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IT'S MORE THAN JUST INTEROCEPTION: THE INSULAR CORTEX INVOLVEMENT IN ALCOHOL USE DISORDER

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ABSTRACT

Understanding brain structures and circuits impacted by alcohol use disorder is critical for improving our future prevention techniques and treatment options. A brain region that has recently gained traction for its involvement in substance use disorder is the insular cortex. This brain region is multi-functional and spatially complex, resulting in a relative lack of understanding of the involvement of the insular cortex in alcohol use disorder. Here we discuss the role of the insular cortex in alcohol use disorder, particularly during periods of abstinence and in response to alcohol and alcohol-related cues and contexts. We also discuss a broader role of the insular in alcohol-associated risky decision making and impulse control. Finally, we canvas potential challenges associated with targeting the insular cortex to treat individuals with alcohol use disorder.

INTRODUCTION

The insular cortex (insula or 'the island of Reil') is a complex brain structure that forms part of the cerebral cortex (Reil 1809). It has distinct anterior-posterior and dorsal-ventral divisions and is known as a cortical integration hub, with connectivity to extensive cortical and subcortical brain regions (reviewed in (Gogolla 2017)). Of note, the insular cortex has numerous connections with the amygdaloid complex and the thalamus (Reep *et al.* 1996). Strong intra-insular networks also exist (Reep *et al*. 1996). The widespread connectivity and spatial organisation of the insular cortex contributes to the relative lack of understanding of this brain structure. Traditionally, the insular had a known role as a visceral-somatic structure, responsible for various sensory and motor responses (Penfield & Boldrey 1937; Rasmussen & Penfield 1947). However, the known functions of the insular cortex have expanded in recent years. The insular is activated by olfactory stimuli, cognitive tasks, perceptions, and emotions (Kurth *et al.* 2010). One well-known function of the insular cortex is in interoception, activated by cues and internal drives to modulate motivated behaviour (Craig 2009).

The involvement of the insular cortex in motivated behaviour suggests that it integrates with key reward circuitry. Indeed, the insular has connections with the ventral striatum, particularly the nucleus accumbens core, and the ventral tegmental area/substantia nigra (Allen *et al.* 1991; Wright & Groenewegen 1996; Ohara *et al.* 2003). Importantly, dopamine, gamma-aminobutyric acid (GABA) and glutamate appear to be key neurotransmitters involved in the function of the insular cortex (Betka *et al.* 2019; Ohara *et al.* 2003). One class of disorders where motivated behaviours are augmented include substance use disorders. Corticostriatal

circuits have long been associated with substance use disorders, however these studies have traditionally focussed on prefrontal and orbitofrontal cortex connections with the striatal complex (Camchong *et al.* 2013; Marchant *et al.* 2015). Evidence for a role of the insular cortex in substance use disorders has begun to emerge within the last two decades.

A seminal finding by Naqvi and colleagues demonstrated that smokers with damage to the insular cortex had a reduction in addictive-like smoking behaviours when compared to smokers who sustained damage to other brain regions (Naqvi *et al.* 2007). Several other studies have implicated a role for the insular cortex in cocaine, methamphetamine and cannabis use (Nestor *et al.* 2010; Stewart *et al.* 2014; Luo *et al.* 2013). Additionally, insular cortex lesions reportedly reduce the distorted cognitive processing typically observed in gamblers (Clark *et al.* 2014). It is important to note that the involvement of the insular in drug abuse is not clear-cut and whether signalling within the insular cortex is enhanced or diminished during drug use, abstinence and relapse is inconsistent across studies. Moreover, studies examining a role for the insular cortex in alcohol use disorder are somewhat sparse.

Recent attention on the role of the insular in substance use disorders has allowed for a more detailed understanding of how this brain region is implicated in these disorders. However, several questions have arisen from this research: Is the role of the insular cortex in substance use purely interoceptive? Is the insular cortex merely a node in "addiction circuitry"? Or is there something more? In this mini review, we will outline the role of the anterior insular cortex in alcohol use disorder and in animal models relevant to alcohol use disorder. We will also discuss recent findings indicating that the insular cortex may have a broader role in alcohol use disorder, focussing on aversion-resistance, risky decision-making and compulsivity.

The role of the anterior insular cortex in alcohol use disorder

Alcohol use disorder is typically associated with reductions in insular cortex volume, however these studies have predominantly measured insular volume during abstinence. For example, individuals with heavy alcohol use and alcohol use disorder exhibit reduced insular cortex grey matter volume and thickness compared to light-drinking or healthy controls (Demirakca *et al.* 2011; Durazzo *et al.* 2011; Durazzo *et al.* 2014; Makris *et al.* 2008; Momenan *et al.* 2012; Senatorov *et al.* 2015; Heikkinen *et al.* 2017; Yang *et al.* 2016; Mechtcheriakov *et al.* 2007). Alcohol use disorder has also been associated with reduced cerebral blood flow in the insular and frank neurodegeneration of the insular cortex (Sullivan *et al.* 2013; Obernier *et al.* 2002).

Reduced anterior insular volume apparently correlates with higher levels of compulsivity and impulsivity in alcohol dependent individuals (Grodin *et al.* 2017).

An emerging role for the insular cortex in alcohol abstinence-associated negative affective states has recently become apparent (Flook *et al.* 2020), consistent with a known role in emotion regulation (for reviews see Craig 2009; Lamm & Singer 2010). In abstinent alcohol use disorder participants experiencing anticipatory anxiety, early life stress was correlated with reduced insular activity (Yang *et al.* 2015). Additionally, reduced insular activity is observed in abstinent alcohol use disorder inpatient participants under stressful conditions (Seo *et al.* 2013). Together these findings suggest that insular activity is downregulated during abstinence and abstinence-associated negative affective states in individuals with alcohol use disorder. From a mechanistic perspective, compulsive alcohol use reduces insular glutamate-glutamine concentrations suggesting that perhaps an excitatory insular pathway may be driving this behaviour (Betka *et al.* 2019). Additionally, Laukkanen et al. (Laukkanen *et al.* 2019) showed increased [^{3}H]quisqualic acid binding (a group I mGlu receptor agonist) in post-mortem anterior insular cortex brain tissue of individuals with alcohol use disorder compared to control brains. This suggests that glutamatergic activity within the insular may play a role in alcohol use disorder and this may vary during different phases of alcohol use disorder.

Some research has shown increased insular cortex activity in a non-abstinent population of alcohol use disorder participants. For example, increased functional connectivity between the insular cortex and the medial orbitofrontal cortex is observed in currently drinking, non-treatment-seeking individuals with alcohol use disorder compared to social drinkers (Halcomb *et al.* 2019). Whilst this result is contrary to other research in the field, these individuals were not in abstinence during testing. The association of the insular with alcohol use disorder is also evident during early life and adolescence. For example, left insular white matter volume has been linked with binge drinking frequency in adolescents via motivation for drinking related to a desired state (Chung & Clark 2014). Additionally, a family history of alcohol use disorder results in male offspring having reduced insular cortex volume compared to males with no family history of alcohol use disorder (Sharma & Hill 2017), although the long-term consequences of this effect remain to be determined.

The insular cortex is also implicated as a biomarker for pharmacotherapeutic interventions targeting alcohol use disorder. Baclofen, a $GABA_B$ agonist, is approved for the treatment of alcohol use disorder in France (Soyka & Muller 2017; Campbell *et al.* 2018). Holla *et al*. (2018)

showed that alcohol use disorder inpatients treated with baclofen had reduced insular cortex activity that was associated with prolonged time to first alcohol drink. However, controls in this experiment did not receive medication and thus did not account for a placebo-effect. Further research using a randomized double-blind, placebo controlled longitudinal study design is required before concrete conclusions can be drawn. Together these results suggest a reduction in anterior insular cortex volume in individuals with alcohol use disorder when tested during a drinking task or binge drinking in adolescence.

The role of the insular cortex in alcohol use disorder is by no means straightforward, particularly if we distinguish research that has examined abstinence from alcohol, alcohol consumption and alcohol-associated cue reactivity. In non-treatment seeking individuals with alcohol use disorder, activation of the insular cortex is observed following exposure to alcohol and an alcohol-related cue compared to a beverage-control cue (Myrick *et al.* 2004). Increased blood oxygen level dependent (BOLD) insular activity is observed in response to alcoholic tastes and to alcohol-associated cues in individuals who escalated their drinking behaviours over a one year period (Filbey *et al.* 2008; Dager *et al.* 2014). Hyperactivation of the insular is also observed following exposure to repeated alcohol images in heavy drinkers versus light drinkers (Dager *et al.* 2013). One study has also shown increased BOLD insular activity in alcohol dependent women in response to alcohol-related cues (Tapert *et al.* 2004). In regard to circuits, increased connectivity following alcohol-associated cue presentation is observed between the right posterior insular and the bilateral anterior cingulate cortex in alcohol dependent patients compared to healthy controls (Bach *et al.* 2020). Importantly, the connectivity between these two brain regions is reduced in individuals treated with the opioid antagonist naltrexone – a potential biomarker for treatment response (Bach *et al.* 2020). Given the broader role of the insular in interoceptive processing, particularly in response to alcohol-associated cues, activity in this brain region is not surprising. However, the divergent activity of the insular during abstinence and in response to alcohol-related cues highlights the heterogeneity and complexity of the involvement of the insular cortex in alcohol use disorder. One possible explanation for this may be due to the compartmentalized nature of the insular cortex, however spatial differentiation in the roles of the insular in alcohol use disorder is yet to be determined.

The role of the anterior insular cortex in animal models of alcohol use disorder

Similar findings to alcohol use disorder are also evident in animal models. In regard to abstinence, aberrant or reduced, connectivity of the insular cortex is observed in post-dependent rats compared to naïve controls (Scuppa *et al.* 2020). Additionally, the insular cortex of conscious rats is activated following acute (six days) Pavlovian conditioning of alcohol-associated cues in an awake fMRI paradigm (Tsurugizawa *et al.* 2012). Some studies have also shown a functional role for the insular cortex in modulating the interoceptive effects of alcohol (Jaramillo *et al.* 2016; Jaramillo *et al.* 2018b). Moreover, functional inactivation of the caudal granular insular cortex with $GABA_A$ and $GABA_B$ agonists reduced alcohol self-administration compared to vehicle control rats (Pushparaj & Le Foll 2015). In contrast, Gq-DREADD mediated activation of the anterior insular cortex reduces both alcohol and sucrose consumption, with Gi-DREADD inactivation having no effect on consumption in a two-bottle choice paradigm (Haaranen *et al.* 2020), but increasing alcohol self-administration under operant conditions (Jaramillo *et al.* 2018a). These differential results could be due to the spatial specificity of insular cortex targeting, designer ligand dose (3mg/kg versus 10mg/kg), or specific behaviour examined. From a circuit perspective, chemogenetic inactivation of the insular cortex to nucleus accumbens core pathway reduces alcohol self-administration (Jaramillo *et al.* 2018a). However, alcohol was available during these tests, making it difficult to tease out the role of the insular in processing gustatory, cognitive or interoceptive information of the alcohol-associated cues or contexts. Animal studies also demonstrate a role for the insular in abstinence-associated negative affective states. For example, chemogenetic activation of the caudal insular cortex to dorsal bed nucleus of the stria terminalis pathway increases latency to feed in a novelty suppressed feeding task after prolonged abstinence following chronic alcohol use in mice (Centanni *et al.* 2019).

Animal studies have also provided insight into the specific receptor subtypes within the insular that may be driving excessive alcohol consumption. Orexin-1 receptors within the anterior insular cortex, receptors known to be critically involved in alcohol seeking (Lawrence *et al.* 2006), do not appear to play a role in driving excessive alcohol consumption in mice (Lei *et al.* 2016). As stated above, there is a strong projection between the insular cortex and the striatum. Indeed, recent imaging studies have shown alcohol-dependent rats have altered insular cortex activity and connectivity which can be partially restored by systemic dopamine D_3 receptor antagonism (Scuppa *et al.* 2020). Alcohol also disrupts μ-opioid receptor mediated long-term synaptic depression at anterior insular cortex-dorsolateral striatum synapses (Munoz *et al.* 2018). Similar to what is observed in humans, chronic, moderate (~3g/kg for 12 months)

alcohol self-administration in male rhesus monkeys results in increased glutamatergic activity in the insular and this may result in compensatory upregulation of presynaptic serotonin (5-HT $_{1A}$) receptors (Alexander *et al.* 2012). Additionally, 24h withdrawal from 10 days of chronic, intermittent alcohol exposure does not alter presynaptic glutamate release from anterior insular inputs to the basolateral amygdala but does increase AMPA receptor-mediated postsynaptic function – the latter of which may relate to withdrawal-induced anxiety (McGinnis *et al.* 2020). Finally, bath-applied ethanol on insular brain slices inhibited evoked, post-synaptic NMDAR-mediated currents but had no effect on spontaneous $GABA_A$-mediated transmission (Shillinglaw *et al.* 2018). Whilst research into the insular cortex and alcohol use disorder is still premature, these studies do suggest that perhaps the involvement of the insular in alcohol use disorder may be glutamate-mediated and involve corticostriatal and corticoamygdaloid circuits.

Combining the human and animal data suggest reduced insular volume and activity in individuals with alcohol use disorder when tested during abstinence but increased insular activity in response to alcohol-associated cues (see Figure 1). A logical next step would be to use repetitive transcranial magnetic stimulation (rTMS) in the insular cortex of individuals with alcohol use disorder. Such an experiment has been recently published where rTMS of the insular cortex did not alter alcohol craving or consumption (Perini *et al.* 2020). This suggests that perhaps it is not the insular alone driving alcohol craving, but instead the insular is a node of a more complex network. Alternatively, the involvement of the insular may depend on the context and/or timing of alcohol use, for example the involvement when alcohol use is associated with a negative consequence or when individuals are examined during periods of withdrawal or abstinence.

The insular, risky decision making and impulse control

A hallmark feature of substance use disorder is continued use despite negative consequences (Koob & Volkow 2010). Individuals with substance use disorder often choose instant gratification of their substance of choice despite known long-term negative consequences. This may be a reflection of impaired cognitive functioning in these individuals, including impaired decision-making processes and diminished control over behaviour. Indeed, individuals with substance use disorder generally adopt risky decision-making behaviours (Verdejo-Garcia *et al.* 2018). Whilst risky decision making can be adaptive in the short term, these behaviours typically result in adverse consequences to the individual.

Interestingly, there is increasing evidence for a role of the insular cortex in risky decision making and response inhibition in alcohol use disorder. For example, in a population of heavy drinkers, activation of the anterior insular was associated with aversion-paired alcohol cues (Grodin *et al.* 2018) (see Figure 1). Interestingly, heavy drinkers showed greater functional connectivity between the right anterior insular cortex and the right nucleus accumbens compared to light drinkers, especially during the threat of an aversive electric shock. Additionally, male adolescents who are more vulnerable to risk insensitivity are more likely to have greater later lifetime alcohol use which is correlated with insular activity (Elder *et al.* 2019). However, not all research is consistent, with reduced insular activity being associated with hazardous drinking during a risk-taking task in heavy drinkers (Claus & Hutchison 2012). Regardless, it appears as though the insular cortex is involved in risk-taking behaviours, at least in heavy drinkers.

A similar trend is observed in rats where activation of the anterior insular cortex is associated with an increased propensity to relapse despite negative consequences in a subset of inbred alcohol preferring iP rats (Campbell *et al.* 2019). Functional inactivation of the anterior insular cortex prevented alcohol seeking despite the potential for adversity. Increased insular activity, as assessed by Fos-protein, is also associated with aversion-resistant alcohol consumption in mice (Chen & Lasek 2020). This activity is further enhanced by the removal of perineuronal nets, the extracellular matrix enclosing fast-spiking inhibitory interneurons, in the insular (Chen & Lasek 2020). From a circuit perspective, insular glutamatergic inputs to the nucleus accumbens core are essential for aversion-resistant alcohol consumption in rats (Seif *et al.* 2013). Together this suggests a growing theme of insular corticostriatal circuit involvement in alcohol abuse in both human and animal studies, particularly in risky alcohol use, and this may be mediated by glutamate (Grodin *et al.* 2018; Seif *et al.* 2013; Jaramillo *et al.* 2018a).

The insular is also involved in alcohol-induced response inhibition. Increased insular activity is observed in heavy drinkers compared to light drinkers during a go/no-go task with alcohol stimuli (Ames *et al.* 2014). Additionally, young adults (male and female) aged 21-24 show diminished response inhibition following alcohol use and this is associated with reduced connectivity between the anterior insular cortex and brain regions associated with executive function including the dorsal anterior cingulate cortex and lateral prefrontal cortex (Sherman *et al.* 2019). Here, the insular is involved in reduced response inhibition, suggesting that alcohol cues might result in attentional bias in heavy drinkers. Individuals with a family history of

alcohol use disorder show increased insular activation during successful inhibitions in an impulse control task (DeVito *et al.* 2013). Weaker functional connectivity between the putamen and anterior insular cortex is observed during response inhibition in individuals with alcohol use disorder (Courtney *et al.* 2013). In addition, Galandra *et al*. (2018) showed reduced insular cortex grey matter in individuals with alcohol use disorder compared to healthy controls which was associated with attentional deficits and reduced working memory. Reduced anterior insular volume apparently correlates with higher levels of compulsivity and impulsivity in alcohol dependent individuals (Grodin *et al.* 2017). In rodents, impulsivity is negatively correlated with insular thickness (Belin-Rauscent *et al.* 2016), suggesting that this measure may also represent a susceptibility factor for alcohol abuse. Therefore, to parse out whether discrete patterns of insular activation during specific behavioural repertoires are a compensatory response to alcohol-induced pathology, or pre-existing and causal (and further impacted by alcohol use) requires longitudinal studies that can differentiate these possibilities. Nevertheless, these data do support a role for the insular cortex in alcohol-induced cognitive changes. Specifically, the insular appears to have a key role in risky decision making and impulse control with respect alcohol use.

Insular-targeted interventions may be useful in individuals with high risk-taking behaviour and associated alcohol use disorder. Similar to Perini *et al*. (2020), Spagnolo *et al.* (2019) recently demonstrated that deep rTMS of the insular cortex using a novel H-coil did not alter risk-taking behaviour in healthy subjects. Success with this form of intervention may require improved technology to properly target such a deep cortical structure, or at least different subdivisions of the insular. Alternatively, whilst the insular cortex does have a role in alcohol use disorder, it is likely that this brain region is a node within a broader circuitry and solely targeting the insular with interventions may not promote abstinence and/or prevent relapse. It also may be that the timing of intervention is critical given the role of the insular in risky decision-making and continued use despite adverse consequences.

CONCLUSION

As outlined above, progress into the role of the insular cortex in alcohol use disorder is still ongoing and the research is somewhat conflicting. This may be partly due to the complex spatial organisation and functional heterogeneity of this brain structure. Indeed, human alcohol use disorder studies have shown that activity in the insular cortex is predominately reduced when individuals are tested during abstinence. However, insular cortex activity appears to be

increased during alcohol consumption or in response to alcohol-associated cues and contexts (see Figure 1). Unfortunately, without techniques that can specifically target different subregions of the insular cortex in humans, the conclusions remain elusive. The animal data are rather consistent with human research and tools that enable us to examine different subregions of the insular cortex have shed light on the possibility of a more complicated role of the insular cortex in alcohol use disorder. For example, functionally inhibiting the caudal portion of the insular cortex results in reduced alcohol consumption whereas functionally inhibiting the anterior insular cortex has no effect on consumption (Pushparaj & Le Foll 2015; Haaranen *et al.* 2020). These studies highlight the need for tools with greater spatial specificity, particularly when considering targeting the insular cortex in human alcohol use disorder. Finally, the last few years have seen an expansion in the understanding of the involvement of the insular cortex in alcohol use disorder and substance use disorder more generally. Indeed, the research highlights that the involvement of the insular cortex in alcohol use disorder is not solely due to the perception of sensations from internal organs. Convincing data suggest that the insular also has a role in risky decision making and impulse control in individuals with alcohol use disorder. The involvement of the insular in these behaviours is critical to understand as these are features that have a substantial impact upon treatment outcomes. However, exactly how the insular impacts these behaviours in the context of reward-related circuitries is yet to be defined.

One aspect that does appear to be consistent across both the human and animal literature is the involvement of insular-striatal pathways in risky alcohol use. Whilst the idea of corticostriatal pathway involvement in substance use disorder is not novel, studies have typically focussed on the prefrontal cortex and orbitofrontal cortex (McFarland *et al.* 2003; Schoenbaum *et al.* 2006). Indeed, prefrontal and orbitofrontal cortex projections to the striatum are involved in goal-directed actions and aversion resistance (Barker *et al.* 2015; Hu *et al.* 2019). As mentioned above, the insular cortex to accumbens pathway has been implicated in alcohol use despite an adverse consequence in both humans and animal models (Grodin *et al.* 2018; Seif *et al.* 2013). Given the importance of risky decision making and impulse control in substance use disorders, insular-striatal pathways should not be ignored when considering treatment options for individuals with alcohol use disorder. Perhaps a more refined circuit-specific approach using current techniques could be used for intervention, as broader targeting of the insular cortex with rTMS does not appear to alter alcohol-associated craving or risk-taking behaviour (Creed *et al.* 2015; Gibson *et al.* 2018; Perini *et al.* 2020; Spagnolo *et al.* 2019). Whilst the literature

to date has honed in on the insular-striatal pathway, it is possible that divergent insular output pathways may have opposing effects on alcohol addiction-relevant behaviours and future research should explore this idea further.

In summary, the insular cortex has known widespread connectivity and complex spatial organisation, features that make examining this brain structure, particularly in humans, challenging. As a field, we need to improve our mechanistic understanding of the insular cortex, particularly during periods of abstinence or alcohol consumption and its involvement in promoting or preventing alcohol use despite negative consequences. Currently, this is only possible using appropriate animal models to specifically delve into the structural organisation of the insular and examine functional circuits with respect to alcohol use disorder. Technological and methodological advances aimed at improving the spatial and circuit specificity of our clinical interventions will considerably advance our understanding of this intricate brain region.

--Human subjects --
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The figure was re-drawn by Laura Hausmann in BioRender (https://biorender.com/) on the basis of a draft provided by the authors.

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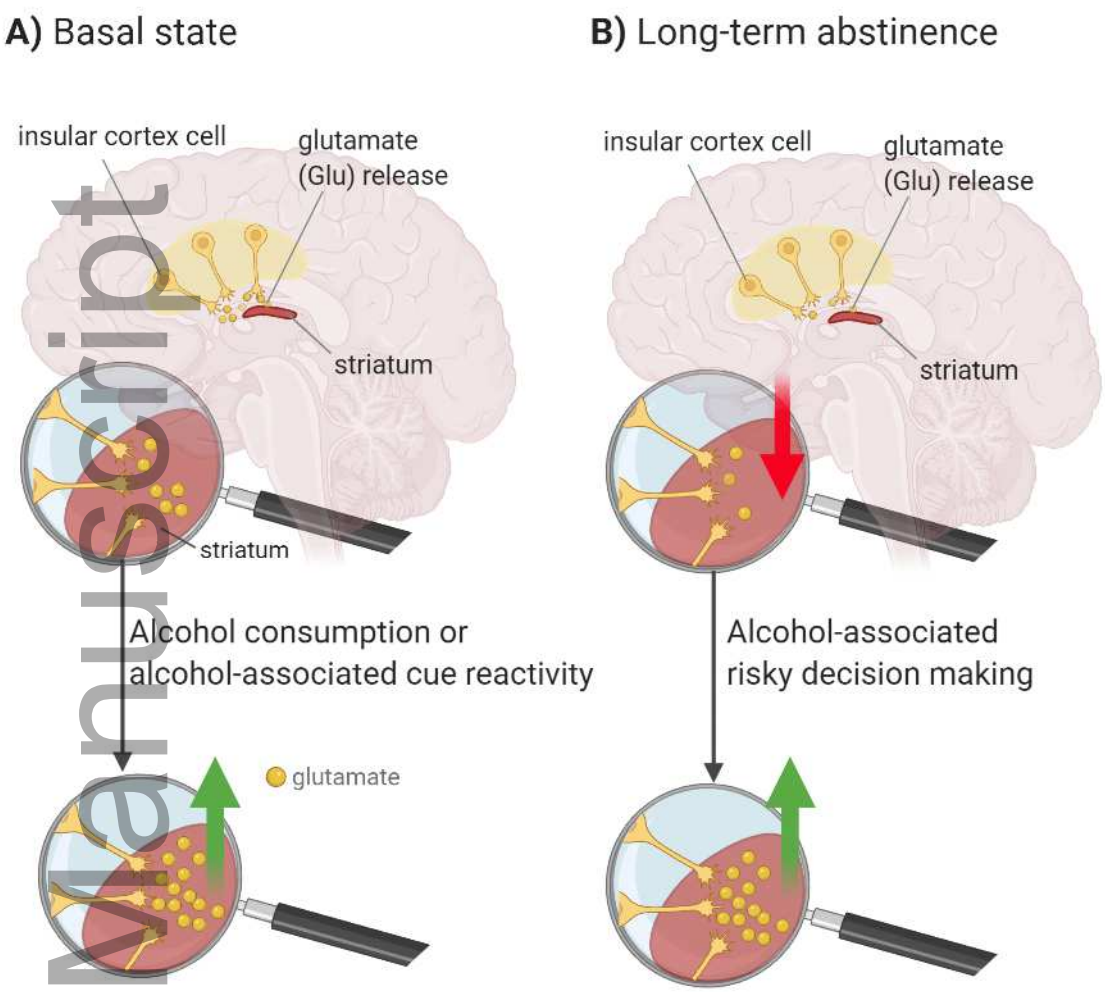
FIGURE LEGEND

Figure 1. The effect of alcohol on the insular cortex. Under basal conditions, the insular provides 'normal' glutamatergic tone onto downstream projection targets such as the striatum (**A**). Based on the literature, we propose that long-term abstinence reduces glutamatergic excitatory drive onto neurons within the striatum (**B**). Alcohol consumption, alcohol-associated

cue-reactivity and alcohol-associated risky decision making increases glutamatergic drive onto downstream neurons within the striatum.



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