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Still a "hidden island"? The rodent insular cortex in drug seeking, reward, and risk

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ABSTRACT

The insular cortex (IC) is implicated in risky decision making and drug-seeking behaviors, in a manner dissociable from natural reward seeking. However, evidence from rodent studies of motivated behaviors suggests that the role of the IC is not always consistent across procedures. Moreover, there is evidence of dissociation of function between posterior (pIC) and anterior (aIC) subregions in these behaviors. Under which circumstances, and by which mechanisms, these IC subregions are recruited to regulate motivated behaviors remains unclear. Here, we discuss evidence of rodent pIC and aIC function across drug-related behaviors, natural reward seeking, and decision making under risk and highlight procedural differences that may account for seemingly conflicting findings. Although gaps in the literature persist, we hypothesize that IC activity is broadly important for selection of appropriate behaviors based on learned action-outcome contingencies and that associated risk is sufficient, but not necessary, to recruit the aIC in reward seeking without involving the pIC.

1. Introduction

The insular cortex (IC) is a relatively recent target of addiction research. In 2007 evidence emerged indicating that cigarette smokers with IC lesions were able to quit smoking easily, without relapse or a persistent urge to smoke (Naqvi et al., 2007). This finding, along with functional imaging studies reporting IC activation in response to drug-associated cues and self-reported craving (Naqvi and Bechara, 2009), ignited increased interest in this region as a potential driver of substance use disorder. Notably, the role of the IC in drug craving appears to be distinct from craving of natural rewards such as food, as evidence indicates that patients with IC lesions have no decrease in food craving, intake, or pleasure in eating (Naqvi et al., 2007). Experiments using rodents have since replicated this dissociable effect on nicotine vs food seeking (Forget et al., 2010; Pushparaj et al., 2015), raising questions for how drugs of abuse uniquely recruit the IC to promote drug-seeking behaviors and how this IC role is related to natural reward seeking processes.

A potentially critical distinction is that drug use is an inherently risky behavior, frequently associated with adverse consequences. Regardless of whether the drug user perceives this risk, clinical evidence suggests that chronic drug use alters the ability to evaluate risks and make sound decisions, promoting further drug use despite the consequences (Rogers et al., 1999). Interestingly, the IC is implicated in risky decision making of all kinds, not only those related to drug use. Evidence indicates that patients with IC lesions perform worse on a gambling task that requires them to adjust their bets based on the odds of winning, suggesting that an intact IC is necessary for appropriate decision making under risk (Clark et al., 2008). Thus, some have proposed that inherent risk, akin to that associated with chronic drug use, is an important factor in recruiting IC activity (Naqvi et al., 2014). Nonetheless, it remains unclear whether this drug-associated risk explains the preferential involvement of the IC in drug vs natural reward seeking. Over the past 15 years, work using rodents has attempted to disentangle the role of the IC in drug seeking, reward, and risk. Despite this increasing interest, significant questions remain regarding IC function across these domains. The purpose of this review is to examine the current rodent literature with regard to these issues and identify critical lacunae in the field that must be addressed to elucidate the role of the IC in specific motivated behaviors.

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2. Anatomy of the insular cortex

2.1. IC organization across species

The 'insula' (Latin for 'island') is so named for its location deep within the lateral sulcus of the primate brain, isolated from the rest of the cerebral cortex (Figs. 1A and 1B). Neuroanatomists divide the insula across species into three subdivisions based on its cytoarchitecture - granular, dysgranular, and agranular IC. These distinctions refer to the presence or absence of the granular cortical layer IV, which is the main target of thalamocortical afferents (Hubel and Wiesel, 1962). The granular (and most dorsal) division contains all six cortical layers, the dysgranular division (ventral to granular) contains a smaller cortical layer IV, and the agranular division (most ventral) lacks a layer IV (Livneh and Andermann, 2021). These cytoarchitectural differences give rise to differences in connectivity. Granular and dysgranular IC divisions, broadly considered to comprise the primary interoceptive cortex, receive direct sensory input from visceral thalamic sensory nuclei (Allen et al., 1991). The agranular division is thought to make up higher-order association sensory cortex and is more reciprocally connected with limbic structures (Allen et al., 1991; Gehrlach et al., 2020; Livneh and Andermann, 2021). Dense projections between IC subdivisions enable the flow of information from primary interoceptive cortex to higher order association areas (Shi and Cassell, 1998). Granular IC projects to dysgranular cortex, which then projects to agranular cortex and back to granular cortex. Agranular IC projects back to granular and dysgranular cortex as well.

2.2. Functional subdivisions of the primate IC

The primate IC can be further divided into anterior and posterior lobes, separated by the central insular sinus in humans, which largely correspond to distinct functional regions (Jakab et al., 2012). Interoceptive signals are carried not only from granular to agranular cortex, but also along the anterior-posterior axis. In human imaging studies, these functional subdivisions are often used rather than the cytoarchitectural ones, based on associated network activity (Droutman et al., 2015). Functional distinctions are sometimes also made between dorsal and ventral anterior IC (Chang et al., 2013), and some have identified a functionally distinct middle IC as well (Cloutman et al., 2012). However,

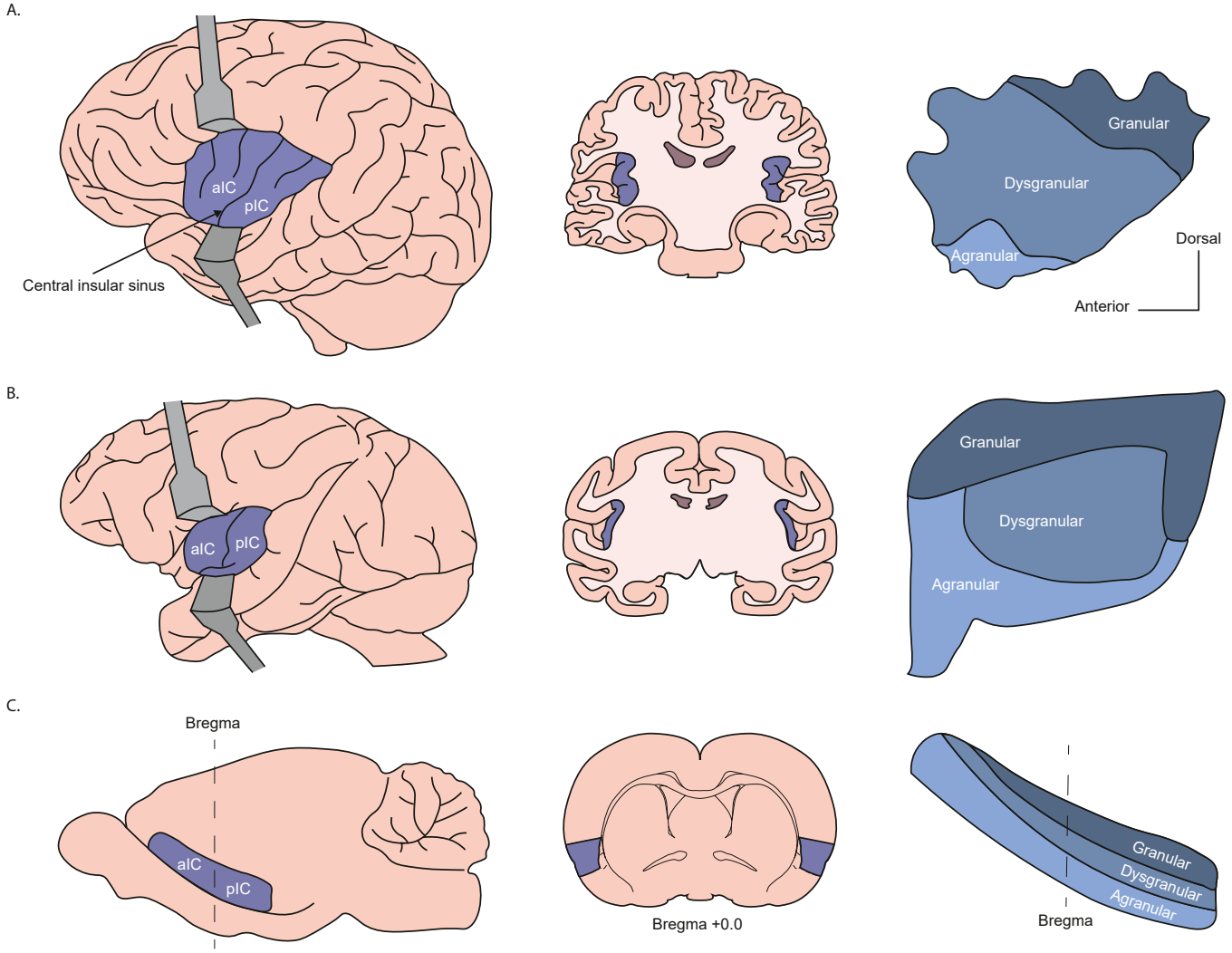

Fig. 1. Comparative neuroanatomy of the IC. A. Neuroanatomy of the human IC. Left, IC location within the lateral sulcus of the human brain. The central insular sinus separates the aIC from the pIC. Middle, coronal section showing the human IC in purple. Right, approximate cytoarchitectural subdivisions of the human IC, in sagittal cross-section. B. Neuroanatomy of the primate (macaque) IC. *Left*, IC location within the lateral sulcus of the macaque brain. *Middle,* coronal section showing the macaque IC in purple. *Right*, approximate cytoarchitectural subdivisions of the macaque IC, in sagittal cross-section. C. Neuroanatomy of the rodent (rat) IC. *Left,* IC location on the lateral surface of the rat brain. The consensus boundary for anterior-posterior subdivisions in the rodent IC is at bregma. *Middle,* coronal section at bregma showing the rat IC in purple. *Right,* cytoarchitectural subdivisions of the rat IC. The pIC and aIC are separated by the dashed line at bregma.

because the field has not established consensus boundaries for these further functional subdivisions, which may not translate across species, we will restrict our discussion to the posterior (pIC) and anterior (aIC) regions, as most commonly defined. These functional subdivisions are not strictly tied to the cytoarchitectural subdivisions. In primates, the pIC contains a larger granular cortex and smaller agranular cortex, whereas the opposite is true in the aIC, reflecting differences in connectivity with thalamic sensory areas (Evrard et al., 2014). Because the pIC contains more granular cortex, it receives more thalamic input and sends projections forward to the largely agranular aIC. However, both the posterior and anterior lobes contain granular, dysgranular, and agranular subdivisions (see Figs. 1A and 1B for anatomical and functional subdivisions).

2.3. IC functional subdivisions are conserved in rodents

In contrast to its location in the sulci of primate brains, the rodent IC lies exposed on the lateral surface of the brain (Gogolla, 2017) (Fig. 1C). Nevertheless, many functions of the IC and its subregions are conserved across species. Despite lacking a central insular sinus, the rodent IC appears to also have posterior and anterior divisions, with the divisions typically made with regard to Bregma. Tracing studies have extensively mapped projections to and from these subregions in rats (Allen et al., 1991; Shi and Cassell, 1998) and mice (Gehrlach et al., 2020). As in primates, the rodent pIC contains a larger granular cortex and receives more direct inputs from visceral thalamic sensory nuclei, as well as primary somatosensory cortex (Gehrlach et al., 2020). The aIC receives inputs from the thalamus as well, but the majority come from the polymodal association group of thalamic nuclei, indicating a role as sensory association cortex. The aIC also has a larger agranular cortex and is more reciprocally connected with limbic structures, including strong connections to the amygdala, hippocampus, medial prefrontal cortex, and particularly the basal ganglia. More than one third of excitatory projections from the aIC terminate in the ventral striatum, with particularly dense innervation in the nucleus accumbens core, implicating a role for the aIC in motivated behavior that is perhaps distinct from the pIC (Gehrlach et al., 2020). Nonetheless, granular, dysgranular, and agranular cortex layers are contained throughout the anterior-posterior extent of the rodent IC, and therefore many structures are connected to both subregions. Despite that, evidence supports distinct functions for the rodent pIC vs aIC, with important implications for the role of the IC in motivated behaviors.

2.4. Theories of IC functional neuroanatomy across species

Researchers have suggested several ideas as to how functions might be organized across the IC. Some propose that the IC is topographically organized by valence, with pIC activity associated with negative valence and aIC activity associated with positive valence (Tye, 2018). Although rodent studies indicate that pIC stimulation is broadly aversive (Gehrlach et al., 2019; Peng et al., 2015; Wang et al., 2018), and aIC stimulation is broadly rewarding (Dolensek et al., 2020), the opposite is true when the animal is already in an extremely aversive or rewarded state (Livneh and Andermann, 2021). Rather than representing the valence of a stimulus, IC activity may help regulate internal body states and facilitate a return to homeostasis. Others argue that the pIC processes a representation of the physiological conditions of the body, whereas the aIC is critical for conscious awareness of the subjective feeling of the body and emotional state – at least in humans (Craig, 2003, 2009). The strength of pIC connections with visceral thalamic sensory nuclei and aIC connections with limbic structures supports this idea, and similarities between primate and rodent IC suggest these functions could be similar across species. Additionally, human imaging studies identify the aIC as a hub of the salience network, suggesting a role in identifying salient events (Uddin, 2015). Although inconclusive, the evidence points to functional dissociations between the pIC and aIC that justify separate interrogations of these subregions in motivated behaviors.

There are potentially distinct roles for the pIC and aIC in drug seeking in particular. Naqvi and colleagues, integrating evidence from human and rodent studies, propose that the pIC is necessary for registering the reinforcing value of drugs and for learning drug-context associations, whereas the aIC is necessary for the retrieval of drug-context associations (Naqvi et al., 2014). Moreover, manipulations in rodent studies of drug and natural reward seeking have revealed distinct (and sometimes conflicting) roles for the pIC and aIC in reward seeking across reward types. Nonetheless, it remains unclear how the functional organization of the IC accounts for these dissociations. A major part of this review is to parse the role of the rodent IC in drug-seeking behaviors, along with natural reward seeking and decision making under risk, in an attempt to reconcile these theories of IC functional topography.

3. Drug seeking

Prior work in rodent models of addiction has used a variety of approaches to investigate the role of the IC. Broadly, the findings from these studies can be divided into four sections based on the measure of drug-seeking behavior: reinforced drug taking, aversion-resistant drug taking, drug seeking (without drug reinforcement), and expression of drug association memories. Each of these measures reveals mechanisms for drug-seeking behavior under distinct circumstances. In drug-taking procedures, instrumental responses are rewarded with drug administration, enabling investigation of the effects of the drug itself on reinforcement mechanisms. In many studies, procedures pair drug taking with aversive outcomes in order to investigate the neurobiological mechanisms that govern the balance between the motivation to obtain the drug vs the motivation to avoid the aversive consequence. Nonetheless, drug-taking procedures measure instrumental responses that are presumably driven by the internal state induced by the drug itself. Drug seeking without drug reinforcement likely serves as a better measure of drug *craving*. Similarly, procedures designed to measure expression of drug association memories, such as conditioned place preference, enable elucidation of these associative learning mechanisms in the absence of drug reinforcement. We will discuss the role of the pIC and aIC in each of these common procedures used to model aspects of drug addiction in rodents.

3.1. Drug taking

3.1.1. The pIC in drug taking

In studies focused on drug-taking procedures, rodents typically learn to perform an instrumental behavior, such as a lever press or nose poke, to self-administer a drug reward (Fig. 2A). As primary interoceptive cortex, the pIC is presumably a critical structure in representing the internal state induced by drug use and, consequently, would be expected to regulate drug self-administration and/or reinforcement mechanisms. Nonetheless, relatively few studies have examined this issue. Early work in rats found that silencing pIC activity via GABA receptor activation or electrical stimulation decreases nicotine self-administration, but *not* food self-administration (Forget et al., 2010; Pushparaj et al., 2013), consistent with the effects of human IC lesions on cigarette smoking while conserving food intake (Naqvi et al., 2007). This dissociation suggests that nicotine taking recruits the pIC in a manner distinct from natural reward or, at minimum, that its role does not generalize to all forms of rewarded instrumental behavior. To our knowledge, only one other study has investigated the pIC in drug taking alone, finding that pIC inactivation with GABA receptor agonists reduces alcohol self-administration in rats (Pushparaj and Le Foll, 2015). Thus, although there appears to be a role for the pIC in drug taking, the lack of comprehensive work does not reveal whether it crosses all drugs of abuse or a variety of measures of drug taking and reinforcement (e.g., breakpoint in a progressive ratio schedule). Moreover, on a more theoretical level, whether the pIC is important for interoceptive awareness of

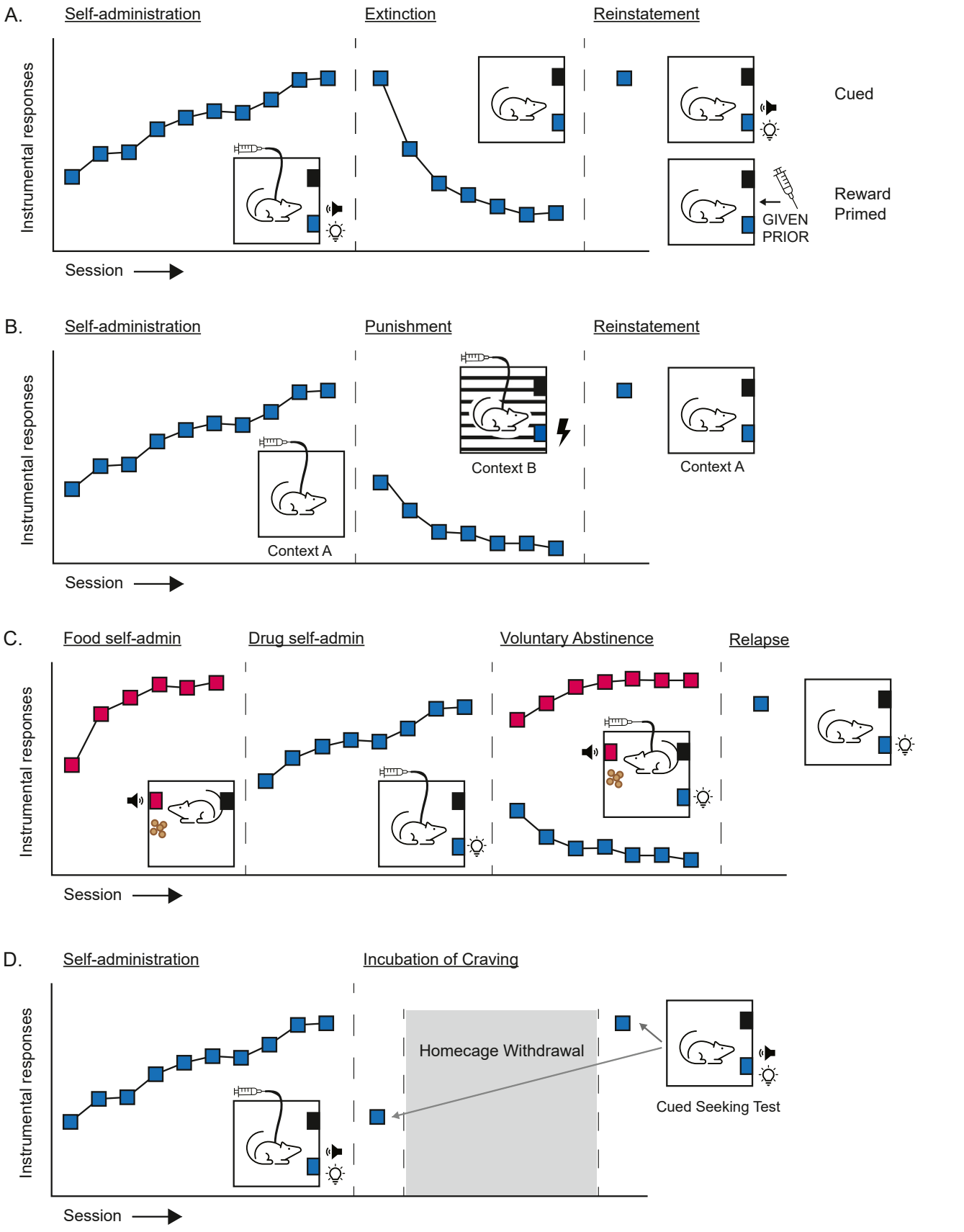

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Fig. 2. A selection of self-administration procedures described in this review. A. Extinction-reinstatement procedures. Left, animals learn to self-administer a reward (drug infusion, alcohol, or food pellet delivery) paired with a light and tone cue by performing a reward-seeking response (lever press or nose poke) on the active lever/port (blue). Responses on the inactive lever/port (black) have no consequence. Middle, removing the reward delivery and associated cues as a consequence of a reward-seeking response causes animals to extinguish responding. Right, reward-seeking is reinstated by bringing back the previously reward-paired light and tone cues on responding (cued reinstatement) or giving a priming injection/delivery of reward before a normal extinction session (reward-primed reinstatement). B. Punishment-induced abstinence procedures as described in (Ghareh et al., 2022). Left, animals learn to self-administer a reward in Context A. Middle, in Context B, reward delivery is pseudo-randomly paired with a punishment (footshock), inducing abstinence from reward-seeking responding. Right, returning animals to reward Context A reinstates reward-seeking. C. "Voluntary abstinence" procedures as described in (Venniro et al., 2017). *Left*, animals learn to self-administer food by responding on the active food lever (red), producing a food pellet reward and paired tone cue. Inactive lever presses (black) have no consequence. *Middle left*, animals learn to self-administer drug in the same context. Responding on the active drug lever (blue) results in a drug infusion and paired light cue. Inactive lever presses (black) have no consequence, and the food lever is unavailable. *Middle right*, "voluntary abstinence" from drug taking is induced by making both the active food (red) and drug (blue) levers available, along with the inactive lever. With both rewards available, animals increase responding for food reward and nearly stop responding for drug reward. *Right*, in the relapse test, only the drug reward lever and inactive lever are available. Pressing the drug reward lever produces a light cue but no drug infusion. In the absence of the food reward option, animals increase their drug-seeking responses. D. Incubation of craving procedures. *Left*, animals learn to self-administer drug as described in *A*. *Right*, drug-seeking responses are measured in a cued seeking test, wherein responding produces drug-paired light and tone cues but no drug infusion. Animals then undergo home cage withdrawal without access to the drug. After a specified number of days, animals return to another cued seeking test identical to that on the first withdrawal day. Animals display increased drug-seeking responses on this second test day, as compared to the first day, a phenomenon known as incubation of craving.

the drug or merely promotes drug-taking behavior also remains unclear.

3.1.2. The aIC in drug taking

In contrast, considerably more work has investigated the aIC in drug-taking procedures, although many of the findings have been inconsistent. Similar to the pIC, prior work indicates that aIC inactivation with GABA receptor agonists reduces nicotine, but not food, self-administration in rats (Pushparaj et al., 2015). However, dissociation of function within the aIC may not be limited to instrumental responding for drug vs natural reward, as there is conflicting evidence for its role across drugs of abuse. Evidence indicates that GABA receptor-based inactivation of the aIC decreases alcohol self-administration in rats (De Oliveira Sergio et al., 2021), yet other studies suggest that chemogenetically inhibiting the aIC either increases (Jaramillo et al., 2018) or does not affect alcohol self-administration (Haaranen, 2020a, 2020b). Evidence also indicates that chemogenetic *activation* of the aIC reduces alcohol self-administration (Haaranen et al., 2020), suggesting that aIC activity *inhibits* alcohol taking in certain circumstances. Nevertheless, it is also possible that such activation disrupted endogenous aIC activity and, thus, produced behavioral outcomes akin to those produced by inhibitory manipulations.

Similarly, evidence with regard to cocaine taking is conflicting. Early work found that aIC inactivation with sodium channel blockers has no effect on cocaine self-administration (Di Pietro et al., 2006), whereas more recent work suggests that excitotoxic aIC lesions made after rats acquire cocaine self-administration decrease subsequent cocaine taking (Rotge et al., 2017). Moreover, Rotge and colleagues (2017) found that aIC lesions made *before* rats learn to self-administer cocaine *increase* the escalation of cocaine taking, although other work found no effect of such pre-training lesions (Pelloux et al., 2013). Despite conflicting findings, these studies indicate that lesion timing relative to acquisition of drug-taking behavior is an important consideration when interpreting the results of similar work.

Interestingly, evidence indicates that post-conditioning, but not pre-conditioning, aIC lesions *potentiate* escalation of heroin self-administration (Joshi et al., 2020), opposing the direction of the effect on cocaine self-administration. This dissociation could be explained by differences in aIC response to distinct interoceptive cues for heroin and cocaine. Whereas cocaine, a psychostimulant and sympathomimetic, produces a variety peripheral effects such as increased blood pressure and heart rate that would be interoceptively detected (Billman, 1995), heroin, a central nervous system depressant, has a less powerful peripheral effect (Thornhill et al., 1989). Thus, it may be that the regulation of cocaine taking is more sensitive to these interoceptive cues, whereas heroin taking is regulated to a greater extent by other factors, such as external cues. However, to our knowledge this is the only study to investigate the aIC in opioid self-administration, and it remains unclear whether the aIC differentially regulates opioid vs psychostimulant taking. Together with the nicotine and alcohol literature, these studies provide a perplexing set of findings that may be explained by different drugs of abuse, drug-taking procedures, or technical differences in how the aIC was manipulated. Moreover, there may be functional heterogeneity within the aIC, and more targeted manipulations may have a greater likelihood of parsing out different effects.

Indeed, several studies have probed specific signaling mechanisms in the aIC that regulate drug taking, most notably dopaminergic signaling, which is important throughout the brain for the reinforcing effects of a variety of drugs (Di Chiara, G, 2000). Evidence indicates that intra-aIC infusions of a dopamine D1, but not D2, receptor antagonist reduce nicotine self-administration in rats (Kutlu et al., 2013), which may reflect the greater expression levels or differences in the site of receptor expression of D1 receptors in the IC (Gaspar et al., 1995). Prior work also indicates that blocking aIC D1 receptors reduces both cocaine and food self-administration in rats (Di Pietro et al., 2008), in contrast to the dissociated effects of global aIC inactivation on cocaine vs food self-administration (Pushparaj et al., 2015; Rotge et al., 2017; Simon et al., 2011). However, this could be a non-specific decrease in motor responding similar to that produced by D1 antagonists in the nucleus accumbens and amygdala (McGregor and Roberts, 1993). Evidence indicates that high doses of a D1 antagonist in the aIC also produce motor impairment (Burkey et al., 1999), making it difficult to draw conclusions on the role of aIC dopaminergic signaling in drug taking vs food taking.

Other signaling mechanisms, including hypocretin and norepinephrine, are also implicated in aIC regulation of drug taking. Hypocretin neurons originate primarily in the lateral hypothalamic area, densely innervate the aIC, and are strongly implicated in drug-seeking behaviors (Hollander et al., 2008). Findings indicate that blocking aIC hypocretin receptors reduces nicotine self-administration under both fixed and progressive ratios of reinforcement, without affecting food self-administration (Hollander et al., 2008). Nonetheless, no studies have yet investigated this specific mechanism for other drugs of abuse. Similarly, there is limited evidence of a role for the aIC noradrenergic system in regulating alcohol taking – De Oliveira Sergio et al. (2021) found that blocking α_1 norepinephrine receptors in the aIC reduces alcohol, but not saccharin, self-administration in rats. This is consistent with a role for the noradrenergic system in regulating alcohol-related behaviors (Vazey et al., 2018), but in the absence of further studies it is unknown whether noradrenergic signaling in the aIC is implicated across drugs of abuse.

Pathway manipulation studies have also identified glutamatergic projections from the aIC to the central amygdala (CeA) and nucleus accumbens core (NAcore) that appear to regulate drug taking. Both regions are densely innervated by the aIC and are involved in the acquisition of drug-taking behaviors and craving for drug rewards, implying

that activity in these pathways might promote drug taking (Scofield et al., 2016; Warlow and Berridge, 2021). However, evidence on this issue is limited and sometimes conflicting. Findings from Haaranen et al. (2020) suggest that chemogenetic activation of the aIC-CeA or aIC-NAcore pathway increases alcohol self-administration in rats, yet, inexplicably, chemogenetic inhibition of either pathway has no effect. Although another study replicated the null effect of aIC-NAcore pathway inhibition with an optogenetic approach (Seif et al., 2013), other work indicates that chemogenetic inhibition of this pathway decreases self-administration, consistent with the activation findings (Jaramillo et al., 2018; Jaramillo, Van Voorhies et al., 2018). These discrepancies could be explained by the limitations of pathway inhibition studies, as the aIC is one of many excitatory inputs to the CeA and NAcore, and thus inhibition of one of these pathways may not be sufficient to reduce alcohol taking. Nevertheless, studies suggest that chemogenetic inhibition of aIC-NAcore projections in rats mimics (or at least heightens) the interoceptive effects of alcohol on both alcohol self-administration (Jaramillo et al., 2018) and discriminative response to alcohol (Jaramillo et al., 2018), indicating that activity in this pathway regulates behavior in response to alcohol-induced internal state. As others have proposed, the effects of IC manipulation may be state-dependent (Livneh and Andermann, 2021). Thus, it is critical to understand how different conditions recruit the aIC and its projections in regulating drug taking.

3.2. Aversion-resistant drug taking

3.2.1. The aIC in aversion-resistant drug taking

Studies indicate that some rodents will continue to self-administer alcohol or cocaine in the face of potentially aversive consequences, such as bitter-tasting quinine (H. Chen and Lasek, 2020; De Oliveira Sergio et al., 2021) or a footshock (Y. Chen et al., 2022) (Fig. 2B). The evidence on whether this aversion-resistant drug taking recruits different aIC mechanisms than drug taking alone, however, remains mixed. De Oliveira Sergio et al. (2021) found that both aIC GABA receptor activation and α_1 norepinephrine receptor inactivation reduce self-administration of alcohol paired with quinine, but also of alcohol alone, indicating that these mechanisms are not unique to aversion-resistant alcohol taking. However, aversion-resistant alcohol taking may be differentially mediated by perineuronal nets, which are extracellular matrix structures that can alter neuron excitability and increase in density in the IC after bouts of binge drinking (H. Chen et al., 2015). Evidence indicates that dissolving perineuronal nets in the mouse aIC reduces self-administration of alcohol paired with quinine, but not of alcohol alone (H. Chen and Lasek, 2020). This suggests that the transition to "compulsive" alcohol use coincides with increased density of perineuronal nets in the aIC, although this mechanism has not been investigated further or for aversion-resistant taking of other drugs of abuse.

Evidence for aIC projections that specifically mediate aversion-resistant alcohol taking is similarly limited and sometimes conflicting. Whereas Seif et al. (2013) found that optogenetic inhibition of aIC-NAcore projections reduces self-administration of alcohol paired with quinine or footshock, but not of alcohol alone, others have found that chemogenetic inhibition of this pathway reduces alcohol self-administration without paired aversive outcomes (Jaramillo et al., 2018; Jaramillo et al., 2018). It is unclear what accounts for this discrepancy and whether aversion-resistant alcohol taking differentially recruits aIC projections compared to alcohol taking alone. Another way that the aIC might specifically promote aversion-resistant alcohol taking is via projections to the locus coeruleus, an area implicated in promoting adaptive stress response as well as the reinforcing effects of alcohol (Vazey et al., 2018). Evidence indicates that optogenetic inhibition of this pathway reduces self-administration of alcohol paired with quinine, but not of alcohol alone (De Oliveira Sergio et al., 2021). In the absence of further studies, it is unknown whether this finding generalizes to other drugs of abuse or aversive stimuli (e.g., footshock).

Evidence indicates that aIC activity regulates aversion-resistant cocaine taking in rats as well, as chemogenetic inhibition or activation of the aIC reduces or increases footshock-resistant cocaine self-administration, respectively (Y. Chen et al., 2022). This study also identified orbitofrontal cortex input to the aIC that regulates this behavior, with chemogenetic inhibition or activation of this pathway producing the same effects as aIC cell body manipulations. Nevertheless, it is unclear whether this is distinct from the aIC activity that regulates cocaine taking without paired aversive outcomes (Rotge et al., 2017). Taken together with the findings described in the "Drug Taking" section, we speculate that, although there are aIC cell populations that mediate aversion-resistant drug self-administration, paired aversive stimuli are not always necessary to recruit these ensembles in drug-taking behavior. Nevertheless, because the IC is interoceptive cortex, it is difficult to disentangle the effects of IC manipulation on drug-taking behavior from interoceptive processing of the drug (or paired aversive stimuli) itself.

3.3. Drug seeking without drug reinforcement

The role of the IC in drug craving, and ultimately relapse to drug seeking, can be better understood by manipulating its activity during drug-seeking tests without drug reinforcement. Studies commonly use extinction of drug-seeking behavior, followed by tests to induce reinstatement of this behavior, to examine such behavior in rodents (Fig. 2A). Extinction learning is accomplished by removing the drug and drug-associated cue reinforcers that were previously associated with an instrumental response, reducing drug-seeking responses over time. Drug seeking is reinstated by bringing back drug-associated cues or contexts, or by giving a priming injection of the drug. Several studies have manipulated activity in the pIC or aIC during these reinstatement tests to investigate their role in drug seeking without drug reinforcement.

3.3.1. The pIC in drug seeking without drug reinforcement

Even in the absence of interoceptive processing of the drug itself, the pIC appears to be necessary for expressing drug-seeking behavior in some circumstances. Evidence indicates that silencing pIC activity via GABA receptor activation or electrical stimulation attenuates both cued and nicotine-primed reinstatement of nicotine seeking, but not food seeking, after extinction learning in rats (Forget et al., 2010; Pushparaj et al., 2013). Given the clinical evidence that IC activity increases in response to drug-associated cues across nicotine, cocaine, and alcohol-dependent individuals (Hanlon et al., 2018), it is perhaps not surprising that nicotine-associated cues appear be sufficient to recruit the pIC in promoting nicotine seeking. On the other hand, a study from our laboratory found that pIC inactivation with GABA receptor agonists had no effect on cued reinstatement of *cocaine* seeking after extinction learning in rats (Cosme et al., 2015). With this limited extant literature, it is unclear whether cued reinstatement of cocaine seeking involves pIC-independent mechanisms that are distinct from those involved in reinstatement of nicotine seeking.

3.3.2. The aIC in drug seeking without drug reinforcement

In contrast to the pIC, the aIC appears to be important for reinstatement of both nicotine and cocaine seeking. Evidence indicates that aIC inactivation with GABA receptor agonists prevents context-induced (Ghareh et al., 2022) and cued reinstatement of nicotine seeking, but not food seeking, after extinction in rats (Pushparaj et al., 2015). This is consistent with the reported effects of pIC inactivation (Forget et al., 2010), indicating no dissociation of function between these subregions as it relates to nicotine seeking. On the other hand, there is a potential dissociation of function between the pIC and aIC in cued reinstatement of *cocaine* seeking. Whereas the pIC does not appear to be necessary for cued reinstatement of cocaine seeking after extinction, evidence indicates that reversible aIC inactivation attenuates cued (Cosme et al., 2015; Di Pietro et al., 2008) and context-induced reinstatement of cocaine seeking (Arguello et al., 2017).

Interestingly, Rotge et al. (2017) found that pre-extinction aIC lesions attenuate *cocaine-primed*, but not cued, reinstatement, in contrast to the finding from our laboratory that reversible aIC inactivation attenuates cued, but not cocaine-primed, reinstatement (Cosme et al., 2015). This perplexing discrepancy could be explained in part by differences in timing of aIC inactivation. Rotge et al. lesioned the aIC after rats had acquired cocaine self-administration, but before extinction learning, whereas our laboratory reversibly inactivated the aIC only during the reinstatement tests. It is possible that aIC activity is modified by extinction learning and that this modified activity is important for responsivity to cocaine-associated cues vs cocaine prime. On the other hand, evidence indicates that pre-extinction aIC lesions *potentiate* cued reinstatement of heroin seeking (Joshi et al., 2020). This is consistent with the opposing effects of aIC lesions on heroin vs cocaine self-administration (Rotge et al., 2017), again pointing to potential heterogeneity of function within the aIC.

Although extinction is a well-established procedure, it is unclear whether it recruits the same neural mechanisms as other methods to suppress drug seeking. To address this, some studies have used punishment or alternative rewards to induce "voluntary abstinence" from drug seeking in rodents (Fig. 2B and C). A recent study directly compared the role of the aIC in contextual reinstatement of nicotine seeking after extinction vs punishment-induced abstinence, using footshock as a consequence of nicotine-seeking responses to induce abstinence in the footshock context. Returning rats to the "safe" context induced reinstatement of nicotine seeking. The findings indicate that chemogenetically inhibiting the aIC attenuates contextual reinstatement of nicotine seeking after both extinction and punishment-induced abstinence (Ghareh et al., 2022). This is a critical finding, as it suggests that the aIC is important for reinstatement regardless of the procedures used to suppress nicotine seeking.

On the other hand, evidence for the role of the aIC in reinstatement of *cocaine* seeking after abstinence has been less conclusive. Findings suggest that pre-cocaine training aIC lesions *potentiate* contextual reinstatement of cocaine seeking after footshock-induced abstinence (Pelloux et al., 2013), in opposition to the *attenuating* effect of reversible aIC inactivation on reinstatement after extinction learning (Arguello et al., 2017). This seems to suggest that cocaine-associated contexts differentially recruit the aIC after abstinence vs extinction. However, the extinction literature indicates that aIC lesions made before contingency learning have a different effect on cued reinstatement of cocaine seeking than reversible aIC inactivation during the reinstatement test (Cosme et al., 2015; Rotge et al., 2017). Although Pelloux and colleagues found no effect of pre-training aIC lesions on cocaine self-administration or footshock learning (Pelloux et al., 2013), it is likely that an intact aIC during these learning processes is important for the region's involvement in contextual reinstatement. For this reason, punishment-induced abstinence studies that reversibly inactivate the aIC during a reinstatement test would provide a better comparison with the cocaine extinction literature.

Although there is a lack of studies investigating the aIC in reinstatement of alcohol seeking after extinction, one study of reinstatement following footshock-induced abstinence provides an interesting comparison to the aversion-resistant alcohol taking literature. Campbell and colleagues found that rats that learned footshock-induced abstinence would reinstate alcohol seeking *in the footshock-paired context* after a further 30 days of forced abstinence (Campbell et al., 2019). Notably, their results indicate that aIC inactivation with GABA receptor agonists prevents reinstatement in the footshock context. This is consistent with a role for the aIC in promoting aversion-resistant alcohol taking (De Oliveira Sergio et al., 2021) and nicotine seeking after footshock-induced abstinence (Ghareh et al., 2022). Further work is needed to determine whether this role is consistent in reinstatement of alcohol seeking after extinction. Moreover, both extinction and punishment-based procedures use negative outcomes to suppress drug seeking behavior. It is possible that other strategies to suppress drug seeking, such as availability of an alternative non-drug reward, recruit different neural mechanisms.

Although limited in scope, findings support the idea that, regardless of which contingency is learned to suppress drug seeking, the aIC is important for expressing the initial drug reward contingency in response to drug-associated cues. Venniro and colleagues developed a "voluntary abstinence" paradigm to abolish methamphetamine-seeking, wherein rats choose to respond for a food pellet over a previously trained methamphetamine reward (Fig. 2C). Rats are then tested for relapse by removing the food reward choice and measuring responses for unreinforced methamphetamine-associated cues. Evidence indicates that inactivating either the aIC or aIC-CeA pathway during the relapse test attenuates methamphetamine seeking (Venniro et al., 2017), suggesting a role for the aIC in reinstatement of methamphetamine seeking that is consistent with much of the extinction and punishment-based literature for other drugs of abuse. Although this is the only study to investigate the role of the aIC-CeA pathway in drug seeking without drug reinforcement, there is evidence that activation of this pathway promotes *alcohol* self-administration in rats (Haaranen et al., 2020). More work is needed to determine whether this pathway promotes drug seeking in general, or whether its role is dependent on the drug of abuse or type of contingency learning. Overall, the evidence suggests that the IC is important for promoting drug seeking without drug reinforcement in some conditions, but the conflicting findings and lack of comprehensive comparisons across drug types and procedures presents challenges in drawing conclusions.

3.4. Expression of drug association memories

Although self-administration models are most commonly used for learning drug associations, other procedures such as conditioned place preference (CPP) are sometimes used to induce drug-context associations *without* instrumental learning in rodents. In these procedures, one compartment of either a two- or three-compartment chamber is paired with drug administration, with the other compartment typically paired with saline. After repeated training over days, the animal is returned to the apparatus to observe expression of the CPP memory, measured by preference for the previously drug-paired compartment. Several studies have used this procedure to probe the role of the pIC and aIC in the expression of drug association memories.

3.4.1. The pIC in expression of drug association memories

Evidence suggests that the pIC is important for CPP memory, although the lack of comprehensive work does not reveal whether there are mechanistic similarities in its involvement across drugs of abuse. Early work indicates that reversible pIC inactivation prevents expression (Contreras et al., 2007) and facilitates extinction (Contreras et al., 2012) of amphetamine-induced CPP in rats. Other studies found that both inhibiting nitric oxide signaling (Ma et al., 2014) and blocking muscarinic acetylcholine receptors (Wu et al., 2014) in the pIC reduce expression of *morphine*-induced CPP in rats. Both of these signaling mechanisms are important for several types of association memories (Itzhak et al., 1998; Miranda and McGaugh, 2004; Naor and Dudai, 1996), although these studies are the first to implicate them in IC-mediated drug association memory. On the other hand, evidence indicates that inhibiting DNA methyltransferases, which are thought to be important for memory formation and maintenance (Zovkic et al., 2013), in the pIC has no effect on the reconsolidation of morphine withdrawal-associated conditioned place aversion (CPA) memory in mice (Liu et al., 2016).

3.4.2. The aIC in expression of drug association memories

In comparison, aIC investigations provide a more compelling examination of this region's role in place association memories. Evidence indicates that pre-conditioning aIC lesions prevent expression of nicotine-induced CPP (Scott and Hiroi, 2011). Interestingly, this study indicates that, although aIC-lesioned mice do not have impaired

expression of food-induced CPP on the first test day, they extinguish this preference more quickly than controls. Moreover, the results suggest that aIC lesions have no effect on expression of nicotine withdrawal-induced CPA. These findings support a role for the aIC in expression of rewarding, but not aversive, drug-place association memories that is distinct from associations with natural reward. Conversely, evidence indicates that aIC manipulations made immediately post-retrieval similarly impair subsequent expression of both morphine-induced CPP (Sun et al., 2020) and withdrawal-induced CPA memories (Liu et al., 2016). Whether this reflects a difference in nicotine vs morphine or expression vs reconsolidation of a withdrawal-associated memory is unclear.

4. Natural reward seeking

Given its role in interoception, one might expect that IC activity is important for seeking and consumption of natural rewards, such as food and water, as well. However, Naqvi and colleagues (2007) identified no effect of IC lesions on self-reported food intake, desire to eat, or pleasure from eating, indicating that an intact IC is not necessary for normal food consumption and taste processing. Nevertheless, it is possible that IC lesions induce changes in food-seeking behaviors that are not captured by self-report data.

4.1. The pIC in natural reward seeking

Despite its role as primary interoceptive cortex, the pIC does not appear to be necessary for natural reward seeking, although specific pIC projections may regulate general consummatory behavior. Evidence indicates that reversible pIC inactivation in rats has no effect on food self-administration (Forget et al., 2010; Pushparaj et al., 2013) or cued or food-primed reinstatement of food seeking (Pushparaj et al., 2013), even though these manipulations attenuate nicotine self-administration and reinstatement. These findings recapitulate those seen in lesion patients and suggest that pIC activity is not necessary for food consumption or food seeking. However, recent evidence indicates that, although optogenetic inhibition of pIC projections to either the NAcore or CeA has no effect on food consumption in mice, brief optogenetic stimulation of either pathway interrupts ongoing food eating and water drinking and *reduces* food and water consumption (Gehrlach et al., 2019). Nonetheless, the results also suggest that optogenetic stimulation of the pIC-CeA pathway produces anxiety-like behaviors and inhibition of ongoing consummatory behaviors more generally, although stimulation of the pIC-NAcore pathway does not produce these non-specific effects. This indicates that, though pIC-NAcore activity is not *necessary* for food or water consumption, activity in this pathway can regulate consummatory behavior. Notably, prior work has investigated the roles of aIC, but not pIC, projections to the NAcore and CeA in drug taking. Therefore, it is unclear whether these pIC pathways have different roles in natural reward vs drug taking.

4.2. The aIC in natural reward seeking

Similar to the pIC, evidence indicates that reversible aIC inactivation in rats has no effect on self-administration of natural rewards including food pellets (Pushparaj et al., 2015) and saccharin (De Oliveira Sergio et al., 2021). However, chemogenetic activation of the aIC reduces *high fat* food self-administration in freely fed rats, but not food-restricted rats, and does not alter self-administration of standard food pellets (Price et al., 2019). To our knowledge, this is the only study that has compared the effect of aIC manipulation on self-administration of high fat vs standard food pellets and across satiety states of the animal. It appears that aIC activation attenuates hedonic, rather than homeostatic, consumption of the highly palatable high fat food reward, which may recruit aIC activity in a manner distinct from standard food. Consistent with this idea, evidence indicates that chemogenetic activation of the aIC reduces sucrose self-administration in rats with free access to water, but not water self-administration in water-restricted rats (Haaranen et al., 2020). This manipulation also reduces alcohol self-administration in the same study, suggesting that aIC activity suppresses both alcohol and natural reward taking in this case. However, chemogenetic inhibition of the aIC has no effect on any of the measures in this study, calling into question whether endogenous aIC activity is normally suppressing these behaviors.

In some cases where specific aIC signaling mechanisms or projections have been implicated in drug taking, the role of these mechanisms in natural reward taking has been investigated for comparison. Evidence indicates that blocking aIC hypocretin (Hollander et al., 2008) or α_1 norepinephrine receptors (De Oliveira Sergio et al., 2021) has no effect on food or saccharin self-administration, respectively, despite reducing nicotine or alcohol self-administration in rats. On the other hand, evidence suggests that blocking aIC dopamine D1 receptors reduces both cocaine and food consumption in rats, although, as discussed previously, this could be a general motor effect of D1 antagonism (Di Pietro et al., 2008). In contrast to the pIC, work has not identified an aIC pathway that regulates natural reward-taking behavior. Evidence indicates that inhibiting aIC-NAcore (Jaramillo et al., 2018) and aIC-locus coeruleus projections (De Oliveira Sergio et al., 2021) does not affect sucrose or saccharin self-administration, respectively, despite modifying alcohol self-administration. It is unclear whether *activation* of these pathways would affect natural reward taking or seeking.

Evidence for aIC involvement in natural reward seeking is similarly limited and inconclusive. Although results from our laboratory indicate that reversible aIC inactivation has no effect on reinstatement of food seeking after extinction (Cosme et al., 2015), other evidence suggests that chemogenetic *activation* of the aIC reduces high fat food seeking one day after self-administration training in rats (Price et al., 2019). This discrepancy could be due to inhibition vs activation of the region, differences in incentive value of the high fat vs standard food pellet reward, or differences in procedures before the seeking tests. Extinction learning and/or prolonged removal from the food-seeking context could have modified the role of the aIC in food seeking in a manner distinct from 24 h of removal from the context, an idea that is supported by the CPP literature. Evidence indicates that pre-conditioning aIC lesions prevent expression of food-induced CPP *after* the first post-conditioning test day, but not acquisition or expression on the first test day, in mice (Scott and Hiroi, 2011). This suggests that aIC activity is either necessary to promote long-term expression of food association memories, or normally works against the extinction of CPP. The same study indicates that pre-conditioning aIC lesions prevent expression of nicotine-induced CPP from the first test day, suggesting that its involvement in expression of nicotine CPP memory is different from that of food CPP memory. Nevertheless, the exact conditions under which aIC activity regulates natural reward seeking remain unclear.

5. Risky decision making

In some reward seeking studies, motivation to obtain the reward is in competition with motivation to avoid aversive consequences. Despite evidence from the drug seeking literature, it remains unclear whether competing motivations recruit different IC mechanisms than reward seeking alone or whether drug use must be perceived as risky to recruit the IC, as some have proposed (Naqvi et al., 2014). In addition to disruptions in drug addiction, evidence indicates that patients with IC lesions have impaired decision making under risk (Clark et al., 2008). Moreover, studies suggest that compulsive drug use itself is associated with impaired decision making (Rogers et al., 1999). Because drug use has inherent risks to human well-being, it can be difficult to disentangle the role of the IC in drug seeking vs risky decision-making processes. Moreover, studies investigating drug seeking in rodents do not necessarily involve the same inherent risks as drug use in humans. Studies investigating IC subregions in natural reward seeking under risk

therefore provide an important comparison to the drug seeking literature and help to disentangle its role in risky decision making more generally.

5.1. The pIC in risky decision making

To our knowledge, only one study has investigated the pIC in natural reward seeking under risk, finding that neither reversible pIC inactivation nor pre-training pIC lesions affects risky decision making in freely fed rats trained on a version of the Iowa Gambling Task (Pushparaj et al., 2015). Responding for a risky option in this task results in low probability of a large food reward and high probability of receiving a long timeout period instead, whereas the safe options results in high probability of receiving a smaller food reward with low probability of a short

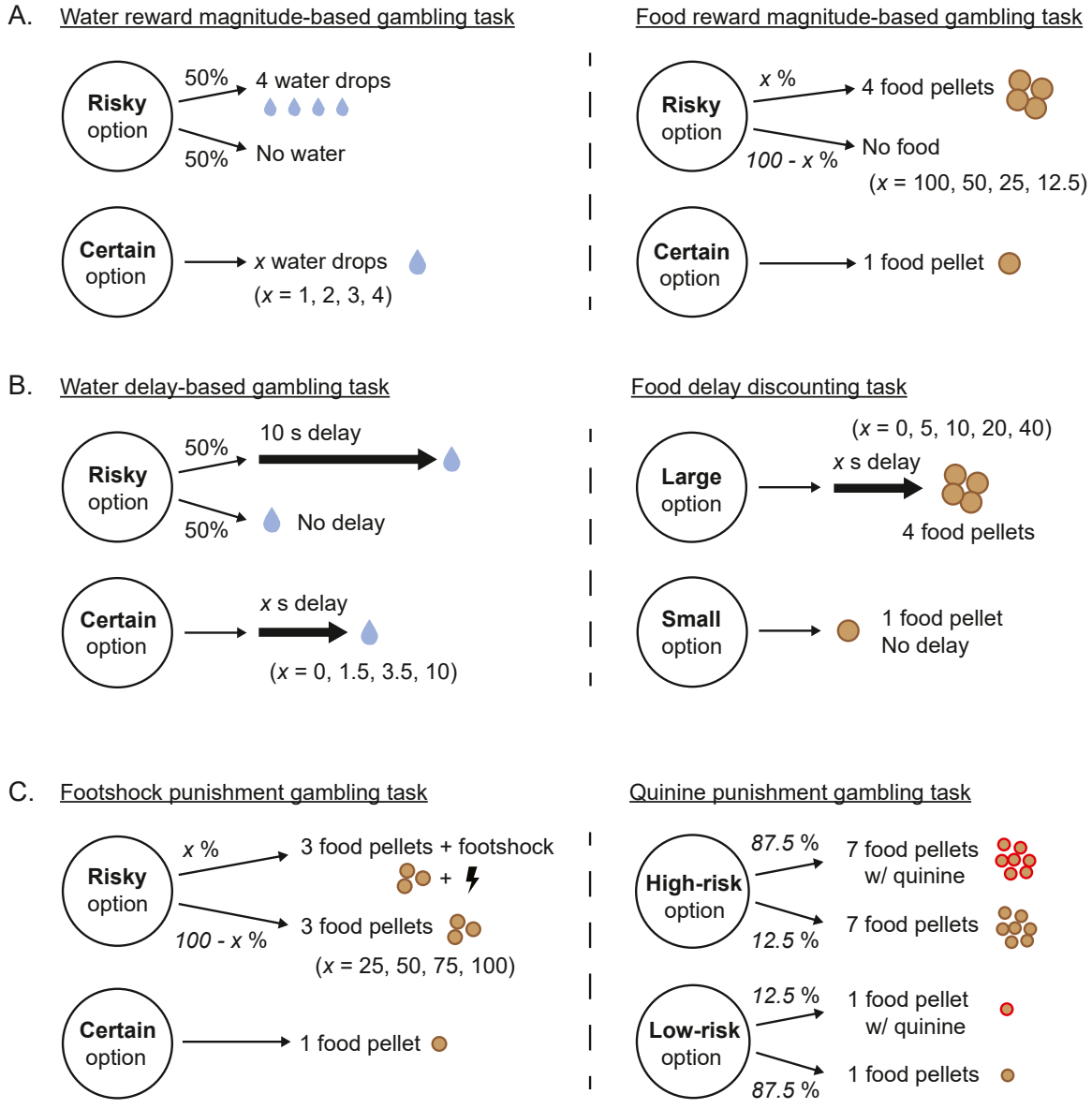

Fig. 3. A selection of comparisons between risky decision-making procedures described in this review where conflicting (or apparently conflicting) findings exist. A. Comparison of two similar reward magnitude-based gambling tasks. Left, procedure described in (Ishii et al., 2012). Rats can press a risky lever, which has a 50% chance of producing either a four-water drop reward, or no water drops. Pressing the certain lever always produces a reward (either 1, 2, 3, or 4 water drops, depending on the trial). Right, procedure described in (St Onge and Floresco, 2010). Rats can press a risky lever, which has some chance (either 100%, 50%, 25%, or 12.5%, depending on the trial) of producing a four-food pellet reward, or otherwise no food pellets. Pressing the certain lever always produces a smaller one-food pellet reward. B. Comparison of two delay-based decision-making tasks. Left, procedure described in (Ishii et al., 2012). Rats can press a risky lever, which has a 50% chance of producing a water reward with either no delay or a 10 s delay. Pressing the certain lever always produces a water reward with some delay (either 0, 1.5, 3.5, or 10 s, depending on the trial). Right, delay discounting procedures described in (Pattij et al., 2014). Rats can nose poke for a large food reward option, which produces four food pellets with some delay (either 0, 5, 10, 20, or 40 s, depending on the trial). A nose poke for the small food reward option produces one food pellet with no delay. C. Comparison of two gambling tasks wherein reward is sometimes paired with an aversive physical outcome. *Left*, procedures described in (Simon et al., 2011). Rats can press a risky lever, which produces three food pellets along with some chance (either 25%, 50%, 75%, or 100%, depending on the trial) of a concurrent footshock. Pressing the certain lever produces one food pellet without footshock. *Right*, procedures described in (Mizoguchi et al., 2015). Rats are placed on an elevated plus maze with access to high-risk and low-risk arms. Rats entering the high-risk arms have a 12.5% chance of finding seven food pellets at the end the arm, and an 87.5% chance of finding seven food pellets coated with bitter-tasting quinine. Rats entering the low-risk arms have an 87.5% chance of finding one food pellet at the end of the arm, and a 12.5% chance of finding one food pellet coated with quinine.

timeout period instead. Although the results indicate that the pIC is not necessary for acquisition or appropriate decision making in this task, there is not enough evidence to comment on the role of this subregion in risky decision making more broadly.

5.2. The aIC in risky decision making

Considerably more work has investigated the aIC in risky decision making, although evidence on its role has been conflicting. In contrast to the pIC, results indicate that both reversible inactivation and pre-training lesions of the aIC *attenuate* responding for risky options on the rat Iowa Gambling Task (Pushparaj et al., 2015), suggesting that aIC activity promotes food seeking under risk. Consistent with this finding, evidence indicates that reversible aIC inactivation reduces risky decision making in two different gambling tasks in water-restricted rats (Ishii, 2012). In a reward magnitude-based gambling task, responding for the risky option has a 50% chance of producing either a large water reward or no reward, whereas responding for the certain option has a 100% chance of producing a smaller water reward. In a delay-based gambling task, responding for the risky option has a 50% chance of producing a water reward with either no delay or a 10 s delay, whereas responding for the certain option always produces a water reward with a shorter than 10 s delay. The results of these studies suggest that involvement of the aIC in risky decision making is sensitive to multiple types of natural reward and aversive outcomes. However, another study, using a nearly identical paradigm to the reward magnitude-based gambling task used by Ishii and colleagues (2012) (Fig. 3A for comparison), indicates no effect of reversible aIC inactivation on risky responding for food reward in mildly food-restricted rats (St Onge and Floresco, 2010). The reason for this difference is unclear, although differences in reward type and level of deprivation might have influenced the perceived risk and reward value in a way that differentially recruited the aIC.

Whether modified dopaminergic signaling in the rat IC is associated with risky decision making is inconclusive. Prior work indicates that increased D1, but not D2, mRNA in the IC is positively associated with risky responding for a large food reward with some probability of concurrent footshock, suggesting that increased dopamine signaling in the IC promotes risk taking (Simon et al., 2011). On the other hand, evidence indicates that intra-aIC infusions of a D1, but not D2, receptor antagonist *increases* impulsive decision making in a delay-discounting task (Pattij, Schetters, and Schoffelmeer, 2014). In this task, responding at one port results in a small food reward with no delay (an impulsive choice), whereas responding at another port results in a larger food reward with a delay. Importantly, an impulsive choice is often the less risky one because there is no guarantee the animal will be able to collect the larger reward after a long delay. Therefore, the result of this experiment is consistent with others indicating increased preference for shorter reward delay as a result of aIC inactivation (Ishii et al., 2012) (Fig. 3B for comparison). Conversely, other evidence suggests that intra-aIC infusions of a D2 receptor antagonist, but not D1 receptor antagonist, *increases* risky responding in the same water reward magnitude-based gambling task by Ishii and colleagues described previously (Ishii et al., 2015). This opposes the direction of the effect when the aIC is inactivated with GABA receptor agonists (Ishii et al., 2012) and contrasts with the reward seeking literature, wherein evidence indicates that blocking D1, but not D2, receptors reduces nicotine, cocaine, and food self-administration (Di Pietro et al., 2008; Kutlu et al., 2013). It is possible that aIC D1- and D2-expressing neurons work in opposition to promote vs inhibit risk taking, again suggesting heterogeneity of function within the aIC.

Evidence suggests subregional differences within the aIC between the more posterior granular vs. more anterior agranular cortex that influence performance in a rat gambling ("slot machine") task (Cocker et al., 2016). In this particular work, rats responded on one of two levers to either collect a food reward or "roll" again, depending on the presence or absence of all three reward-associated light cues. The findings indicate that inactivating the agranular, but not granular, aIC with GABA receptor agonists impairs performance on this task, whereas D4 receptor activation in the granular, but not agranular, aIC improves performance. Based on these results, agranular aIC, but not granular, appears to be critical for appropriately responding to (potentially conflicting) visual stimuli. However, the results also indicate that potentiating dopamine signaling within the granular aIC *enhances* correct responding to these salient stimuli. Although the mechanisms underlying these intriguing results are unclear, the evidence nonetheless points to critical subregional and neurotransmitter contributions to assessing external stimuli and responding to produce the most beneficial outcome.

There may be individual variability in aIC activity related to risky decision making as well. Evidence indicates that post-training aIC lesions reduce risky responding in rats that are initially risk-prone and increase risky responding in rats that are initially risk-averse, on a version of the Iowa Gambling Task with food reward (Daniel et al., 2017). This suggests that aIC activity is important for maintaining established behaviors and that lesions shift behavior to alternate strategies, an idea that is consistent with some of the drug-seeking literature (Pelloux et al., 2013; Rotge et al., 2017). Moreover, evidence indicates that drug use itself alters IC risk evaluation processes for natural reward. Prior work suggests that rats treated with methamphetamine for 7–15 days choose a high-risk option more frequently than control rats in a gambling task, wherein the high-risk option is a larger food reward with higher probability of quinine coating than the low-risk option (Mizoguchi et al., 2015). These methamphetamine-treated rats also have increased c-Fos expression in the IC and NAcore. Interestingly, the results indicate that chemogenetic aIC inhibition reduces risky decision making in methamphetamine-treated rats but *increases* risky decision making in control rats. This finding suggests that methamphetamine use alters the role of the aIC in risky decision-making, and mimics the bidirectional effects identified by Daniel and colleagues (2017). In contrast, evidence indicates that systemic amphetamine reduces preference for a risky choice involving a potential footshock (Simon et al., 2011). However, in this study, rats were given a one-time injection of amphetamine immediately before testing. We speculate that the difference in results is more likely attributable to one-time vs repeated drug administration, rather than differences in reward size, punishment modality, or outcome probability (Fig. 3C for comparison). It is unknown whether these findings translate to other drugs of abuse, but it seems likely that drug use alters IC activity in a way that drives risky decision making in general and not exclusively decisions related to drug taking.

6. General discussion

Fifteen years after the groundbreaking finding of Naqvi and colleagues, there are still large gaps in our knowledge of how the IC regulates drug and natural reward seeking, as well as risky decision making more generally. In particular, it remains unclear under which circumstances, and by which mechanisms, IC activity regulates these motivated behaviors. Naqvi and colleagues argue that drug-associated cues and contexts activate the IC under conditions in which drug taking is perceived as "risky" or is in conflict with other goals or contingencies (Naqvi et al., 2014). Although some evidence supports this idea, many studies suggest that these conditions are not *necessary* to recruit the IC in drug seeking. For example, several studies indicate an effect of pIC and aIC manipulation during drug taking, in the absence of any competing goals or contingencies (Forget et al., 2010; Pushparaj et al., 2015). Nonetheless, this may be an effect on interoceptive processing of the drug itself that is dissociable from the processing of drug-associated cues in the absence of drug reinforcement. Whether perceived risk or competing contingencies are necessary conditions for drug-associated cues to recruit the IC in drug seeking without drug reinforcement remains a critical yet open question. Although several studies have manipulated the IC during seeking tests after contingency alterations

(extinction and aversion-induced abstinence), none have yet manipulated it after a period of prolonged withdrawal without contingency alterations (e.g., incubation of craving procedures).

Significant evidence suggests that drug seeking in response to drug-associated cues increases over extended withdrawal periods (Grimm, 2001; Tran-Nguyen et al., 1998). This phenomenon, known as incubation of craving, occurs across drugs of abuse and is conserved across species. By removing access to the drug after a period of self-administration, and then reintroducing drug-associated cues in a seeking (incubation) test days to months later, these procedures measure craving after extended withdrawal without any additional contingency learning (Fig. 2D). Evidence indicates that the incubation of nicotine craving is associated with upregulated dopamine signaling in the rat aIC (Abdolahi et al., 2010). However, no studies have yet manipulated the IC during incubation tests to determine its role in drug-associated cue processing after prolonged withdrawal. This is a critical body of work missing from the literature, as it could assess whether competing contingencies are necessary to recruit the IC when processing unreinforced drug-associated cues.

Lesion timing relative to the acquisition of drug seeking also appears to be important for determining the effect on this behavior, as made evident by the bidirectional effects of pre- vs post-training aIC lesions on cocaine seeking (Rotge et al., 2017). This finding has important implications for interpretation of IC lesion studies, but there is a lack of similar pre- and post-training lesion comparisons in the literature across drugs of abuse. It would also be important to determine whether IC lesion patients without a history of substance abuse have any change in susceptibility to substance use disorders post-lesion. One hypothesis is that IC activity is important for managing the utility of action-outcome associations and that IC lesions disrupt this association (Balleine and Dickinson, 1998; Parkes and Balleine, 2013; Parkes, Bradfield, and Balleine, 2015). Supporting this further, evidence indicates that aIC lesions have bidirectional effects on risky decision making in a gambling task, depending on whether the rat favors a risky or risk-averse strategy pre-lesion (Gehrlach et al., 2019). For individuals with a history of drug use or other risky behaviors, IC lesions may disrupt the established action-outcome association, decreasing craving and risk seeking. This is consistent with findings that drug use in humans alters risky decision making and correlates of neural activity throughout the IC (Bi et al., 2017; Rogers et al., 1999; Sutherland et al., 2013).

There is a notable dearth of in vivo electrophysiological recording studies that might explain how drug use alters IC activity. We hypothesize that repeated drug use induces aberrant activation of the IC under certain circumstances, which may account for changes in reward-seeking behavior. Indeed, limited evidence suggests that the response of aIC neurons to cocaine-predictive cues changes with self-administration learning, and that this cue-responsive activity correlates with measures of motivation for cocaine (Moschak et al., 2018). Other work indicates that chronic cocaine self-administration produces long-lasting changes in aIC neuron responsivity to sucrose reward and predictive cues as well (Pribut et al., 2021). Future studies should record from IC subregions during drug taking and drug seeking without drug reinforcement to determine whether IC neuronal activity is altered by drug infusions, drug-paired cues and contexts, or response-outcome contingency learning. Comparing IC activity during drug seeking vs natural reward seeking, with or without associated risk, would help elucidate which circumstances are necessary to recruit IC activity. These findings could help explain why IC inactivation does not consistently reduce drug taking and seeking. Additionally, studies can use multi-site electrophysiological recording to determine which conditions induce coherent activity in the aIC-NAcore, aIC-CeA, and aIC-locus coeruleus pathways, which are all implicated in aversion-resistant drug taking or seeking. If there is heterogeneity of function within the aIC, as evidence suggests, there should also be populations of aIC neurons that exert inhibitory control over drug seeking. One candidate downstream target of the aIC is the infralimbic cortex, a region that is important for extinction and ongoing inhibition of drug seeking (Gutman et al., 2017; Peters et al., 2008). It is possible that coherent activity between these regions predicts inhibition of drug seeking. Indeed, human imaging studies indicate a negative correlation between aIC-PFC resting state functional connectivity and nicotine use and craving (Bi et al., 2017; Sutherland et al., 2013).

Broadly, the evidence suggests that manipulating aIC or pIC activity has no effect on natural reward taking or seeking, with few exceptions. Future work should investigate the role of the IC in highly palatable food taking/seeking to validate the findings of Price and colleagues, which indicate that high-fat food and associated cues *can* recruit aIC activity to regulate food seeking (Price et al., 2019). High-fat food pellets may be a highly salient reward more akin to drugs of abuse. Evidence also indicates that aIC activity is important for natural reward seeking in risky decision-making tasks. It may be that natural reward seeking under risk creates a stronger action-outcome association, which aIC activity is necessary to maintain. Highly salient outcomes, and not necessarily inherent risk, may be the critical factor in recruiting the aIC in reward seeking. This idea is consistent with evidence that the aIC is a hub of the salience network (Uddin, 2015). Nevertheless, it is unclear why aIC activity does not always appear to be important for drug taking in the absence of paired aversive outcomes across drugs of abuse. This is complicated by evidence indicating that some drugs, like cocaine, have both appetitive and aversive properties themselves (Ettenberg et al., 1999).

In the few instances where there appear to be disparate roles for the IC in seeking of different drugs of abuse, distinct mechanisms of drug action might account for the differences. As discussed, evidence indicates that aIC lesions made after acquisition of cocaine or heroin self-administration produce effects in opposite directions on subsequent escalation of drug taking (Joshi et al., 2020; Rotge et al., 2017). One possible explanation is that an intact aIC is necessary to promote cocaine taking in the face of its aversive effects (Ettenberg et al., 1999), whereas the short-term effects of heroin are more purely rewarding and rely on other circuitry to promote self-administration (Badiani et al., 2011). These differences could also reflect the degree to which hedonic vs homeostatic mechanisms control cocaine and heroin self-administration. Whereas rats regulate their heroin intake to maintain an allostatic set point, rats will continue to self-administer cocaine to the point of overdose, indicating that cocaine relies more on hedonic mechanisms to promote intake (Bozarth and Wise, 1985). Somewhat perplexingly, evidence indicates that chemogenetic activation of the aIC suppresses hedonic, but not homeostatic, intake of highly palatable natural rewards (Haaranen et al., 2020; Price et al., 2019), inconsistent with the reduced cocaine self-administration produced by aIC lesions. However, as noted earlier, it is possible that chemogenetic activation disrupts endogenous aIC activity to produce behavioral outcomes akin to those produced by inhibitory manipulations. Regardless, it appears that aIC activity can both suppress and promote drug-seeking behavior depending on the properties of the drug. Further work is needed to determine the specific IC pathways and mechanisms that promote vs suppress drug seeking across drugs of abuse.

A unifying theory of pIC vs aIC function in drug seeking, reward, and risk processing remains elusive, largely due to a deficit of studies investigating the pIC. Notably, dissociation of function between the pIC and aIC cannot be fully explained by learning vs retrieval of drug-context associations, as earlier theorized (Naqvi et al., 2014), because pIC activity appears to be necessary for expression of drug association memories in at least some circumstances (Ma et al., 2014; Wu et al., 2014). Nevertheless, limited evidence suggests that there are differences in pIC vs aIC function in drug seeking and natural reward seeking under risk. Evidence indicates that aIC, but not pIC, inactivation reduces cued reinstatement of cocaine seeking after extinction learning (Cosme et al., 2015), whereas the pIC and aIC both appear to be important for cued reinstatement of nicotine seeking (Pushparaj et al., 2015). We posit that the aversive properties of cocaine create directly competing motivations

and that selecting for cocaine seeking selectively recruits the aIC. This selectivity could be explained by the greater connectivity of the aIC with limbic structures that are sensitive to aversive stimuli. Associated risk may explain subregional differences in natural reward seeking as well, as evidence indicates that aIC, but not pIC, inactivation reduces risky responding for a food reward (Pushparaj et al., 2015). While associated risk may not be a necessary factor to recruit the IC in reward seeking, it appears to be sufficient to preferentially recruit the aIC.

This theory is tenuous in the absence of studies investigating the pIC in aversion-resistant drug taking. Future work should manipulate pIC activity during cocaine or alcohol taking paired with footshock for comparison with the aIC literature. Evidence indicates that pIC c-Fos expression is unchanged by footshock-resistant cocaine self-administration, suggesting that pIC activity is unrelated to this behavior (Y. Chen et al., 2022). Nonetheless, it would also be useful to manipulate pIC activity during cocaine taking without paired aversive stimuli to determine whether this behavior is pIC-independent when reinforced with cocaine alone. Moreover, there may be pIC projections that have a distinct role in promoting (or inhibiting) drug-seeking behavior, which have not been explored. Parsing these subregional differences in IC functioning could be critical to developing therapeutic treatments for substance use disorder. Evidence indicates that silencing pIC neuronal activity via electrical stimulation reduces nicotine taking in rats (Pushparaj et al., 2013), and deep brain stimulation can be used to similarly alter neural activity in awake patients (Wang, 2018). Considerably more work is needed to understand how subregional differences in IC activity across risk and reward seeking gives rise to these complicated behaviors.

Finally, the vast majority of extant work has been conducted only in male animals. Given the evidence for sex differences in reward systems and risky decision making (Orsini et al., 2022), it is possible that IC subregions are differentially involved in drug and natural reward seeking across sexes, too. More work is needed to determine how individual differences in IC activity correlate with motivated behaviors.

7. Conclusions

Taken together, the literature indicates that the IC regulates drug-seeking behavior in a manner that is often, but not always, distinct from natural reward seeking. Drugs of abuse may preferentially recruit the IC in creating more powerful action-outcome associations that can be disrupted by IC manipulations. Nonetheless, certain conditions, such as a highly salient reward or associated risk, appear to recruit the IC in natural reward seeking as well. Whether the IC is important for learning these associations or merely expressing the reward-seeking behavior, remains unexplored, as do the mechanisms that might underlie this learning. Moreover, although evidence indicates that associated risk may preferentially recruit the aIC in reward seeking, a lack of comprehensive comparisons in the pIC precludes any confident unifying theory of pIC vs aIC function in complex motivated behaviors.

Interestingly, the evidence suggests that the aIC promotes *or suppresses* drug taking, drug seeking, and natural reward seeking under risk, pointing to heterogeneity of function within the subregion. Further work is needed to determine whether distinct aIC cell populations account for these different behaviors, and which conditions and mechanisms might select for one population over another. Competing external motivations may not even be a necessary condition to recruit the IC in motivated behaviors. Rather, we propose that IC activity selects for behaviors that will facilitate a return to a desired internal state, based on highly salient learned action-outcome contingencies.

Declarations of Interest

none.

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References

Abdolahi, A., Acosta, G., Breslin, F.J., Hemby, S.E., Lynch, W.J., 2010. Incubation of nicotine seeking is associated with enhanced protein kinase A-regulated signaling of dopamine- and cAMP-regulated phosphoprotein of 32 kDa in the insular cortex. Eur. J. Neurosci. 31 (4), 733–741. https://doi.org/10.1111/j.1460-9568.2010.07114.x.

Allen, G.V., Saper, C.B., Hurley, K.M., Cechetto, D.F., 1991. Organization of visceral and limbic connections in the insular cortex of the rat. J. Comp. Neurol. 311 (1), 1–16. https://doi.org/10.1002/cne.903110102.

Arguello, A.A., Wang, R., Lyons, C.M., Higginbotham, J.A., Hodges, M.A., Fuchs, R.A., 2017. Role of the agranular insular cortex in contextual control over cocaine-seeking behavior in rats. Psychopharmacology 234 (16), 2431–2441. https://doi.org/10.1007/s00213-017-4632-7.

Badiani, A., Belin, D., Epstein, D., Calu, D., Shaham, Y., 2011. Opiate versus psychostimulant addiction: the differences do matter. Nat. Rev. Neurosci. 12 (11), 685–700. https://doi.org/10.1038/nrn3104.

Balleine, B.W., Dickinson, A., 1998. Goal-directed instrumental action: contingency and incentive learning and their cortical substrates. Neuropharmacology 37 (4–5), 407–419. https://doi.org/10.1016/s0028-3908(98)00033-1.

Bi, Y., Yuan, K., Guan, Y., Cheng, J., Zhang, Y., Li, Y., Tian, J., 2017. Altered resting state functional connectivity of anterior insula in young smokers. Brain Imaging Behav. 11 (1), 155–165. https://doi.org/10.1007/s11682-016-9511-z.

Billman, G.E., 1995. Cocaine: a review of its toxic actions on cardiac function. Crit. Rev. Toxicol. 25 (2), 113–132. https://doi.org/10.3109/10408449509021610.

Bozarth, M.A., Wise, R.A., 1985. Toxicity associated with long-term intravenous heroin and cocaine self-administration in the rat. JAMA 254 (1), 81–83.

Burkey, A.R., Carstens, E., Jasmin, L., 1999. Dopamine reuptake inhibition in the rostral agranular insular cortex produces antinociception. J. Neurosci. 19 (10), 4169–4179. https://doi.org/10.1523/JNEUROSCI.19-10-04169.1999.

Campbell, E.J., Flanagan, J.P.M., Walker, L.C., Hill, M., Marchant, N.J., Lawrence, A.J., 2019. Anterior insular cortex is critical for the propensity to relapse following punishment-imposed abstinence of alcohol seeking. J. Neurosci. 39 (6), 1077–1087. https://doi.org/10.1523/JNEUROSCI.1596-18.2018.

Chang, L.J., Yarkoni, T., Khaw, M.W., Sanfey, A.G., 2013. Decoding the role of the insula in human cognition: functional parcellation and large-scale reverse inference. Cereb. Cortex 23 (3), 739–749. https://doi.org/10.1093/cercor/bhs065.

Chen, H., Lasek, A.W., 2020. Perineuronal nets in the insula regulate aversion-resistant alcohol drinking. Addict. Biol. 25 (6), e12821 https://doi.org/10.1111/adb.12821.

Chen, H., He, D., Lasek, A.W., 2015. Repeated binge drinking increases perineuronal nets in the insular cortex. Alcohol Clin. Exp. Res 39 (10), 1930–1938. https://doi.org/10.1111/acer.12847.

Chen, Y., Wang, G., Zhang, W., Han, Y., Zhang, L., Xu, H., Shi, J., 2022. An orbitofrontal cortex-anterior insular cortex circuit gates compulsive cocaine use. Sci. Adv. 8 (51), eabq5745. https://doi.org/10.1126/sciadv.abq5745.

Clark, L., Bechara, A., Damasio, H., Aitken, M.R., Sahakian, B.J., Robbins, T.W., 2008. Differential effects of insular and ventromedial prefrontal cortex lesions on risky decision-making. Brain 131 (Pt 5), 1311–1322. https://doi.org/10.1093/brain/awn066.

Cloutman, L.L., Binney, R.J., Drakesmith, M., Parker, G.J., Lambon Ralph, M.A., 2012. The variation of function across the human insula mirrors its patterns of structural connectivity: evidence from in vivo probabilistic tractography. Neuroimage 59 (4), 3514–3521. https://doi.org/10.1016/j.neuroimage.2011.11.016.

Cocker, P.J., Lin, M.Y., Barrus, M.M., Le Foll, B., Winstanley, C.A., 2016. The agranular and granular insula differentially contribute to gambling-like behavior on a rat slot machine task: effects of inactivation and local infusion of a dopamine D4 agonist on reward expectancy. Psychopharmacol. (Berl.) 233 (17), 3135–3147. https://doi.org/10.1007/s00213-016-4355-1.

Contreras, M., Ceric, F., Torrealba, F., 2007. Inactivation of the interoceptive insula disrupts drug craving and malaise induced by lithium. Science 318 (5850), 655–658. https://doi.org/10.1126/science.1145590.

Contreras, M., Billeke, P., Vicencio, S., Madrid, C., Perdomo, G., Gonzalez, M., Torrealba, F., 2012. A role for the insular cortex in long-term memory for context-evoked drug craving in rats. Neuropsychopharmacology 37 (9), 2101–2108. https://doi.org/10.1038/npp.2012.59.

Cosme, C.V., Gutman, A.L., LaLumiere, R.T., 2015. The dorsal agranular insular cortex regulates the cued reinstatement of cocaine-seeking, but not food-seeking, behavior in rats. Neuropsychopharmacology 40 (10), 2425–2433. https://doi.org/10.1038/npp.2015.92.

Craig, A.D., 2003. Interoception: the sense of the physiological condition of the body. Curr. Opin. Neurobiol. 13 (4), 500–505. https://doi.org/10.1016/s0959-4388(03)00090-4.

Craig, A.D., 2009. How do you feel–now? The anterior insula and human awareness. Nat. Rev. Neurosci. 10 (1), 59–70. https://doi.org/10.1038/nrn2555.

Daniel, M.L., Cocker, P.J., Lacoste, J., Mar, A.C., Houeto, J.L., Belin-Rauscent, A., Belin, D., 2017. The anterior insula bidirectionally modulates cost-benefit decision-

making on a rodent gambling task. Eur. J. Neurosci. 46 (10), 2620–2628. https://doi.org/10.1111/ejn.13689.
De Oliveira Sergio, T., Lei, K., Kwok, C., Ghotra, S., Wegner, S.A., Walsh, M., Hopf, F.W., 2021. The role of anterior insula-brainstem projections and alpha-1 noradrenergic receptors for compulsion-like and alcohol-only drinking. Neuropsychopharmacology 46 (11), 1918–1926. https://doi.org/10.1038/s41386-021-01071-w.
Di Chiara, G, 2000. Role of dopamine in the behavioural actions of nicotine related to addiction. Eur. J. Pharm. 393 (1–3), 295–314. https://doi.org/10.1016/s0014-2999(00)00122-9.
Di Pietro, N.C., Black, Y.D., Kantak, K.M., 2006. Context-dependent prefrontal cortex regulation of cocaine self-administration and reinstatement behaviors in rats. Eur. J. Neurosci. 24 (11), 3285–3298. https://doi.org/10.1111/j.1460-9568.2006.05193.x.
Di Pietro, N.C., Mashhoon, Y., Heaney, C., Yager, L.M., Kantak, K.M., 2008. Role of dopamine D1 receptors in the prefrontal dorsal agranular insular cortex in mediating cocaine self-administration in rats. Psychopharmacol. (Berl.) 200 (1), 81–91. https://doi.org/10.1007/s00213-008-1149-0.
Dolensek, N., Gehrlach, D.A., Klein, A.S., Gogolla, N., 2020. Facial expressions of emotion states and their neuronal correlates in mice. Science 368 (6486), 89–94. https://doi.org/10.1126/science.aaz9468.
Droutman, V., Read, S.J., Bechara, A., 2015. Revisiting the role of the insula in addiction. Trends Cogn. Sci. 19 (7), 414–420. https://doi.org/10.1016/j.tics.2015.05.005.
Ettenberg, A., Raven, M.A., Danluck, D.A., Necessary, B.D., 1999. Evidence for opponent-process actions of intravenous cocaine. Pharm. Biochem Behav. 64 (3), 507–512. https://doi.org/10.1016/s0091-3057(99)00109-4.
Evrard, H.C., Logothetis, N.K., Craig, A.D., 2014. Modular architectonic organization of the insula in the macaque monkey. J. Comp. Neurol. 522 (1), 64–97. https://doi.org/10.1002/cne.23436.
Forget, B., Pushparaj, A., Le Foll, B., 2010. Granular insular cortex inactivation as a novel therapeutic strategy for nicotine addiction. Biol. Psychiatry 68 (3), 265–271. https://doi.org/10.1016/j.biopsych.2010.01.029.
Gaspar, P., Bloch, B., Le Moine, C., 1995. D1 and D2 receptor gene expression in the rat frontal cortex: cellular localization in different classes of efferent neurons. Eur. J. Neurosci. 7 (5), 1050–1063. https://doi.org/10.1111/j.1460-9568.1995.tb01092.x.
Gehrlach, D.A., Dolensek, N., Klein, A.S., Roy Chowdhury, R., Matthys, A., Junghanel, M., Gogolla, N., 2019. Aversive state processing in the posterior insular cortex. Nat. Neurosci. 22 (9), 1424–1437. https://doi.org/10.1038/s41593-019-0469-1.
Gehrlach, D.A., Weiand, C., Gaitanos, T.N., Cho, E., Klein, A.S., Hennrich, A.A., Gogolla, N., 2020. A whole-brain connectivity map of mouse insular cortex. Elife 9. https://doi.org/10.7554/eLife.55585.
Ghareh, H., Alonso-Lozares, I., Schetters, D., Herman, R.J., Heistek, T.S., Van Mourik, Y., Marchant, N.J., 2022. Role of anterior insula cortex in context-induced relapse of nicotine-seeking. Elife 11. https://doi.org/10.7554/eLife.75609.
Gogolla, N., 2017. The insular cortex. Curr. Biol. 27 (12), R580–R586. https://doi.org/10.1016/j.cub.2017.05.010.
Grimm, J.W., Hope, B.T., Wise, R.A., Shaham, Y., 2001. Neuroadaptation. Incubation of cocaine craving after withdrawal. Nature 412 (6843), 141–142. https://doi.org/10.1038/35084134.
Gutman, A.L., Nett, K.E., Cosme, C.V., Worth, W.R., Gupta, S.C., Wemmie, J.A., LaLumiere, R.T., 2017. Extinction of cocaine seeking requires a window of infralimbic pyramidal neuron activity after unreinforced lever presses. J. Neurosci. 37 (25), 6075–6086. https://doi.org/10.1523/JNEUROSCI.3821-16.2017.
Haaranen, M., Scuppa, G., Tambalo, S., Jarvi, V., Bertozzi, S.M., Armirotti, A., Hyytia, P., 2020. Anterior insula stimulation suppresses appetitive behavior while inducing forebrain activation in alcohol-preferring rats. Transl. Psychiatry 10 (1), 150. https://doi.org/10.1038/s41398-020-0833-7.
Haaranen, M., Schafer, A., Jarvi, V., Hyytia, P., 2020. Chemogenetic stimulation and silencing of the insula, amygdala, nucleus accumbens, and their connections differentially modulate alcohol drinking in rats. Front Behav. Neurosci. 14, 580849 https://doi.org/10.3389/fnbeh.2020.580849.
Hanlon, C.A., Dowdle, L.T., Gibson, N.B., Li, X., Hamilton, S., Canterberry, M., Hoffman, M., 2018. Cortical substrates of cue-reactivity in multiple substance dependent populations: transdiagnostic relevance of the medial prefrontal cortex. Transl. Psychiatry 8 (1), 186. https://doi.org/10.1038/s41398-018-0220-9.
Hollander, J.A., Lu, Q., Cameron, M.D., Kamenecka, T.M., Kenny, P.J., 2008. Insular hypocretin transmission regulates nicotine reward. Proc. Natl. Acad. Sci. 105 (49), 19480–19485. https://doi.org/10.1073/pnas.0808023105.
Hubel, D.H., Wiesel, T.N., 1962. Receptive fields, binocular interaction and functional architecture in the cat's visual cortex. J. Physiol. 160, 106–154. https://doi.org/10.1113/jphysiol.1962.sp006837.
Ishii, H., Ohara, S., Tobler, P.N., Tsutsui, K., Iijima, T., 2012. Inactivating anterior insular cortex reduces risk taking. J. Neurosci. 32 (45), 16031–16039. https://doi.org/10.1523/JNEUROSCI.2278-12.2012.
Ishii, H., Ohara, S., Tobler, P.N., Tsutsui, K., Iijima, T., 2015. Dopaminergic and serotonergic modulation of anterior insular and orbitofrontal cortex function in risky decision making. Neurosci. Res 92, 53–61. https://doi.org/10.1016/j.neures.2014.11.009.
Itzhak, Y., Martin, J.L., Black, M.D., Huang, P.L., 1998. The role of neuronal nitric oxide synthase in cocaine-induced conditioned place preference. Neuroreport 9 (11), 2485–2488. https://doi.org/10.1097/00001756-199808030-00011.
Jakab, A., Molnar, P.P., Bogner, P., Beres, M., Berenyi, E.L., 2012. Connectivity-based parcellation reveals interhemispheric differences in the insula. Brain Topogr. 25 (3), 264–271. https://doi.org/10.1007/s10548-011-0205-y.
Jaramillo, A.A., Agan, V.E., Makhijani, V.H., Pedroza, S., McElligott, Z.A., Besheer, J., 2018. Functional role for suppression of the insular-striatal circuit in modulating interoceptive effects of alcohol. Addict. Biol. 23 (5), 1020–1031. https://doi.org/10.1111/adb.12551.
Jaramillo, A.A., Van Voorhies, K., Randall, P.A., Besheer, J., 2018. Silencing the insular-striatal circuit decreases alcohol self-administration and increases sensitivity to alcohol. Behav. Brain Res 348, 74–81. https://doi.org/10.1016/j.bbr.2018.04.007.
Jaramillo, A.A., Randall, P.A., Stewart, S., Fortino, B., Van Voorhies, K., Besheer, J., 2018. Functional role for cortical-striatal circuitry in modulating alcohol self-administration. Neuropharmacology 130, 42–53. https://doi.org/10.1016/j.neuropharm.2017.11.035.
Joshi, D.D., Puaud, M., Fouyssac, M., Belin-Rauscent, A., Everitt, B., Belin, D., 2020. The anterior insular cortex in the rat exerts an inhibitory influence over the loss of control of heroin intake and subsequent propensity to relapse. Eur. J. Neurosci. 52 (9), 4115–4126. https://doi.org/10.1111/ejn.14889.
Kutlu, M.G., Burke, D., Slade, S., Hall, B.J., Rose, J.E., Levin, E.D., 2013. Role of insular cortex D(1) and D(2) dopamine receptors in nicotine self-administration in rats. *Behav. Brain Res* 256, 273–278. https://doi.org/10.1016/j.bbr.2013.08.005.
Liu, P., Zhang, J., Li, M., Sui, N., 2016. Distinctive Roles of 5-aza-2′-deoxycytidine in Anterior Agranular Insular and Basolateral Amygdala in Reconsolidation of Aversive Memory Associated with Morphine in Rats. Front Behav. Neurosci. 10, 50. https://doi.org/10.3389/fnbeh.2016.00050.
Livneh, Y., Andermann, M.L., 2021. Cellular activity in insular cortex across seconds to hours: Sensations and predictions of bodily states. Neuron 109 (22), 3576–3593. https://doi.org/10.1016/j.neuron.2021.08.036.
Ma, P., Liu, H., Li, H., Huang, X., Chen, L., Zhai, H., 2014. Involvement of the insular nitric oxide signaling pathway in the expression of morphine-induced conditioned place preference in rats. Neuroreport 25 (9), 641–646. https://doi.org/10.1097/WNR.0000000000000146.
McGregor, A., Roberts, D.C., 1993. Dopaminergic antagonism within the nucleus accumbens or the amygdala produces differential effects on intravenous cocaine self-administration under fixed and progressive ratio schedules of reinforcement. Brain Res 624 (1–2), 245–252. https://doi.org/10.1016/0006-8993(93)90084-z.
Miranda, M.I., McGaugh, J.L., 2004. Enhancement of inhibitory avoidance and conditioned taste aversion memory with insular cortex infusions of 8-Br-cAMP: involvement of the basolateral amygdala. Learn Mem. 11 (3), 312–317. https://doi.org/10.1101/lm.72804.
Mizoguchi, H., Katahira, K., Inutsuka, A., Fukumoto, K., Nakamura, A., Wang, T., Yamada, K., 2015. Insular neural system controls decision-making in healthy and methamphetamine-treated rats. Proc. Natl. Acad. Sci. USA 112 (29), E3930–E3939. https://doi.org/10.1073/pnas.1418014112.
Moschak, T.M., Wang, X., Carelli, R.M., 2018. A Neuronal Ensemble in the Rostral Agranular Insula Tracks Cocaine-Induced Devaluation of Natural Reward and Predicts Cocaine Seeking. J. Neurosci. 38 (39), 8463–8472. https://doi.org/10.1523/JNEUROSCI.1195-18.2018.
Naor, C., Dudai, Y., 1996. Transient impairment of cholinergic function in the rat insular cortex disrupts the encoding of taste in conditioned taste aversion. Behav. Brain Res 79 (1–2), 61–67. https://doi.org/10.1016/0166-4328(95)00262-6.
Naqvi, N.H., Bechara, A., 2009. The hidden island of addiction: the insula. Trends Neurosci. 32 (1), 56–67. https://doi.org/10.1016/j.tins.2008.09.009.
Naqvi, N.H., Rudrauf, D., Damasio, H., Bechara, A., 2007. Damage to the insula disrupts addiction to cigarette smoking. Science 315 (5811), 531–534. https://doi.org/10.1126/science.1135926.
Naqvi, N.H., Gaznick, N., Tranel, D., Bechara, A., 2014. The insula: a critical neural substrate for craving and drug seeking under conflict and risk. Ann. N. Y Acad. Sci. 1316, 53–70. https://doi.org/10.1111/nyas.12415.
Orsini, C.A., Brown, T.E., Hodges, T.E., Alonso-Caraballo, Y., Winstanley, C.A., Becker, J. B., 2022. Neural Mechanisms Mediating Sex Differences in Motivation for Reward: Cognitive Bias, Food, Gambling, and Drugs of Abuse. J. Neurosci. 42 (45), 8477–8487. https://doi.org/10.1523/JNEUROSCI.1378-22.2022.
Parkes, S.L., Balleine, B.W., 2013. Incentive memory: evidence the basolateral amygdala encodes and the insular cortex retrieves outcome values to guide choice between goal-directed actions. J. Neurosci. 33 (20), 8753–8763. https://doi.org/10.1523/JNEUROSCI.5071-12.2013.
Parkes, S.L., Bradfield, L.A., Balleine, B.W., 2015. Interaction of insular cortex and ventral striatum mediates the effect of incentive memory on choice between goal-directed actions. J. Neurosci. 35 (16), 6464–6471. https://doi.org/10.1523/JNEUROSCI.4153-14.2015.
Pattij, T., Schetters, D., Schoffelmeer, A.N., 2014. Dopaminergic modulation of impulsive decision making in the rat insular cortex. Behav. Brain Res 270, 118–124. https://doi.org/10.1016/j.bbr.2014.05.010.
Pelloux, Y., Murray, J.E., Everitt, B.J., 2013. Differential roles of the prefrontal cortical subregions and basolateral amygdala in compulsive cocaine seeking and relapse after voluntary abstinence in rats. Eur. J. Neurosci. 38 (7), 3018–3026. https://doi.org/10.1111/ejn.12289.
Peng, Y., Gillis-Smith, S., Jin, H., Trankner, D., Ryba, N.J., Zuker, C.S., 2015. Sweet and bitter taste in the brain of awake behaving animals. Nature 527 (7579), 512–515. https://doi.org/10.1038/nature15763.
Peters, J., LaLumiere, R.T., Kalivas, P.W., 2008. Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. J. Neurosci. 28 (23), 6046–6053. https://doi.org/10.1523/JNEUROSCI.1045-08.2008.
Pribut, H.J., Vazquez, D., Brockett, A.T., Wei, A.D., Tennyson, S.S., Roesch, M.R., 2021. Prior Cocaine Exposure Increases Firing to Immediate Reward While Attenuating Cue and Context Signals Related to Reward Value in the Insula. J. Neurosci. 41 (21), 4667–4677. https://doi.org/10.1523/JNEUROSCI.3025-20.2021.
Price, A.E., Stutz, S.J., Hommel, J.D., Anastasio, N.C., Cunningham, K.A., 2019. Anterior insula activity regulates the associated behaviors of high fat food binge intake and

cue reactivity in male rats. Appetite 133, 231–239. https://doi.org/10.1016/j.appet.2018.11.011.
Pushparaj, A., Le Foll, B., 2015. Involvement of the caudal granular insular cortex in alcohol self-administration in rats. Behav. Brain Res 293, 203–207. https://doi.org/10.1016/j.bbr.2015.07.044.
Pushparaj, A., Hamani, C., Yu, W., Shin, D.S., Kang, B., Nobrega, J.N., Le Foll, B., 2013. Electrical stimulation of the insular region attenuates nicotine-taking and nicotine-seeking behaviors. Neuropsychopharmacology 38 (4), 690–698. https://doi.org/10.1038/npp.2012.235.
Pushparaj, A., Kim, A.S., Musiol, M., Trigo, J.M., Le Foll, B., 2015. Involvement of the rostral agranular insular cortex in nicotine self-administration in rats. Behav. Brain Res 290, 77–83. https://doi.org/10.1016/j.bbr.2015.04.039.
Pushparaj, A., Kim, A.S., Musiol, M., Zangen, A., Daskalakis, Z.J., Zack, M., Le, Foll, B., 2015. Differential Involvement of the Agranular vs Granular Insular Cortex in the Acquisition and Performance of Choice Behavior in a Rodent Gambling Task. Neuropsychopharmacology 40 (12), 2832–2842. https://doi.org/10.1038/npp.2015.133.
Rogers, R.D., Everitt, B.J., Baldacchino, A., Blackshaw, A.J., Swainson, R., Wynne, K., Robbins, T.W., 1999. Dissociable deficits in the decision-making cognition of chronic amphetamine abusers, opiate abusers, patients with focal damage to prefrontal cortex, and tryptophan-depleted normal volunteers: evidence for monoaminergic mechanisms. Neuropsychopharmacology 20 (4), 322–339. https://doi.org/10.1016/S0893-133X(98)00091-8.
Rotge, J.Y., Cocker, P.J., Daniel, M.L., Belin-Rauscent, A., Everitt, B.J., Belin, D., 2017. Bidirectional regulation over the development and expression of loss of control over cocaine intake by the anterior insula. Psychopharmacol. (Berl.) 234 (9–10), 1623–1631. https://doi.org/10.1007/s00213-017-4593-x.
Scofield, M.D., Heinsbroek, J.A., Gipson, C.D., Kupchik, Y.M., Spencer, S., Smith, A.C., Kalivas, P.W., 2016. The Nucleus Accumbens: Mechanisms of Addiction across Drug Classes Reflect the Importance of Glutamate Homeostasis. Pharm. Rev. 68 (3), 816–871. https://doi.org/10.1124/pr.116.012484.
Scott, D., Hiroi, N., 2011. Deconstructing craving: dissociable cortical control of cue reactivity in nicotine addiction. Biol. Psychiatry 69 (11), 1052–1059. https://doi.org/10.1016/j.biopsych.2011.01.023.
Seif, T., Chang, S.J., Simms, J.A., Gibb, S.L., Dadgar, J., Chen, B.T., Hopf, F.W., 2013. Cortical activation of accumbens hyperpolarization-active NMDARs mediates aversion-resistant alcohol intake. Nat. Neurosci. 16 (8), 1094–1100. https://doi.org/10.1038/nn.3445.
Shi, C.J., Cassell, M.D., 1998. Cortical, thalamic, and amygdaloid connections of the anterior and posterior insular cortices. J. Comp. Neurol. 399 (4), 440–468. https://doi.org/10.1002/(sici)1096-9861(19981005)399:4<440::aid-cne2>3.0.co;2-1.
Simon, N.W., Montgomery, K.S., Beas, B.S., Mitchell, M.R., LaSarge, C.L., Mendez, I.A., Setlow, B., 2011. Dopaminergic modulation of risky decision-making. J. Neurosci. 31 (48), 17460–17470. https://doi.org/10.1523/JNEUROSCI.3772-11.2011.
St Onge, J.R., Floresco, S.B., 2010. Prefrontal cortical contribution to risk-based decision making. Cereb. Cortex 20 (8), 1816–1828. https://doi.org/10.1093/cercor/bhp250.
Sun, K., Mu, Q., Chang, H., Zhang, C., Wang, Y., Rong, S., Sun, T., 2020. Postretrieval Microinjection of Baclofen Into the Agranular Insular Cortex Inhibits Morphine-Induced CPP by Disrupting Reconsolidation. Front Pharm. 11, 743. https://doi.org/10.3389/fphar.2020.00743.
Sutherland, M.T., Carroll, A.J., Salmeron, B.J., Ross, T.J., Stein, E.A., 2013. Insula's functional connectivity with ventromedial prefrontal cortex mediates the impact of trait alexithymia on state tobacco craving. Psychopharmacol. (Berl.) 228 (1), 143–155. https://doi.org/10.1007/s00213-013-3018-8.
Thornhill, J.A., Townsend, C., Gregor, L., 1989. Intravenous morphine infusion (IMF) to drug-naive, conscious rats evokes bradycardic, hypotensive effects, but pressor actions are elicited after IMF to rats previously given morphine. Can. J. Physiol. Pharm. 67 (3), 213–222. https://doi.org/10.1139/y89-036.
Tran-Nguyen, L.T., Fuchs, R.A., Coffey, G.P., Baker, D.A., O'Dell, L.E., Neisewander, J.L., 1998. Time-dependent changes in cocaine-seeking behavior and extracellular dopamine levels in the amygdala during cocaine withdrawal. Neuropsychopharmacology 19 (1), 48–59. https://doi.org/10.1016/S0893-133X(97)00205-4.
Tye, K.M., 2018. Neural Circuit Motifs in Valence Processing. Neuron 100 (2), 436–452. https://doi.org/10.1016/j.neuron.2018.10.001.
Uddin, L.Q., 2015. Salience processing and insular cortical function and dysfunction. Nat. Rev. Neurosci. 16 (1), 55–61. https://doi.org/10.1038/nrn3857.
Vazey, E.M., den Hartog, C.R., Moorman, D.E., 2018. Central Noradrenergic Interactions with Alcohol and Regulation of Alcohol-Related Behaviors. Handb. Exp. Pharm. 248, 239–260. https://doi.org/10.1007/164_2018_108.
Venniro, M., Caprioli, D., Zhang, M., Whitaker, L.R., Zhang, S., Warren, B.L., Shaham, Y., 2017. The Anterior Insular Cortex–>Central Amygdala Glutamatergic Pathway Is Critical to Relapse after Contingency Management. e418 Neuron 96 (2), 414–427. https://doi.org/10.1016/j.neuron.2017.09.024.
Wang, L., Gillis-Smith, S., Peng, Y., Zhang, J., Chen, X., Salzman, C.D., Zuker, C.S., 2018. The coding of valence and identity in the mammalian taste system. Nature 558 (7708), 127–131. https://doi.org/10.1038/s41586-018-0165-4.
Wang, T.R., Moosa, S., Dallapiazza, R.F., Elias, W.J., Lynch, W.J., 2018. Deep brain stimulation for the treatment of drug addiction. Neurosurg. Focus 45 (2), E11. https://doi.org/10.3171/2018.5.FOCUS18163.
Warlow, S.M., Berridge, K.C., 2021. Incentive motivation: 'wanting' roles of central amygdala circuitry. Behav. Brain Res 411, 113376. https://doi.org/10.1016/j.bbr.2021.113376.
Wu, W., Li, H., Liu, Y., Huang, X., Chen, L., Zhai, H., 2014. Involvement of insular muscarinic cholinergic receptors in morphine-induced conditioned place preference in rats. Psychopharmacol. (Berl.) 231 (21), 4109–4118. https://doi.org/10.1007/s00213-014-3550-1.
Zovkic, I.B., Guzman-Karlsson, M.C., Sweatt, J.D., 2013. Epigenetic regulation of memory formation and maintenance. Learn Mem. 20 (2), 61–74. https://doi.org/10.1101/lm.026575.112.