Neural circuit adaptations during drug withdrawal — Spotlight on the lateral habenula

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A B S T R A C T

Withdrawal after drug intake triggers a wealth of affective states including negative feelings reminiscent of depressive symptoms. This negative state can ultimately be crucial for relapse, a hallmark of addiction. Adaptations in a wide number of neuronal circuits underlie aspects of drug withdrawal, however causality between cellular modifications within these systems and precise behavioral phenotypes remains poorly described. Recent advances point to an instrumental role of the lateral habenula in driving depressive-like states during drug withdrawal. In this review we will discuss the general behavioral features of drug withdrawal, the importance of plasticity mechanisms in the mesolimbic systems, and the latest discoveries highlighting the implications of lateral habenula in drug addiction. We will further stress how specific interventions in the lateral habenula efficiently ameliorate depressive symptoms. Altogether, this work aims to provide a general knowledge on the cellular and circuit basis underlying drug withdrawal, ultimately speculating on potential treatment for precise aspects of addiction.

1. The cyclical tale of drug addiction

Drug addiction is a chronic psychiatric disorder defined by the repetitive compulsive intake of addictive substances, despite the negative consequences this behavior may cause (Koob and Le Moal, 2001). One of the hallmarks of drug addiction is its cyclical nature, comprising recurring periods of drug seeking, withdrawal, and relapse. Unintended relapse may occur both shortly after cessation of drug use (a period colloquially referred to as the “crash”), but can also take place after years of abstinence (Pickens et al., 2011). Interventions aimed at preventing relapse represent a particularly challenging task, but are also necessary to interrupt the addiction cycle (O’Brien, 2008).

Prior to substance dependence, most addictive drugs are typically mainly consumed to obtain rewarding effects such as elevated mood and pleasure (i.e. positive reinforcement). After binge intake, the absence of the drug from the system produces a negative withdrawal state (including symptoms reminiscent of clinical depression), which can potentially motivate drug intake to temporarily alleviate the aversive symptoms (i.e. negative reinforcement) (Barr et al., 2002; Everitt and Robbins, 2005; Koob and Le Moal, 2001). The experience of the withdrawal state is therefore not merely a self-inflicted byproduct of drug

addiction. Instead, it has a prominent role in the pathology, playing a key role in maintaining the cyclical nature of the addiction, and poses a serious hindrance for successful anti-addiction therapy. In this review we address the nature of the withdrawal state, assess its contributions to drug addiction pathology, and explore the neural substrates that underlie it.

1.1. Withdrawal as a driving force for relapse

Research in humans with drug addiction has outlined the symptomatology associated with the withdrawal state. The strength of this state depends on several factors, including the amount of drug that was consumed, the rate at which this occurred, which drug was taken, the time point within the withdrawal period, and individual differences (Piazza and Le Moal, 1996; Sinha, 2007; West and Gossop, 1994). Nevertheless, some common features exist. Typically, the withdrawal state is comprised of a combination of physical and psychological symptoms (Koob and Le Moal, 2001; West and Gossop, 1994). Prominent examples of the latter category are mood disturbances such as dysphoria (an intense state of distress) and anhedonia (a diminished capacity to experience or seek pleasure). These have been reported to

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occur during withdrawal from psychostimulants, opiates, cannabinoids, nicotine and alcohol (Barr et al., 2002; Hatzigiakoumis et al., 2011; Koob and Le Moal, 2001; Schuckit, 2016), representing common core symptoms for drug withdrawal. The intensity of these symptoms can even reach levels akin to those seen in major depressive disorder (Barr et al., 2002).

Symptoms of withdrawal arise quickly after abated drug intake. For alcohol and heroin, withdrawal symptoms peak around 72 h after the last dose. Similarly, for cocaine the short-lived pleasurable effects, which last mere minutes, can be rapidly followed by withdrawal symptoms that peak in the following hours and days (Kosten and O'Connor, 2003; Barr et al., 2002). While these symptoms decline over time as the abstinence progresses, it may still take several weeks or even months before withdrawal-associated mood disturbances go into full remission (Barr et al., 2002; Gawin and Kleber, 1986; Kosten and O'Connor, 2003).

Drug withdrawal-induced mood disturbances have been postulated to be a potent driving force for resumed drug use (Koob et al., 2014). In subjects with substance use disorder, positive correlations have been reported between the intensity of withdrawal symptoms such as anhedonia, and the degree of drug craving (Hatzigiakoumis et al., 2011). Anhedonia scores may also be predictive for the occurrence of relapse (Garfield et al., 2014; Hatzigiakoumis et al., 2011). Moreover, certain antidepressant drugs may have beneficial preventive effects on drug relapse (O'Brien, 2008). Finally, there is a considerable amount of literature from animal studies to suggest that drug withdrawal processes play a causal role in relapse behavior.

1.2. Animal studies on drug withdrawal and relapse

Research in animals can model important aspects of the course of drug addiction in humans. Indeed, several read-outs have shown that rodents that are forced to abstain from the intake of addictive drugs, exhibit signs of anhedonia. One seminal approach has been to gauge hedonic processes by implanting self-stimulation electrodes in reward-encoding brain regions of animals, like the posterior lateral hypothalamus (intracranial self-stimulation; ICSS). By titrating the available intensity of the self-stimulation, thresholds for reward detection can be determined. Rodents in withdrawal from psychostimulants, opiates, cannabinoids, nicotine or alcohol, all show increases in ICSS reward thresholds (Bruijnzeel et al., 2009; Cryan et al., 2003; Kenny and Markou, 2006; Koob et al., 2014; Ahmed et al., 2002). Moreover, drug withdrawal reduces the pleasurable effects of natural rewards, such as sucrose (Creed et al., 2016). While such studies show that hedonic processing is diminished in rodents undergoing withdrawal, they do not directly show that the withdrawal state is associated with an aversive dysphoric state for these animals. However, such evidence has been provided by alternative behavioral tests, such as conditioned place preference/aversion paradigms. When rodents repeatedly experience the pleasurable effect of various addictive drugs in a particular compartment, they develop a conditioned place preference for this environment. Instead, an opposite conditioned place aversion for an environment occurs when it is paired with the withdrawal state for a variety of drug classes, including psychostimulants, opiates and nicotine (Ettenberg et al., 1999; Zhou et al., 2013; Kenny and Markou, 2001; Schulteis and Koob, 1994). Yet another class of behavioral paradigms has been used to assess how animals in withdrawal cope with undesirable circumstances. For instance, in the forced swim test (FST) and the tail suspension test (TST), the extent of immobility that an animal exhibits when placed in an undesirable situation (i.e. being placed upside down in the TST, or being placed in water in the FST) is assessed. High immobility in these tests is thought to reflect behavioral despair, reminiscent of depressive-like symptoms (Castagné et al., 2011). During psychostimulant withdrawal, mice and rats display heightened immobility in both the TST and the FST (Cryan et al., 2003; Meye et al., 2015; Meye et al., 2016). Similar heightened immobility has been

observed with withdrawal for opiates, nicotine and alcohol (Anraku et al., 2001; Hirani et al., 2002; Roni and Rahman, 2014). In summary, a variety of behavioral tests suggest the occurrence of mood disturbances of anhedonic and dysphoric nature in rodents in withdrawal from several classes of addictive drugs.

Several animal studies link the mood disturbances during the drug withdrawal phase with a heightened proclivity for drug seeking. For instance, elevations in ICSS reward thresholds during cocaine withdrawal in rats highly correlate with the occurrence of escalated cocaine intake (Ahmed et al., 2002). Interestingly, the withdrawal state sensitizes behavioral responses to subsequent stressors, which can potentially rekindle drug reward seeking (Breese et al., 2005; Koob and Le Moal, 2001; Mantsch et al., 2016; Shaham et al., 2003; Sinha, 2007). Accordingly, several studies indicate that stressors experienced during the withdrawal state can produce anhedonic/dysphoric effects, as well as enhance drug seeking. One such study paired neutral cues to precipitated opiate withdrawal in rats. Presenting these aversive cues induced a conditioned negative withdrawal state, heightened ICSS thresholds, and caused large increases in self-administered heroin infusions (Kenny and Markou, 2006). Moreover, rats in withdrawal from self-administered nicotine exhibit elevated ICSS reward thresholds, and display an increased likelihood of reinstatement of nicotine seeking after a footshock stressor. Interestingly, pharmacological blockade of the corticotropin releasing factor (CRF) stress system was able to prevent both increased reward thresholds, and reinstated drug seeking (Bruijnzeel et al., 2009). Conditioned place preference tests have also been utilized to show that stressors during drug withdrawal trigger relapse behavior (Mantsch et al., 2016). Following this paradigm, in a recent study, mice were conditioned to associate a specific compartment to the rewarding effects of cocaine. This association was then extinguished by means of saline injections to the previously cocaine-paired environment. Subsequently, during cocaine withdrawal, these mice were exposed to swim stress. This reinstated a clear preference for the previously cocaine-paired room, with the extent of this reinstatement being positively correlated to the degree of the immobility response during the swim episode. Preventing this behavioral despair during withdrawal, by rebalancing affected neural circuitry (see Section 3), also prevented the drug reinstatement behavior (Meye et al., 2016).

Overall these studies show that the negative emotional state of drug withdrawal is an important contributor to renewed drug seeking, and as such plays a pivotal role in maintaining the drug addiction cycle. It is therefore of importance to unravel the neural substrates that are involved in the aversive withdrawal state. In the following sections we address the involvement of brain regions classically implicated in drug withdrawal, like the accumbens, the dopamine system, and the extended amygdala but also a newly identified prominent player, the lateral habenula.

2. Neural circuits adaptations in drug withdrawal

Drug withdrawal is characterized by diminished hedonic processing, dysphoria and enhanced stress-sensitivity. Analogously, during drug withdrawal, adaptations occur in neural circuits that encode rewards, but also in neural circuits encoding stress and emotions (Koob and Volkow, 2016). Together, these persistent multi-scale adaptations in a variety of brain structures alter the functionality of reward and stress-encoding, setting the basis for the negative symptoms emerging during withdrawal (Koob and Volkow, 2016).

A neural circuit with a prominent role in reward encoding is the midbrain dopamine system. Indeed, all classes of addictive drugs exert their reinforcing effects by targeting this neural circuit, and causing rapid transient increases in dopamine signaling in target areas, like the nucleus accumbens (NAc) (Di Chiara and Imperato, 1988; Lüscher and Ungless, 2006). During withdrawal, important alterations occur at different levels of function of the midbrain dopamine system, including synapses, ion channels, and structural compartments. Such

modifications also contribute to reorganize stress-encoding circuits (Koob and Volkow, 2016). In the following sections we describe how withdrawal from two major drug classes, namely psychostimulants and opiates, affects reward- and stress-encoding neural circuitry.

2.1. Alterations in the mesolimbic reward system during cocaine withdrawal

During drug withdrawal, dopamine (DA) signaling from midbrain structures is modified in striatal targets (Volkow and Koob, 2015). This points to important modifications at the level of the mesolimbic reward system.

Indeed, several studies have shown that cocaine withdrawal (1, 7, or 14 days after the last dose) leads to a reduction of basal dopamine levels within the nucleus accumbens (NAc) in rats (Puig et al., 2012; Robertson et al., 1991). Another study, showed that this decrease in dopamine levels occurred along with adaptations in NAc dopamine D1 and D2 receptors (D1R, and D2R) (Neisewander et al., 1994). Consistently, in humans, discontinuation of drug intake in cocaine abusers reduced striatal dopamine levels, as revealed by D2 receptor binding using positron emission tomography (Volkow et al., 1997). The exact nature of alterations in striatal dopamine signaling during withdrawal remains a debated topic however. Several reports indicated that DA levels in the ventral striatum are unchanged (Robinson et al., 1988; Kalivas and Duffy, 1993) or even increased during cocaine withdrawal (Imperato et al., 1992). The technological advances of modern neuroscience may allow to tackle again this issue and provide a defined solid description on whether levels of DA change during drug withdrawal.

In addition, synaptic changes in the VTA and the NAc during withdrawal have been abundantly described. Initial experimental evidence reported that cocaine exposure alters excitatory synaptic transmission onto VTA DA neurons one day after a single injection, indicating that drug experience profoundly modifies synaptic function (Ungless et al., 2001). The same type of plasticity occurred after cocaine self-administration persistent up to a month after the last infusion, supporting the idea that withdrawal from drug exposure hijacks VTA circuits (Chen et al., 2008). These findings indicate that initial excitatory synaptic changes onto VTA DA neurons are independent from the frequency of cocaine intake as a single dose produces adaptations similar to the extended access to the drug. The persistence of plasticity in the VTA is instrumental for adaptations occurring in downstream structures such as the NAc. A report in mice demonstrated that controlling the longevity of drug-evoked plasticity within the VTA decides whether or not adaptations occur in the NAc (Mameli et al., 2009).

In addition, alterations occur at glutamatergic synapses onto NAc medium spiny neurons (MSNs). In studies using self-administration of cocaine in rats, a portion of animals developed addiction-like traits (high motivation for cocaine, irrespective of negative consequences). Following a period of withdrawal these animals lacked NMDA receptor-dependent long-term depression (LTD) in the NAc, a form of plasticity that was instead preserved in non-addicted animals (Kasanez et al., 2010). Although these plastic changes were linked to the transition to addiction, their causality for withdrawal symptoms still needs to be clarified. Other studies, performed in mice instead, showed that strengthening of glutamatergic transmission occurs onto NAc (both in the core and the shell) MSNs during cocaine withdrawal (Wolf and Ferrario, 2010). At similar time points, AMPA receptor-mediated transmission is potentiated at excitatory synapses onto MSNs (Boudreau et al., 2007; Conrad et al., 2008; Kourrich et al., 2007; Mameli et al., 2009). This drug withdrawal-linked strengthening of AMPAR-mediated glutamatergic transmission occurred along with a switch in AMPAR subunit composition, from GluA2-containing to calcium-permeable GluA2-lacking ones (Conrad et al., 2008). Importantly, potentiation at excitatory synapses in the NAc occurs specifically at D1R- rather than D2R-expressing MSNs (Pascoli et al., 2011; Pascoli et al., 2014; MacAskill et al., 2014). Interestingly, input-specific drug-withdrawal-

evoked plasticity at excitatory synapses onto MSNs underlies susceptibility to relapse in mice (Conrad et al., 2008; Pascoli et al., 2014).

2.2. Alterations in the mesolimbic reward system during opiate withdrawal

Morphine withdrawal may be associated with reduced activity of DA neurons (Diana et al., 1995; Diana et al., 1999; Georges et al., 2006). Moreover, decreased levels of dopamine occur in the NAc during opiate withdrawal (Espejo et al., 2001). This is likely in part because morphine withdrawal causes synaptic potentiation of GABAergic inhibitory transmission onto DA neurons within the ventral tegmental area (Bonci and Williams, 1997; Madhavan et al., 2010; Meye et al., 2012).

Opiate withdrawal is also linked to increased glutamatergic signaling in the NAc, involving enhanced glutamate release and post-synaptic insertion of GluA2-lacking AMPARs (Hearing et al., 2016; Russell et al., 2016; Sepulveda et al., 1998). Interestingly, the strengthening of glutamatergic transmission and insertion of GluA2-lacking AMPARs during morphine withdrawal occurs particularly onto D1-MSNs in the NAc shell (Hearing et al., 2016). This may contribute to the manifestation of withdrawal symptoms and consequent relapse. Indeed, pharmacologically blocking glutamatergic signaling in the NAc in morphine-dependent animals in withdrawal, diminishes the manifestation of anhedonic (elevated ICSS thresholds) and dysphoric (conditioned place aversion) symptoms (Russell et al., 2016). The same study showed that even specifically blocking GluA2-lacking AMPARs in the NAc in the opiate withdrawal state is sufficient to attenuate the anhedonic symptom of ICSS threshold elevations.

2.3. Alterations in the stress system during drug withdrawal

A large set of studies has highlighted the contribution of the stress system to the symptoms of psychostimulant and opiate withdrawal. In particular, the stress signals dynorphin and CRF have been heavily implicated in mediating aversive withdrawal symptoms. These signals exert their effects within the mesolimbic system, but also in other circuits, especially in the extended amygdala (Koob and Volkow, 2016; Muschamp and Carlezon, 2013). Dynorphin signaling acts on Kappa opioid (K) receptors with anti-reward and aversive properties (Muschamp and Carlezon, 2013), and can be released by various neuronal populations during withdrawal. Chronic drug abuse, via a phasic surge in DA release, stimulates DA receptors in the NAc shell. Specifically, D1 receptor activation leads to cyclic adenosine monophosphate (cAMP)- and protein kinase A (PKA)-dependent production of dynorphin (Girault and Greengard, 2004). It has been proposed that by acting within the ventral striatum, dynorphin may reduce dopamine signaling, contributing to anhedonic properties emerging during drug withdrawal (Muschamp and Carlezon, 2013). Moreover, dynorphin can also be released from extended amygdalar neuronal populations to affect local neural circuitry (Crowley et al., 2016). A proposed hypothesis is that during drug withdrawal, elevated dynorphin-K receptor signaling in regions like the extended amygdala and ventral striatum, produces anxiety and mood disturbances that may contribute to relapse (Koob and Volkow, 2016; Shippenberg et al., 2007; Wee and Koob, 2010). Consistent with this, inhibition of K opioid receptors reduced drug withdrawal symptoms including anxiety, place preference/aversion and depressive-like symptoms (Kelsey et al., 2015; Mague et al., 2003; Pliakas et al., 2001; Schank et al., 2012).

Concomitantly with K opioid signaling, drug withdrawal engages the pivotal stress neuropeptide CRF, which acts via CRF1 and CRF2 receptors. CRF can be released from hypothalamic sites at the level of the paraventricular nucleus to engage the HPA-axis, but also from extrahypothalamic regions such as the extended amygdala. While both of these systems are affected by drug withdrawal, particularly the elevated CRF levels in extrahypothalamic regions have been linked to the emergence of the aversive negative withdrawal state (Logrip et al.,

2011).

CRF levels are indeed increased in extended amygdala nuclei like the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST) during withdrawal from various drugs, including psychostimulants and opiates (Logrip et al., 2011; Zorrilla et al., 2014). Importantly, causal links have been shown between the elevated CRF signaling, particularly through CRF1 receptors, in the extended amygdala and the occurrence of withdrawal symptoms and drug relapse. For instance, the infusion of CRF1 receptor antagonists in the CeA reduces heightened ICSS thresholds (Bruijnzeel et al., 2012) and heightened sensitivity to pain during nicotine withdrawal (Baiaamonte et al., 2014). Moreover, there are various studies indicating that antagonism of CRF signaling in the extended amygdala can prevent drug relapse during withdrawal (Zorrilla et al., 2014). Still, the relation between CRF levels in the extended amygdala and the emergence of withdrawal symptoms is likely complex. One study observed that virally-mediated overexpression of CRF in the CeA diminished ICSS threshold elevations during nicotine withdrawal. This may have been brought about by a preferential shift towards CRF-CRF2 receptor signaling (Qi et al., 2014).

Overall these findings are consistent with the idea that the drug withdrawal state and relapse are characterized by alterations in neural circuits linked to emotion and reward processing. In this light it is interesting that recent advances have also elucidated the involvement of previously unexplored neuronal circuits that crucially contribute to the negative aversive states, produced especially by withdrawal from psychostimulants. Namely, drug withdrawal-induced maladaptations in the lateral habenula (LHb) represent a neuronal substrate for the cocaine withdrawal state. The next paragraph will describe the synaptic and cellular modifications that occur within the LHb during drug withdrawal, and their repercussions for addictive behaviors.

3. A suspect for the cocaine crash: the lateral habenula

The lateral habenula (LHb) is a phylogenetically conserved structure located in a complex named the *epithalamus*. The LHb has a pivotal role in reward and aversion processing (Hikosaka, 2010) and has lately received attention because of its implications in neuropsychiatric disorders including depression and addiction (Lecca et al., 2014).

Indeed, initial studies suggested the occurrence of LHb neuronal hyperactivity in depressed patients (Morris et al., 1999). Moreover, in vivo electrophysiological studies in monkeys showed that the LHb played an important role in encoding information pertaining to punishments (Matsumoto and Hikosaka, 2007). These studies were a major drive for clinicians to test the efficiency of deep-brain stimulation in LHb for symptoms in mood disorders. In an initial single-case study, a depressed patient that was insensitive to pharmacological treatment, showed a striking amelioration during the continuous deep-brain stimulation of the LHb (Sartorius et al., 2010). These studies were the backbone for following work that intended to understand the neurobiological mechanisms behind the implication of the LHb in mood disorders. Using a strain of rats exhibiting congenital learned helplessness, Li et al. (2011) demonstrated that synaptic excitatory transmission onto LHb neurons is stronger in this animal model for depression. As outlined above, depressive-like negative emotional states, including anhedonia and dysphoria, are behavioral phenotypes that also emerge during drug withdrawal, with a key role in relapse behavior. This raised the hypothesis that specific aspects of addiction, and especially withdrawal symptoms, may rely on adaptations at the level of the LHb.

3.1. Altered synaptic inputs and firing of LHb neurons during cocaine withdrawal

Several studies have described that cocaine experience can alter both synaptic inputs to, and firing patterns of, LHb neurons. Recordings

in acute slices from mice prepared during withdrawal from repeated daily cocaine injections showed an increase in the strength of excitatory transmission onto LHb neurons that specifically send axons to the rostromedial tegmental nucleus (RMTg), rather than to the VTA. Namely, the amplitude of miniature excitatory postsynaptic currents, but not the frequency, was higher in cocaine treated mice (Maroteaux and Mameli, 2012; Meye et al., 2015). The expression mechanism of such cocaine-evoked plasticity was postsynaptic and involved the increase of GluA2-lacking, GluA1-containing, AMPA receptors at synapses onto LHb neurons (Maroteaux and Mameli, 2012; Meye et al., 2015). Moreover, the strengthening of glutamatergic inputs onto LHb neurons was persistent, lasting at least 14 days during the withdrawal period (Meye et al., 2015).

In a different study the authors examined the impact of cocaine exposure on LHb neuronal firing in anesthetized rats. Systemic cocaine injections led, although in a minor population of neurons, to an initial reduction in LHb activity (coinciding with the euphoric 'high') followed by an increase in activity at later time points (coinciding with the 'crash' period) (Jhou et al., 2013). Studies have also found increases in c-Fos/FOS in LHb neurons during cocaine withdrawal, including in those neuronal populations projecting to the RMTg, suggesting that this population of neurons may be hyperactive during withdrawal (Jhou et al., 2013; Zhang et al., 2013). Accordingly, in vivo recordings from anesthetized mice in cocaine withdrawal, showed elevated activity levels of LHb neurons that specifically projected to the RMTg rather than to other targets (Meye et al., 2015). The latter study also revealed that an important mechanism driving this hyperactivity may be a reduction in K^+ conductance, resulting in a higher membrane resistance in LHb neurons during cocaine withdrawal, a modification requiring GluA1-containing AMPA receptor trafficking (Meye et al., 2015). Converging evidence for this has come from a study in which rats were allowed to self-administer cocaine. Upon 7 days of withdrawal, recordings on brain slices from these animals revealed an increase in excitability and higher membrane resistance of LHb neurons (Neumann et al., 2014). These data, obtained in slices and in anesthetized animals in withdrawal from operator-mediated or voluntary drug administration, illustrate that cocaine withdrawal reliably leads to LHb overactivity.

A question arising from these studies is whether these synaptic modifications broadly occur at all LHb inputs, or at specific ones. A LHb afferent of particular note is the entopeduncular nucleus (EPN) (the rodent homolog of the primate globus pallidus interna), an output station of the basal ganglia. This habenular input region plays a prominent role in encoding aversive information (Hong and Hikosaka, 2008; Stephenson-Jones et al., 2016) and, likely through the habenula, gates neuronal responses in the midbrain to psychostimulants (Sasaki et al., 1990). Moreover, the EPN, which sends axons to the LHb capable of co-releasing glutamate and GABA, produces dysphoric phenotypes, when optogenetically stimulated (Shabel et al., 2012). Together these findings made the EPN a likely candidate LHb input at which the previously mentioned glutamatergic alterations would occur during withdrawal. However, we recently found that glutamate signaling at EPN to LHb synapses remains in itself unaffected during cocaine withdrawal, leaving the origin of such changes an open question (Meye et al., 2016). Nevertheless, we found that EPN output (a mixture of partially co-released GABA and glutamate) to the LHb was altered during cocaine withdrawal in an important but different way. During withdrawal, the GABA/AMPA ratio at EPN-LHb synapses was reduced, suggesting a diminished inhibitory control in this afferent projection. Along with these changes, the vesicular GABA transported (VGAT) diminished at EPN-LHb synapses, without modification in postsynaptic GABA_A receptor function. A reduced VGAT expression would be expected to functionally reduce vesicular GABA content, diminishing GABA release (Wang et al., 2013). Indeed, when challenged with trains of stimulation at 4 Hz, EPN-LHb GABA currents recorded in slices from cocaine withdrawal mice were profoundly reduced. This effect was promptly rescued when VGAT was virally-overexpressed specifically at EPN to

LHb synapses (Meye et al., 2016). It is possible that deficits at EPN-LHb GABAergic signaling during withdrawal extend beyond VGAT reductions. One study found that withdrawal from cocaine resulted in a decreased density of GABA immunolabeling in nerve terminals contacting LHb neurons (Meshul et al., 1998). This might suggest that aside from diminished transport of GABA into vesicles, also a reduction in GABA levels could occur. Together these results show that psychostimulant withdrawal coincides with multifactorial alterations in the LHb at the level of GABAergic, glutamatergic and intrinsic plasticity, which in conjunction make LHb neurons more excitable. We now turn to the ramifications that these LHb alterations may have for the emergence of withdrawal symptoms.

3.2. LHb hyperactivity as a cause for cocaine withdrawal symptoms

Optogenetic studies have shown that stimulation of LHb populations that control the midbrain results in aversive effects (Lammel et al., 2012; Stamatakis and Stuber, 2012). Consequently, counteracting the synaptic and output alterations in LHb neurons during withdrawal would be expected to diminish both aversive withdrawal symptoms, and relapsed drug seeking. Indeed, several lines of evidence support this idea, providing causal links between LHb hyperactivity and the aversive withdrawal state and relapse. First, pharmacological inactivation of the LHb of rats in cocaine withdrawal during heightened stress, caused reduced anxiety responses in the elevated plus maze, and reduced cue-triggered cocaine reinstatement (Gill et al., 2013). Furthermore, particular deep-brain stimulation protocols of the LHb also lead to reduced reinstated cocaine seeking (Friedman et al., 2010). Contrarily however, a recent study reported that pharmacological LHb inhibition may not be successful in diminishing foot shock-induced drug reinstatement, and instead can cause perseverant cocaine seeking, when the drug is not available (Zapata et al., 2017).

Second, preventing the potentiation of glutamatergic synapses onto LHb neurons during cocaine withdrawal (by means of overexpression of a dominant negative peptide), prevented the expression of behavioral despair in the forced swim test and tail suspension test in mice (Meye et al., 2015). Third, reverting diminished GABAergic signaling at EPN to LHb synapses during cocaine withdrawal (by means of synapse-specific VGAT overexpression) abolished both measures of behavioral despair, and proneness to drug reinstatement behavior as a consequence of stress in a conditioned place preference test (Meye et al., 2016).

While these convergent glutamatergic and GABAergic synaptic modifications in the LHb are crucial for the emergence of aversive effects during withdrawal, they appear not to be implicated in the rewarding properties of cocaine. Modification of neither the glutamatergic, nor the GABAergic LHb plasticity affected cocaine-induced place preference (Meye et al., 2015; Meye et al., 2016).

4. Conclusions and perspectives

The behavioral ramifications of drug withdrawal are complex, and along with it, the reorganization of neural circuits is also an intricate process. Despite major efforts from basic and clinical research, the neurobiological basis of drug withdrawal remains incompletely understood, and several important questions remain open. One question is through which exact mechanisms withdrawal-encoding brain regions are recruited by the withdrawal from addictive substances. Certainly for the LHb this is of interest, as it is debated if and how dopamine may directly act in the LHb. Although evidence of tyrosine hydroxylase positive terminals and dopamine transporters within the LHb has been reported, the functional aspects around DA release in this structure and its implications for drug-evoked adaptations remain unclear (Lammel et al., 2015; Root et al., 2015; Stamatakis et al., 2013). Moreover, given the importance of the stress system in mediating the withdrawal state (Section 2.3), how and if stress related peptides or stress hormones may

directly act on the LHb contributing to such state remains poorly addressed (Zhang et al., 2013).

Secondly, while the literature around the role of the LHb in cocaine withdrawal has evolved in the last few years, very little is still known about whether this structure represents a common substrate for withdrawal from drugs other than psychostimulants. As discussed above, opiates trigger profound negative affective states, however the contribution of the LHb in these remains elusive. Only recently it has been investigated how LHb neurons acutely react to activation of mu opioid receptors. The mu opioid receptor agonist DAMGO led to mixed responses spanning from hyperpolarization to depolarization in the LHb (Margolis and Fields, 2016). While this shows that opiates may directly act on the LHb, the functional, synaptic and behavioral repercussions of opiate exposure and withdrawal on habenular circuits remains an open issue.

Thirdly, how can we translate these results from fundamental research to implement therapeutical interventions? Approaches including deep brain stimulation (DBS) or transcranial magnetic stimulation appear to be particularly promising to treat aspects of addiction, allowing researchers and clinicians to directly target and remodel maladapted circuits (Creed et al., 2015; Kravitz et al., 2015). This is especially interesting in the context of LHb dysfunction. DBS within the LHb has already been shown to produce therapeutically beneficial effects in the context of depression, which is a disorder with a high comorbidity with drug addiction (Li et al., 2011; Sartorius et al., 2010). DBS appears to exert its beneficial effects by suppressing the hyperactivity that occurs in the LHb during depressive states (Lecca et al., 2016). Given that hyperactivity of the LHb also occurs during drug withdrawal, it would be interesting to test the efficiency of DBS-like approach within the LHb to ameliorate drug withdrawal symptoms. Indeed, some tentative evidence already suggests that DBS of the LHb may be beneficial in limiting drug intake in rodents (Friedman et al., 2010).

In conclusion, the field should put effort in providing a precise dissection of cellular mechanisms and a refined knowledge of the anatomical substrates ultimately required for the development of better interventions.

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