A Biological Framework for Emotional Dysregulation in Alcohol Misuse: From Gut to Brain

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ABSTRACT

Alcohol use disorder (AUD) has been associated with impairments in social and emotional cognition that play a crucial role in the development and maintenance of addiction. Repeated alcohol intoxications trigger inflammatory processes and sensitize the immune system. In addition, emerging data points to perturbations in the gut microbiome as a key regulator of the inflammatory cascade in AUD. Inflammation and social cognition are potent modulators of one another. At the same time, accumulating evidence implicates the gut microbiome in shaping emotional and social cognition, suggesting the possibility of a common underlying loop of crucial importance for addiction. Here we propose an integrative microbiome neuro-immuno-affective framework of how emotional dysregulation and alcohol-related microbiome dysbiosis could accelerate the cycle of addiction. We outline the overlapping effects of chronic alcohol use, inflammation and microbiome alterations on the fronto-limbic circuitry as a convergence hub for emotional dysregulation. We discuss the interdependent relationship of social cognition, immunity and the microbiome in relation to alcohol misuse-from binge drinking to addiction. Additionally, we emphasize adolescence as a sensitive period for the confluence of alcohol harmful effects and emotional dysregulation in the developing gut-brain axis.

1. INTRODUCTION

Alcohol use disorder (AUD) is a multifaceted psychiatric condition influenced by environmental factors [1] and genetic components [2], that has been associated with widespread cognitive deficits [3]. Only recently, impairments in social and emotional functioning -accompanied by alterations in underlying fronto-limbic circuits- have emerged as a key factor for the development and chronicity of the disorder [4-6].

Over the past number of years there is a growing realisation that repeated drinking and withdrawal cycles enhance neuroimmune signaling in the brain and peripheral inflammation [7]. Alterations in dopamine, glutamate and γ-aminobutyric acid (GABA) release are linked to chronic alcohol exposure [8]. In particular, dopaminergic decreases in the reward system and recruitment of brain stress neurotransmitters, play a role in the progression form positive to negative reinforcement [9]. These psychoneuroimmunological neuroadaptations promote emotional dysregulation -and further inflammation- contributing to the development of AUD and related affective co-morbidities [10, 11].

New data point to perturbations in the gut microbiome- the trillions of microorganisms residing in our gut- and intestinal permeability as key regulators of the inflammatory cascade in AUD [10, 12]. Indeed, we are witnessing the rise of studies that aim to understand the role of the gut microbiota in drug addiction, and particularly in AUD [13, 14]. At the same time, it is being increasingly recognized that gastrointestinal microbes can influence the social brain and behaviour [15, 16]. The fronto-limbic circuitry constitutes a crucial target for low-grade systemic inflammation [17]. This brain network -and in particular the amygdala- is also a focal point through which the gut microbiota modulates social behaviour [18]. Perturbations in both the intestinal mucosa and the community of microorganisms colonizing the gut [19] might accelerate the cycle of addiction via metabolic and inflammatory pathways that translate into augmented emotional and social cognition impairments, promoting negative reinforcement processes. Here we propose a microbiome neuro-immuno-affective framework to advance the understanding of the mechanisms that might fuel the transition from binge to addiction by linking the social brain and the distant gut microbiome.

The social brain shows marked development during adolescence [20], which is considered to last up to 24 years of age in terms of both biological growth and major social role transitions [21]. Among young people, drinking to intoxication or binge drinking (BD) is the most prevalent pattern of alcohol consumption (National Institute on Alcohol Abuse and

Alcoholism [NIAAA], 2004) [22]. Previous studies have demonstrated the particular sensitivity of the adolescent brain to repeated alcohol intoxications and the risk for future addiction [23]. Although the literature on the neurocognitive consequences of BD has traditionally focused on emotion-independent cognitive functions; the study of deficits in social cognition in binge drinkers (BDs) has gained increased interest [24]. Fronto-limbic brain regions implicated in emotional functioning -including the amygdala- exhibit dramatic neuromaturational changes during adolescence [20, 25] and have known connections with the immune system and the microbiome, both of which are still developing [26, 27]. The adolescent years are characterized by emotional fluctuations and increased stress reactivity, which creates the perfect context for disturbances [28]. Therefore, BD-related emotional dysregulation is likely to be augmented by both the disruption of neurodevelopmental processes and overlooked factors in the refinement of the gut-brain axis communication, together with the added effect of typical emotional fluctuations and the co-occurrence of social stressors.

In this review, we synthetize the literature -from BD to AUD- describing deficits in social cognition and emotional functioning and the underling alterations in fronto-limbic circuitries. Then, we provide an overview of the links between alcohol and the immune system and the implications of the co-regulation of inflammation, drinking and social behaviour. Based on the emerging biology of the gut-brain communication, we discuss its role in shaping cognitive networks that encompass social and emotional functioning. We present an in-depth analysis of alcohol-related alterations in the gut microbiota and their clinical implications. By integrating seemingly disparate areas of research (e.g., microbiology, psychology, immunology) we explain how fronto-limbic anomalies are at the centre of a feed-forward loop that is likely to accelerate loss of control over drinking and promote co-occurring mood disorders. We discuss the implications of our framework for the aetiology and pathogenesis of AUD in the light of classical addiction theories to delineate future directions. Finally, we highlight the special case of adolescence as a period in which alcohol-related social deficits are likely to be amplified causing an allostatic load that may act as an open door to addiction and psychopathology.

2. SOCIAL COGNITION, GENERATION AND REGULATION OF EMOTIONS

Central to our framework is the concept of emotional dysregulation which has served as an umbrella to unify varied alterations in emotional functioning in many psychopathological disorders (e.g., anxiety or personality disorders) including AUD. In order to overcome the non-specific nature of this term, we describe alterations across all facets of emotional dysregulation -social cognition, generation and regulation of emotions-, both in AUD and BD, and the implications for drinking escalation.

2.1 SOCIAL COGNITION

Social cognition refers to the ability to make sense of the world through processing signals generated by other members of the same species and modifying our behaviour accordingly [29]. Social cognition encompasses two main components, namely emotional processing and theory of mind [30] and involves a network of fronto-limbic brain areas (principally dorsolateral prefrontal cortex, orbitofrontal cortices, amygdala and anterior cingulate cortex) [25].

2.1.1 *EMOTIONAL PROCESSING*

Emotion decoding skills are crucial for navigating social interactions. Impairments in decoding basic and especially complex emotions have been consistently reported in individuals with AUD, in terms of overestimation of socio-affective information signaling social threat and misinterpretation of emotional facial expressions as negative [4, 31, 32]. Accordingly, a recent meta-analysis found that facial emotion recognition was significantly impaired in patients with AUD for negative emotions, principally for anger, but also for disgust and fear [33]. In addition, others have argued that when using more complex paradigms patients show impairments not only for negative emotions but also for positive ones [34]. These emotional decoding disruptions are not exclusive to visual modality, in fact, impairments in recognizing emotions have also been observed for voices, body postures and musical excerpts [35-37]. In the same line, individuals with AUD do not seem to benefit from crossmodal processing facilitation (i.e., emotional information presented through multiple sensory modalities) [38-40]; which, together, suggest a generalized emotional decoding impairment in AUD individuals.

Alterations in brain activity associated with emotional facial processing -specially for negative emotions- have also been reported by electrophysiological studies in AUD [41,42], that seem to persist with midterm abstinence [43]. These deficits originate earlier in the

cognitive stream, at the attentional level (N2b/P3a attention orienting complex) and extend to later decisional levels (P3b) [41, 42]. The greater the deficit for N2b/P3a (indexing the recruitment of attentional resources to switch and orient the attentional focus towards new or relevant stimuli) in AUD individuals, the greater the deficit for P3b, suggesting an accumulative influence of early attentional difficulties on later decisional processes [42].

Neuroimaging studies in AUD have also shown structural and functional abnormalities in brain regions involved in emotional processing, such as the prefrontal cortex (PFC; particularly the orbitofrontal region), the limbic system (including the hippocampus and amygdala), and the insula [44-46]. The amygdala is a critical structure of the social brain implicated in processing salient stimuli, especially negative stimuli, and it is also related to stress responsiveness through activation of neurohormonal systems [47]. Convergent evidence shows that alcohol (both acute and long-term consumption) alters amygdala reactivity to emotional stimuli [46, 48-51]. The severity of the disorder appears to be particularly associated with greater hyper-responsiveness of the amygdala to emotional stimuli [52], suggesting that repeated cycles of intoxication and withdrawal lead to increased bottom-up responsiveness and hyperexcitability [53]. Some inconsistencies (e.g., over-responsiveness versus under-responsiveness) may respond to long-term neural adaptations in the extended amygdala [53].

Preliminary findings in young BDs seem to mirror –to a lower degree- the difficulties in emotion decoding found in AUD. Indeed, BDs present poor emotional decoding performance, especially in the recognition of negative stimuli [24, 40, 54-56] but also positive stimuli [57]. Adolescents who started drinking alcohol at an early age have increased threat-related amygdala and ventral striatal activity and higher levels of stress, indicative of relevant developmental effects [6, 58]

2.1.2 *THEORY OF MIND*

Theory of mind (ToM), which is the ability to attribute mental states (intentions, feelings or beliefs) to self and others, enables individuals to successfully adapt to social interactions [59]. ToM is a broad concept usually subdivided into two different aspects: affective and cognitive ToM, which involve tasks requiring the decoding of others' emotional or cognitive states, respectively [33, 59]. AUD individuals appear to display a particularly severe disruption for affective ToM [4, 60]. Findings are in accordance with brain imaging studies revealing structural and functional changes in critical networks for ToM (namely, ventromedial and

dorsolateral prefrontal cortex, anterior cingulate cortex, hippocampus, amygdala and insula) [61-63]. The strong links among ToM compromise and chronicity, amount of alcohol consumed or craving, underscore the clinical prognostic relevance [4, 64]. Emerging findings in BD [65, 66] also point to a dissociation between cognitive and affective ToM in this population, the latter being the most affected [66]. This apparent "continuum" reinforces the hypothesis that similar deficits are expected across different alcohol-related disorder stages.

2.2 EMOTIONAL REACTIVITY

Research in the area of emotional reactivity (i.e., intensity of emotions) show that individuals with AUD present higher subjective emotional reactivity than controls in response to aversive tasks [67,68], basal hyperactivity of peripheral stress markers, accompanied by hypoactivity in response to stress and alcohol cues [69]. A lower ability to withstand negative emotional states, also termed distress tolerance [70], has been frequently reported in AUD [71, 72]. Individuals with lower distress tolerance may attempt to avoid aversive states by pursuing negative reinforcement opportunities (i.e., escape/avoidance) through drinking intoxications, resulting in a vicious circle [70]. Similarly, adolescent BDs appear to display heightened emotional reactivity and poor distress tolerance to stressful tasks [73, 74], that improved after an abstinence period [75], suggesting a causative effect.

2.3 EMOTIONAL REGULATION

Emotion regulation is a multifaceted process by which individuals successfully modulate the intensity of emotions and modify emotional reactions to accomplish goals [76]. Substance use can be a maladaptive emotional regulation strategy [77], and impulsive drinking is one of the most common examples [78]. Some individuals show a tendency to act rashly when experiencing strong positive or negative emotions, referred to as positive and negative urgency [79]. While BD has been associated with both forms of impulsivity [79, 80], negative urgency seems to be more strongly linked with problematic alcohol consumption [81, 82].

On the continuum of impulsive/controlled emotional regulation strategies [83], the pole in which controlled processes dominate, refers to affective control (i.e., application of cognitive control to affective contexts [84]). Strong evidence supports the association of AUD and BD with impairments in controlled processes, principally in response inhibition and interference control, but also in self-monitoring processes in working memory and cognitive flexibility

[45, 85]. The imbalance in affective control systems (enhanced bottom-up emotional processing and diminished top-down regulation) has been extensively studied in relation to cue (drug/alcohol) reactivity (e.g., dual process model [86]); but is relatively unexplored in relation to emotional reactivity. Emerging data points to difficulties in affective control of emotional stimuli in both AUD individuals and BDs [87-90]. In this line, neuroimaging studies have revealed that alcohol-related impairments in executive functions seem to contribute to the dysregulation of the extended amygdala [91, 92], which may promote negative emotional states difficult to inhibit at later stages of the addiction cycle [9]. Like alcohol-related stimuli, emotional stimuli might activate craving-related regions in AUD, and the urge to consume alcohol [93]. Paralleling findings from AUD, young BDs also display decreased recruitment of executive control regions due to the interference of negative content [94, 95]. A deeper understating of the role of affective control deficits in relapse risk for AUD and a better characterization of such difficulties in young BDs from a developmental perspective are needed (see Table 1 for proposed future directions on this topic).

3. INFLAMMATION: FOCUS ON THE SOCIAL BRAIN

3.1. ALCOHOL AND THE IMMUNE SYSTEM

Innate immune signaling (pathways of immune-to-brain communication can be found in Table 2) is an important feature in the pathophysiology of many disorders, including AUD. Ethanol activates the peripheral and central immune systems in multiple ways [7]. Even after a short exposure, there are significant changes in the upregulation of immunity, such as increased microglial markers [96], that seem to persist over time [97]. After chronic administration, alcohol sensitizes the neuroimmune system to subsequent inflammatory stimuli [98]. Apart from a direct interaction of ethanol with neuronal and immune brain cells, a large contribution of neuroinflammation originates in the periphery [7, 19]. The most extensively studied inflammatory pathway in AUD is the Toll-like receptor 4 (TLR4) signaling pathway, which is critical for ethanol-induced neuroinflammation, brain injury, and possible neurodegeneration [99, 100]. Part of the pro-inflammatory effects of chronic alcohol are due to impairments of the gut barrier function or “leaky gut” [19]. Alcohol disrupts gut tight junctions, allowing the passage of bacteria and endotoxins (such as lipopolysaccharide [LPS]) across the gut wall, that enter the liver via portal circulation and release of proinflammatory cytokines into systemic circulation (for gut-liver-brain interactions, see

[101]). The peripheral inflammatory response can then impact brain and behaviour through several routes (see Figure 1). AUD individuals present an increase in blood LPS levels and low-grade inflammation, both in non-cirrhotic and cirrhotic populations, compatible with increased intestinal permeability [10, 12]. It appears that a single BD episode in healthy subjects is enough to cause a rapid increase in serum endotoxin levels [102], whereas a regular BD pattern was associated with elevated pro-inflammatory markers in -otherwise-healthy young individuals [103]. In a sample of moderate drinkers, a single binge alcohol intoxication elicited changes in pro-inflammatory cytokines (IL-8 and TNF-α), six hours after the drinking episode [104]. Moreover, an earlier age of BD onset may be a risk factor for increased inflammation [100].

Following a different approach, other researchers have suggested co-regulation, or in other words, immune signaling activation promotes alcohol consumption. Preclinical findings have showed that immune activation might be a relevant factor driving alcohol-seeking behaviour. Interestingly, inflammatory activation (a single injection of LPS) can produce long-lasting increases in alcohol consumption (up to 3 months) [105]. Peripheral inflammation plays an integral role during withdrawal, inflammatory markers (i.e., IL-1β and IL-8) have been shown to positively correlate with scores of anxiety and alcohol craving in detoxified AUD patients [12]. This association might be already present at early stages of alcohol misuse. In fact, changes in pro-inflammatory cytokines (i.e., IL-6) predicted alcohol craving in BDs [106]. Moreover, alcohol intoxication is a potent activator of the stress system (hypothalamic–pituitary–adrenal axis [HPA axis]), which in turn, results in failure to down-regulate the inflammatory response [69], adding another factor to this negative feedback self-regulation.

3.2. BRAIN TARGETS FOR INFLAMMATION

Numerous studies have confirmed the negative effects of peripheral inflammation in key brain areas for social cognition [17] (for immune-to-brain communication see Table 2). The amygdala is a central hub in this “neuroimmune network” [17, 107]. Acute inflammation has been shown to increase amygdala reactivity in response to stress [108]. Similarly, endotoxin-induced inflammation enhanced neural responsivity in threat-related (e.g., bilateral amygdala) and reward-related (e.g., ventral striatum) brain regions, as well as in a region implicated in inferring mental states of others (dorsomedial prefrontal cortex [DMPFC]) [109, 110]. Focusing on ventral striatum as a key area for reward anticipation, another study

demonstrated that individuals exposed to endotoxin challenge showed significant reductions in activity related to reward cues [111]. The insula is a brain area highly involved in emotional processing of negative emotions [112] and has also been shown to be activated under inflammatory conditions [113]. Another major target for cytokines is the anterior cingulate cortex (ACC), a region involved in cognitive control processes, including performance monitoring and inhibitory control, with a crucial role in compulsive drug-seeking behaviours [114, 115]. In addition, this region plays a role in regulating autonomic and neuroendocrine outflow- and, in turn, constitutes a potential neural hub to influence inflammation in the periphery [107]. Experimental data linking inflammation to deficits in social cognition are sparse. Exposure to an endotoxin inflammatory challenge (versus placebo) led to changes in ToM (Reading the Mind in the Eyes test) [116]. However, non-social cognition was not assessed, limiting the specificity of the assumptions. From a network perspective, fronto-limbic regions implicated in social behaviour (including the amygdala, hippocampus, hypothalamus, insula, ventral striatum, medial prefrontal and anterior cingulate cortex) have been consistently involved in peripheral inflammatory physiology across neuroimaging studies [17], which appears to contribute to sensitization towards social threats and altered reward-related neural responding. This interconnected circuitry is a probable hub where the effects of alcohol and peripheral inflammation converge to produce disordered socio-affective behaviour.

4.THE MICROBIOTA-GUT-BRAIN AXIS

The microbiota-gut-brain axis is a bidirectional pathway of communication that encompasses the central nervous system, the autonomic and enteric nervous system, and the neuroendocrine and neuroimmune systems [117, 118]. It plays a key role in brain development and neuroinflammatory responses influencing cognition and emotional behaviour [16]. The bottom-up crosstalk between the gut microbes and the host implicates a vast array of signaling pathways, from neurotransmitters to inflammatory cytokines [119]. Conversely, neural signaling through top-down pathways can disrupt the intestinal barrier and alter the composition and function of the gut microbiota (for gut-brain communication pathways, see Table 3) [118].

4.1 INTESTINAL MICROBES AND BRAIN FUNCTION

4.1.1 *NEUROBIOLOGICAL LINKS*

The gut microbiome and its metabolites are long-overlooked modulators of immune function [120]. In the first instance, the microbiome has a vital role in the early development of the immune system [121, 122]. For example, microglia from germ-free (GF) mice exhibited alterations that resulted in an impaired innate immune response, which was restored after treatment with microbial-produced metabolites (e.g., short-chain fatty acids [SCFAs]) [123]. Without the microbiota, certain TLRs are not fully expressed in the gut [120]. Compelling evidence points to gastrointestinal microbes as mediators of sustained inflammatory activation in mood disorders [119]. The microbiota has a profound effect in the development of the HPA axis and the stress response, a cross-talk that is bidirectional in nature [119]. In a milestone study, GF mice exhibited inflated HPA axis hyperactivity with elevated corticosterone levels in response to stressors [124]. Interestingly, this exaggerated HPA stress response was reversed by microbiome reconstitution at an early age but not later in life [124]. This time-sensitive efficacy hinted at the existence of neurodevelopmental windows. Microbiome-derived dysregulation of the stress response further impacts the immune response, causing an allostatic load [125]. Allostasis is a term that describes body adjustments in order to maintain homeostasis and it is thought to be coordinated by the activation of the HPA axis and its main end hormone cortisol. Such adjustments include shutting down activation of some immune mechanisms and regulation of systemic low-grade inflammation [126]. Altogether, there is strong evidence supporting that dysfunction of stress - and immune- systems may be dependent on a healthy gastrointestinal microbiota.

Furthermore, gut microbes contribute to the production of potentially neuroactive molecules, including GABA and serotonin [127]. Of great importance for addiction is the increasing evidence showing that changes in gut microbiota composition influence dopaminergic neurotransmission [13, 128]. Dopamine is regarded as a main regulator of the mesocorticolimbic circuit, which is involved in reward responses. A recent investigation has found a gut-to-brain neural circuit that establishes vagal neurons as an essential component of the reward neuronal pathway, linking sensory neurons in the upper gut to striatal dopamine release [129]. Preliminary evidence indicates that drug-induced perturbations in the gut microbiota are linked to dopamine reductions in the striatum [130] and causally relate to neuroinflammation and deficits in reward responding [131]. Studies examining neurobehavioural responses to drugs showed that microbiome-depleted animals exhibited enhanced sensitivity to reward [132] and altered brain responses during withdrawal [133],

which indicates the need for research investigating the role of microbiome alterations in driving reward-seeking behaviours.

4.1.2 *THE FRONTO-LIMBIC SYSTEM*

There is an emerging consensus, at least from animal studies, that the gut microbiota plays a role in regulating early brain development [26, 134]. One brain region shown to be regulated by the microbiome is the amygdala [135]. The absence of the microbiome results in perturbations in the amygdaloid complex, such as enlarged amygdala volume and dendritic hypertrophy, and an overall increase in neural hyperactivity [136]. Several studies in GF and microbiome-depleted mice have shown changes in the amygdalar concentration of various brain chemicals and receptors, including brain-derived neurotrophic factor (BDNF) and N-methyl-D-aspartate (NMDA) receptors, that contributed to increased risk-taking behaviour [135-137].

The hippocampus is another limbic region tightly connected to the amygdala that also depends on input from the microbiome for normal development [138]. While the hippocampus is most notably known for its role in episodic and spatial memory; it also plays an essential role in emotional behaviours and neuroendocrine responses, modulating emotional regulation [139]. Early antibiotic depletion of the gut microbiome led to significant reductions in BDNF and monoamine neuromodulation function in the hippocampus (e.g., increased tryptophan and decreased kynurenine) [140], which could represent early evidence of microbial-neural critical windows. Similarly, manipulating the microbiome has been shown to alter neurogenesis and hippocampal gene expression involved in neural plasticity [141-143].

Another aspect of neurodevelopment shown to be partially regulated by the microbiome is prefrontal cortical myelination [142, 144]. The PFC is essential in cognitive flexibility and inhibitory control but also in emotion regulation [145]. Gut microbiota can modify the synthesis of key metabolites affecting gene expression in the PFC, subsequently altering social behaviour [146, 147]. Following these early rodent models, further replication will allow confirmation of the apparent contribution of gut microbes in shaping the development of the central nervous system.

4.1.3 *COGNITION AND BEHAVIOUR*

Although inconsistencies are evident, the social dimension appears to be among the behaviours most intimately connected to a functional microbiome [16], including a pivotal role in disorders with persistent deficits in social skills (e.g., autism spectrum disorders [134]). Different studies using GF mice have demonstrated that animals completely lacking microbiota have impaired sociability and present deficits in the identification of social novelty [148, 149]. These findings have been corroborated in antibiotic depletion models across species [16]. Social behaviour and emotional functioning have been further investigated through the use of probiotics, i.e., live bacteria that, when ingested in adequate amounts, produce health benefits [150]. In different mouse models of autism, a probiotic administration could reverse observed social behaviour deficits and restore plasticity [151]. In a human placebo-controlled study, a probiotic intervention in stressed adults resulted in reduced levels of proinflammatory cytokines, lower anxiety scores and faster reaction times in an emotional recognition task [152]. In healthy participants, 4-week administration of probiotics was associated with changes in brain activation patterns related to emotional memory and emotional decision-making tasks [153]. Similarly, in healthy women the consumption of a probiotic for one month reduced task-related responses in a distributed brain network involved in emotional processing, including the insula and the PFC [154]. Animal studies have also shown that the microbiome may be necessary for adequate learning-related plasticity in fear extinction behaviour [155, 156]. For example, GF mice show impaired maintenance of fear stimuli–response associations, regulated by altered gene expression in the amygdala, and these anomalies were partially reversed after microbial colonization [157, 158]. Microbiome manipulations reinforce the idea that microbial signals are important for the healthy neurodevelopment and programming of social cognition.

Therefore, the fronto-limbic circuitry appears to be a convergence hub for the overlapping effects of alcohol misuse, inflammation and microbiome alterations, constituting an intertwined matrix that is likely to amplify emotional dysregulation (for proposed overlapping hubs see Figure 2).

4.2 ALCOHOL EFFECTS ON GUT MICROBIOME

Chronic alcohol consumption induces intestinal inflammation through various pathways, including increased permeability of the intestinal mucosa and changes in intestinal microbiota composition and function [159, 160]. Despite some inconsistencies, AUD seems to alter the balance between bacterial strains, decreasing the presence of beneficial bacteria (e.g.,

Lactobacillus and *Bifidobacterium*) and increasing abundance of pro-inflammatory bacteria (e.g., *Proteobacteria*) [161-166], which create a state of microbial dysbiosis. Alterations in microbiota-generated metabolites have been reported (e.g., lower levels of indoles and reduced tryptophan biosynthesis [167]). Here, we will focus on AUD individuals without alcohol-related liver disease, as cirrhosis, rather than alcohol use, may be the primary factor influencing microbiome disturbances in such patients (for liver disease and gut microbiota see [101, 167]).

The literature arising from preclinical studies has suggested a role for gut microbiota in the pathophysiology of alcohol addiction. Mice exposed to ethanol over four weeks showed notable changes in many bacterial taxa, particularly significant reductions in the genera *Clostridium* and a significant decrease in alpha diversity (an index of species richness) [164]. Mixed findings have been found in relation to alpha diversity (e.g., no differences [130] or higher species richness [162]). In a similar study, many genus level bacteria in order *Clostridiales*, family *Ruminococcaceae* and *Lachnospiraceae*, were positively associated to the severity of alcohol seeking [130]. Interestingly, microbiome alterations correlated with increased impulsive and compulsive behaviours, as well as with dopamine receptors type 2 in the striatum (i.e., D2R decreased mRNA expression) [130]. Reductions in striatal D2R has been proposed to modulate negative reinforcement processes implicated in impulsive and compulsive behaviours via striato-cortical pathways [114, 168]. In this vein, a recent study reported significant correlations between bacteria belonging to the families *Ruminococcaceae* and *Lachnospiraceae* with several addiction-related behaviours, principally increased impulsivity, inattention deficits and reward learning [169].

Only one study so far has investigated this issue in relation to a BD pattern during adolescence [170]. Using an intermittent ethanol model to mimic human adolescent BD, the authors found massive BD-derived microbial dysbiosis during adolescence. Importantly, some of these changes persisted into adulthood (e.g., decreased abundance of microbes from the *Firmicutes* and *Bacteroidetes* phylum) [170]. Another work on adult rats reported a decrease in gut microbial alpha diversity and *Bacteroidales* abundance after alcohol binge exposure [171]. This preliminary evidence seems to be in line with findings in chronic alcohol use [13, 162].

Although human studies are scarce, current data indicate that gut dysbiosis might be related to AUD symptomatology, especially craving and withdrawal responses [10]. Moreover, gut dysbiosis correlated with the duration of sobriety, suggesting long-lasting alterations that persist despite abstinence periods [163]. This assumption was corroborated in an elegant detoxification study [162]. After three weeks of detoxification, patients' intestinal permeability was recovered but gut-microbiota composition and functionality remained altered. Those individuals with high intestinal permeability and microbiome dysbiosis (principally decreases in genera from the *Ruminococcaceae* family) also presented the highest scores of alcohol craving and anxiety [162]. This aligns with other studies showing alterations in bacteria from the family *Ruminococcaceae* associated with alcohol severity [130, 172]. Overall, these findings lead the authors to suggest that microbiota changes could have a role - together with inflammation - in negative reinforcement processes driving alcohol consumption [12, 162]. In this sense, many outstanding questions remain unanswered: Is it possible to identify a microbiome signature in AUD? Are microbial alterations in AUD associated with neurocognitive deficits in social cognition and executive functions? Can alterations in the gut microbiome -and its immunomodulatory metabolites- drive alcohol-seeking behaviours?

As a causal tool to analyze the role of gut microbiota in brain functioning, two studies have employed a faecal matter transplant (FMT) from alcohol-fed mice to normal healthy control mice. This manipulation remarkably shaped the composition of gut bacteria and elicited withdrawal-anxiety signs [165]. Similarly, mice transplanted with microbiota from patients with severe alcohol-related hepatitis exhibited increased intestinal permeability and inflammation (in the liver and intestines) [172]. Intriguing results come from a recent FMT trial in males with AUD-related cirrhosis [173]. Patients were randomized into placebo or FMT enriched in *Lachnospiraceae* and *Ruminococcaceae* (deficient taxa in this population). The FMT was associated with short-term (15 days) increases in beneficial and butyrate-producing genera accompanied by higher plasma butyrate. This intervention resulted in short-term improvement in inhibitory control (measured by a Stroop task that lacks the classical interference index [neutral-conflict]) and reduced craving that negatively correlated with *Ruminococcaceae* genera. However, the long-term (6 months) stability of the findings was not assessed and no effects were observed in abstinence levels in the long-term [173]. Although promising, larger trials are needed to confirm and extend these (phase 1) findings. Finally, other interventions targeting the gut microbiota (psychobiotics) have reversed some

sequalae of alcohol misuse, which could hold potential for translation into new treatments (see Table 4).

5.THE SPECIAL CASE OF ADOLESCENCE

5.1 VULNERABILITY PERIOD FOR BINGE DRINKING

Adolescence is a sensitive period for neuromaturational changes that are usually accompanied by unique stereotypical behaviours. The imbalance between a rapidly changing limbic circuitry and a relatively slower developing prefrontal circuitry leads to heightened limbic reactivity that is not effectively down-regulated, resulting in an immature affective control [77, 174]. These maturational processes are thought to underlie common adolescent characteristics such as emotional fluctuations, increased emotional reactivity, higher levels of negative mood and enhanced reward sensitivity [23, 174]. Affective processing– of both positive (e.g., rewards) and negative (e.g., threatening) stimuli- seems to peak in mid-adolescence [174]. Compared with children and adults, adolescents show heightened amygdala activity to emotional cues and decreased fear extinction, mediated by changes in PFC-amygdala connectivity [20, 175]. Due to ongoing neuromaturational processes, the adolescent brain is particularly sensitive to repeated alcohol intoxications or BD [23]**.** Therefore, when combined with alcohol neurotoxicity, heightened emotional reactivity and poor affective control could create a perfect context for the emergence and exacerbation of emotional dysregulation; particularly, in the light of other relevant -but usually overlooked- developmental processes that are taking place during this sensitive window, that is, immune and microbiome changes.

An additional vulnerability factor for the effects of BD might be the expected maturational changes of neuroimmune interactions during this period (e.g., synaptic pruning or fluctuations in neurotransmitter systems co-regulate immune signals) [176]. For example, the active pruning of glutamatergic synapses may be one potential mechanism by which blood brain barrier permeability could be altered during this period; facilitating the passage of immune molecules into the brain [176]. Adolescence is also characterized by developmental variations in sex hormones, which have well-known modulatory effects on brain function and physiological stress responses [177]. Another significant change is the marked shifts in HPA axis reactivity. Indeed, cortisol levels experience normative changes in adolescence, resulting in a prolonged HPA axis response compared to adults [28]. In addition, it is compelling to

note that these same brain-immune systems are - at the same time- involved in normal microbiome development; constituting another overlooked developmental factor.

Although scarce, the studies comparing adolescents with children and adults revealed that adolescents' microbiomes were distinguishable by changes in relative abundance and specific taxa in the gut, indicative of a gradual transition from childhood to adulthood rather than a distinct adolescent microbiome profile [178-180]. Based principally in early life studies, researchers have begun to propose microbial-neural critical windows [26, 124]. While more human studies are evidently needed, rodent models suggest that adolescence could represent a critical window during which the gut microbiome's colonization might impact the ongoing refinement of the central nervous system and the future emergence of socio-affective and stress□associated behaviours [27, 181, 182]. For example, antibiotic-induced depletion of the microbiota from adolescence alters immunological responses [183], monoamine and neuromodulation in the hippocampus and amygdala resulting in anxiety-like behaviours and social deficits in adulthood (altered social motivation and novelty preference) [140]. Adolescence may be also a period of opportunity for gut-brain connections [184, 185]. In this sense, GF mice that are colonized with commensal bacteria by adolescence- but not during adulthood- showed amelioration in different facets of socio-affective behaviour (e.g., reduced stress hyperresponsivity) [148]. These studies support the concept that homeostasis of central and peripheral neuroendocrine and immune responses, relies -at least partially- on the presence of a functional microbiota during critical windows of neurodevelopment.

5.2. IMPLICATIONS FOR BINGE DRINKING

Adolescent alcohol drinking contributes to the development and severity of AUD later in adulthood, particularly in those individuals who start drinking at an early age [23]. Rodent studies have demonstrated that adolescent alcohol misuse can reprogram brain development not only through inflammatory processes but also by decreasing neurogenesis and inducing changes in gene expression through epigenetic mechanisms (e.g., BDNF expression) in the amygdala and PFC [99, 186, 187], linked to persistent anxiogenic behaviour [188]. Human studies have also shown that a BD pattern of alcohol consumption during adolescence might alter developmental trajectories causing structural and functional alterations in prefrontal regions, accompanied by neuropsychological deficits in executive functions, particularly in inhibitory control [85, 189]. A growing number of studies in young BDs suggest specific difficulties for processing negative stimuli [24, 40, 54, 55], greater emotional interference [94, 95] and heightened reactivity [74]. In this vein, impairments in already immature

prefrontal control regions are likely to further fuel emotional dysregulation through poor affective control (see Table 4). Furthermore, alcohol is a potent deregulator of the HPA axis, a response that becomes adapted after chronic use resulting in blunted activity [69]; a similar disruption might occur in regular BDs [190]. Blunted cortisol responses have been associated with increasing alcohol craving and intake [190, 191] and are likely to play a role in alcohol-related emotional dysregulation. This gains importance in the context of adolescence as a stressful developmental phase.

Adolescence has been termed a "storm and stress" period, as young people usually have to face a variety of challenges and stressors, especially in the social domain [192]. Mild social stressors have rendered animals more sensitive to the rewarding properties of alcohol [193, 194]. Stress and alcohol activate common neural circuits [195] that impact sensitivity to alcohol [193]. When experienced together, alcohol and stress might exert a cumulative effect altering reward sensitivity via perturbations in the mesocorticolimbic dopaminergic system, governing salience attribution and impacting alcohol sensitization [196], of crucial importance at early stages of drug use [196, 197]. Functional connectivity studies have revealed stress-induced hyperconnectivity between striatal and limbic networks but hypoconnectivity between striatal and control prefrontal networks, which contributes to sensitization and maladaptive responses to alcohol [198]. Neural sensitization leads to strong impulsive reactions (e.g., attentional biases and approach tendencies) to classically conditioned cues that signal alcohol or drugs, which might occur more rapidly during adolescence due to top-down imbalances in control vs. affective systems [199], in line with animal research [193].

The impact of stress in the immune system is a cornerstone of the gut-brain axis literature [125]. Potential alcohol-related perturbations in gut bacteria may further disrupt the homeostasis of central and peripheral neuroendocrine and immune responses, contributing to altering the trajectory of brain development and augmenting the vulnerability to emotional disturbances and loss of control over drinking. Human studies have shown that even a single BD episode was able to increase peripheral endotoxin levels [102], whereas a regular BD pattern was associated with alterations in cortisol levels and peripheral inflammatory markers and endotoxemia (e.g., LPS, cytokine IL-6) that correlated with poor executive functions [103]. Elevated levels of plasma endotoxin in BDs could be compatible with intestinal alterations both in terms of permeability and microbial dysbiosis (as seen in AUD [162]). However, this remains unexplored in young people with a BD pattern. Further support to this

hypothesis comes from a recent study revealing, for the first time, microbiome alterations in an animal model of adolescent BD, and part of these microbiome alterations appear to be relatively permanent, lasting until adulthood [170]. Therefore, it is plausible that even before a severe AUD develops gut permeability and microbiome dysbiosis are already present. This hypothesis holds relevant implications for adolescence as a "gut-brain" vulnerability window in which repeated alcohol intoxications are the most common pattern of alcohol consumption among youth.

6. MODEL-BASED INSIGHTS

The critical insight of this framework is an appreciation of microbiome-immune disruptions as mediators of fronto-limbic anomalies - especially in the amygdala- and derived emotional dysregulation (Figure 3). We situate these dysfunctions at the centre of a feed-forward loop that is likely to accelerate loss of control over drinking and promote comorbid psychopathology. We have based the present framework in the classical three-stage cycle model of addiction: binge/intoxication stage, withdrawal/negative affect stage and the preoccupation/anticipation stage [200, 201]. This model conceptualizes addiction as a cycle of spiralling dysregulation of brain reward and stress systems, resulting in compulsive drug use [202].

A) The *binge/intoxication stage* is characterized by the positive reinforcing effects of drugs and increased salience [203], mediated by the mesocorticolimbic dopamine system [9].

B) In the *withdrawal/negative affect stage*, negative emotional states such as dysphoria, anxiety or irritability -principally related to withdrawal- engage the activation of the extended amygdala, which drives negative reinforcement processes. Aversive emotional states are mediated by decreases in reward function and increases in the stress function (mainly the HPA axis) [204]. Stress is further argued to impact the addiction cycle creating an emotional dysregulation or allostasis underlying the pathology of addiction [202, 205].

C) The *preoccupation/anticipation stage* involves prefrontal dysfunction that results in deficits in executive functions and failure in the down-regulation of reward responses [53]. This imbalance contributes to cue-induced craving (as the

central focus of most theories), a key element for the relapsing nature of the disorder [191].

Previous considerations to expand the conceptualization of addiction have been focused principally on prefrontal dysfunctions (e.g., self-control [114] or impaired insight [206]). Here, we have moved beyond the prefrontal dysfunction hypothesis and deconstructed the concept of emotional dysregulation across all facets -social cognition, emotional reactivity and regulation. We overcome the non-specific nature of emotional dysregulation as an umbrella term by describing individual and added contributions of different cognitive functions and circuitry alterations that may underly *negative reinforcement*, from binge drinking to severe AUD. Hypoactivity of top-down control regions and hyperactivity bottom-up salience regions such as the amygdala, seem to be a signature of addiction [207], resulting in poor affective control. We argue for a better understanding of affective control over stress/emotional stimuli (in contrast to the extensively studied drug-related cues) as a domain dysfunction that may hold promising clinical applicability, especially with regard to *emotional*/*stress-induced craving* and *relapse*.

Following the concept of *allostatic load*, classically linked to malfunctions in the stress response, we have integrated the latest research in immune and microbiome disruptions as important and overlooked drivers of this gradual accumulation of alterations. We have further bridged the gap between the brain and the body by moving beyond the stress response and integrating putative mechanisms in the microbiota-immune matrix that may influence emotional dysregulation. From this broad conceptualization, we have described the effects of inflammation, microbiota dysbiosis and chronic alcohol use in the fronto-limbic circuitry as a proposed underlying hub for the summative effects of these forces in the *spiralling cycle* of addiction.

Using this framework in the context of BD during adolescence, we articulated how this mutual-maintenance loop might explain addiction susceptibility. During the sensitive period of adolescence, overlooked factors in the refinement of the gut-brain axis, normative changes in emotional reactivity and expected stressors might contribute to the vulnerability of the adolescent brain to repeated alcohol intoxications and the risk of addiction (Figure 4).

Although a strong case can be made for adopting this framework to improve our understanding of alcohol-related disorders, caution is advised for causality assumptions regarding microbiome-cognition associations. A promising future direction could be the

adoption of standardised multi-centre studies that allow for larger human data sets. A deeper characterization of the gut microbiome using shot-gun sequencing (as opposed to 16S sequencing) is encouraged. Among other advantages, it will provide a much more detailed profiling of the taxonomy of communities, particularly at the species and strain level [160]. Incorporation of nutritional information and collection of repeated faecal samples to account for intra-individual variability will progressively become the norm [208, 209]. The contribution of computational approaches [210] such as machine learning will allow for methodological advances in the integration of neuropsychological and multimodal imaging data with multiomics (e.g., metagenomic [composition] and metabolomics [function]) that will help elucidate patterns of microbiome-brain communication.

7. CONCLUDING REMARKS

Seemingly distant, yet deeply intertwined, the social brain, the immune system and the gut microbiome constitute a complex matrix that deserves further investigation in addiction. Departing from the classical theories of addiction, we have integrated the latest findings on immunology, microbiology and neuropsychology in relation to emotional dysregulation, in order to shed light on the neurobiology of drinking escalation and the development of comorbid psychopathology. The central consideration of this framework, is the argument that alcohol-related microbiome dysbiosis, together with social cognition deficits, might accelerate the transition to addiction through immuno-affective pathways. In addition, we provide a compelling argument for how this loop is likely to be amplified during the sensitive period of adolescence in the context of binge drinking. We argue that overlooked factors in the refinement of the gut-brain axis communication, together with typical emotional fluctuations and the co-occurrence of social stressors might contribute to the vulnerability of the adolescent brain to repetitive alcohol intoxications and the increased risk for addiction and mood disorders during these years. This framework will be a novel and important mean of testing hypotheses aimed at unravelling the interdependencies between these seemingly disparate domains and designing new therapeutic approaches that could have profound implications for advancing the understanding of addiction.

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9. CONFLICT OF INTEREST

All authors declare that they have no conflicts of interest.

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Figure Legends

Figure 1. Effects of alcohol misuse in the gut-brain axis. Chronic alcohol consumption may impact inflammation directly at the brain level and in the periphery. Alcohol induces intestinal permeability and microbial dysbiosis that contribute to the inflammatory cascade. Damage to the intestinal epithelial layer causes the leakage ("leaky gut") of bacterial products such as lipopolysaccharide (LPS) that can enter the blood and reach the liver. In response, immune cells secrete cytokines are transported via the blood stream to the brain causing neural damage that leads to cognitive and emotional impairments and sensitization of stress-response pathways (hypothalamic pituitary adrenal [HPA] axis). Alcohol-derived alterations in stress and inflammatory responses may contribute to fronto-limbic alterations, resulting in emotional dysregulation. Alcohol-derived imbalances in the peripheral homeostasis might communicate to the brain through different routes, such as the vagus nerve, neurotransmitters or gut-derived metabolites such as short-chain fatty acids (SCFAs), see Table 3 for further details regarding communication pathways.

Figure 2. Overlapping brain hubs for the interlinked effects of alcohol misuse, inflammation and gut microbes. Alcohol misuse, inflammation and microbiome alterations are likely to impact the fronto-limbic circuitry as a convergence hub for emotional dysregulation. We highlight the summative effects on probable hubs including the amygdala, prefrontal cortex, hippocampus and the hypothalamic pituitary adrenal (HPA) axis, which at the same time, coincide with the main nodes for socio-affective networks. *A) Alcohol misuse: Effects of alcohol misuse on socio-affective networks.* AUD has been associated with deficits in emotional processing (misinterpretation and overestimation of emotional signals), social cognition (affective ToM) and exaggerated emotional reactivity. These difficulties are associated with abnormalities in different brain regions, principally orbitofrontal, ventrolateral and dorsolateral prefrontal cortex [oPFC, vPFC, dPFC], hippocampus [Hippo], hypothalamus [HPA axis], amygdala [Amg], anterior insula [Ant In], anterior cingulate cortex [ACC]). *B) Inflammation: Brain targets for peripheral inflammation.* Peripheral inflammation has been found to negatively affect several brain areas: bilateral amygdala, hippocampus, hypothalamus, ventral striatum (VS), insula, medial prefrontal cortex (mPFC) and anterior cingulate cortex. *C) Microbiome: Gut microbes and derived metabolites as regulators of brain function.* Gut microbiota and its metabolites appear to play a role in regulating social behaviour and brain development including: normal HPA axis and amygdala development, hippocampal monoamine concentrations and gene expression, prefrontal cortical myelination and dopaminergic neurotransmission in the mesocorticolimbic circuit.

Figure 3. Integration of the microbiome neuro-immuno-affective framework in the addiction cycle. The critical construct of this framework is an acknowledgement of microbiome-immune disruptions as potential mediators of fronto-limbic anomalies and derived emotional dysregulation in the alcohol addiction cycle. This model reconceptualizes emotional dysregulation in terms of deficits in social cognition, emotional processing and affective control that influence the development of alcohol-related disorders. At the binge/intoxication stage, alcohol use is mainly motivated by positive reinforcement (i.e., sensitization of incentive salience). Repeated intoxications might initiate microbiome neuro-immune-affective imbalances that progressively feeds allostasis, contributing to alterations in fronto-limbic networks and early emotional dysregulation. At the withdrawal/negative affect stage, both negative affect related to acute withdrawal and lasting emotional disturbances may drive negative reinforcement processes that might be further exaggerated by microbiome-immune interdependencies. At the preoccupation/anticipation stage, impairments in top-down connectivity result in weakened affective control, with relevant implications for stress-induced craving and relapse. Underlying alcohol-related microbiome alterations might, therefore, contribute to a vicious circle of emotional dysregulation that is likely to accelerate the transition to compulsive alcohol use.

Figure 4. Adolescent vulnerability to binge drinking from a microbiome neuro-immuno-affective perspective. Early binge drinking is a risk factor for the development of AUD (e.g. via epigenetic reprograming). During adolescence, maturational imbalances in fronto-limbic networks contribute to hyperemotionality that is not effectively down-regulated, resulting in poor affective control. The immaturity of the adolescent brain makes it more vulnerable to insults such as binge drinking (BD), which has been associated with alterations in fronto-limbic networks accompanied by poor executive functions and deficits in emotional processing and social cognition. The ongoing development of the gut microbiome-brain communication appears to have important modulatory effects on immune and neuroendocrine responses, as well as on the normal development of socio-affective behaviour and fronto-limbic circuits. BD-related microbiome disruptions could act as mediators of fronto-limbic anomalies and derived emotional dysregulation via neuro-immuno-affective pathways. This mind-body conceptualization gains importance in the context of adolescence as a stressful developmental phase. When experienced together, alcohol and stress might exert a cumulative effect altering neuroendocrine responses and salience attribution, fuelling further central and peripheral (i.e., immune/microbiome) alterations. Therefore, BD-related emotional dysregulation is likely to be augmented due to the disruption of neurodevelopmental processes and overlooked factors in the refinement of the gut-brain axis communication, together with the co-occurrence of typical emotional fluctuations and social stressors. These interactions might contribute to the increased risk of mood disorders and addiction susceptibility.

Table 1. Affective control in the context of alcohol misuse

Affective control, or the down-regulation of automatic emotional responses, is central to emotional regulation and mental health [84]. Here we highlight the implications of affective control over emotional/stressful stimuli (as opposed to drug-related stimuli) for alcohol relapse and BD during adolescence. Although inhibition of emotional information is one of the main facets, it also encompasses various higher order cognitive functions, such as updating or shifting between emotional events, working memory (e.g., avoiding negative recurrent thoughts) and episodic memory control (e.g., controlling the retrieval of intrusive memories) [84, 211]. We propose to expand and adapt this topic to the alcohol field: **(I)** by addressing the effects of BD in the mechanisms supporting affective control. Affective control develops throughout adolescence until early adulthood [77]; therefore, frequent alcohol intoxications during this period may be a threat to future successful regulatory processes. **(II)** By exploring the role of affective control in emotional/stress-induced craving in relation to the escalation of addictive behaviour and relapse. **(III)** By investigating the potential efficacy of affective control training in the reduction of alcohol craving and immune/endocrine alterations. Cognitive control of emotional information could potentially be enhanced through neuropsychological training to reduce emotional/stress-induced craving and cytokine reactivity to emotional stress [106, 212].

Prolonged alterations in social cognition and emotional regulation have been shown to alter immune signaling [213], exaggerated inflammatory responses, in turn, further dysregulate fronto-limbic systems in a vicious circle [17]. Some researchers have gone a step further on this complex co-regulation and investigated if greater efficiency in affective control could lead to reduced inflammation. Apparently, the answer might be yes; an efficient cognitive control of emotional stimuli could lead to reduced inflammatory responses and less reactivity to stress. For example, better cognitive control (emotional Stroop task) following a stressor (emotionally evocative video) was associated with lower pro-inflammatory cytokine reactivity to such emotional stressor, revealing the importance of control processes not only for self-regulation but also for inflammatory reactivity [212, 214]. An intriguing question is whether potential improvements in affective control training would be translated into amelioration of immune/endocrine alterations and reduced craving in AUDs and BDs. Examination of the generalisability of these findings to the alcohol field could hold clinical applicability.

Table 2. Immune-to-brain communication pathways

A typical inflammatory response consists of inflammatory inducers, sensors detecting the inducers (i.e., receptors expressed by immune cells, including Toll-like receptors [TLRs]); and the release of inflammatory mediators such as cytokines and chemokines, (e.g., interleukin [IL]-1β, IL-6 or TNFα) [215]. The central nervous system has its own immune cells called microglia- resident macrophages of the brain- that respond to insults and peripheral inflammation up-regulating a number of cell surface receptors and increasing the production of cytokines and chemokines [216]. This neuroimmune activation, as well as the peripheral immune molecules that activate the immune cells in the brain, can cause significant tissue damage and cell death, particularly within the social brain [107, 215]. Brain responses to peripheral immune activity occur either via vagal afferents, directly at the blood brain barrier (BBB), or at circumventricular organs [17]. The vagus nerve is able to expresses cytokine receptors, is activated by peripheral inflammation, and this signal is transmitted to central brain regions involved in the regulation of emotional functioning [117]. Another potential route is via the BBB; peripheral cytokines can cross the BBB either by transport proteins or by diffusion in regions where the barrier is leaky such as the circumventricular organs [216]. Cytokines have been shown to deplete tryptophan and subsequently decrease serotonin production [217].

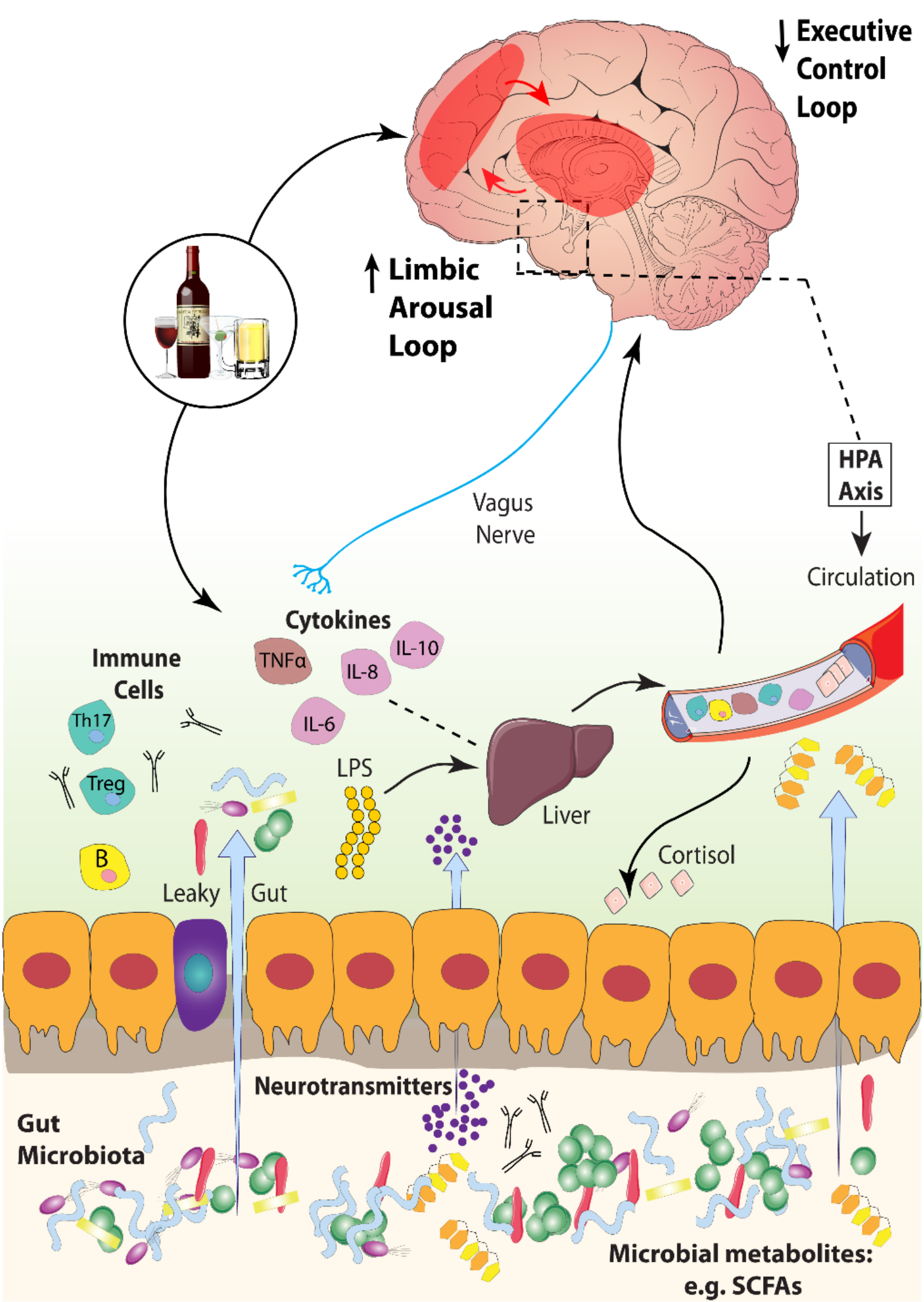
Within the brain, inflammatory signals from the periphery influence local physiological processes such as neurotransmitter metabolism, long-term potentiation and synaptic plasticity [218]. Heightened activity of the immune system seems to particularly affect the structure and function of fronto-limbic brain regions, mainly through changes in levels of serotonin and kynurenine pathway metabolites and the dysregulation of the HPA axis [215].

Table 3. Bidirectional pathways of the Microbiota-Gut-Brain axis
The microbiota is a diverse community of trillions of microorganisms and their genes that reside in the gastrointestinal tract, including bacteria, fungi, and archaea [219], and outnumber human genes [220]. Communication via this axis influences health through vagal, neural, endocrine, and/or immune pathways [117]. Although, much remains to be understood about the mechanisms behind this cross-talk, proposed pathways include the vagus nerve, production of neurotransmitters, and gut-derived peptides such as ghrelin [221, 222], the stimulation of cytokine expression, and immune activation.
The vagus nerve: The vagus nerve is a key branch of the parasympathetic nervous system and constitutes one of the main routes of bidirectional communication between the brain and the gut microbiome. The vagus nerve receives and responds to signals from bacterial metabolites and to the release of neurotransmitters such as serotonin; and its activation has marked anti-inflammatory effects [223].
Cortisol: The hypothalamic pituitary adrenal (HPA) axis represents the main efferent route from the brain to the gut. When activated, the resulting secretion of cortisol affects immune cell activity, locally and systemically [224]. Cytokines (particularly IL-1 and IL-6) produced in the gastrointestinal tract travel via the bloodstream to the brain and activate the HPA axis. Whereas pathogenic bacteria migrating out of the gut (through a weakened gut barrier or "leaky gut") can trigger a proinflammatory cascade, beneficial bacteria can also trigger the release of anti-inflammatory cytokines [224].
Short-chain fatty acids (SCFAs): SCFAs, which include propionate, butyrate and acetate, are important microbial metabolites generated via the fermentation of non-digestible dietary fibers. SCFAs enter systemic circulation and have been shown to regulate the activity of the sympathetic nervous system [225]. SCFAs promote gut barrier integrity, gut immune homeostasis and modulate cytokine production. These metabolites are able to cross the BBB and have been shown to alter the maturation of microglia [226]. SCFAs might influence psychological functioning directly via humoral/immune effects or indirectly via interactions with histone deacetylases, G protein- coupled receptors, hormonal pathways [225]
Tryptophan: Tryptophan is an essential amino acid precursor to many biologically active molecules such as serotonin. The enteric nervous system (ENS) produces an estimated 95% of the body's serotonin [118]. An adequate balance in the transformation of tryptophan into its metabolites kynurenine and serotonin is considered to play an important role in bacteria–brain signaling, as microbes are important contributors in this metabolic pathway [226].
Neurotransmitters: Certain bacteria have the capacity to generate neurotransmitters and neuromodulators including GABA (e.g., produced by *Lactobacillus*); serotonin (e.g., by *Enterococcus*) and dopamine (e.g., by *Bacillus*). However, their impact on brain function is likely to be indirect by acting on the ENS [127].

Table 4. Towards psychobiotics in alcohol use disorder

Psychobiotics are live bacteria (probiotics) or dietary supplements (prebiotics), that when ingested, confer cognitive and mental health benefits [150, 227]. The restoration of the beneficial bacteria altered by chronic alcohol use (e.g., *Bifidobacterium* and *Lactobacillus*) via probiotic supplementation could ameliorate alcohol-derived damage [228]. Indeed, probiotic administration in mice prevents alcohol-derived inflammation, gut permeability and microbiome dysbiosis [161, 229, 230]. The very few studies conducted in humans show amelioration of alcohol-induced liver damage in patients with alcohol cirrhosis after probiotic interventions [231, 232]. In the alcohol field, less attention has been devoted to prebiotics. Prebiotics are dietary fibres that serve as nutrients for beneficial gut microbes (especially *Bifidobacterium* and *Lactobacillus*) and exert their effects through the production of beneficial metabolites such as short-chain fatty acids (e.g., butyrate) [233]. In rats, prebiotics improved alcohol-induced liver damage and bacterial overgrowth [234, 235]. Prebiotics have been used in combination with probiotics to obtain a synergistic effect, referred to as synbiotics [236]. Synbiotics have been showed to improve hepatic function in patients suffering from cirrhosis [236]. Collectively, these studies support the therapeutic effects of psychobiotics as a co-adjuvant strategy in the management of some aspects of AUDs pathophysiology. However, no study to date has investigated the cognitive and psychological effects of psychobiotics in alcohol misuse.

In healthy subjects, emerging studies investigating the link between pyschobiotics and cognition have focused principally on emotional functioning [227]. These preliminary studies suggest improvements in emotional processing [153, 154, 237] through modulation of emotional networks implicated in attentional bias and emotional reactivity [227]. Improvements have also been reported for reduced inflammation, lower stress reactivity and self-reported mood [152, 238, 239]. Nevertheless, considerable variability exists. Inconsistencies might respond to differences in doses or strain/composition, duration of the intervention and cognitive tasks employed. Larger sample sizes and randomized-placebo-controlled trials employing sensitive neuropsychological tasks (e.g., alternate forms to avoid ceiling and practice effects) are needed. Future movement towards greater specificity will require replicable strain-dependent knowledge and new studies that mirror the microbial deficiencies of particular disorders for elaboration of precision probiotics [240].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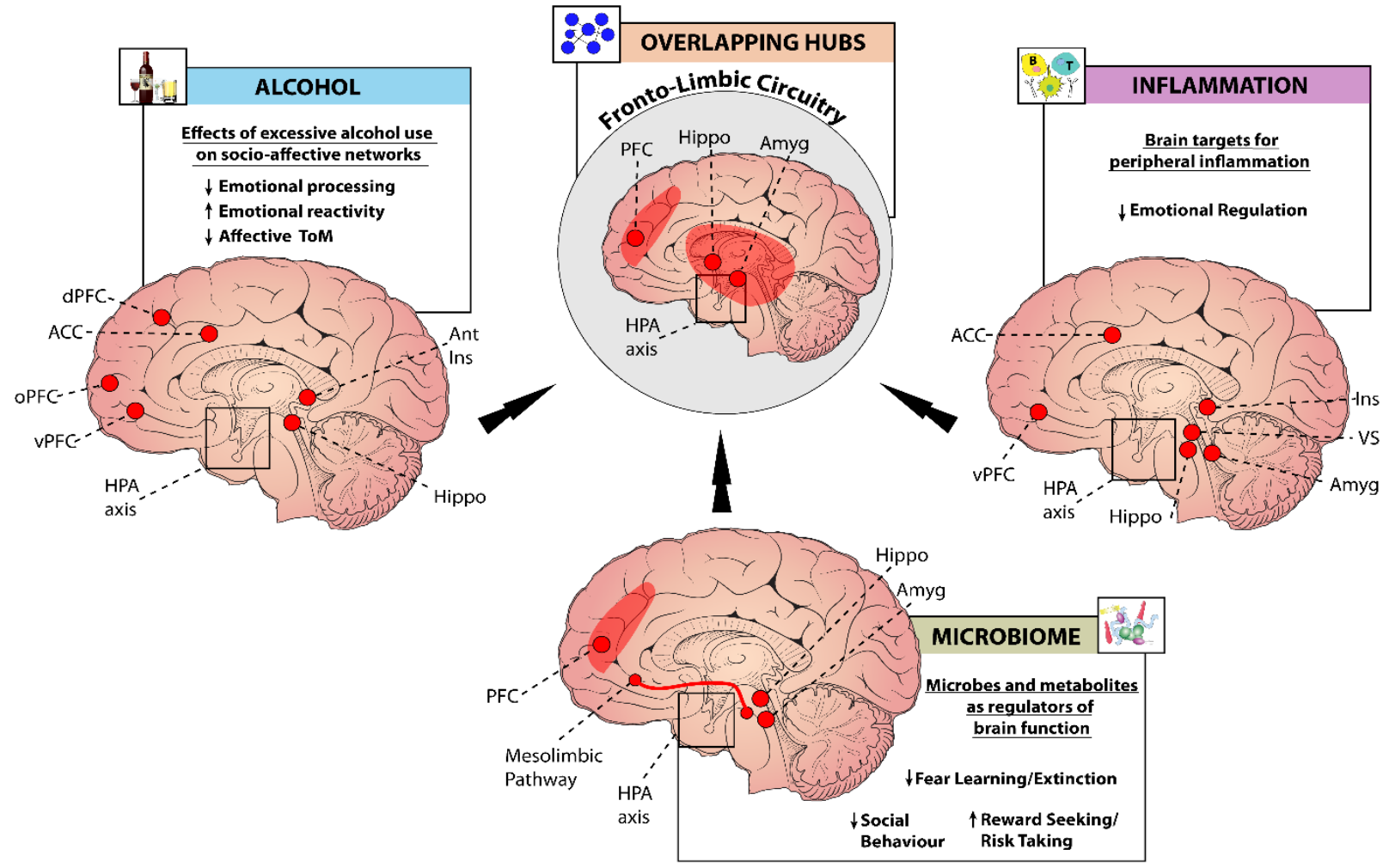
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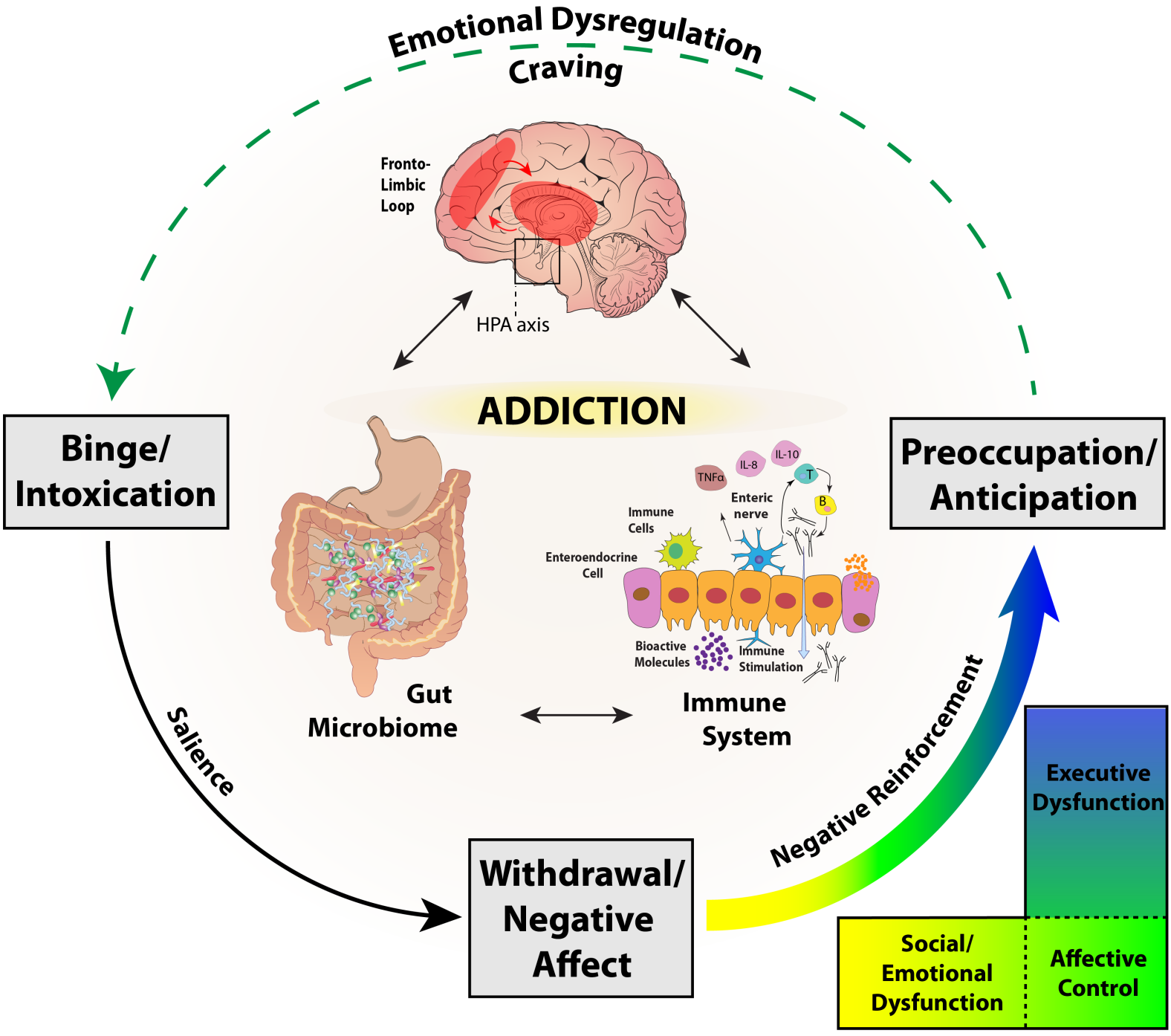
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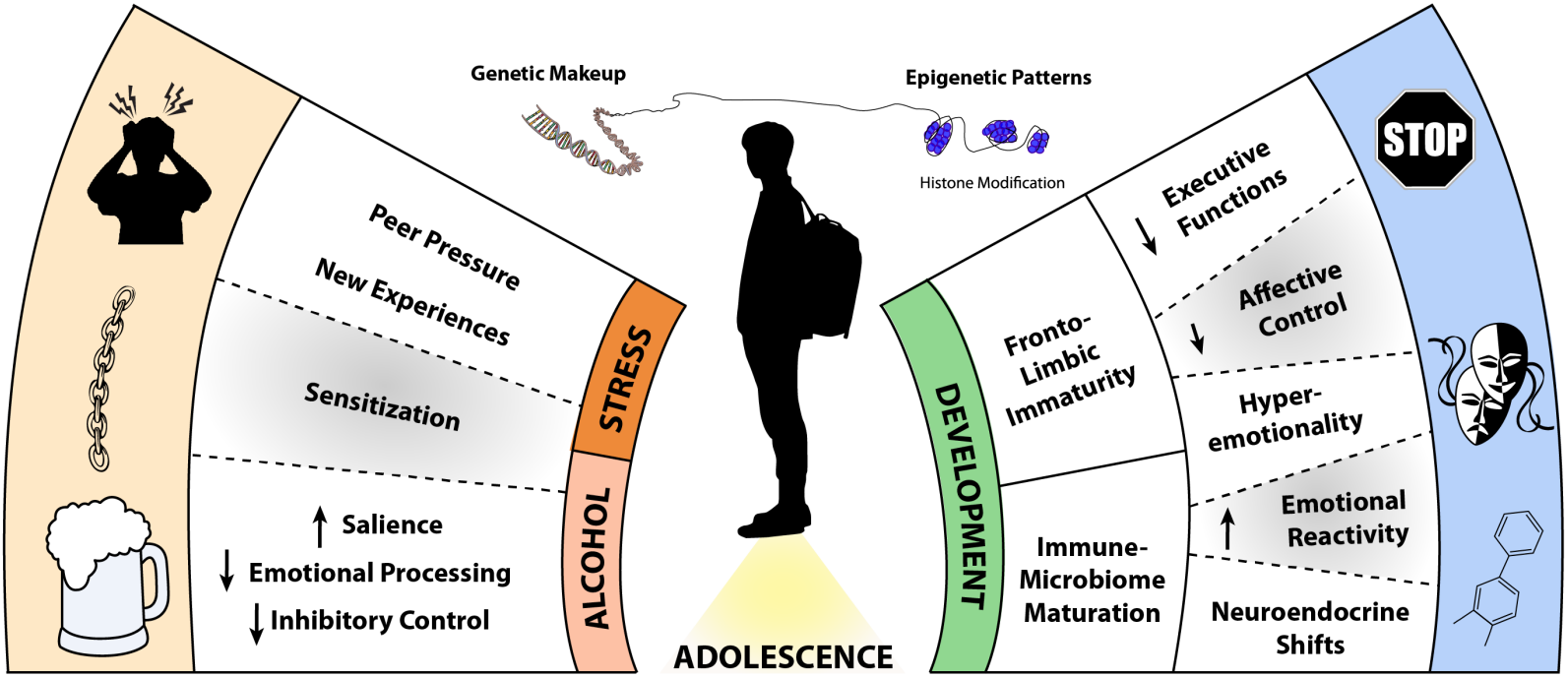
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DEVELOPMENT
Fronto-Limbic Immaturity
Immune-Microbiome Maturation
Executive Functions
Affective Control
Hyper-emotionality
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Neuroendocrine Shifts
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