–1049. [PubMed: 27931744]
44. Sanchez-Catalan MJ, et al. Response of the Tail of the Ventral Tegmental Area to Aversive Stimuli. *Neuropsychopharmacology.* 2017; 42(3):638–648. [PubMed: 27468916]
45. Kaufling J, et al. Pharmacological recruitment of the GABAergic tail of the ventral tegmental area by acute drug exposure. *Br J Pharmacol.* 2010; 161(8):1677–91. [PubMed: 21087442]
46. Matsui A, et al. Separate GABA afferents to dopamine neurons mediate acute action of opioids, development of tolerance, and expression of withdrawal. *Neuron.* 2014; 82(6):1346–56. [PubMed: 24857021]
47. Kaufling J, Aston-Jones G. Persistent Adaptations in Afferents to Ventral Tegmental Dopamine Neurons after Opiate Withdrawal. *J Neurosci.* 2015; 35(28):10290–303. [PubMed: 26180204]
48. Glover EJ, et al. Role for the Rostromedial Tegmental Nucleus in Signaling the Aversive Properties of Alcohol. *Alcohol Clin Exp Res.* 2016; 40(8):1651–61. [PubMed: 27388762]
49. Edwards NJ, et al. Circuit specificity in the inhibitory architecture of the VTA regulates cocaine-induced behavior. *Nat Neurosci.* 2017; 20(3):438–448. [PubMed: 28114294]
50. Gibson GD, et al. Distinct Accumbens Shell Output Pathways Promote versus Prevent Relapse to Alcohol Seeking. *Neuron.* 2018
51. Zhu Y, et al. A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature.* 2016; 530(7589):219–22. [PubMed: 26840481]
52. Harris GC, Aston-Jones G. Involvement of D2 dopamine receptors in the nucleus accumbens in the opiate withdrawal syndrome. *Nature.* 1994; 371(6493):155–7. [PubMed: 7915401]
53. Root DH, et al. The ventral pallidum: Subregion-specific functional anatomy and roles in motivated behaviors. *Prog Neurobiol.* 2015; 130:29–70. [PubMed: 25857550]
54. Tooley J, et al. Glutamatergic Ventral Pallidal Neurons Modulate Activity of the Habenula-Tegmental Circuitry and Constrain Reward Seeking. *Biol Psychiatry.* 2018
55. Bocklisch C, et al. Cocaine disinhibits dopamine neurons by potentiation of GABA transmission in the ventral tegmental area. *Science.* 2013; 341(6153):1521–5. [PubMed: 24072923]
56. Creed M, et al. Convergence of Reinforcing and Anhedonic Cocaine Effects in the Ventral Pallidum. *Neuron.* 2016; 92(1):214–226. [PubMed: 27667004]
57. Heinsbroek JA, et al. Loss of Plasticity in the D2-Accumbens Pallidal Pathway Promotes Cocaine Seeking. *J Neurosci.* 2017; 37(4):757–767. [PubMed: 28123013]
58. Stekete JD, Kalivas PW. Drug wanting: behavioral sensitization and relapse to drug-seeking behavior. *Pharmacol Rev.* 2011; 63(2):348–65. [PubMed: 21490129]
59. Knowland D, et al. Distinct Ventral Pallidal Neural Populations Mediate Separate Symptoms of Depression. *Cell.* 2017; 170(2):284–297 e18. [PubMed: 28689640]
60. Koo JW, et al. Loss of BDNF signaling in D1R-expressing NAc neurons enhances morphine reward by reducing GABA inhibition. *Neuropsychopharmacology.* 2014; 39(11):2646–53. [PubMed: 24853771]
61. Kennedy PJ, et al. Class I HDAC inhibition blocks cocaine-induced plasticity by targeted changes in histone methylation. *Nat Neurosci.* 2013; 16(4):434–40. [PubMed: 23475113]
62. Kaila K, et al. Cation-chloride cotransporters in neuronal development, plasticity and disease. *Nat Rev Neurosci.* 2014; 15(10):637–54. [PubMed: 25234263]

63. Taylor AM, et al. Microglia disrupt mesolimbic reward circuitry in chronic pain. *J Neurosci.* 2015; 35(22):8442–50. [PubMed: 26041913]
64. Thomas AM, et al. Adolescent Nicotine Exposure Alters GABAA Receptor Signaling in the Ventral Tegmental Area and Increases Adult Ethanol Self-Administration. *Cell Rep.* 2018; 23(1): 68–77. [PubMed: 29617674]
65. Nelson AC, et al. Ventral Tegmental Area GABA Neurons Are Resistant to GABA(A) Receptor-Mediated Inhibition During Ethanol Withdrawal. *Front Neurosci.* 2018; 12:131. [PubMed: 29556175]
66. Taylor AM, et al. Neuroimmune Regulation of GABAergic Neurons Within the Ventral Tegmental Area During Withdrawal from Chronic Morphine. *Neuropsychopharmacology.* 2016; 41(4):949–59. [PubMed: 26202104]
67. Vargas-Perez H, et al. BDNF signaling in the VTA links the drug-dependent state to drug withdrawal aversions. *J Neurosci.* 2014; 34(23):7899–909. [PubMed: 24899712]
68. Versace F, et al. Prequit fMRI responses to pleasant cues and cigarette-related cues predict smoking cessation outcome. *Nicotine Tob Res.* 2014; 16(6):697–708. [PubMed: 24376278]
69. Koob GF, Volkow ND. Neurobiology of addiction: a neurocircuitry analysis. *Lancet Psychiatry.* 2016; 3(8):760–73. [PubMed: 27475769]
70. Wang GJ, et al. Decreased dopamine activity predicts relapse in methamphetamine abusers. *Mol Psychiatry.* 2012; 17(9):918–25. [PubMed: 21747399]
71. Peachatka AL, et al. Cigarette craving is associated with blunted reward processing in nicotine-dependent smokers. *Drug Alcohol Depend.* 2015; 155:202–7. [PubMed: 26233484]
72. Audrain-McGovern J, et al. Where is the pleasure in that? Low hedonic capacity predicts smoking onset and escalation. *Nicotine Tob Res.* 2012; 14(10):1187–96. [PubMed: 22387990]
73. Buchel C, et al. Blunted ventral striatal responses to anticipated rewards foreshadow problematic drug use in novelty-seeking adolescents. *Nat Commun.* 2017; 8:14140. [PubMed: 28221370]
74. Janes AC, et al. Reward Responsiveness Varies by Smoking Status in Women with a History of Major Depressive Disorder. *Neuropsychopharmacology.* 2015; 40(8):1940–6. [PubMed: 25662839]
75. Taylor SR, et al. GABAergic and glutamatergic efferents of the mouse ventral tegmental area. *J Comp Neurol.* 2014; 522(14):3308–34. [PubMed: 24715505]
76. Brown MT, et al. Ventral tegmental area GABA projections pause accumbal cholinergic interneurons to enhance associative learning. *Nature.* 2012; 492(7429):452–6. [PubMed: 23178810]
77. Stamatakis AM, et al. A unique population of ventral tegmental area neurons inhibits the lateral habenula to promote reward. *Neuron.* 2013; 80(4):1039–53. [PubMed: 24267654]
78. Lammel S, et al. Diversity of transgenic mouse models for selective targeting of midbrain dopamine neurons. *Neuron.* 2015; 85(2):429–38. [PubMed: 25611513]
79. Zhang YF, Cragg SJ. Pauses in Striatal Cholinergic Interneurons: What is Revealed by Their Common Themes and Variations? *Front Syst Neurosci.* 2017; 11:80. [PubMed: 29163075]
80. Jennings JH, et al. Distinct extended amygdala circuits for divergent motivational states. *Nature.* 2013; 496(7444):224–8. [PubMed: 23515155]
81. Nieh EH, et al. Inhibitory Input from the Lateral Hypothalamus to the Ventral Tegmental Area Disinhibits Dopamine Neurons and Promotes Behavioral Activation. *Neuron.* 2016; 90(6):1286–98. [PubMed: 27238864]
82. Hewitt SA, et al. Altered chloride homeostasis removes synaptic inhibitory constraint of the stress axis. *Nat Neurosci.* 2009; 12(4):438–43. [PubMed: 19252497]
83. MacKenzie G, Maguire J. Chronic stress shifts the GABA reversal potential in the hippocampus and increases seizure susceptibility. *Epilepsy Res.* 2015; 109:13–27. [PubMed: 25524838]
84. Silvestre de Ferron B, et al. Increase of KCC2 in hippocampal synaptic plasticity disturbances after perinatal ethanol exposure. *Addict Biol.* 2017; 22(6):1870–1882. [PubMed: 27778437]
85. Ko MC, et al. Bumetanide blocks the acquisition of conditioned fear in adult rats. *Br J Pharmacol.* 2017

86. Tao R, et al. Transcript-specific associations of SLC12A5 (KCC2) in human prefrontal cortex with development, schizophrenia, and affective disorders. *J Neurosci.* 2012; 32(15):5216–22. [PubMed: 22496567]
87. Ben-Ari Y. NKCC1 Chloride Importer Antagonists Attenuate Many Neurological and Psychiatric Disorders. *Trends Neurosci.* 2017; 40(9):536–554. [PubMed: 28818303]
88. Shibasaki M, et al. Involvement of the K⁺-Cl⁻ co-transporter KCC2 in the sensitization to morphine-induced hyperlocomotion under chronic treatment with zolpidem in the mesolimbic system. *J Neurochem.* 2013; 125(5):747–55. [PubMed: 23565710]
89. Li Y, et al. Resveratrol modulates cocaine-induced inhibitory synaptic plasticity in VTA dopamine neurons by inhibiting phosphodiesterases (PDEs). *Sci Rep.* 2017; 7(1):15657. [PubMed: 29142291]
90. Creed M. Current and emerging neuromodulation therapies for addiction: insight from pre-clinical studies. *Curr Opin Neurobiol.* 2018; 49:168–174. [PubMed: 29524847]
91. Milosevic L, et al. Neuronal inhibition and synaptic plasticity of basal ganglia neurons in Parkinson's disease. *Brain.* 2018; 141(1):177–190. [PubMed: 29236966]
92. Lenz M, et al. Repetitive magnetic stimulation induces plasticity of inhibitory synapses. *Nat Commun.* 2016; 7:10020. [PubMed: 26743822]
93. Menegas W, et al. Dopamine neurons projecting to the posterior striatum form an anatomically distinct subclass. *Elife.* 2015; 4:e10032. [PubMed: 26322384]
94. Faget L, et al. Afferent Inputs to Neurotransmitter-Defined Cell Types in the Ventral Tegmental Area. *Cell Rep.* 2016; 15(12):2796–808. [PubMed: 27292633]
95. Omelchenko N, Sesack SR. Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. *J Comp Neurol.* 2005; 483(2):217–35. [PubMed: 15678476]
96. Nugent FS, et al. PKG and PKA signaling in LTP at GABAergic synapses. *Neuropsychopharmacology.* 2009; 34(7):1829–42. [PubMed: 19194373]
97. Castillo PE. Presynaptic LTP and LTD of excitatory and inhibitory synapses. *Cold Spring Harb Perspect Biol.* 2012; 4(2)
98. Mele M, et al. Role of GABAA R trafficking in the plasticity of inhibitory synapses. *J Neurochem.* 2016; 139(6):997–1018. [PubMed: 27424566]
99. Fiumelli H, et al. Modulation of GABAergic transmission by activity via postsynaptic Ca²⁺-dependent regulation of KCC2 function. *Neuron.* 2005; 48(5):773–86. [PubMed: 16337915]
100. Woodin MA, et al. Coincident pre- and postsynaptic activity modifies GABAergic synapses by postsynaptic changes in Cl⁻ transporter activity. *Neuron.* 2003; 39(5):807–20. [PubMed: 12948447]
101. Staley KJ, et al. Ionic mechanisms of neuronal excitation by inhibitory GABAA receptors. *Science.* 1995; 269(5226):977–81. [PubMed: 7638623]
102. Doyon N, et al. Chloride Regulation: A Dynamic Equilibrium Crucial for Synaptic Inhibition. *Neuron.* 2016; 89(6):1157–1172. [PubMed: 26985723]
103. Kyzozis A, Reichling DB. Perforated-patch recording with gramicidin avoids artifactual changes in intracellular chloride concentration. *J Neurosci Methods.* 1995; 57(1):27–35. [PubMed: 7540702]
104. Ostroumov A, et al. Cystic fibrosis transmembrane conductance regulator modulates synaptic chloride homeostasis in motoneurons of the rat spinal cord during neonatal development. *Dev Neurobiol.* 2011; 71(3):253–68. [PubMed: 21308994]
105. Kahle KT, et al. Modulation of neuronal activity by phosphorylation of the K-Cl cotransporter KCC2. *Trends Neurosci.* 2013; 36(12):726–737. [PubMed: 24139641]

Highlights

- Circuits within the mesocorticolimbic system play a critical role in shaping behavior.
- Experience-dependent, activity-dependent synaptic plasticity has most commonly been associated with excitatory neurotransmission, but inhibitory transmission also undergoes diverse forms of plasticity
- Addictive drugs and aversive stimuli trigger multiple forms of plasticity at inhibitory synapses leading to maladaptive behaviors.
- Experience-dependent alteration in chloride homeostasis is a form of inhibitory plasticity that mediates blunted reward signaling and increases intake of addictive drugs.
- Normalizing inhibitory transmission within the circuitry of the mesocorticolimbic system is a potential therapeutic target for treating mental illness and addiction.

Outstanding questions

What are the specific presynaptic and postsynaptic mechanisms that underlie the various forms of inhibitory plasticity?

How does inhibitory synaptic plasticity interact with excitatory plasticity during experience-dependent circuit remodeling?

How does ongoing neuronal activity regulate chloride homeostasis via transporters such as KCC2?

To what extent do changes in chloride homeostasis alter GABAergic signaling, moving it between the extremes from strongly inhibitory to excitatory? On what time scales do these changes in GABAergic signaling operate?

Beyond the midbrain GABAergic circuitry, what other circuits have alterations in chloride homeostasis following drugs of abuse or stress?

Box 1**Anatomical distribution of VTA inputs**

Besides DA neurons, the VTA contains neurons that release GABA, glutamate, and a combination of those neurotransmitters. Anatomical studies indicate that despite neurotransmitter type heterogeneity, VTA neurons receive qualitatively similar inputs with the largest projections coming from the striatum, the ventral pallidum and the dorsal raphe [12, 93, 94]. However, more detailed quantitative analysis reveals substantial differences within many projection regions. For example, striatal and globus pallidus neurons provide larger inputs onto DA neurons, whereas cortical and hindbrain areas project preferentially onto VTA glutamate and GABA neurons respectively [94]. Furthermore, DA neurons that project to different targets receive largely biased monosynaptic inputs from dorsal raphe and distinct subregions of the nucleus accumbens [12]. Additional differences can be observed at the cellular level within each input. For example, optogenetic and electron microscopy studies indicate that inhibitory projections from the lateral hypothalamus and laterodorsal tegmentum synapse preferentially on GABA, not DA, neurons in the VTA [81, 95]. In contrast, glutamatergic neurons from the laterodorsal tegmentum and neurons that release neuropeptides from the lateral hypothalamus exhibit preference for DA neurons in the VTA [12, 95]. To some degree, these anatomical studies support the currently prevailing view that distinct populations of VTA neurons participate in separate, but interconnected, circuits through their biased input and diverse output pathways [11, 12, 19, 76]. This view has been challenged, however, by findings suggesting that instead of integrating disparate signals from separable circuits, DA neurons receive highly redundant information that is mixed and distributed among multiple projections to the VTA [13]. The redundancy enhances reliability of information flow to the VTA, as it is less dependent on the precise synaptic connectivity between inputs and DA neurons [13].

Box 2**Forms of inhibitory synaptic plasticity**

Plasticity in inhibitory synapses can be presynaptic or postsynaptic. Long-lasting increases or decreases in presynaptic GABA release largely depend on two distinct intracellular pathways: activation of the adenylate cyclase/cAMP/PKA cascade and activation of the guanylate cyclase/cGMP/PKG cascade [20, 21, 24, 96, 97]. Activity-dependent activation or inhibition of these cascades can occur entirely at the presynaptic terminal, independent of postsynaptic activation [55, 97]. But in many cases, inhibitory plasticity is induced in a heterosynaptic manner – glutamate afferents activate NMDA or metabotropic receptors, leading to the release of retrograde messengers [24, 25]. Nitric oxide (NO), endocannabinoids, and BDNF are typical examples of retrograde messengers that activate intracellular pathways in presynaptic terminals and alter the probability of GABA release.

Postsynaptic mechanisms of inhibitory plasticity typically involve activity-dependent intracellular signaling cascades, such as protein kinases A and C, Ca^{2+} /calmodulin dependent kinases, and tyrosine kinases [98]. These mechanisms regulate GABA_A receptor membrane expression, subunit composition, phosphorylation, and trafficking. In addition, various environmental stimuli or drug exposure paradigms recruit epigenetic mechanisms that regulate GABA_A receptor subunit gene expression [61].

Another postsynaptic form of inhibitory plasticity is mediated by long-lasting shifts in the transmembrane gradient for Cl^- ions [32, 64, 99, 100]. The Cl^- gradient determines the reversal potential (E_{GABA}) for GABA_A receptor-mediated currents, and E_{GABA} relative to the cell's resting potential determines the strength of GABAergic inhibition. More negative values of E_{GABA} underlie the normally hyperpolarizing, inhibitory action of GABA_A receptor activity. In contrast, a depolarized E_{GABA} decreases GABA_A receptor-mediated inhibition, and in some cases, leads to paradoxical GABAergic excitation [32, 101]. Regulation of the Cl^- gradient across the cell membrane depends on the activity of anion transporters [102]. The K^+ , Cl^- co-transporter KCC2 is the main mechanism that extrudes Cl^- ions from neurons, whereas the Na^+ , K^+ , Cl^- co-transporter, NKCC1, loads Cl^- into neurons. Activity-dependent decreases in the strength of inhibitory transmission in the adult nervous system have been largely attributed to the functional down-regulation of KCC2 or up-regulation of NKCC1.

Box 3**Measuring Cl^- gradients at GABAergic synapses**

Given that even small changes in the intracellular Cl^- concentration lead to significant shifts in the GABA_A reversal potential (E_{GABA}), measuring these shifts requires preservation of the neuronal Cl^- levels. During electrophysiological recordings using patch-clamp techniques, the intracellular Cl^- concentration can be preserved by filling the patch pipette with the antibiotic gramicidin [103]. Gramicidin forms pores in the cell membrane that are permeable to cations, but not to anions, thus permitting an accurate estimation of E_{GABA} (Figure I-A), which is the hold potential (V_h) where the GABA_A current reverses direction from inward to outward. The main limitation of this method is that gramicidin-perforated patch-clamp recordings alter the intracellular potassium concentration, indirectly affecting the activity of KCC2 and other potassium-dependent Cl^- co-transporters. Therefore, in addition to direct measurements of the GABA_A reversal potential, investigators can estimate Cl^- transporter function. An established electrophysiological approach for comparing the efficiency of Cl^- -extruding transporters between different preparations involves repetitive stimulation of GABAergic inputs to produce high Cl^- influx into the cell (Figure I-B) [32, 82]. At a depolarized holding potential (e.g., $V_h=0\text{mV}$), Cl^- enters the cell, as indicated by an outward positive current (upward-deflection, Figure 1-B, left). A rapid reduction in the IPSC amplitude during repetitive stimulation is indicative of impaired chloride extrusion. In addition to functional measurements, expression of Cl^- transporters may be assessed directly using various molecular biology techniques. For example, using specific primers and antibodies, researchers demonstrate changes in the KCC2 and NKCC1 genes and protein expression during development and under certain pathological conditions [62, 104]. However, physiological stimuli, including stress and increased neuronal activity, modulate transporter function via post-translational modifications, such as phosphorylation or oligomerization of Cl^- transporters [105]. Direct measurements of protein post-translational modifications are often laborious, but the increasing availability of phospho-specific antibodies simplifies the detection of Cl^- transporter phosphorylation. In summary, a combination of electrophysiological and molecular approaches is an appropriate methodology used to detect changes in GABAergic signaling.

Figure I. Measuring Cl^- homeostasis in neurons

(A) To preserve the intracellular Cl^- concentration, evoked inhibitory postsynaptic currents (eIPSCs) are measured using whole-cell, gramicidin perforated-patch recordings at different holding potentials (V_h). The eIPSCs reverse direction at the E_{GABA} . (B) eIPSCs are measured in the whole-cell mode under the conditions that favor Cl^- entry ($V_h = 0$ mV). Normal regulation of chloride homeostasis involves the mechanism that extrudes Cl^- out of the neuron, thereby preventing intracellular chloride accumulation during repetitive GABA_A receptor activation. Preventing Cl^- accumulation maintains a high driving force for Cl^- entry and, therefore, high amplitude of GABA_A postsynaptic currents (black). Compromised Cl^- extrusion capability leads to intracellular Cl^- accumulation, a decreased driving force for Cl^- to enter the cell, and a progressive decrease in GABAergic postsynaptic currents (red). Modified from [32, 64].

Figure 1. Main GABAergic inputs onto VTA DA neurons

DA neurons (green) receive inhibitory inputs from local VTA GABA neurons (blue) and from GABA neurons that originate in the RMTg and NAc. Recent evidence indicates that distinct subregions of the NAc target different microcircuits in the VTA. D1-MSNs in the NAc medial shell recruit GABA_A and GABA_B receptors to inhibit DA neurons that project to the NAc medial (m) and lateral (lat) shell, respectively. D1-MSNs in the NAc lateral shell project predominantly to VTA GABA neurons, exerting indirect influence over inhibitory input onto DA neurons that send axons to the NAc lateral shell. Abbreviations: lat shell, nucleus accumbens lateral shell; m shell, nucleus accumbens medial shell; core, nucleus accumbens core; NAc, nucleus accumbens; RMTg, rostromedial tegmental nucleus; VTA, ventral tegmental area.

Figure 2. Divergent forms of cocaine-mediated synaptic plasticity in NAc target regions

D1-MSNs in the NAc project to VTA GABA neurons and to VTA-projecting neurons in the VP. D2-MSNs in the NAc also project to VTA-projecting neurons in the VP. Repetitive cocaine exposure induces LTP of GABA release from D1-MSN terminals in the VTA and VP. In contrast, similar exposure to cocaine leads to LTD of GABA release from D2-MSN terminals. The transmitter phenotype of VTA-projecting neurons in the VP is unknown (shown in black). Abbreviations: NAc, nucleus accumbens; VP, ventral pallidum; VTA, ventral tegmental area.

Figure 3, Key figure. KCC2-mediated synaptic plasticity in VTA GABA neurons

(A) Cl^- entering a VTA GABA neuron via the GABA_A receptor is transported out by the K^+ , Cl^- transporter, KCC2. In VTA GABA neurons, KCC2 maintains low intracellular Cl^- , which is required for the hyperpolarizing, inhibitory activity of GABA_A receptors. Stress, pain, withdrawal from nicotine and morphine (rightward arrow) all attenuate Cl^- extrusion capacity via decreased function of KCC2, leading to accumulation of Cl^- inside VTA GABA neurons (also see Box 2). (B) GABAergic activity is usually inhibitory (letter “i” in black circle). In response to stimulation of GABAergic input (yellow lightning bolt), VTA GABA neuron activity is decreased (“i” in circle). Decreased Cl^- extrusion (rightward arrow) shifts GABA_A receptor signaling from inhibition toward paradoxical GABAergic excitation of VTA GABA neuron (letter “e” in red circle). In this case, GABAergic input causes a depolarizing excitation (“e” in circle). In response to stimulation of GABAergic input (yellow lightning bolt), VTA GABA neuron activity is increased (“e” in circle). Inset currents modified from [32]. (C) In addition to causing local inhibition of DA neurons, VTA GABA neurons project to multiple regions within the mesocorticolimbic system. Many of these regions send reciprocal inhibitory projections back to the VTA GABA neurons. Abbreviations: BNST, bed nucleus of the stria terminalis; LDTg, laterodorsal tegmental nucleus; LH, lateral hypothalamus; LHb, lateral habenula; NAc, nucleus accumbens; PFC, prefrontal cortex; VP, ventral pallidum.