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Neural systems mediating the inhibition of cocaine-seeking behaviors

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Abstract

Over the past decades, research has targeted the neurobiology regulating cocaine-seeking behaviors, largely in the hopes of identifying potential targets for the treatment of cocaine addiction. Although much of this work has focused on those systems *driving* cocaine seeking, recently, studies examining the *inhibition* of cocaine-related behaviors have made significant progress in uncovering the neural systems that attenuate cocaine seeking. Such systems include the infralimbic cortex, nucleus accumbens shell, and hypothalamus. Research in this field has focused largely on the infralimbic cortex, as activity in this region appears to attenuate cocaine seeking during reinstatement and contribute to extinction learning. However, an overarching theory of function for this region that includes its role in other types of reward seeking and learning remains to be determined. Furthermore, the precise relationship between other regions involved in attenuating cocaine-seeking behavior and the infralimbic cortex remains unclear. Recent advances in the use of viral vectors combined with optogenetics, chemogenetics, and other approaches have greatly affected our capacity to investigate those systems inhibiting behavior dependent on cocaine-associated memories. This review will present current understanding regarding the neurobiology underlying the inhibition of such behaviors, especially focusing on the extinction of such memories, and explore how viral-vector targeting of specific brain circuits has begun to alter, and will continue to enrich, our knowledge regarding this issue.

Keywords

optogenetics; chemogenetics; extinction; reinstatement; infralimbic cortex

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1. Introduction

Cocaine addiction is characterized by cycles of relapse and repeated failure to maintain abstinence (Gass and Chandler, 2013; Kalivas, 2009; Volkow et al., 2016); as a result, much effort has been directed toward elucidating the neural mechanisms underlying cocaine-seeking behavior. Although most studies have specifically focused on those systems *driving* cocaine seeking, research over the past decade has increasingly investigated those systems responsible for *inhibiting* such behavior. In particular, work using animal models of cocaine seeking has significantly expanded our knowledge of the neurobiology underlying the extinction and inhibition of these behaviors, and thus most, though not all, of the findings presented herein are derived from such models.

For the sake of brevity, this review will use the term “cocaine-associated memories” to refer, in general, to memories and their associated behaviors dependent on a history of cocaine exposure or self-administration, including cocaine-seeking and -taking behaviors, as observed in both instrumental and associative learning paradigms. Much of the work examining cocaine-associated memories has depended on traditional pharmacological approaches of investigating the brain, which present significant limitations. With the advent and expansion of viral-based targeting combined with optogenetics, chemogenetics, and other approaches, which allow for investigation of the role of specific cell types, brain regions, and pathways with high temporal precision, researchers have increasingly adopted these techniques to broaden the range of questions that can be asked. The goals of the present review are to (1) describe the findings of animal studies that explore the brain regions involved in inhibition of cocaine-seeking behaviors; (2) examine how different neural structures interact to inhibit such behaviors; and (3) discuss how viral-vector targeting of specific brain circuits has altered our understanding of systems that inhibit cocaine seeking and how these techniques can open the door to new questions.

2. The use of animal models in studying the inhibition of behaviors dependent on cocaine-associated memories

Cocaine addiction is an exclusively human disease (Moorman et al., 2015) that involves a complexity of factors, from the social and economic to the developmental and molecular. Consequently, it is impossible for an animal model to capture the full complexity of factors involved in addiction; nevertheless, animal models have been especially useful in understanding specific components underlying addiction. In particular, such models have given us a greater understanding of the basic neural mechanisms involved in cocaine-taking and cocaine-seeking behavior. Although numerous methods of assessing cocaine-seeking behaviors in animals exist (Ahmed, 2012; Moorman et al., 2015), the following paragraphs provide an overview of approaches used to model drug taking and seeking, as well as those approaches used to understand the inhibition of behaviors dependent on cocaine-associated memories, particularly with relation to the findings discussed in this review.

Much research on the induction of cocaine-associated memories derives from two major approaches: Conditioned place preference (CPP) and cocaine self-administration. In CPP, a cocaine injection is repeatedly paired with a specific context in a two- or three-chambered

apparatus, whereas a vehicle injection is paired with the other chamber. After repeated pairings, rodents will “prefer” the cocaine-paired context when they are given access to the full apparatus, as indicated by an increase in the amount of time spent in that area (Gourley and Taylor, 2016; Shukla et al., 2017). A major drawback of CPP models is that experimenters administer cocaine to the animal and, consequently, the animal has no choice in whether to receive the drug. In contrast, in cocaine self-administration (see figure 1), animals engage in an instrumental behavior, often a nose-poke or a lever press for rodents, that produces the delivery of cocaine and, frequently, neutral stimuli, such as light and tone cues, that become associated with cocaine delivery. It is worth noting that, in examining the inhibition of cocaine seeking, some studies use cocaine-associated cues during the self-administration period (e.g. Gutman et al., 2017), whereas other do not (e.g. Peters et al., 2008a). Furthermore, there is also variability in precisely how the cues are used across different studies. Whether these differences in approaches alters the underlying neurobiology for inhibiting cocaine seeking remains unclear. Regardless, self-administration models enable animals to “choose” to engage in drug-seeking behavior and permit investigation into questions such as the strength of the motivation to obtain the drug.

However, CPP and self-administration both enable investigation into inhibitory processes related to the cocaine-associated behavior. Indeed, researchers frequently use extinction training, defined as “the omission of a previously delivered [...] reinforcer” (Lattal and Lattal, 2012), following self-administration or CPP training to examine the development of inhibitory neural systems in the face of a new contingency (i.e., lack of cocaine reward). Current theories of extinction learning suggest that, except in limited instances such as in the developing brain and during the reconsolidation window, extinction involves the formation of a new memory (Milad and Quirk, 2012; Myers and Davis, 2002; Otis et al., 2015; Quirk et al., 2010; Rescorla, 2001). Some of the most compelling arguments for this theory include work on subcellular correlates of learning and memory that show that extinction training brings about the same changes seen in other forms of new memory formation (Kelley, 2004). However, behavioral evidence also supports the theory that extinction learning involves the formation of a new memory. For example, spontaneous recovery, in which previously extinguished behaviors are expressed once more after a temporal delay, suggests that the original memory remains intact following extinction (Rescorla, 2001).

Researchers can also examine relapse-like behaviors, termed reinstatement, in both self-administration and CPP following either extinction-withdrawal or homecage-withdrawal from cocaine administration. To reinstate the original place preference in CPP following extinction, a single “priming” injection of cocaine is given to the animal. Alternatively, spontaneous recovery of the preference can occur if the animal is kept in its homecage for a sufficient amount of time before being placed back into the apparatus. Within self-administration paradigms, similar procedures are frequently used to induce reinstatement and assess relapse-like behaviors. Following extinction training, animals can be given priming injections of cocaine in order to drive cocaine seeking. Animals can also undergo cue-driven reinstatement, in which lever presses produce the cocaine-associated cues during the reinstatement session (but not the drug itself). Similarly, contextual associations can be used to induce reinstatement (or renewal) of cocaine-seeking behavior. Reinstatement by exposure to cocaine-associated cues, contexts, or the drug itself is intended to mirror the

human condition in which drug-addicted individuals report feelings of craving and higher motivation to obtain the drug when in the presence of drug paraphernalia or in contexts that remind them of the drug (Bonson et al., 2002; Crombag et al., 2008).

It should be noted that variations based on the self-administration model, including use of punishers and discriminative stimuli (DSs), provide other avenues for addressing the inhibition of cocaine taking and seeking. Paradigms that include punishers may be well suited for studying the suppression of cocaine-seeking responses in the face of adverse consequences. In punishment-based paradigms, cocaine-seeking responses can lead to the delivery of a noxious stimulus along with, or instead of, the drug and associated cues (Chen et al., 2013; Deroche-Gamonet et al., 2004; Pelloux et al., 2007; Vanderschuren and Everitt, 2004). Previous work using this approach reports that a minority of rats continue to engage in lever pressing for cocaine despite the potential for a negative consequence (Pelloux et al., 2007). Interestingly, resistance to punishment does not develop in rodents with only a brief history of drug taking, but only occurs in rodents with a long history of drug taking, suggesting that the motivation of such animals has been altered and arguably enhanced and/or that the inhibitory processes that would normally occur in the face of punishment have been altered (Pelloux et al., 2007). A significant caveat of punishment models is that the negative consequences of drug use in humans (e.g., loss of a job, imprisonment) frequently occur long after a drug-taking period and typically involve adverse consequences of a psychosocial, rather than physically painful, nature.

Like punishers, DSs can also be used in self-administration paradigms to investigate the inhibition of cocaine-associated behavior. However, in contrast to punishers, DSs indicate the availability, or lack thereof, of the cocaine reward thereby modulating drug-seeking and drug-taking behavior. Specifically, rodents can be trained to distinguish between a DS+, which indicates that an instrumental response will produce the cocaine reward, and a DS-, which indicates that the same response will not produce the cocaine reward (Gutman et al., 2016; Kearns et al., 2005; Yun and Fields, 2003). In some cases (Di Pietro et al., 2006), the DS+ and DS- serve as “background” stimuli that remain on for long periods of time (e.g., 30 min). In other cases, such as in our recent work (Gutman et al., 2016), the DS+ or DS- only appears for each discrete trial, forcing the animal to attend to the specific DS present on a trial-by-trial basis. Although there is no punishment for an incorrect instrumental response, rats demonstrate a clear ability to distinguish between the DSs and make the appropriate response (which includes no response for the DS-) with high accuracy (e.g., Gutman et al., 2016).

Although the neural systems underlying the inhibition of behaviors dependent on cocaine-associated memories in particular have received less attention than those driving cocaine-related behaviors, CPP, self-administration, and variations on these paradigms have allowed researchers to closely examine the neurobiological substrates associated with the inhibition of drug-seeking behaviors, as will be discussed in the next section (section 3).

3. Circuitry involved in the inhibition of behaviors dependent on cocaine-associated memories

3.1. Medial Prefrontal Cortex

3.1.1 The human medial prefrontal cortex—The prefrontal cortex (PFC) has long been associated with control over executive function and motivational processes. Complex cognitive skills, such as response inhibition, attention, cognitive control, and decision making, are all linked to this brain region (Bechara and Damasio, 2002; Bechara et al., 1996; Brewer et al., 2008). The ventromedial prefrontal cortex (vmPFC) is of particular interest in studies of relapse, as it is involved in decision making and suppression of inappropriate behaviors (Bechara et al., 1999; Bechara et al., 1998). Whereas functional imaging studies of individuals addicted to cocaine indicate that cocaine-associated cues increase activity in the anterior cingulate cortex and amygdala (Bonson et al., 2002; Childress et al., 1999; Garavan et al., 2000; Goldstein and Volkow, 2002), similar studies suggest that such cues decrease activity in the vmPFC (Bonson et al., 2002). Although it is difficult to directly examine neural correlates of relapse in humans, these findings raise the possibility that vmPFC functioning, or lack thereof, is an important determinant in the ability to inhibit cocaine use. Indeed, evidence indicates that substance-dependent (alcohol, methamphetamine or cocaine) individuals perform similarly to individuals with vmPFC lesions on a gambling task, indicating that vmPFC dysfunction and substance abuse are correlated with similar impulsive behavior (Bechara et al., 2001). Moreover, Brewer and colleagues (2008) found that vmPFC activity during a cognitive control task (stroop test) predicts the duration of abstinence in cocaine-addicted individuals, suggesting a direct relationship between vmPFC function and the inhibition of cocaine-seeking behavior.

When drawing comparisons between human and rodent work on the PFC, it is important to consider that, as the prefrontal cortex is considerably larger and shows a commensurate increase in the number of subregions in primates compared to rodents (Wise, 2008), it is difficult to create one-to-one comparisons between rodent and human PFC subregions. Some regions of primate PFC, such as dorsolateral PFC, may not truly exist in the rodent PFC (Wise, 2008). Moreover, functions housed within a single rodent PFC region may be spread across several human PFC regions. Indeed, evidence suggests that other regions of the human frontal cortices may play a role in inhibitory control. For example, evidence from studies using functional imaging and transcranial direct current stimulation suggest that the right inferior frontal gyrus is involved in response inhibition in stop-signal tasks (Li et al., 2006; Stramaccia et al., 2015). As poor inhibitory control is often a characteristic of human drug addiction, further investigation of this region, as well as other human PFC regions, may yield new insights into changes in response inhibition in humans dependent on cocaine use (for a more detailed discussion, see Beveridge et al., 2008). Finally, it is important to consider that even distinct regions in humans such as vmPFC may contain subregions that are difficult to isolate using current neuroimaging techniques. Due in part to this issue, different laboratories and publications may use “vmPFC” to refer to slightly different anatomical regions. Thus, although functional homologies are frequently identified between different species, caution is warranted in trying to determine whether such homologies are

also truly anatomical ones and whether anatomical homologies necessarily connote functional ones.

3.1.2. The rodent medial prefrontal cortex—Because the mPFC is a key node in the mesocorticolimbic circuitry regulating motivated behavior, including cocaine seeking (Kalivas, 2008; Kalivas et al., 2009), studies using animal models of cocaine seeking have closely examined the role of the mPFC in the control of behaviors dependent on cocaine-associated memories. The rodent mPFC is a collection of structures located in the medial anterior wall (Gass and Chandler, 2013; Heidbreder and Groenewegen, 2003; Moorman et al., 2015). It receives dopaminergic inputs from the ventral tegmental area, as well as glutamatergic inputs from thalamus, hippocampus, and amygdala (Hitchcott et al., 2007; Hoover and Vertes, 2007). The ventral portion of the mPFC, encompassing the IL and parts of the orbitofrontal cortex, projects preferentially to the nucleus accumbens (NA) shell (Gorelova and Yang, 1997) and prelimbic cortex (PL) (Fisk and Wyss, 1999; Ji and Neugebauer, 2012), as well as to a variety of limbic regions including the hypothalamus and amygdala (Gourley and Taylor, 2016; Killcross and Coutureau, 2003). The dorsal portion of the mPFC, composed of the PL and the anterior cingulate cortex, projects preferentially to the NAc core (Gorelova and Yang, 1997) and the dorsomedial striatum (McGeorge and Faull, 1989). The projections from the PL and IL to the NA are of particular importance for examining cocaine-associated memories, as evidence suggests that these differential projections allow dichotomous control over the NA and associated cocaine-seeking behavior (Gass and Chandler, 2013; Kalivas, 2009; Moorman et al., 2015; Peters et al., 2009).

3.1.3. The IL and the inhibition of behaviors dependent on cocaine-associated memories—Early evidence for the role of the IL (or vmPFC) in the extinction or inhibition of previously learned behaviors emerged from work with auditory fear conditioning where findings suggested that the IL is necessary, and encodes, for such extinction learning (Do-Monte et al., 2015; Laurent and Westbrook, 2009; Milad and Quirk, 2002; Sierra-Mercado et al., 2011; Vidal-Gonzalez et al., 2006). Of notable relevance to the importance of using more sophisticated approaches to these questions, recent work using viral vector targeting with optogenetics found that optically inhibiting IL during extinction training for auditory fear conditioning impaired the formation of the extinction memory, but, in contrast to prior thinking about the role of the IL, such inhibition during retrieval did not alter the extinction expression (Do-Monte et al., 2015).

Nonetheless, based on the early fear conditioning findings, work expanded into the investigation of the role of the IL in extinction and inhibition of cocaine seeking. Prior work found that IL inactivation, via intra-IL administration of the GABA agonists baclofen and muscimol, has no effect on cocaine-induced reinstatement (McFarland and Kalivas, 2001), suggesting that IL activity does not *drive* cocaine seeking. Based on these findings, as well as the dearth of systematic investigations into the neural systems underlying the expression of extinction for cocaine seeking, Peters et al. (2008a) directly examined whether the IL mediates the extinction of cocaine seeking. The results indicated that IL inactivation, via baclofen and muscimol, increases active lever presses during a standard extinction session (i.e., no reinstatement triggers present) in rats that have undergone cocaine self-

administration and extinction training. Within the same behavioral paradigm, however, PL and basolateral amygdala (BLA) inactivation have no effect on cocaine seeking during extinction. Indeed, PL or BLA inactivation *prevents* the increased lever pressing induced by IL inactivation. Similarly, other findings indicate that IL inactivation potentiates the spontaneous recovery of cocaine seeking (Peters et al., 2008b).

Conversely, evidence suggests that IL activation, via administration of AMPA or PEPA (an allosteric AMPA receptor potentiator), reduces cocaine-primed and cue-induced reinstatement, respectively (LaLumiere et al., 2012; Peters et al., 2008a). Together, these results suggest that, at least following extinction training, IL activity acts like a brake on the cocaine-seeking system and that removal of the brake leads to “normal” cocaine seeking dependent on structures already known to drive this behavior. Moreover, as PEPA only potentiates AMPA receptor activity in the presence of the endogenous ligands (i.e., glutamate), these findings strongly indicate that, following extinction training, the IL normally acts to suppress cocaine seeking and that such activity can be enhanced to “override” the reinstatement of this behavior. Furthermore, evidence using a DS-based cocaine self-administration task suggests that IL inactivation increases responding to the DS – (i.e., pressing the lever when no cocaine infusion is available) (Gutman et al., 2016). These results suggest the potential for a broader role for the IL in inhibiting instrumental responses when such responses are not adaptive. Studies using other paradigms and approaches have generally confirmed the basic findings regarding the IL and cocaine seeking (see table 1).

Although the studies described above primarily focused on the role of the IL in the ongoing inhibition of cocaine seeking following extinction training, these results do not indicate whether the IL is involved in extinction learning itself – i.e., during early extinction training when the animal learns the new contingencies regarding lever presses. Investigating this issue, later work found that potentiating and inhibiting IL activity immediately after each extinction session for the first five days of extinction enhances and impairs, respectively, the retention of the extinction learning for cocaine seeking (LaLumiere et al., 2010), suggesting a role for the IL in the consolidation of early extinction learning. Similarly, evidence suggests that blocking and potentiating NMDA receptors in the IL impairs and enhances, respectively, the consolidation of extinction for cocaine seeking (Hafenbreidel et al., 2014). Findings also indicate that activation and blockade of β -adrenergic receptors in the IL enhances and impairs, respectively, the retention of the extinction for cocaine seeking (LaLumiere et al., 2010), consistent with a role for the IL noradrenergic system in the extinction of tone fear conditioning (Mueller et al., 2008). Studies using cocaine CPP have also found a role for the IL in extinction learning, as optogenetic inhibition of IL principal cells during extinction training impairs extinction learning, as indicated by increased preference for the cocaine-paired side of the chamber even following extinction training (Van den Oever et al., 2013).

Employing viral vector targeting, recent work from our laboratory used a “closed-loop” optogenetic approach to determine whether specific temporal periods of IL activity are necessary for the extinction of cocaine-seeking behavior (Gutman et al., 2017). Rats underwent cocaine self-administration, followed by shortened extinction training sessions, during which active lever presses triggered optical inhibition of the IL for 20 s. We found

that such inhibition increased active lever pressing during the extinction session and led to higher levels of active lever pressing during subsequent cue-induced cocaine seeking during which no inhibition was provided. Non-contingent inhibition – that is, inhibition given in a manner unrelated to the lever pressing – had no effect on cocaine seeking. These results suggest that IL activity during a temporally limited window following unreinforced active lever presses is involved in the extinction “encoding” and provide further support for IL involvement in the new contingency learning that underlies the extinction of cocaine seeking. However, *how* the IL encodes such learning remains unclear due to the paucity of studies examining this issue. In vivo electrophysiological recordings in the IL have identified neurons that significantly alter their firing following rewarded lever presses during self-administration and also unrewarded lever presses during a single cued cocaine-seeking session, suggesting the possibility that such activity is encoding the extinction contingencies (West et al., 2014). However, the authors of this study focused on mPFC (IL and PL) encoding for incubation of cocaine craving, rather than extinction, and thus interpreted their findings in that light, making it difficult to draw stronger conclusions regarding specific IL activity and the extinction of cocaine seeking.

Other studies, focusing on the connections between the mPFC subregions and NA subregions, provide further support for the involvement of the IL in the inhibition of cocaine seeking and extend this work to systems-level analyses. Using Cre-dependent Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), Augur et al. (2016) found that selective activation of IL projections to the NAc attenuates cue-induced, but not cocaine-primed, reinstatement. Other work targeting this pathway examined synaptic changes in mPFC neurons projecting to the NA following 6-hour cocaine self-administration and a long home-cage withdrawal period as part of an “incubation of craving” paradigm (Ma et al., 2014). Their findings indicate that IL-to-NAc synapses are unsilenced following the incubation period through the insertion of calcium-permeable AMPA receptors and that reversal of this unsilencing using a long-term depression protocol increases cocaine seeking. Of particular interest, this study did not use extinction training and yet observed changes in the IL-NAc circuit that appear to underlie the inhibition of cocaine seeking. In contrast, other evidence suggests that IL inactivation on the first day of extinction training does not alter cocaine seeking (Peters et al., 2008a). Similarly, findings indicate that chemogenetic activation of the IL reduces cue-primed reinstatement following extinction training but not following home-cage withdrawal (Augur et al., 2016). Thus, whether extinction training is *necessary* for the IL to inhibit cocaine seeking remains unclear. Nonetheless, accumulating evidence points to a critical role for the IL in inhibiting cocaine-seeking behaviors.

Despite many studies indicating a role for the IL in the suppression of cocaine seeking, as noted above, not all studies investigating the role of the IL link this region to the inhibition of cocaine seeking. Koya and colleagues (2009) found that IL inactivation, via GABA agonists, *decreases* responding on the active lever during a context-induced reinstatement session following a 30 day withdrawal period after long access (6 hours/day) cocaine self-administration. Similarly, findings regarding dopaminergic activity within the IL have also revealed a more complex picture of IL control over cocaine seeking. Recent work from our laboratory indicates that D1, but not D2, receptor blockade in the IL reduces cue-induced, but not cocaine-primed, reinstatement of cocaine seeking (Cosme et al., 2016). Outside of

this recent study, however, relatively little work has investigated the role of IL dopamine with regard to the inhibition of cocaine-associated memories, and further work in this area may yield important insights into the IL function in directing reward-seeking behavior (Peters et al., 2013). The reasons for the differences in the role of the IL in cocaine seeking discussed above are not clear but likely involve a variety of factors, including the precise type of manipulation and paradigm differences.

Relatedly, it is critical to note that, despite evidence for a role of the PL in *driving* cocaine seeking (Gutman et al., 2017; McFarland and Kalivas, 2001; McFarland et al., 2003; Peters et al., 2008a; Stefanik et al., 2013), some studies suggest a role for the PL in *inhibiting* cocaine-seeking. Chen and colleagues (2013) used a punishment-based self-administration paradigm in which 30% of cocaine-seeking responses resulted in the delivery of a footshock. Their findings indicate that optogenetic stimulation and inhibition of the PL suppresses and promotes, respectively, cocaine seeking in rats that continue to engage in cocaine self-administration despite the footshock. Similarly, in our work using a DS-based cocaine self-administration paradigm, we found that pharmacological inactivation of the PL (in addition to the IL) decreases overall task accuracy and increases responding to the DS– (Gutman et al., 2016). Using a similar DS paradigm, Mihindou and colleagues (2013) also found that pharmacological inactivation of the PL disinhibits responding during the DS–, suggesting that the PL is necessary for inhibitory control of cocaine seeking in at least some circumstances. Using a sucrose self-administration task, Trask and colleagues (2017) found that PL pharmacological inactivation suppresses operant responding when animals are tested in the original acquisition context, but not in a new context, suggesting that the capacity of the PL to attenuate operant responding is dependent on context. These findings raise the distinct possibility that the roles of the mPFC subdivisions (IL and PL) in extinction learning and cocaine seeking are not as clearly dichotomous as some of the evidence might suggest and that the precise nature of each subdivision's role likely depends on the type of task being used.

3.1.4. Role of IL activity in regulating motivated behavior for other drugs of abuse and “natural” rewards—Although this review focuses on the inhibition of behaviors dependent on cocaine-associated memories specifically, the question of whether the role for the IL in the inhibition of cocaine seeking generalizes to other drugs of abuse or to “natural” rewards is an especially salient issue. Nonetheless, the role of the IL in these other domains is not as clear. Studies of alcohol self-administration suggest that activity-dependent ablation of selective IL sub-populations increases alcohol seeking during cue-induced reinstatement (Pfarr et al., 2015). The authors did not observe the same effect when the entire structure was ablated. Furthermore, the ablation of cells involved in cue-induced reinstatement did not increase levels of stress-induced reinstatement. This suggests the involvement of precise circuits within the IL in the inhibition of alcohol seeking and possibly drug seeking as a whole. Other work on ethanol seeking suggests that restoration of normal mGluR2 expression in the IL abolishes escalation of ethanol seeking (Meinhardt et al., 2013). Similarly, findings suggest that mGluR5 activation via systemic administration of mGluR5 positive allosteric modulator CDPBB decreases active lever responding and increases extinction learning for alcohol seeking, effects that are dependent on the mGluR5

receptors in the IL (Gass et al., 2014). Evidence from work with alcoholic-beer seeking indicates that extinction training activates projections from the IL to the medio-dorsal hypothalamus (Marchant et al., 2010), consistent with a role for this hypothalamic region in extinction and inhibition, as described more below. However, other evidence indicates that pharmacological inactivation of the IL has no effect on extinction learning, context-induced reinstatement, or the reacquisition of beer seeking (Willcocks and McNally, 2013).

Likewise, the evidence regarding how IL activity influences opiate-seeking behavior is not consistent. For example, work suggests that IL activation with PEPA facilitates extinction learning and attenuates cue-primed reinstatement of heroin seeking (Chen et al., 2016). Furthermore, IL inactivation with GABA agonists in a heroin CPP model elicits reinstatement of place preference following extinction (Ovari and Leri, 2008). However, other studies suggest that the IL is involved in *driving* heroin seeking. IL inhibition with GABA agonists decreases heroin seeking in cue-induced and drug-primed reinstatement (Rogers et al., 2008). Work using retrograde tracing indicates a role for IL neurons projecting to the NAc shell in context-induced reinstatement of heroin seeking (Bossert et al., 2012), suggesting that the same pathway (IL-NAc shell) differentially modulates drug seeking for cocaine and heroin.

With regard to natural reward seeking, work using a DS-based task for either standard food pellets or sucrose indicates that IL inactivation increases responding to the DS- (Gutman et al., 2016; Ishikawa et al., 2008), suggesting that IL activity inhibits maladaptive responding for natural rewards. However, recording studies indicate more heterogeneity of IL activity during sucrose seeking. In vivo electrophysiological recordings suggest that, during a DS-based sucrose-seeking task, IL neurons are active both during periods where rats should press the lever in order to obtain a reward and when rats should withhold lever pressing when no reward is available (Moorman and Aston-Jones, 2015). In a separate experiment, authors also found that pharmacological inactivation of the IL, but not PL, reduces sucrose seeking during the DS-based task and yet that IL and PL inhibition during extinction training for this task *impairs* retention of extinction learning. However, in our recent work in which we found a temporally restricted window of IL activity important for the extinction of cocaine seeking (discussed earlier), we used our closed-loop optogenetic paradigm to examine the extinction of food seeking and found no effect of silencing IL activity immediately after unreinforced lever presses during extinction training (Gutman, 2015).

Because it is highly unlikely that the IL functions solely to suppress cocaine seeking, it is important to consider the cocaine-seeking literature in the broader context of studies that use different paradigms to examine IL function. Yet, the evidence described above points to a complex picture in which caution is warranted before reaching wide-ranging conclusions regarding the IL and the inhibition/extinction of drug and natural reward seeking. Several points should be made regarding this issue. First, many studies examining the IL and drug or reward seeking have used different manipulations and different behavioral procedures to do so. These discrepancies raise the potential problem that, while subtle differences may exist between how the IL regulates the extinction of different drugs of abuse or natural rewards, the larger differences in the approaches used to address those questions may exacerbate and/or mask the underlying similarities and differences in the neurobiology for each. For

example, it may be that chronic cocaine and opiates have slightly different effects on basic IL functioning and that these differences are magnified by the relatively imprecise approaches that have been used in the past. Small differences in procedures may also explain the apparent discrepancies within studies using the same drug (e.g., opiate studies). Second, with regard to natural reward seeking vs. drug seeking, considerable evidence points to non-cortical systems playing a critical role in food-seeking behavior vis-à-vis their role in drug-seeking behavior, most likely due to the important nature of homeostatic control for natural rewards (DiLeone et al., 2012; Volkow et al., 2013). Indeed, akin to the discrepancies with regard to the IL, prior work has found that PL inactivation does not alter the reinstatement of food-seeking behavior (McFarland and Kalivas, 2001). Therefore, the roles of mPFC, and the IL specifically, in natural reward-seeking behavior may require careful behavioral manipulations in order to bring the mPFC “online”. For example, the use of satiated rodents along with highly palatable natural rewards may be of particular importance. Finally, and perhaps most importantly, the advent of advanced approaches, such as the viral targeting approaches discussed herein, will likely play a critical role in resolving the discrepancies.

3.1.5. Theories of IL function—As a prefrontal cortical structure, the IL likely has a role in complex processing beyond merely the inhibition of cocaine seeking, though elucidating the core function(s) of the IL remains difficult. Studies examining habit formation suggest that the IL is especially important for the expression of habitual behavior. Killcross and Coutureau (2003) found that rats with selective IL lesions remain goal-directed despite overtraining on an operant task for food or sucrose reward, whereas those with PL lesions, but an intact IL, become insensitive to goal devaluation, suggesting that the switch to habit-based behavior may be an IL function. Other work has used T-maze training, in which rats are overtrained to run to a particular arm in response to a specific cue, to examine the role of the IL in habit formation. Findings using this task suggest that optogenetic disruption of IL activity impairs the expression of habitual behavior (Smith et al., 2012), in accordance with previous work using in vivo electrophysiology suggesting that IL activity is necessary for the formation and expression of habit (Smith and Graybiel, 2013).

The role of the IL in habit formation vs. the extinction/inhibition of cocaine seeking creates an apparent conflict, as these two functions would appear to be incompatible with one another. Barker et al. (2014) has proposed that IL function is similar in both extinction learning and habit formation, but the confines of task designs serve to create an artificially distinct dichotomy of function. The authors suggest that the IL may actively inhibit established action-outcome mediated behavior, promoting the acquisition of habitual behavior as well as extinction learning. Nonetheless, a problem in trying to resolve these issues lies in the comparison between IL functioning in “normal” learning tasks vs. its functioning in animals that have been exposed to chronic cocaine, as evidence indicates that cocaine self-administration produces regressive plasticity in the mPFC (Radley et al., 2015). It should be noted that theories of IL function are not limited to models of reward seeking or regulation of behavioral flexibility. Other investigators propose that the IL has a prominent role in the expression of executive function (Cassaday et al., 2014). Yet others, propose that, through its connections with regions that signal outcomes such as reward or pain, the central role of the IL lies in the regulation of adaptive emotional responses in processes of decision

making (Euston et al., 2012). How these roles fit into a larger theory of IL functioning that adequately explains the diversity of findings, including the inhibition of cocaine seeking, remains to be determined via future work.

3.2. Other regions involved in the inhibition of behaviors dependent on cocaine-related memories

3.2.1. Nucleus Accumbens—The NA receives inputs from limbic and cortical regions including the amygdala, hippocampus and PFC, and projects to downstream motor-related regions. Activation of medium-spiny neurons, the principal cells in the nucleus accumbens, is critical for execution of motivated behavior including cocaine seeking (Wolf, 2010). Evidence suggests that the core and shell subdivisions of the NA function in considerably different manners to influence cocaine-seeking behavior (LaLumiere et al., 2012; McFarland and Kalivas, 2001; McFarland et al., 2003; Peters et al., 2008a). The NAc core receives mPFC inputs primarily from the PL and anterior cingulate cortex, and evidence suggest that the PL-NAcore pathway, as well as the NAc core itself, promotes cocaine-seeking behavior (Stefanik et al., 2013). In contrast, the IL projects (preferentially) to the NAc shell and, as noted above, this pathway appears to be important for the inhibition of cocaine-seeking behavior (Augur et al., 2016; Ma et al., 2014).

Early work suggested a critical role for the NAc shell in the extinction of cocaine seeking, as findings indicated that extinction training following cocaine self-administration decreases AMPA receptor expression in the NAc shell and that overexpression of these receptors facilitates the extinction of cocaine-seeking behavior (Sutton et al., 2003). Subsequent work found that, as with IL manipulations, NAc shell activation and inactivation decreases and increases, respectively, cocaine seeking (LaLumiere et al., 2012; Peters et al., 2008a). However, it should be noted that other work has found that activation and blockade of dopamine receptors in the NAc shell promotes and inhibits cocaine seeking, respectively (Anderson et al., 2003; Anderson et al., 2008; Bachtell et al., 2005; Schmidt et al., 2006), indicating heterogeneity in how the NAc shell regulates cocaine-seeking behavior. Findings indicate that the NAc shell modulates cocaine seeking depending on which receptors are activated (glutamatergic vs. dopaminergic), suggesting that the activity of different inputs and, therefore, the activation of different neuronal ensembles within the NAc shell drives these different effects on cocaine seeking (LaLumiere et al., 2012).

Indeed, the use of viral vector targeting to control activity in specific pathways confirms that, at minimum, the glutamatergic projection from the IL to the NAc shell inhibits cocaine seeking (Augur et al., 2016; Ma et al., 2014).

Recent work also indicates that optical stimulation and inhibition of NA cholinergic neurons enhances and impairs, respectively, the extinction of cocaine CPP (Lee et al., 2016). Although the medial NA (i.e., primarily shell) was targeted, it is difficult to know whether these findings are specific to the NAc shell due to the relatively small size of the NA in mice and the spread of virus and illumination. Nonetheless, and of considerable interest, the authors found that both CPP extinction alone and optical stimulation of NA cholinergic neurons during CPP extinction reduce glutamatergic presynaptic strength onto the MSNs of the NA, suggesting that this may be a critical mediator of the extinction of behaviors

dependent on cocaine-associated memories. If so, it is not clear how this fits with findings suggesting that the strength of IL-NAshell inputs is positively related to the inhibition of cocaine seeking (e.g., Ma et al., 2014). However, it is possible that the reduction in synaptic strength observed by Lee et al. reflects non-IL glutamatergic inputs that promote cocaine seeking.

Of note, some evidence suggests a role for the NAc_{core} in the extinction of cocaine-related memories. Indeed, work indicates that D-cycloserine enhances the ability of extinction to become context-independent when administered into the NAc_{core} following cue extinction sessions after cocaine self-administration (Torregrossa et al., 2010). Moreover, rats that have undergone extinction, rather than homecage withdrawal, following cocaine self-administration have increased levels of several synaptic plasticity-associated proteins in the NAc_{core}, but not the shell (Knackstedt et al., 2010). These findings suggest that, as with PL and IL, the relationship between NAc_{core} and shell with regard to the inhibition of cocaine seeking is likely more complicated than a simple dichotomy may suggest.

3.2.2. Hypothalamus—The hypothalamus has long been implicated in reward and food-seeking behaviors (Marchant et al., 2012), and recent work has examined its potential role in the extinction and inhibition of cocaine seeking. Whereas the lateral hypothalamus, particularly orexin neurons, drive the reinstatement of cocaine seeking (Marchant et al., 2012), the medial hypothalamus is involved in extinction learning of alcohol and sucrose seeking (Marchant et al., 2010; McNally, 2014; Millan et al., 2011). Indeed, mediodorsal hypothalamic infusion of CART, an inhibitory neuropeptide, induces alcohol seeking in rats that previously underwent alcoholic beer self-administration and extinction training (Marchant et al., 2010). Because the medial dorsal hypothalamus receives direct projections from the IL, this hypothalamic region is likely involved in the extinction of cocaine-seeking behaviors, a matter that should be examined by future studies (Millan et al., 2011).

3.2.3. Hippocampus—As the hippocampus plays a critical role in spatial/contextual learning and evidence indicates that extinction is context dependent (Fuchs et al., 2005), the hippocampus would seem to be a likely candidate region involved in the inhibition of behaviors dependent on cocaine-associated memories. Yet relatively little research has explored the role of this region in the inhibition of cocaine seeking. Studies indicate that rats that receive extinction training have decreased c-Fos expression in the CA1 region of the hippocampus in response to the cocaine self-administration environment (Neisewander et al., 2000). Furthermore, blocking activity in the dorsal hippocampus or BLA during the reconsolidation window disrupts cocaine seeking and incubation of cocaine craving in rats, suggesting that coordinated activity between these two regions is necessary to drive some forms of cocaine-seeking (Wells et al., 2011). Evidence also suggests that simultaneous activity between the dorsal subiculum and the BLA is necessary for extinction of cocaine-seeking behaviors, as bilateral and asymmetrical pharmacological disconnection of these two regions slows cued-extinction learning (Szalay et al., 2011). Whether the hippocampus plays a more focused role in the inhibition of cocaine-related memories beyond contextual/spatial signaling in rodents is unclear and remains to be explored by future work.

4. Viral vector targeting as a tool for future research

Although the findings described above indicate that studies have begun to establish the contours for the neural systems involved in the inhibition of cocaine-seeking behaviors, considerable work needs to be completed in order to develop a more comprehensive understanding of these processes. Indeed, as alluded to in the above sections, several outstanding issues, such as the precise mechanisms and circuits that enable the IL to inhibit behaviors dependent on cocaine-associated memories, the role of the IL in the extinction of other drugs and rewards, and a more cohesive theory of IL functioning, remain unresolved. Many of the advances in this domain over the next several years will likely arrive through approaches using viral vector targeting of different neural systems. The improved precision of such approaches, through targeting of specific cell types and projections (LaLumiere, 2011; Stefanik et al., 2013; Stuber et al., 2011) and millisecond-second control over neuronal activity (Aston-Jones and Deisseroth, 2013; Cassaday et al., 2014; Gutman et al., 2017), will undoubtedly be a significant tool in resolving the issues noted above and in addressing seemingly intractable questions.

Viral vector targeting is not a single approach but, as an umbrella, covers the use of viral vectors in neurobiological investigations and is typically used as a tool for introducing genes into different cells. Most commonly in current research, the gene products enable investigators to gain control over neuronal activity either via light (optogenetics, see Figure 2) or drugs (chemogenetics, see Figure 3) or, more recently, to monitor activity in specific populations of transduced cells (calcium imaging). However, considering the pace of change in the field, there may be significant advances in our technical capabilities in the next decade that are not yet foreseeable. Nonetheless, three examples from this review help to illustrate how viral vector targeting may be used to further elucidate the systems inhibiting cocaine seeking.

As discussed earlier, Lee et al. (2016) found that activating and inhibiting cholinergic neurons in the NA enhances and impairs, respectively, the extinction of cocaine CPP. In order to target these neurons selectively, the authors used a line of transgenic mice in which Cre was expressed in ChAT-expressing neurons (i.e., cholinergic cells) and then injected a virus expressing Cre-dependent opsins into the NA. As a result, the experimenters gained selective optogenetic control over the cholinergic neurons in the target region. Such procedures will be critical for elucidating the functional roles of specific cell types in modulating both behavior and synaptic plasticity, as the authors did in their work.

Targeting pathways, rather than entire brain regions, Ma et al. (2014) found different synaptic changes in the IL-to-NAshell and PL-to-NAcore pathways following cocaine self-administration and home cage withdrawal. Furthermore, researchers demonstrated that the changes in the IL-NAshell pathway mediated inhibition of cocaine seeking. To conduct these studies, the authors transduced IL neurons with channelrhodopsin (ChR2) and applied light to the NAshell, enabling selective control over those particular axonal terminations. In this case, this was especially important as the IL and PL project preferentially to the NAshell and core, respectively, but also provide some projections to the other NA subregion. Figure 3B illustrates this approach.

Similarly, Augur et al. (2016) reported that chemogenetically activating the pathway from the IL to the NAsHells reduces cue-induced reinstatement. For this work, authors used a dual-virus approach in order to achieve selective DREADD expression in only those IL neurons projecting to the NAsHells. The authors injected a Cre-dependent DREADD-containing virus into the IL and a Cre-containing virus (CAV2) into the NAsHells. The CAV2 virus has been found to have significant retrograde transduction of cells (Junyent and Kremer, 2015) and, thus in this case, led to Cre expression in IL neurons that terminated in the NAsHells. As a result, DREADDs were only expressed in dually infected neurons, enabling the authors to administer a systemic injection of clozapine-n-oxide (CNO) that selectively activated those neurons. This dual-virus approach provides researchers with a significant technical advantage, as a drug can be administered systemically and yet have highly selective and precise effects. The findings from Augur et al. provide considerably clearer and more definitive evidence of the critical nature of the IL-to-NAsHells pathway in inhibiting cocaine seeking, whereas prior studies, through traditional pharmacological approaches, often provided less direct evidence of the role of this pathway.

Figure 3C illustrates this approach, part of a larger set of INTRSECT (INTronic Recombinase Sites Enabling Combinatorial Targeting)-related constructs (Fenno et al., 2014).

5. Conclusions

As the fundamental problem in cocaine addiction rests in the inability to successfully inhibit the drive to engage in drug seeking and use, especially following periods of withdrawal, it is essential to continue to focus on understanding the neural systems responsible for such inhibition. Overall, the evidence points to a critical role for the ventromedial portion of the PFC – the IL – and, likely, its downstream projection target the NAsHells, in inhibiting cocaine-seeking behavior. Other regions are also likely involved, though relatively less research has been devoted to examining how these regions interact with structures such as the IL and NAsHells to inhibit cocaine seeking. Although a large body of literature link the IL and NAsHells to the inhibition of cocaine seeking, not all studies are in complete agreement on the role of the IL and NAsHells, and significant questions still exist regarding these structures and their relationships with the PL and NAcCore. Furthermore, a number of issues remain that require resolution in order to better understand the larger circuitry that enables the inhibition of cocaine-related memories. For example, despite the work described throughout this review, it is unclear how the systems responsible for *suppressing* cocaine seeking interfere with those systems *promoting* cocaine seeking. Similarly, considering that the IL has larger functions beyond inhibiting cocaine seeking, it has been difficult to formulate a single theory of IL function. The concomitant development of viral-based targeting strategies will undoubtedly aid in the efforts to answer these questions and enable a more careful and thorough parsing of the neural systems involved in the inhibition of behaviors dependent on cocaine-associated memories.

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Highlights

- Structures including infralimbic cortex and nucleus accumbens shell inhibit cocaine seeking.
- How such structures interact with other regions to inhibit cocaine seeking is under investigation.
- Viral vector targeting presents advantages in studying systems inhibiting cocaine seeking.

Figure 1.

Depiction of self-administration, extinction, and reinstatement. The Y-axis represents lever pressing, which is used as an index of cocaine seeking during extinction and reinstatement procedures. During extinction training, rats learn to inhibit cocaine-seeking responses. Reinstatement tests enable the investigation of the neural systems that promote cocaine seeking in a model of relapse. That different “triggers” can induce reinstatement provides evidence that extinction involves new learning, rather than erasure of the original cocaine-seeking memory.

Figure 2.

Schematic diagram demonstrating the use of optogenetics in investigating neuronal structures and pathways. The examples above involve the IL and NAsH to depict optogenetic approaches combined with viral vector targeting. **A.** IL neurons are transduced with adenoassociated virus (AAV) to express the desired opsin, and an optical fiber is permanently implanted to provide illumination to the IL. Photo stimulation/inhibition will affect all transduced IL neurons. **B.** IL neurons are transduced with AAV to express the desired opsin and an optical fiber is permanently implanted to provide illumination to the NAsH. Photo stimulation/inhibition will affect only axons projecting from the IL to the NAsH. **C.** Neurons in the IL are transduced with Cre-dependent AAV to express the desired opsin. A retrograde virus (e.g., CAV2) containing Cre is injected into the NAsH and is taken up by IL terminals. This leads to selective expression of the opsin in those IL neurons that project to the NAsH. In contrast to panel B, illumination can be provided to the IL itself and yet provide selective pathway stimulation/inhibition.

Figure 3.

Schematic diagram demonstrating the use of chemogenetics in investigating neuronal structures and pathways. CNO can be administered through microinjections or systemically (inset in panels A and C) and will affect only the brain region that expresses the receptor. Shown are the IL and NAc shell, areas which are involved in the inhibition of cocaine-seeking, however a variety of structures and pathways can be investigated using this method. **A.** IL neurons are transduced with AAV to express the desired DREADD. Systemic administration of CNO results in the activation/inhibition of IL neurons only. **B.** Neurons in the IL are transduced with AAV to express the desired DREADD. Intra-NAc shell micro-injections of CNO result in the activation/inhibition of projection neurons from the IL to the NAc shell. **C.** IL neurons are transduced with Cre-dependent DREADDs. A retrograde virus (e.g., CAV2) containing Cre is injected into the NAc shell and is taken up by IL terminals. This leads to selective expression of the DREADD in those IL neurons that project to the NAc shell. Systemic administration of CNO results in the selective activation/inhibition of projection neurons from the IL to the NAc shell.

Table 1

Studies supporting a role for the IL attenuating cocaine seeking.

Manipulated structure	Method	Manipulation	Stage of Manipulation	Result	Citation
IL	Self-administration	Inactivation via baclofen + muscimol	Extinction training	Increased cocaine seeking	Peters et al., 2008a
IL	Self-administration	Inactivation via baclofen + muscimol	Spontaneous recovery following extinction	Decreased cocaine seeking	Peters et al., 2008b
IL	Self-administration	Inactivation via baclofen + muscimol	Extinction training	Impaired extinction learning	LaLumiere et al., 2010
IL	Self-administration	Activation via PEPA	Extinction training	Facilitated extinction learning	LaLumiere et al., 2010
IL	Self-administration	Activation via DREADDs	Cue-induced reinstatement following extinction	Decreased cocaine seeking	Augur et al., 2016
IL	Self-administration	Inactivation via ArchT3.0	Early extinction training	Increased cocaine seeking	Gutman et al., 2017
IL	Self-administration	Inactivation via ArchT3.0	Cued reinstatement	Increased cocaine seeking	Gutman et al., 2017
IL	Conditioned Place-Preference	Activation via Chr2	Extinction training	Facilitated extinction learning	Van den Oever et al., 2013
IL	Conditioned Place-Preference	Inactivation via Halorhodopsin (NpHR)	Extinction training	Impaired extinction learning	Van den Oever et al., 2013
IL	DS based self-administration	Inactivation via baclofen + muscimol	Self-administration	Increased DS-responding	Gutman et al., 2016
IL to NAc shell projection	Self-administration	Reversal of synaptic changes via Chr2	Cue-induced reinstatement following withdrawal	Increased cocaine seeking	Ma et al., 2012
IL to NAc shell projection	Self-administration	Activation via DREADDs	Cue-induced reinstatement following extinction	Decreased cocaine seeking	Augur et al., 2016