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The Effect of Tetrahydrocannabivarin (THCV) and Cannabidiol (CBD) on Voluntarily Alcohol Drinking in Male Rats

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List of Abbreviations

2BC Two-Bottle Choice

2-AG 2-Arachidonoylglycerol

ADE Alcohol Deprivation Effect

ADH Alcohol Dehydrogenase

ALDH Aldehyde Dehydrogenase

AEA Anandamide

ANOVA Analysis of Variance

AUD Alcohol Use Disorder

BNST Bed Nucleus of the Stria Terminalis

CB1 / CB2 Cannabinoid Receptor Type 1 / Type 2

CBD Cannabidiol

CeA Central Nucleus of the Amygdala

CRF Corticotropin-Releasing Factor

DSM-5 Diagnostic and Statistical Manual of Mental Disorders, 5th Edition

ECS Endocannabinoid System

FAAH Fatty Acid Amide Hydrolase

GABA Gamma-Aminobutyric Acid

i.p. Intraperitoneally

ILI Inter-Lick Interval

NAc Nucleus Accumbens

NAM Negative Allosteric Modulator

NMDA N-Methyl-D-Aspartate

PFC Prefrontal Cortex

SEM Standard Error of the Mean

THCV Delta-9-Tetrahydrocannabivarin

VTA Ventral Tegmental Area

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Introduction

Alcohol remains one of the most widely consumed drugs in the world despite its recognised toxicity, holding a significant role in culture and social life, while interfering with many fundamental metabolic processes, resulting in stress and impaired recovery. General alcohol use is shaped by contextual interactions within society; however, alcohol ranks among the top five most addictive substances, and with its challenging comorbidity profile, prolonged exposure can often result in Alcohol Use Disorder (AUD) across a wide severity spectrum. AUD is not simply about heavy drinking; it is defined by a loss of control over consumption that persists despite recognised harmful consequences, leading to greater susceptibility to comorbidities and accumulated toxicity, ultimately reaching a point where the individual loses the ability to make sound decisions independently. As such, external intervention can be crucial to any effective treatment strategy. Three medications are currently approved for AUD treatment: naltrexone, acamprosate, and disulfiram, each working through a different mechanism and each carrying its own limitations in efficacy and long-term adherence (Kranzler and Soyka, 2018). Relapse rates after treatment remain high across all three, which clearly points to the need for compounds targeting different parts of the system driving compulsive alcohol use.

To treat AUD effectively, it is important to recognise that two fundamental concepts are often conflated: the pleasure derived from drinking and the compulsive drive to drink. Contrary to common belief, addiction is not simply about enjoying how something feels. As AUD develops, alcohol produces rapid and intense dopamine activity in reward circuits; the brain adapts to this, resulting in a state where the urge to drink persists even when drinking provides little to no pleasure (Robinson and Berridge, 1993). Physical withdrawal, intense craving that overrides judgment, and continued use despite harmful consequences are all products of this neurobiological progression and not a matter of willpower or choice.

Robinson and Berridge (1993, 2008) proposed a distinction between craving and pleasure in addiction, now known as the incentive-sensitization theory. This framework separates reward into two dissociable components: *wanting* the motivational drive to seek and initiate contact with a reward, mediated by mesolimbic dopaminergic circuits and *liking*; the hedonic pleasure generated during consumption, mediated by opioidergic and GABAergic mechanisms in specific regions of the nucleus accumbens and ventral pallidum (Berridge et al., 2010). With repeated exposure, wanting becomes sensitised independently of liking. This is why individuals with severe AUD often report strong urges to drink while deriving little actual pleasure from it. The distinction is not merely theoretical but also testable in animal models through detailed analysis of not only how much, but also how animals drink.

The endocannabinoid system (ECS) offers a well-supported target for intervening in alcohol reinforcement. Cannabinoid type-1 (CB1) receptors are distributed across circuits that drive both the motivational and hedonic components of alcohol consumption (Parsons and Hurd, 2015). The CB1 inverse agonist rimonabant showed strong anti-alcohol effects in animal studies but was withdrawn from clinical use due to serious psychiatric side effects arising from its constitutive suppression of CB1 signalling below baseline levels (Sam et al., 2011). This pointed toward a more targeted approach: compounds capable of modulating CB1 without reducing basal receptor tone. In this study, two such compounds were tested for their effects on drinking components; delta-9-tetrahydrocannabivarin (THCV), a neutral CB1 antagonist that blocks agonist-driven signalling without reducing constitutive activity, and cannabidiol (CBD), a negative allosteric modulator (NAM) of CB1 that attenuates agonist-stimulated signalling through an allosteric site while preserving baseline receptor function.

The relevance of this study lies in its direct comparison of these two compounds using drinking microstructure analysis within the same experimental model. No published study has yet performed this comparison using lickometer-based bout decomposition, making this the first microstructure-level investigation of whether neutral CB1 antagonism and CB1 negative allosteric modulation produce dissociable effects on the motivational and hedonic components of voluntary alcohol drinking. A previous study from this laboratory (Poceviciute et al., 2026) confirmed that both compounds reduce 24-hour voluntary alcohol intake without negatively altering locomotor activity or emotional state, establishing the basis for the present microstructure investigation.

The central question of this thesis is: does THCV and CBD differentially affect the motivational and hedonic components of voluntary alcohol drinking in male Wistar rats, and if so, what does this suggest about their distinct pharmacological profiles?

The aim of the thesis is to evaluate the differential effects of THCV and CBD on voluntary alcohol drinking microstructure in male Wistar rats following long-term two-bottle choice exposure. To achieve this aim, the following objectives were formulated:

1. To establish a validated lickometer-based microstructure protocol following long-term voluntary alcohol exposure in male Wistar rats;

2. To characterise the effects of THCV (5 and 20 mg/kg i.p.) on the bout number, bout size, bout duration, and lick rate of both alcohol and water;

3. To characterise the effects of CBD (20 and 40 mg/kg i.p.) on the bout number, bout size, bout duration, and lick rate of both alcohol and water.

1. ALCOHOL USE DISORDER: CLINICAL DEFINITION AND NEURAL CIRCUITS

1.1. Diagnostic Criteria and Severity Classification (DSM-5)

Alcohol use disorder is defined as a pattern of problematic drinking resulting in meaningful functional impairment or psychological distress seen within the eleven diagnostic criteria described in DSM-5 (American Psychiatric Association, 2013). These eleven criteria include: (1) mainly, impairment of control where consumption of alcohol exceeds the main limits or for longer durations than originally planned; (2) persistent desire or unsuccessful efforts to cut down: ongoing wish to reduce drinking or multiple failed attempts to do so; (3) time spent: considerable time devoted to alcohol-related activities, including acquisition, consumption, and recovery; (4) craving: experiencing intense urges or compulsions to consume alcohol; (5) failure to fulfil major role obligations: alcohol-related interference with fulfilling key responsibilities in professional, academic, or domestic settings; (6) social or interpersonal problems: maintaining drinking behaviour despite interpersonal conflicts or relationship difficulties attributable to alcohol; (7) activities given up: withdrawing from valued social engagements, work commitments, or leisure pursuits because of drinking; (8) Hazardous use: consuming alcohol in contexts that pose physical danger; (9) physical or psychological problems: persisting with alcohol use while recognizing that it perpetuates or worsens health problems; (10) tolerance: requiring progressively larger quantities of alcohol to produce the same subjective effects, or experiencing reduced effects from consistent doses; and (11) withdrawal: developing physiological withdrawal symptoms during abstinence, or drinking to prevent or alleviate such symptoms. The disorder is graded by symptom count: mild severity corresponds to two to three criteria, moderate to four or five, and severe to six or more (Hasin et al., 2013). The severe range is particularly significant in this context, as it represents the stage where volitional control over intake has deteriorated most profoundly and where the pleasure-seeking and motivational aspects of drinking have become functionally independent.

This classification suggests a change in how AUD is normally understood; it is not a condition someone either has or does not have, but a spectrum of neurobiological change progressing across three overlapping stages that are defined as follows: binge and intoxication, where reinforcing effects drive initial heavy use; withdrawal and negative affect, where distress during abstinence motivates further drinking for relief; and lastly preoccupation and anticipation, of which craving and reactivity to alcohol-associated cues sustain compulsive seeking even without active intoxication (Koob and Volkow, 2016). The progression from social drinking habits to AUD is not simply about drinking more because it just feels better; Robinson and Berridge (1993) showed that incentive salience, the

so-called motivational pull assigned to reward-predictive stimuli, can become sensitised independently of hedonic experience. Wise and Koob (2014) demonstrated that negative reinforcement, which means drinking to relieve withdrawal distress, progressively replaces positive reinforcement as the primary driver of use. By the time AUD becomes severe, the person is not primarily drinking because it feels good but the circuits driving the urge to drink have been recalibrated, and not drinking produces a state that is itself feels aversive.

1.2. Neural Circuits Underlying Alcohol Reinforcement

1.2.1. The Mesolimbic Dopamine Pathway

The mesolimbic dopamine system is composed of dopaminergic neurons projecting from the ventral tegmental area (VTA) to the nucleus accumbens (NAc) shell, as well as to the prefrontal cortex, amygdala, and hippocampus; it is the primary circuit that carries out reward processing and reinforcement learning (Koob and Volkow, 2016). Acute ethanol exposure increases dopamine release in the NAc through two routes: direct facilitation of VTA dopamine neuron firing, and indirect disinhibition achieved by suppressing local GABAergic interneurons within the VTA (Di Chiara and Imperato, 1988; Gessa et al., 1985). The resulting dopamine signal encodes the reinforcing value of the drinking episode and drives the progressive formation of alcohol-stimulus associations underlying craving (criterion 4). As shown in **Fig. 1**, repeated ethanol exposure engages mesolimbic reward circuitry, promoting the progression from recreational use to craving, withdrawal, and loss of control.

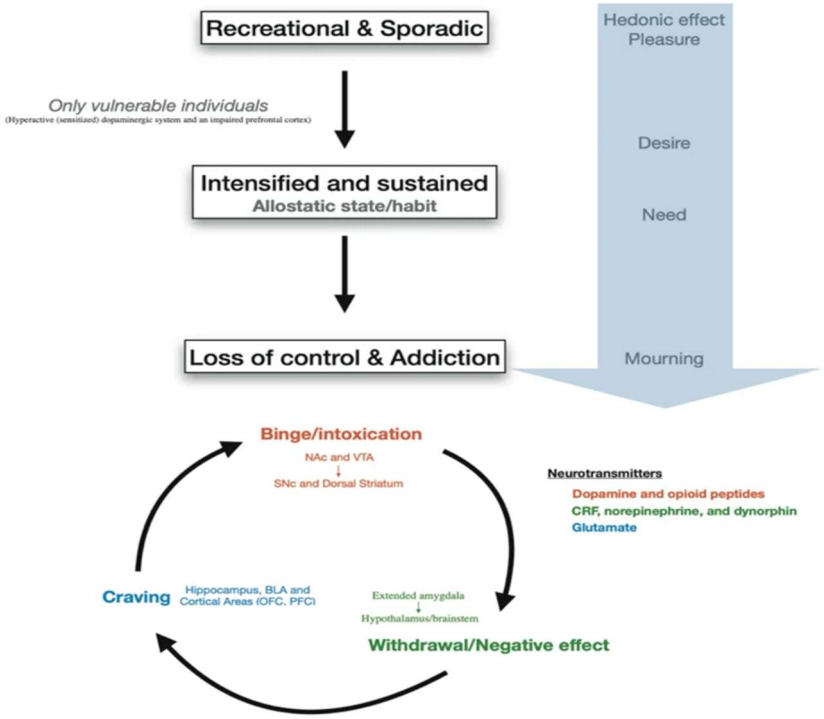


Figure. 1 The model of the progression from recreational alcohol use to addiction via mesolimbic reward circuitry and allostatic neuroadaptation. Adapted from Julian Cheron & Alban de Kerchove d'Exaerde (2021).

The pharmacokinetics of ethanol bear directly on this. In rats, peak brain concentrations are reached within 30 to 60 minutes of ingestion. This is slower than smoked or injected drugs, but it is fast enough to activate mesolimbic circuits reliably and produce sensitisation with repeated exposure

(Spanagel, 2009). Chronic heavy drinking leads to a compensatory reduction in striatal D2 receptor availability, documented both in people with severe AUD and in rodent long-term drinking models (Volkow et al., 2002; Hirth et al., 2016). The result is a reduced baseline hedonic tone that drives escalating consumption simply to achieve a subjective state that was previously the baseline, reflecting impaired control (Criterion 1), excessive time spent in alcohol-related activities (Criterion 3), and tolerance (Criterion 10). This is a substrate for compulsive drinking that has nothing to do with pleasure-seeking and everything to do with the consequences of prior exposure manifesting as continued use despite social problems (Criterion 6) and known physical or psychological harm (Criterion 9).

1.2.2. Cortico-Striatal-Limbic Circuits (Extended Amygdala, BNST, CeA)

Beyond the mesolimbic core, the extended amygdala, including the central nucleus of the amygdala (CeA) and the bed nucleus of the stria terminalis (BNST), mediates the negative reinforcement mechanisms that sustain drinking during withdrawal and stress (Koob, 2008). These structures receive strong input from corticotropin-releasing factor (CRF), neuropeptide Y, and dynorphin systems, all of which are dysregulated by chronic ethanol exposure (Roberto et al., 2012). During withdrawal, CRF hyperactivity in the CeA generates anxiety, dysphoria, and heightened stress reactivity. Drinking provides temporary relief seen in Criterion 11 withdrawal. This relief-seeking mechanism operates through pathways that are separate from, and often opposite to, the hedonic circuits activated during acute intoxication, which is why treating only the reward side of AUD is insufficient.

The prefrontal cortex (PFC), particularly its orbitofrontal and anterior cingulate regions, exerts top-down inhibitory control over alcohol-seeking behavior through direct projections to the nucleus accumbens and basolateral amygdala (Holmes et al., 2012; Moorman, 2018). Repeated heavy alcohol exposure progressively impairs PFC function, which diminishes inhibitory control and enhances reactivity to alcohol-associated cues (Abernathy et al., 2010; Goldstein & Volkow, 2011).

This neurobiological process underlies one of the most clinically recognizable features of severe alcohol use disorder (AUD): continued compulsive drinking despite full awareness of its negative consequences. The behavior cannot be explained primarily by the pursuit of pleasure; instead, it reflects a failure of executive control over sensitized reward and habit circuits (Moorman, 2018; Abernathy et al., 2010).

1.2.3. Hedonic Hotspots: Opioid and GABAergic Signalling

The hedonic impact of alcohol is not distributed uniformly across reward circuits. It is generated within anatomically specific hedonic hotspots: a rostral subregion of the NAc shell and a posterior patch of the ventral pallidum, where activation of mu-opioid receptors amplifies positive affective reactions to rewarding stimuli (Smith and Berridge, 2007; Berridge et al., 2010). Ethanol activates

these circuits partly through direct opioidergic mechanisms and partly through facilitation of GABAergic disinhibition in connected structures (Oswald and Wand, 2004). Additionally, ethanol positively modulates delta-subunit-containing GABA-A receptors at extra synaptic sites in the CeA, VTA, and dorsal striatum, contributing to anxiolytic and sedative properties that are experienced as pleasurable (Olsen, 2018). With prolonged exposure, tolerance develops to these hedonic effects. The pleasurable experience of drinking diminishes while the motivational drive persists, which is the dissociation that incentive-sensitization theory was built to explain.

1.3. The Incentive-Sensitization Model Dissociating Wanting from Liking

Robinson and Berridge (1993, 2008) proposed that reward consists of two separable components served by different neural systems. Wanting, or the incentive salience, is the motivational pull toward a reward, mediated by mesolimbic dopaminergic circuits. Liking, or hedonic impact, is the pleasure generated during consumption, mediated by opioidergic and GABAergic mechanisms in the hedonic hotspots described above (Berridge et al., 2010). The theory predicts that repeated drug exposure sensitises the wanting system through mesolimbic neuroplasticity, amplifying the compulsive motivational pull toward alcohol independently of changes in liking. The person craves and compulsively seeks alcohol while deriving progressively less subjective pleasure from it (Berridge and Robinson, 2016).

1.4 The Three Stages of Alcohol Use Disorder

The neurobiological components described above mesolimbic dopaminergic sensitisation, hedonic opioid signalling, extended amygdala stress dysregulation, and prefrontal inhibitory failure do not operate independently. They are the mechanistic substrates of a single, iterative pathological process. Koob and Le Moal (1997) formalised this as a three-stage addiction cycle binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation and subsequent convergent evidence from rodent self-administration models and human neuroimaging has mapped each stage onto discrete, though interacting, circuit-level changes (Koob and Volkow, 2016). What the framework reveals is not three separate disorders but a self-reinforcing cascade in which each cycle of heavy drinking reconfigures the very circuits that might otherwise constrain it.

The binge/intoxication stage is where this cascade begins, and it is where the mesolimbic system and hedonic hotspots described in sections 1.2.1 and 1.2.3 are most directly implicated. The acute dopamine surge in the NAc and concurrent mu-opioid activation in the ventral pallidum encode not just the hedonic value of the episode but also the predictive weight of the cues surrounding it (Di Chiara and Imperato, 1988; Smith and Berridge, 2007). With repeated exposure, Robinson and Berridge's (1993, 2008) incentive sensitisation framework predicts that mesolimbic reactivity to these

cues amplifies, even as the hedonic response to alcohol itself diminishes. In parallel, Everitt and Robbins (2005) demonstrated in rodent models that repeated drug-associated learning progressively transfers behavioural control from ventromedial to dorsolateral striatal circuits — that is, from goal-directed action, still sensitive to outcome value, toward stimulus-response habits that are not. By the time drinking has become habitual, its continuation no longer depends on pleasure. This is not a trivial distinction; it means that interventions targeting only acute reward will fail to interrupt drinking that has already shifted to dorsal striatal control.

The withdrawal/negative effect stage recruits an entirely separate set of circuits, and Solomon and Corbit's (1974) opponent process theory anticipated why. Every bout of intoxication triggers an opposing negative affective process that, with repeated exposure, grows in magnitude and duration, eventually outlasting the positive hedonic response that initiated it. Neurobiologically, this manifests as the CRF and dynorphin hyperactivity in the CeA and BNST described in section 1.2.2, which Koob (2008, 2013) characterised as an allostatic shift in the brain's reward and stress set points which is what he termed the dark side of addiction. The anxiety, dysphoria, and stress hyperreactivity of withdrawal are not simply the absence of reward; they are an actively generated aversive state, and ethanol's capacity to transiently suppress it becomes its dominant reinforcing property (Roberto et al., 2012). At this stage, drinking to avoid suffering has displaced drinking for pleasure entirely.

The preoccupation/anticipation stage is where the circuitry described in section 1.2.2 specifically the deteriorating PFC and its projections to the NAc and basolateral amygdala determines whether the cycle continues or is interrupted. Volkow and colleagues documented, through PET imaging in individuals with AUD, that striatal D2 receptor downregulation reduces baseline dopaminergic tone while simultaneously weakening PFC inhibitory output (Volkow et al., 2002). The result is a brain in which sensitised wanting circuits operate with reduced cortical constraint, and in which alcohol-associated cues acquire disproportionate motivational salience relative to the diminished capacity to resist them. Wise and Koob (2014) described this as the convergence of reward deficit and stress surfeit: a state in which neither the prospect of pleasure nor the exercise of control is sufficient to interrupt the cycle.

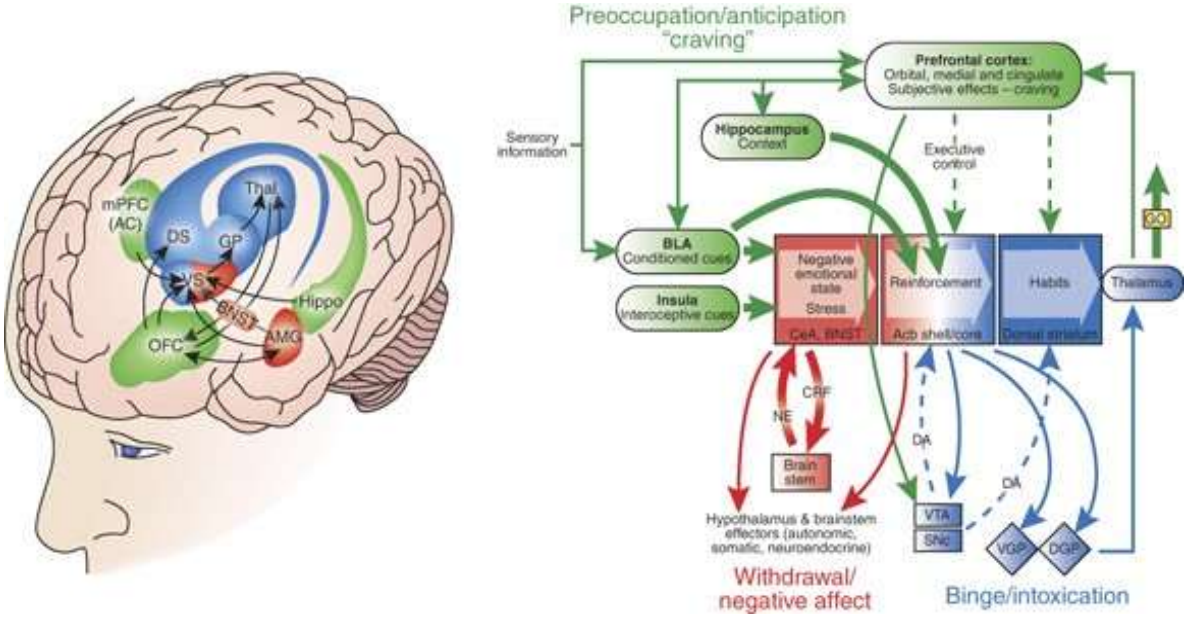


Figure. 2 Neurocircuitry of addiction in the addicted state. Cues and stress activate striatal and extended amygdala circuits; frontal cortex function is compromised; dopamine and CRF systems are dysregulated. *Adapted from Koob & Volkow (2010), Neuropsychopharmacology.*

This architecture matters for the present study because it determines what voluntary drinking microstructure can and cannot tell us. In a non-dependent Wistar rat model without imposed withdrawal, the animal is operating primarily within the binge/intoxication stage: mesolimbic dopaminergic and opioidergic circuits are engaged, with the ventral tegmental area and ventral striatum serving as the focal point for this stage (Koob & Volkow, 2010; Koob & Volkow, 2016), incentive sensitisation is underway (Robinson & Berridge, 1993; Berridge & Robinson, 2016), and the transition toward dorsal striatal habit consolidation is in its early stages (Everitt & Robbins, 2005; Everitt & Robbins, 2013). The extended amygdala stress systems that dominate dependent drinking are not yet the primary driver (Koob, 2009; Koob & Le Moal, 2001). Measures of drinking microstructure; lick patterns, bout structure, and consumption dynamics therefore index the positive reinforcement mechanisms that initiate the cycle, providing a window into the circuit-level processes that pharmacological intervention must target (Weiner et al., 2015; Samson, 2000) before allostatic dysregulation forecloses the possibility of voluntary control (Koob & Le Moal, 2001; Koob & Volkow, 2016).

1.5. The Role of Negative Reinforcement in Severe AUD

While positive reinforcement drives the initial acquisition and maintenance of alcohol drinking, in severe, dependent individuals, a second process emerges: drinking to relieve withdrawal. Koob and colleagues (Koob, 2013; Koob and Volkow, 2016) describe this as a shift from reward-seeking to relief-seeking, mediated by dysregulation of stress systems including CRF and dynorphin signalling in the extended amygdala and noradrenergic circuits in the bed nucleus of the stria terminalis

(BNST). This negative affective state is characterized by anxiety, dysphoria, and stress hyperreactivity that is temporarily alleviated by alcohol consumption. However, not all individuals with AUD are driven primarily by withdrawal relief. Many continue to drink due to sensitized reward-seeking and craving (positive reinforcement), particularly in non-dependent or early-stage AUD (Heilig et al., 2010). Understanding how pharmacological interventions modulate positive reinforcement mechanisms remains a critical first step in developing treatments that can prevent progression to dependence. The present study focuses on positive reinforcement mechanisms in a voluntary drinking model without imposed withdrawal, allowing for the characterization of how THCV and CBD affect reward-seeking and hedonic components of alcohol consumption.

2. CURRENT PHARMACOLOGICAL TREATMENTS FOR AUD

2.1. Approved Medications in AUD

Three drugs currently hold regulatory approval for AUD treatment. Naltrexone is a competitive antagonist at mu-opioid receptors (Swift, 1999). It works by blocking the opioidergic hedonic response to alcohol in NAc hedonic hotspots, reducing the pleasurable component of drinking and attenuating cue-induced craving (Oswald and Wand, 2004). The COMBINE trial confirmed significant reductions in heavy drinking days and time to first relapse versus placebo (Anton et al., 2006). Extended-release injectable formulations address adherence but carry hepatotoxicity risk at higher doses. Acamprosate is thought to normalise the glutamatergic hyperexcitability that emerges during protracted abstinence, with proposed mechanisms including NMDA receptor antagonism and metabotropic glutamate receptor modulation (Kiefer and Mann, 2010). A sex-stratified individual patient data meta-analysis found significant benefit for sustained abstinence with a number needed to treat of approximately nine at twelve months (Mason and Lehert, 2012). It requires three-times-daily dosing, which limits adherence in practice. Disulfiram inhibits ALDH irreversibly, causing acetaldehyde accumulation on ethanol ingestion and an aversive reaction meant to deter drinking (Fuller et al., 1986). Without supervised administration, trial evidence shows no advantage over placebo.

2.2. Limitations of Current Pharmacotherapies

Even in controlled trials, the number needed to treat to prevent one additional relapse is approximately nine for both naltrexone and acamprosate at one year (Roozen et al., 2006). In real clinical settings, fewer than 30% of patients continue any of these medications beyond six months (Mark et al., 2003). Mechanistically there is gap. Naltrexone targets the hedonic liking side through opioid receptor blockade but does not directly attenuate the sensitised mesolimbic wanting circuits that drive craving independently of pleasure (Berridge and Robinson, 2016). Acamprosate addresses glutamate-mediated hyperexcitability during abstinence but has no effect during active drinking.

2.3. Novel Therapeutic Targets

Two strategic directions have accumulated support in the search for novel AUD pharmacotherapy. The first is targeting systems specifically dysregulated by chronic ethanol exposure, including CRF, dynorphin, nociceptin, and endocannabinoid pathways (Koob, 2008). The second is identifying compounds with established human safety data that can modulate relevant circuits without the adverse effects seen with first-generation candidates like rimonabant. The endocannabinoid system satisfies both criteria simultaneously. It is altered by chronic alcohol use at multiple circuit levels (Basavarajappa & Hungund, 2002; Parsons & Hurd, 2015), and pharmacological modulation of CB1/CB2 signalling has repeatedly been shown to reduce voluntary ethanol intake and relapse-like behaviour in rodents (Colombo et al., 1998; Cippitelli et al., 2005; Aracil-Fernandez et al., 2012). Critically, non-psychotropic cannabinoids such as cannabidiol and tetrahydrocannabivarin offer a safer pharmacological profile than first-generation CB1 antagonists (Izzo et al., 2009; Thomas et al., 2005), with emerging preclinical evidence supporting their potential to attenuate ethanol consumption without inducing aversion or motor impairment (Viudez-Martínez et al., 2018; Poceviciute et al., 2026).

3. THE ENDOCANNABINOID SYSTEM IN AUD

3.1. CB1 Receptors: Motivation, Reinforcement, and Synaptic Plasticity

CB1 receptors are among the most densely expressed G-protein-coupled receptors in the mammalian brain, with particularly high concentrations in the basal ganglia, cerebellum, hippocampus, neocortex, and limbic structures (Herkenham et al., 1990). Their primary function is retrograde synaptic modulation: postsynaptic depolarisation stimulates synthesis of endocannabinoids, primarily anandamide (AEA) and 2-arachidonoylglycerol (2-AG), which travel back across the synapse and activate presynaptic CB1 receptors to suppress neurotransmitter release (Kano et al., 2009). Through this mechanism CB1 receptors regulate both glutamatergic excitation and GABAergic inhibition in circuits relevant to alcohol reinforcement, including the VTA, NAc, PFC, and CeA.

In the VTA, CB1 receptors on GABAergic interneurons, when activated, suppress GABA release onto VTA dopamine neurons, removing tonic inhibition and allowing those neurons to fire more readily (Szabo et al., 2002). Ethanol promotes endocannabinoid mobilisation in VTA circuits and uses this CB1-mediated disinhibition to amplify mesolimbic dopamine output, which is one mechanism by which alcohol produces its rewarding dopamine surge (Parsons and Hurd, 2015). Chronic heavy drinking downregulates CB1 receptor expression and reduces AEA and 2-AG levels in limbic regions (Vinod and Hungund, 2006), contributing to heightened anxiety, lower hedonic tone, and stronger craving during abstinence.

3.2. CB2 Receptors: Reward System Alterations and Relapse

CB2 receptors were long considered exclusively peripheral but have now been identified on dopaminergic neurons in the VTA, on microglia, and on astrocytes throughout reward-relevant circuitry (Zhang et al., 2017). Their activation reduces voluntary alcohol self-administration and attenuates stress-driven reinstatement in rodent models, possibly through modulation of neuroinflammatory signalling contributing to both compulsive drinking and withdrawal-related negative affect (Aracil-Fernandez et al., 2012; Ishiguro et al., 2007). THCV acts as a partial agonist at CB2 receptors in its lower dose range (Pertwee et al., 2007), meaning its effects on alcohol drinking may include a CB2-mediated component in addition to its primary CB1 antagonist activity, a dimension that compounds acting only at CB1 do not have.

3.3. CB1 Antagonists: Preclinical Efficacy and Clinical Failure of Rimonabant

Rimonabant (SR141716A) is a selective CB1 inverse agonist (Rinaldi-Carmona et al., 1994). In animal studies, it consistently reduced voluntary alcohol intake, preference, and the alcohol deprivation effect (Colombo et al., 1998; Lallemand and De Witte, 2005). Microstructure analysis showed that rimonabant reduced bout number more than lick rate, consistent with attenuation of mesolimbic incentive salience rather than palatability (Arnone et al., 1997). Phase III trials showed efficacy for obesity and smoking cessation, but post-marketing data revealed serious psychiatric adverse effects, depression, anxiety, and suicidality, leading to European market withdrawal in 2008 and FDA rejection (Sam et al., 2011). The reason is now understood: as an inverse agonist, rimonabant reduces constitutive CB1 signalling below basal endocannabinoid tone, producing CB1 hypoactivity in mood-regulatory circuits beyond what simple competitive blockade achieves (Christopoulos, 2002). This distinction between inverse agonism and neutral antagonism, illustrated in **Figure. 3**, is mechanistically explained by Sideris et al. (2023): inverse agonists reduce constitutive CB1 activity below basal levels, whereas neutral antagonists block agonist-driven signalling without affecting baseline receptor tone. What is understood in this case is that CB1 is a valid target but the mode of action matters. Neutral antagonists and negative allosteric modulators are predicted to produce safer profiles (Pertwee, 2010). Both THCV and CBD fall into these categories.

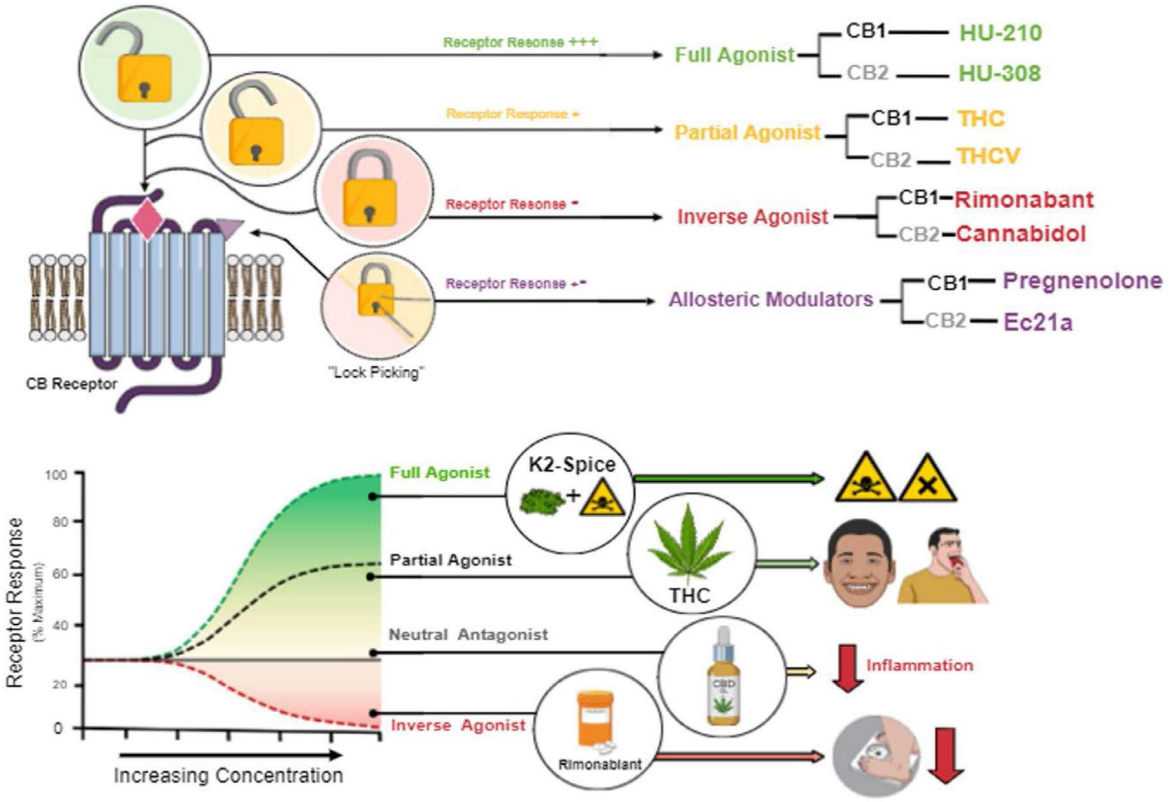


Figure. 3 Orthosteric and allosteric modes of CB1 receptor modulation. Full agonists (e.g., HU-210) produce maximal receptor response. Partial agonists (e.g., THC) produce submaximal response. Inverse agonists (e.g., rimonabant) suppress constitutive activity below baseline. Neutral antagonists (e.g., THCV) block agonist-driven signalling without reducing basal tone. Negative allosteric modulators (e.g., CBD) bind to a distinct site and attenuate agonist efficacy without directly activating or suppressing the receptor. Adapted from Sideris et al. (2023).

4. PHYTOCANNABINOIDS AS CANDIDATE COMPOUNDS

4.1.Delta-9-Tetrahydrocannabivarin (THCV) A Neutral CB1 Antagonist

Delta-9-Tetrahydrocannabivarin (THCV) is a naturally occurring phytocannabinoid found mainly in cannabis strains from equatorial Africa and Asia (Pertwee et al., 2007). Structurally, it is a propyl homologue of delta-9-THC, with a three-carbon rather than five-carbon side chain at the C-3 position (Izzo et al., 2009). At doses up to approximately 3 to 5 mg/kg in rodents, THCV acts as a neutral competitive CB1 antagonist: it occupies the orthosteric binding site and blocks agonist-driven signalling without reducing constitutive recept or activity below its basal level (Pertwee et al., 2007; Bolognini et al., 2010). At the same doses, it acts as a partial agonist at CB2 receptors. At substantially higher doses, partial CB1 agonist activity may emerge.

The critical distinction from rimonabant is that THCV does not reduce basal CB1 tone. It only blocks agonist-driven signalling. This predicts attenuation of CB1-mediated amplification of meso-

limbic dopamine release following ethanol exposure, reducing incentive salience, without the constitutive CB1 suppression in mood circuits that generated rimonabant's adverse effects (Christopoulos, 2002). Riedel et al. (2009) showed THCV reduces food intake and body weight in obese mice through a CB1-dependent mechanism without rimonabant-like anxiety. Thomas et al. (2005) demonstrated antagonism of delta-9-THC effects without intrinsic anxiogenic properties. A prior study in this laboratory confirmed that THCV reduces 24-hour voluntary alcohol intake without altering locomotor activity (Poceviciute et al., 2026), establishing the safety and selectivity profile that forms the basis for the present microstructure investigation.

4.2. Cannabidiol A Negative Allosteric Modulator of CB1

Cannabidiol (CBD) is the major non-psychoactive phytocannabinoid in Cannabis sativa L. (Mechoulam et al., 2007). It acts as a negative allosteric modulator (NAM) at both CB1 and CB2 receptors: it binds to an allosteric site distinct from the orthosteric site and reduces the binding affinity and maximal efficacy of orthosteric agonists, without itself acting as a direct agonist or inverse agonist (Laprairie et al., 2015). This mechanism is subtler and more gradual in its effects on CB1 signalling than competitive antagonism. Outside the cannabinoid system, CBD acts as an agonist at 5-HT1A serotonin receptors, antagonises GPR55, modulates TRPV1 channels, inhibits FAAH, and has anti-inflammatory effects through PPARgamma (Bisogno et al., 2001; Russo et al., 2005).

Gonzalez-Cuevas et al. (2018) found that CBD significantly reduced alcohol self-administration and relapse-like reinstatement in rats with prior heavy drinking history, with effects persisting for several weeks after treatment. Hamelink et al. (2005) documented protective effects against alcohol-induced neurodegeneration. Yang et al. (2014) reported hepatoprotective effects in binge alcohol-induced liver injury models. CBD's FDA approval for epilepsy treatment (Epidiolex) substantially reduces the translational barrier for clinical testing. Turna et al. (2019) concluded CBD is a pharmacologically credible AUD treatment candidate.

4.3. Predicted Effects in AUD Treatment

The mechanistically distinct CB1 modulation profiles of THCV, as a neutral CB1 antagonist at VTA GABAergic interneurons, is predicted to block ethanol-driven endocannabinoid mobilisation that disinhibits VTA dopamine neurons, attenuating the mesolimbic signal encoding incentive salience and driving bout initiation. The specific prediction would be reduced bout number without proportionally reduced within-bout lick rate. The decision to start drinking is suppressed; the hedonic character of the drinking that does occur is unchanged. CBD, as a CB1 NAM with additional 5-HT1A and potential opioidergic modulatory actions, is predicted to have a complementary profile; its subtler CB1 modulation may not substantially affect mesolimbic incentive signalling at the doses tested, but

its serotonergic actions could attenuate the within-bout hedonic evaluation of alcohol's sensory properties (Russo et al., 2005). The prediction for CBD is preserved or minimally affected bout number with reduced within-bout lick rate and shorter bout duration.

5. PRECLINICAL MODELS OF ALCOHOL DRINKING BEHAVIOR

5.1. Validity of Preclinical Models and the Two-Bottle Choice Paradigm

The usefulness of any animal model in addiction research depends on how well it maps onto the human condition it is meant to represent. Three complementary criteria are used to evaluate this: face validity, construct validity, and predictive validity (Belzung et al., 2011). Face validity refers to whether the model produces observable features that look like human addiction such as drinking despite negative consequences, escalation over time, or relapse after abstinence. Construct validity asks whether the underlying mechanisms driving that behaviour in the animal align with what is known about the mechanisms in humans. Predictive validity is perhaps the most practically important: does the model respond to pharmacological treatments in the same direction and proportion as humans do? No single model ticks all three boxes perfectly, and Sanchis-Segura and Spanagel (2006) were clear that what researchers usually get is a reasonable approximation of one or two criteria, with trade-offs depending on the paradigm chosen.

The two-bottle choice (2BC) paradigm gives the animal a genuine choice between water and ethanol, which is its main advantage: drinking that occurs with a freely available non-alcoholic alternative present is voluntary, and patterns of ethanol preference that emerge reflect the reinforcing properties of ethanol rather than deprivation-driven consumption (Tabakoff and Hoffman, 2000). The procedure requires no food restriction and no operant training, avoiding the motivational confounds introduced by hunger or specific apparatus demands. Individual differences in ethanol intake stabilise over weeks to months of continuous access, which is itself important: under similar exposure conditions, only a minority of outbred animals such as Wistar rats develop the kind of high, stable voluntary intake that resembles chronic heavy drinking in humans, while others remain low consumers (Crabbe et al., 2011). This spontaneous variability within a genetically heterogeneous population is not a flaw to be averaged away; it enhances face validity by mirroring the fact that most people who drink alcohol do not develop AUD. Selecting the highest-drinking animals for pharmacological testing, as done here, produces a sample whose intake patterns are stable enough to detect pharmacological reductions reliably (Simms et al., 2008). In this study, animals were given eight to ten months of two-bottle choice access before lickometer recording began, to produce a sample of well-established, chronic voluntary drinkers.

5.2. Relapse Models: The Alcohol Deprivation Effect (ADE)

The alcohol deprivation effect (ADE), first described by Sinclair and Senter (1968), is a transient but consistent increase in voluntary ethanol intake after a period of enforced abstinence, and is considered one of the more translationally credible models of relapse drinking (Spanagel and Holter, 1999). The face validity argument rests on clinical observations that alcoholics frequently experience episodes of heavy drinking upon relapse, which tend to diminish over time (Larimer et al., 1999). Crucially, pharmacotherapies effective in humans; naltrexone and acamprosate also attenuate the ADE in rodents (Spanagel and Zieglgänsberger, 1997), which is precisely the kind of predictive validity that makes a model useful for drug screening. Limitations do exist like the magnitude of the ADE varies across strains and species, with inconsistent results in mice and opposite effects reported in hamsters (Sinclair and Sheaff, 1973; Spanagel and Sanchis-Segura, 2003), which means the ADE cannot be assumed to generalise across all preclinical systems.

The present study uses continuous access rather than an ADE design, which limits conclusions about anti-relapse potential. This is a deliberate trade-off rather than an oversight. Continuous access produces the stable within-subject baseline that microstructure analysis requires; the day-to-day consistency of licking patterns under continuous access is what makes it possible to detect subtle changes in bout structure that a post-ADE spike in intake would obscure. The question being asked here is not whether THCV or CBD prevents relapse which requires a deprivation design; but whether they alter the motivational and hedonic structure of ongoing voluntary drinking in established long-term drinkers. Limitations like these are discussed in Section 8.

5.3 Incentive-Sensitization

The theoretical framework is the incentive-sensitization theory of addiction (Robinson and Berridge, 1993, 2008; Berridge and Robinson, 2016). Within this framework, compulsive alcohol use in AUD results from sensitisation of mesolimbic dopaminergic wanting systems by repeated ethanol exposure, producing a dissociation between the drive to drink and the pleasure derived from drinking. Effective pharmacotherapy for AUD could work through two separable mechanisms: reducing sensitised incentive salience, which would be reflected in reduced bout number, or attenuating the hedonic palatability sustaining drinking within episodes, which would be reflected in reduced lick rate and shorter bout duration (Davis et al., 2009; Dwyer et al., 2014). Identifying which pathway a compound engages, or whether it engages both, is the core question this study is designed to answer. THCV, as a neutral CB1 antagonist at VTA GABAergic interneurons, is theoretically positioned to reduce incentive salience. CBD, as a CB1 NAM and additionally at 5-HT1A and TRPV1 receptors, is theoretically positioned to attenuate within-bout hedonic reinforcement. In the Section 1.3 Incentive-Sensitization model was introduced. In animal models, these components are separable through

drinking microstructure analysis (Davis et al., 2009; Dwyer et al., 2014). Each time an animal approaches and begins drinking, it is making a motivational decision driven by mesolimbic incentive signals that precede any sensory contact with the fluid. The number of these decisions per session, operationalised as bout number, indexes wanting. Once drinking has started, the rate at which the animal licks, the within-bout lick rate or its inverse inter-lick interval (ILI), reflects ongoing hedonic evaluation of the sensory properties of the fluid. Lick rate is reliably sensitive to palatability: more palatable fluids produce faster, more rhythmic licking (Davis, 1996; Glendinning et al., 2002).

6. MATERIALS AND METHODS

6.1. Animals

Sixty male Wistar rats (2 months old) from the in-house breeding colony at Vilnius University (Lithuania) were used in the study. The animals were housed individually in standard polycarbonate cages under a controlled 12-hour light/dark cycle (lights on at 08:00) at a constant temperature of 22 ± 1 degrees Celsius. Rats had ad libitum access to standard laboratory chow and tap water throughout the experimental procedures. Body weight was recorded weekly. All experimental procedures were approved by the State Food and Veterinary Service of the Republic of Lithuania (permit number G2-155) and conducted in accordance with national regulations and the European Directive 2010/63/EU on the protection of animals used for scientific purposes.

6.2. Voluntary Alcohol Consumption Procedure

Following a short taste habituation phase (rats had access to water and 5% (v/v) ethanol for one week and then 8% (v/v) ethanol for another week), all 60 animals were given simultaneous access to two bottles mounted on the home cage, one containing tap water and one containing 10% ethanol (v/v), for 6 to 8 months. Ethanol solutions were prepared from 99% ethanol (Honeywell, Germany) diluted with tap water. This extended habituation period allowed stable, chronic voluntary alcohol preference to develop. Fluid intake was recorded gravimetrically three times per week. Following the habituation period, the animals with the highest mean daily ethanol intake over the final two weeks were selected for transfer to the lickometer chambers. The experimental design is summarised in the **Figure. 4.** Ethanol is metabolised hepatically via alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) following zero-order kinetics at concentrations reached during voluntary drinking (Lieber, 2004). Rats clear ethanol approximately 2–3 times faster than humans (0.3–0.4 vs. 0.1–0.15 g/kg/h; Gilpin & Koob, 2008), meaning that the 10% solution used here produces blood alcohol levels that engage mesolimbic reinforcement circuits without producing severe acute intoxication at the intake levels typical of Wistar rats in long-term drinking models (Simms et al., 2008). This pharmacokinetic profile supports the use of continuous-access two-bottle choice

paradigms to study positive reinforcement mechanisms prior to the development of dependence-related neuroadaptations.

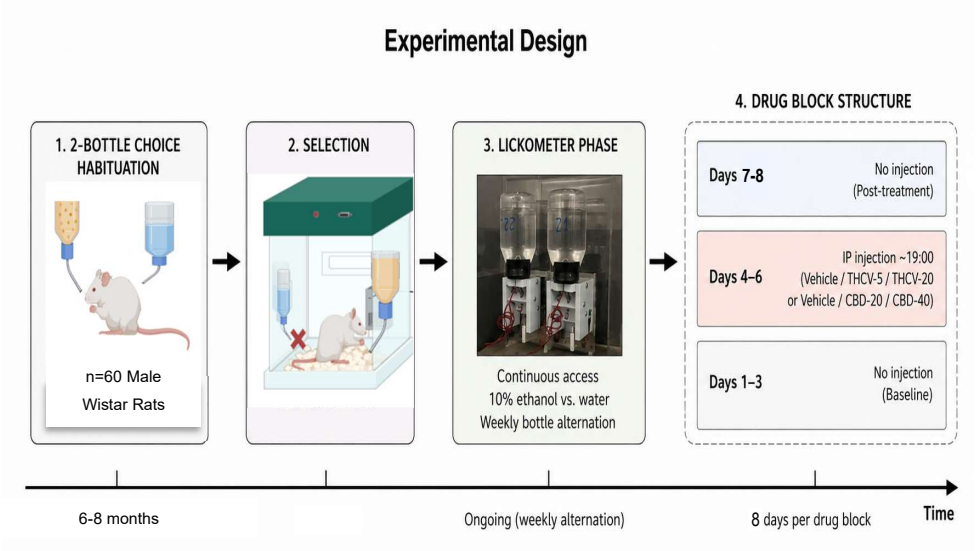


Figure. 4 Experimental design overview: 6 to 8 month two-bottle choice habituation phase, selection of the highest-drinking animals, and lickometer drug-testing phase with block structure. Separate cohorts were used for THCV and CBD experiments.

6.3. Lickometer Apparatus

Licking behaviour was assessed using transparent Plexiglas chambers (40 × 40 cm floor area, 50 cm height) equipped with ventilated lids and two drinking ports mounted on the back wall. Lickometer systems typically employ either contact-based detection, which relies on completion of a voltage-divider circuit upon tongue contact, or optical detection using an infrared beam interrupted by the tongue. The present study utilised an optical detection system (Coulbourn Instruments, Holliston, USA) to avoid signal artefacts from incidental fur or whisker contact and to eliminate variability arising from differences in tongue conductivity across animals or sessions. Each port was fitted with a sipper tube connected to an optical sensor; an infrared beam transmitted via glass rods across the tube tip was interrupted upon tongue contact and recorded using GraphicState 4.1.06 software at a resolution of up to 25 lick events per second.

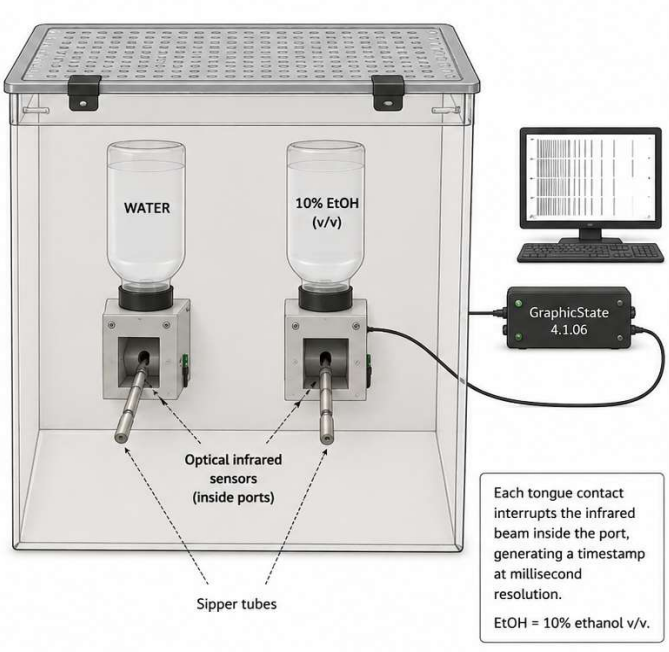


Figure. 5 Schematic of the lickometer apparatus. The Plexiglas chamber, optical infrared lick sensors, GraphicState 4.1.06 data acquisition, and custom Python bout-extraction pipeline.

This millisecond-level temporal precision is essential for inter-lick interval (ILI) analysis and palatability assessment, as lower-resolution contact-based systems would blur the rapid lick sequences that carry critical hedonic information. Given that rats are larger than mice and produce larger lick volumes per contact, chamber dimensions, sipper tube bore, and bottle volumes were specifically selected to ensure reliable detection and prevent fluid depletion during extended drinking bouts. The apparatus is illustrated schematically in the **Figure. 5.**

Within the lickometer chambers, animals received continuous access to both tap water and 10% ethanol (v/v). To prevent the development of location-based port preferences, bottle positions were alternated weekly throughout the lickometer phase. Animals were given a minimum of one week to adapt to lickometer housing before drug testing commenced.

6.4. Drinking Microstructure Analysis and Parameters

Total fluid intake, measured as mL/kg per day, tells us that a drug treatment reduced alcohol consumption, yet it does not tell you why this happens. Two animals that drink the same total volume can arrive at that number through entirely different behavioural routes: one might make fewer decisions to approach the sipper but drink a lot per visit, another might approach constantly but take only a few licks each time. These patterns reflect different underlying processes: motivational drive, hedonic palatability, within-bout satiation; and a treatment that changes one without affecting the others is doing something mechanistically specific. Drinking microstructure analysis, which decomposes raw lick timestamps into discrete bouts and their properties, is the tool that makes this dissection possible (Davis et al., 2009; Dwyer et al., 2014). The parameters used in this study are described below, along with what each one indexes and why it matters for separating THCV and CBD effects.

6.4.1. Bout Number as a Measure of Motivational Drive

Each discrete drinking bout represents a behavioural decision to approach and engage with the alcohol sipper. This decision is made before any sensory contact with the fluid and is driven by mesolimbic incentive salience signals that have accumulated since the last drinking episode (Davis et al., 2009). Bout number per session is the operational index of motivational wanting within the incentive-sensitization framework. A compound reducing mesolimbic dopaminergic incentive signalling, such as a CB1 antagonist blocking VTA GABAergic disinhibition, would be expected to reduce the frequency of these initiation decisions without necessarily changing the character of the drinking that takes place once a bout has started (Arnone et al., 1997; Dwyer et al., 2014). A reduction in bout number that is specific to alcohol, with water bout number either unaffected or increased, is the clearest microstructure signature of attenuated incentive salience for ethanol specifically.

6.4.2. Bout Size and Within-Bout Persistence

Bout size, the mean number of licks per bout, reflects how much the animal drinks once a drinking episode has started. It integrates hedonic motivation to continue drinking and sensitivity to post-ingestive satiety signals (Spector et al., 1998). Large bout sizes suggest sustained hedonic engagement, reduced sensitivity to post-ingestive feedback, or both. In the context of distinguishing THCV and CBD effects, bout size is useful in combination with lick rate and bout duration: it helps discriminate between patterns of frequent, brief, exploratory sipper contacts and patterns of fewer but more sustained and high-rate drinking episodes. A drug that reduces bout size without reducing bout number is cutting short the within-episode reward signal rather than dampening the drive to seek alcohol in the first place.

6.4.3. Bout Duration as an Integrated Measure

Bout duration, measured from the first to the last lick of a discrete drinking episode, integrates lick rate and bout size into a single temporal measure of within-bout engagement (Dwyer et al., 2014). It is useful for detecting pharmacological dissociations: a treatment that reduces bout duration while leaving bout number and lick rate intact suggests animals are terminating episodes earlier, possibly because the reward signal diminishes faster within each bout, even if the rate of licking within that shorter period is unchanged. Conversely, a drug that reduces both bout duration and lick rate is most likely acting on the palatability of the fluid where the animal is both drinking more slowly and giving up sooner.

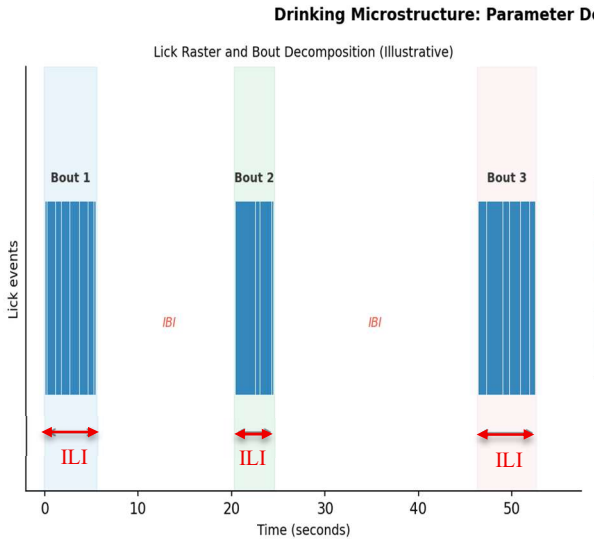
6.4.4. Lick Rate, Inter-Lick Interval, and the Distinction from Motor Effects

The rhythm of licking within an active drinking bout is governed by the biomechanics of the tongue-jaw system and by orosensory feedback from the fluid being consumed (Travers et al., 1997; Yamamoto et al., 1982). In rats the drinking preferred fluids, the modal inter-lick interval (ILI) falls between 125 and 150 milliseconds, producing a fast and regular licking pattern (Davis, 1996). When palatability decreases whether through lower sucrose concentration, sweetener substitution, or adulteration with bitter compounds like quinine; the ILI distribution shifts toward longer intervals and greater variability (Spector et al., 1998; Glendinning et al., 2002). A drug slowing within-bout lick rate without reducing bout number is therefore attenuating the hedonic evaluation of the fluid during consumption, specifically the opioidergic and GABAergic signalling in hedonic hotspots that generates the pleasurable component of drinking (Berridge et al., 2010).

Because licking in rats is a stereotyped, rhythmically constrained motor behaviour with a tightly bounded normal frequency (Spector et al., 1998; Davis, 1996), a drug producing generalised motor suppression or sedation would slow lick rate across both alcohol and water bouts indiscriminately (Davis, 1996) and would likely also reduce locomotor activity. If lick rate for water is unaffected

while alcohol lick rate changes, or if both change in opposite directions, that rules out non-specific motor suppression as an explanation (Sanchis-Segura & Spanagel, 2006; Dwyer et al., 2014). This is why tracking lick rate simultaneously for alcohol and water rather than for alcohol alone is essential for interpreting any drug effect on the microstructure level (Dwyer et al., 2014; Doyle et al., 2023). In a compound like THCV, for which locomotor data from a prior study (Poceviciute et al., 2026) already establish the absence of motor suppression at the doses tested, a change in alcohol lick rate that is absent for water would constitute strong evidence of a pharmacologically specific hedonic effect rather than a confound.

The inter-lick interval (ILI) is the within-bout complement to bout number: it points what happens between each lick during an active drinking episode rather than between episodes. The ILI distribution is typically unimodal and narrow for palatable fluids, reflecting the rhythmic, driven quality of motivated consumption. As palatability or reward value decreases, the distribution spreads and the modal ILI lengthen. Because ILI and lick rate are mathematical inverses of one another, they carry the same information. The convention in this study is to report lick rate (licks per minute) for interpretive convenience but increases in ILI and decreases in lick rate are the same finding expressed differently and are treated as equivalent throughout.



Parameter-to-Theory Mapping

Parameter	Measurement	Theoretical Construct	CB1 Target
Bout Number	N bouts/day	Motivational 'Wanting'	THCV (neutral antagonist)
Bout Size	Licks/bout	Persistence + Hedonic driv	THCV + CBD
Bout Duration	Seconds/bout	Integrated engagement	CBD (NAM)
Lick Rate (ILI)	Licks/min (ms/lick)	Hedonic 'Liking'	CBD (NAM)
Preference %	EtOH licks / total licks x100	Relative motivation	Both

Figure. 6 Drinking microstructure parameter definitions and mapping to incentive-sensitization constructs: illustrative lick raster with bout decomposition. Left panel shows illustrative lick raster with bouts separated by inter-bout intervals (IBI). Right panel maps each parameter to its incentive-sensitization construct and the compound predicted to selectively affect it.

6.4.5. Inter-Bout Interval and the Temporal Structure of a Drinking Session

The inter-bout interval (IBI) is the gap between the end of one drinking bout and the start of the next. Where bout number tells you how many drinking decisions were made in a session, IBI tells you how far apart those decisions were spaced in time. A drug that increases IBI without reducing bout number is delaying the re-emergence of the motivational signal driving approach behaviour but

not eliminating it. In practice, IBI is most useful as a confirmatory measure: when bout number decreases, IBI is expected to increase proportionally, and mismatches between the two offer clues about whether the effect is specifically on motivation or on the animal's capacity to organise its behaviour over the session.

6.4.6. Alcohol Versus Water Microstructure: The Selectivity Check

Every microstructure parameter described above is computed separately for alcohol and water licking. This simultaneous comparison is not a secondary analysis; it is essential for interpreting results. If a drug reduces alcohol bout number and also reduces water bout number, the most parsimonious explanation is a non-specific reduction in ingestive drive or motor capacity, not a selective effect on ethanol reinforcement. If it reduces alcohol bout size and increases water bout size, the animal is compensating by redirecting consummatory behaviour, which is a much stronger signal of alcohol-specific hedonic disruption. The water channel therefore functions as a within-subject, within-session control that no separate vehicle group can fully replicate, because it is measuring exactly the same motor output at exactly the same time under exactly the same pharmacological conditions. This design choice is what makes lickometer-based microstructure analysis substantially more informative than total intake measures alone (Barkley-Levenson and Crabbe, 2012).

6.5. Drugs and Administration

THCV (generously provided by Sanobiotec, Lithuania) was dissolved in Tween 80 (Sigma-Aldrich, Germany) and then diluted with 0.9% saline to a final Tween concentration of 5%, and administered intraperitoneally (i.p.) at a volume of 2 mL/kg. Three conditions were used: vehicle (5% Tween 80 in 0.9% saline), 5 mg/kg THCV (THCV-5), and 20 mg/kg THCV (THCV-20).

CBD (THC Pharm GmbH, Germany) was suspended in 0.9% saline containing 0.5% methylcellulose (Sigma-Aldrich, Germany) and administered i.p. at a volume of 3 mL/kg. Three conditions were used: vehicle (0.5% methylcellulose in 0.9% saline), 20 mg/kg CBD (CBD-20), and 40 mg/kg CBD (CBD-40).

In order to study the effect of THCV and CBD treatment on baseline alcohol and water drinking, rats were divided into groups (n=7–9 per treatment condition) so that mean alcohol and water intake were matched. Before each drug treatment, stable baseline drinking was monitored for at least three days.

All injections were given approximately one hour before lights off, coinciding with the onset of the dark phase during which rats concentrate the majority of their voluntary drinking activity.

Each drug testing block comprised eight consecutive recording days structured as three phases: three days of no-injection baseline recording (Days 1 to 3), three days of treatment with daily injec-

tions (Days 4 to 6), and two days of post-treatment recording without injections (Days 7 to 8). Lickometer recording continued continuously throughout each 24-hour period, capturing the full dark-phase drinking session.

6.6. Data Collection and Behavioural Parameters

Fluid intake was calculated daily from the difference in bottle weight at the end of each 24-hour recording period, normalised to body weight, and expressed as g of pure ethanol/kg of body weight per day, g/kg/day or ml of water /kg/day. Evaporation was minimized by use of special bottle caps.

Lick-based microstructure parameters were extracted from raw timestamp files using a custom Python script. A drinking bout was defined as a sequence of at least 20 licks with no inter-lick interval exceeding 60 seconds, following the validated criteria of Barkley-Levenson and Crabbe (2012). The following parameters were computed separately for alcohol and water licking: number of bouts per day; bout size, defined as the mean number of licks per bout; bout duration, measured in seconds from the first to the last lick of each bout; and bout lick rate, expressed as licks per minute within bouts. The microstructure parameter definitions and their mapping to incentive-sensitization theory constructs are shown in **Fig. 6**.

6.7. Statistical Analysis

All statistical analyses were performed using SigmaPlot 15 (Systat Software, Inc., San Jose, CA, USA). Data are expressed as mean ± standard error of the mean (SEM). Sample sizes were n = 8–9 per group for THCV experiments and n = 7 per group for CBD experiments, based on prior power calculations and consistent with similar microstructure studies (Barkley-Levenson & Crabbe, 2012).

Baseline differences between alcohol and water licking microstructure parameters were assessed using independent two-tailed t-tests. Effect sizes for t-tests are reported as Cohen's d.

Drug effects on gravimetric intake and each lick-based microstructure parameter (bout number, bout size, bout duration, within-bout lick rate) were analysed separately for alcohol and water using two-way repeated-measures analysis of variance (ANOVA), with treatment group (vehicle, low dose, high dose) as the between-subjects factor and day (baseline days 1–3, injection days 4–6, post-treatment days 7–8) as the within-subjects factor. Mauchly's test was used to assess sphericity; where violated, Greenhouse-Geisser corrections were applied. Partial eta-squared (ηp^2) is reported as a measure of effect size for ANOVA terms.

Significant main effects or Group × Day interactions were followed by Student Newman Keuls post-hoc comparisons to identify specific day-wise or group-wise differences.

The significance threshold for all tests was set at $p < 0.05$. No imputation was used for missing data; animals with incomplete recording sessions were excluded from analysis on a per-parameter basis (final n per analysis reported in figure legends).

7. RESULTS

7.1. Baseline Drinking Microstructure: Alcohol versus Water

All animals were selected for the pharmacological testing phase based on high baseline alcohol intake following an extended two-bottle choice consumption period. Drug effects were assessed using the lickometer apparatus, allowing simultaneous gravimetric and lick-based measurement throughout each 8-day testing blocks (three days baseline, three days injection, two days post-treatment) for microstructure analysis. Before any drug treatment, baseline microstructure was assessed across three recording days.

7.1.2. Baseline Drinking Microstructure: Alcohol versus Water

Prior to pharmacological testing, lickometer-based drinking microstructure was analysed using baseline fluid intake data. An independent two-tailed t-test revealed that the number of alcohol bouts did not differ from the water bouts [$p=0.88$]. However, alcohol bouts were significantly longer in duration [$t(21)=6.74$, $p<0.001$], larger in size [$t(21)=6.55$, $p<0.001$], and higher in within-bout lick rate [$t(21)=3.92$, $p<0.001$] than water bouts (**Fig. 7**), consistent with previously described differences in the consummatory structure of ethanol and water intake in rodents (Barkley-Levenson & Crabbe, 2012). These differences show that the apparatus was sensitive to the motivational properties of ethanol under the conditions used here and provide a basis for interpreting treatment-related changes in microstructure parameters.

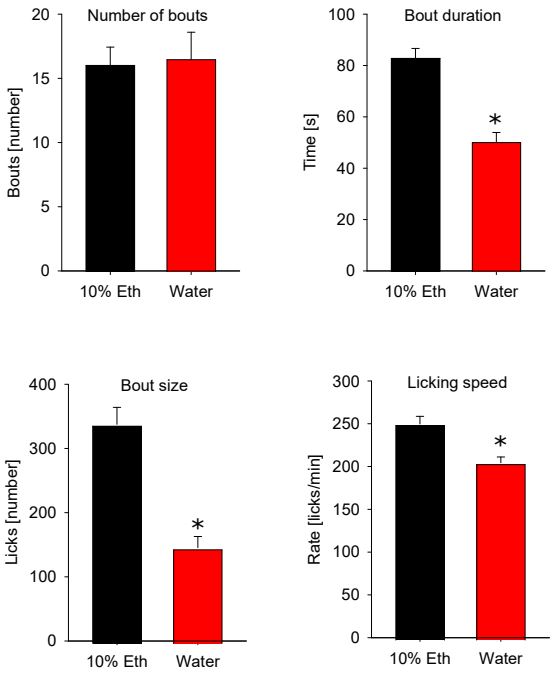


Figure. 7 Baseline ethanol vs. water bout microstructure (n = 22–25). Data are shown as mean ± SEM. * denotes significant difference from the ethanol; $p < 0.05$.

These results confirm that the selected animals had developed stable long-term alcohol preference with a clear hedonic signature in their drinking microstructure: larger, longer, and faster-licked bouts for alcohol than for water, consistent with alcohol carrying greater incentive salience and hedonic palatability in this group.

7.2. Effects of THCV on Voluntary Alcohol and Water Intake

7.2.1. Gravimetric alcohol and water consumption

Vehicle-treated animals maintained relatively stable alcohol intake throughout the testing period. Lower dose of THCV (5 mg/kg, THCV-5) produced a modest, non-significant downward trend during the injection phase. In contrast, 20 mg/kg THCV (THCV-20) produced a more pronounced suppression of alcohol consumption during treatment, with the strongest reduction observed during the third THCV administration day, followed by partial recovery during post-treatment days (**Fig. 8**). Hence, two-way repeated-measures ANOVA revealed a significant main effect of Day $F(7,154) = 8.00$, $p<0.001$ and a significant Group × Day interaction $F(14,154) = 3.89$, $p<0.001$, while the main effect of Group was not significant $F(2,22) = 1.12$, $p=0.345$. Post hoc comparisons demonstrated significant within-group reductions relative to baseline in the THCV-20 group, as well as a significant difference between THCV-20 and vehicle-treated animals on the 3rd injection day ($p = 0.034$). Water consumption tended to increase but was not significantly affected by THCV treatment (Group × Day interaction: $p = 0.054$).

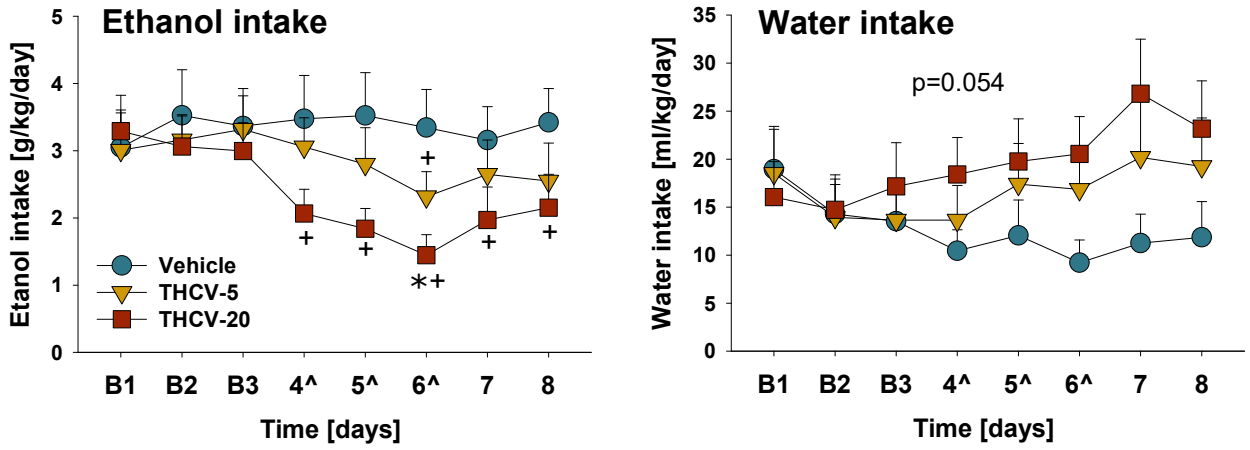


Figure. 8 Effect of THCV (5 mg/kg and 20 mg/kg, i.p.) on 24-hour gravimetric ethanol (g/kg/day; left) and water (ml/kg/day; right) intake across baseline (B1–B3), injection (4^–6^), and post-treatment (7–8) days. n = 8–9 per group. Data are shown as mean ± SEM. + denotes significant difference from group's own baseline; * denotes significant difference from the vehicle group; $p < 0.05$.

7.2.2. Lick-based microstructure of alcohol and water drinking

Analysis of the number of alcohol bouts per day revealed that treatment had no significant effect on how many times animals initiated drinking [Group effect $F(2,22) = 0.22$, $p = 0.803$ and Group × Days interaction $F(16,176) = 0.96$, $p = 0.498$], and only a marginal main effect of Days [$F(8,176) = 2.23$, $p < 0.05$]. All three groups showed comparable bout counts across all phases (vehicle baseline: 16.8 ± 2.5 bouts/day; injection phase: 15.2–16.7 bouts/day; THCV-20 baseline: 15.3 ± 2.9; injection: 11.8–13.6 bouts/day), with the modest numerical decline in the high-dose group not reaching significance. Water bout counts were likewise unaffected [Group × Days interaction $p = 0.64$] (**Fig. 9**).

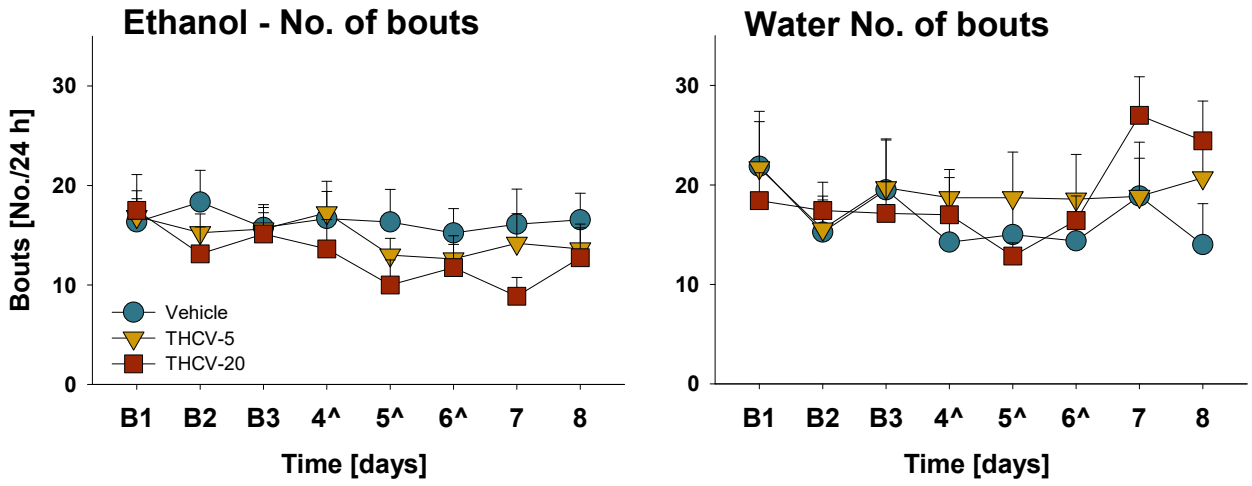


Figure. 9 Effect of THCV (5 mg/kg and 20 mg/kg, i.p.) on the number of ethanol (left) and water (right) drinking bouts per 24-hour period during baseline (B1–B3), injection (4^–6^), and post-treatment (7–8) days. n = 8–9 per group. Data are shown as mean ± SEM.

Bout duration (s) of alcohol drinking was not significantly affected by either dose of THCV (Group × Days interaction $p = 0.451$; **Fig.10**, left). A significant effect was detected for water bout duration [Group effect $F(2,109) = 9.28$, $p < 0.01$; **Fig.10**, right), with post-hoc analysis revealing a significant increase in both THCV treated groups, which did not persist across post-treatment days.

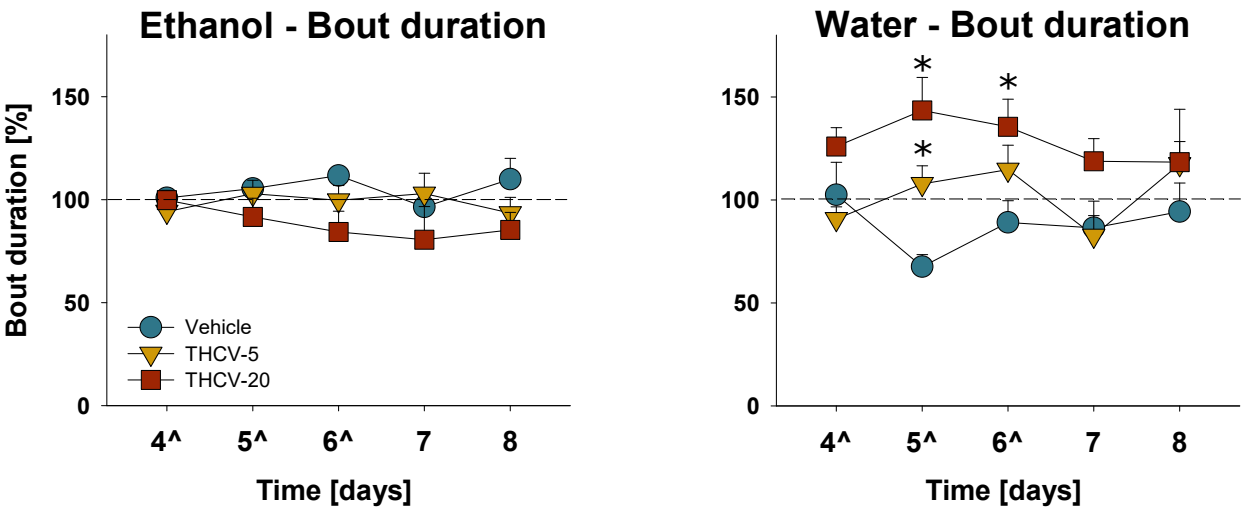


Figure. 10 Effect of THCV (5 mg/kg and 20 mg/kg, i.p.) on ethanol (left) and water (right) bout duration during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 8–9 per group. Dashed line at 100% represents bout duration baseline. Data are shown as mean ± SEM. * denotes significant difference from the vehicle group; $p < 0.05$.

Alcohol bout size (i.e., the number licks per bout) was significantly reduced by THCV treatment [Group effect $F(2,124) = 3.5$, $p < 0.05$] (**Fig.11**, left). Simultaneously, water bout size was significantly increased during treatment days [Group effect $F(2,109) = 4.02$, $p < 0.05$] (**Fig.11, right**). Post hoc analysis showed that this effect was significant only for THCV-20 group (**Fig.11**).

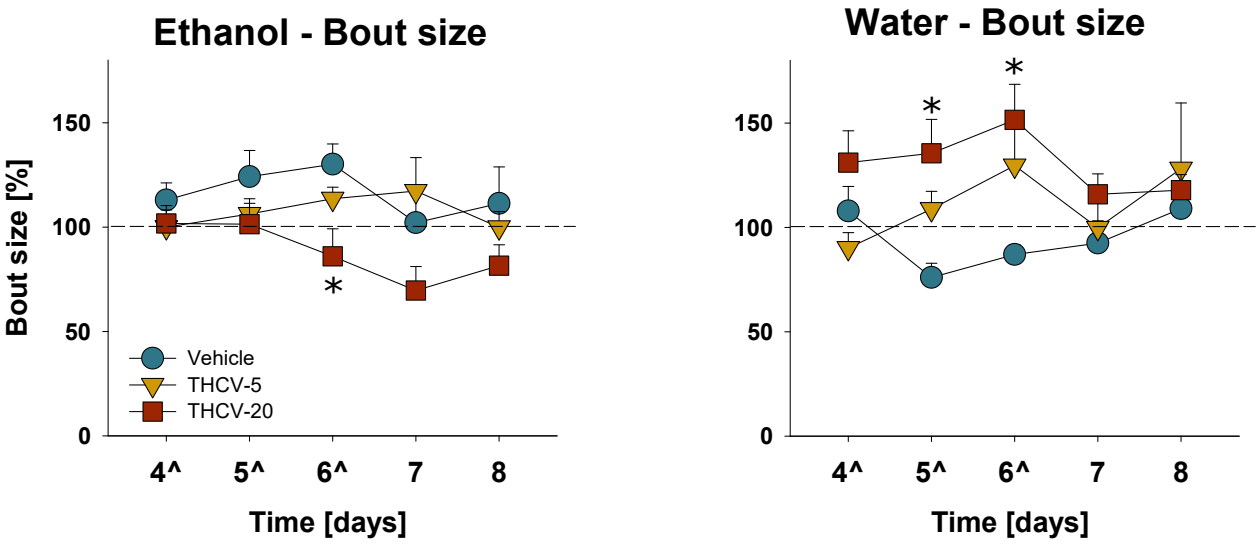


Figure. 11 Effect of THCV (5 mg/kg and 20 mg/kg, i.p.) on ethanol (left) and water (right) bout size (licks per bout) during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 8–9 per group. Dashed line at 100% represents baseline. Data are shown as mean ± SEM. * denotes significant difference from the vehicle group; $p < 0.05$.

Alcohol lick rate within the bout was not significantly affected by THCV at either dose [Group effect p = 0.5] (**Fig.12, left**). Water lick rate was also not significantly affected by THCV treatement [p = 0.96] (**Fig.12**, **right**).

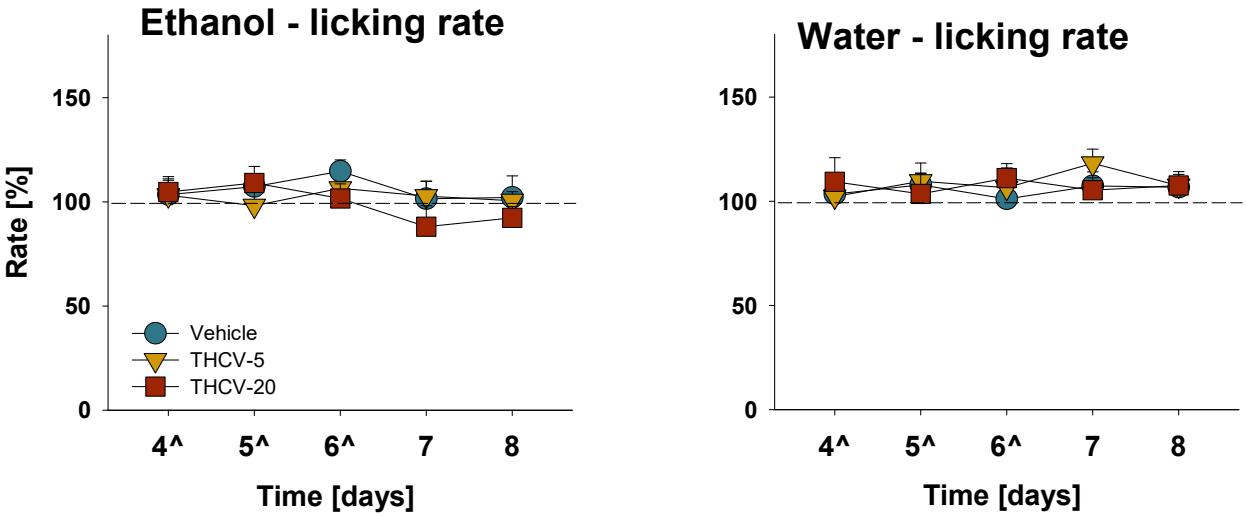


Figure. 12 Effect of THCV (5 mg/kg and 20 mg/kg, i.p.) on ethanol (left) and water (right) within-bout lick rate (licks/min) during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 8–9 per group. Dashed line at 100% represents baseline. Data are shown as mean ± SEM.

7.3. Effects of CBD on Voluntary Alcohol and Water Intake

7.3.1. Gravimetric alcohol and water consumption

Vehicle-treated animals in the CBD experiment showed stable daily alcohol consumption across all phases (baseline B1–B3: 2.38 ± 0.34, 2.54 ± 0.15, 2.66 ± 0.25 g/kg/day; injection days: 2.30 ± 0.26, 2.08 ± 0.29, 2.22 ± 0.19 g/kg/day; post-treatment: 2.23 ± 0.28, 2.44 ± 0.29, 2.43 ± 0.18 g/kg/day. Both CBD-treated groups showed pronounced within-group reductions in ethanol intake during the injection phase relative to baseline values. CBD 20 mg/kg animals showed injection-phase intakes of 1.62 ± 0.40, 1.19 ± 0.36, and 1.60 ± 0.36 g/kg/day, compared to their baseline mean of ~3.00 ± 0.36 g/kg/day. CBD 40 mg/kg animals showed a comparable pattern (injection phase: 1.82 ± 0.37, 1.11 ± 0.25, 1.34 ± 0.23 g/kg/day; baseline mean ~2.80 ± 0.37 g/kg/day), with both groups recovering toward pre-treatment levels in the post-treatment phase.

Two-way repeated-measures ANOVA of gravimetric alcohol intake revealed a significant main effect of Day [$F(7,126) = 18.03$, $p < 0.001$] and a significant Group × Day interaction [$F(14,126) = 2.05$, $p < 0.05$], indicating differential temporal patterns of ethanol consumption across treatment groups. The main effect of Group was not significant [$F(2,18) = 0.37$, $p = 0.694$]. Post-hoc analyses showed that both CBD-treated groups exhibited significant reductions in ethanol intake during the

injection phase relative to baseline values, with consumption recovering toward baseline during the post-treatment phase. Water intake was not significantly affected by CBD treatment, with no significant Group × Day interaction observed (p = 0.762; **Fig.13, right**).

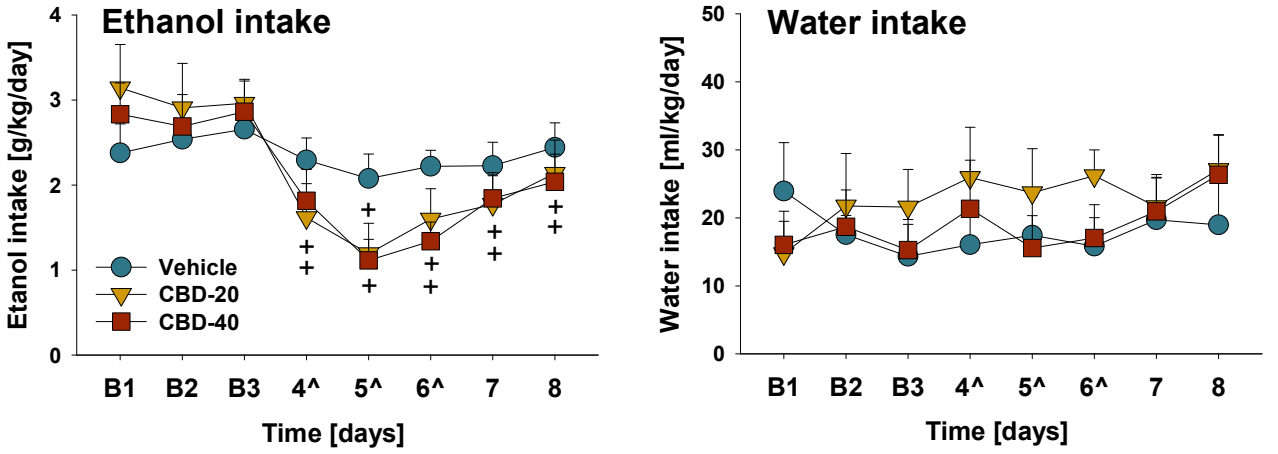


Figure. 13 Effect of CBD (20 and 40 mg/kg, i.p.) on 24-hour gravimetric ethanol intake (g/kg/day; left) and water intake (ml/kg/day; right) across baseline (B1–B3), injection (days 4–6), and post-treatment (days 7–8) phases. n = 7 per group. Data are shown as mean ± SEM. + denotes significant difference from group's own baseline; $p < 0.05$.

7.3.2. Lick-based microstructure of alcohol and water drinking

The number of alcohol bouts per day showed a pronounced numerical reduction during the injection phase in both CBD-treated groups: CBD 20 mg/kg fell from a baseline mean of 15.0 ± 3.1 to 6.0–7.4 bouts/day during injection, and CBD 40 mg/kg fell from 14.5 ± 1.0 to 5.6–9.6 bouts/day, compared to the stable vehicle group (11.5–13.1 bouts/day). However, these changes did not reach statistical significance due to high between-subject variability [Group effect: $F(2,18) = 1.95$, $p = 0.172$; Group × Day interaction: $F(14,126) = 0.91$, $p = 0.547$]. The Day main effect was significant [$F(7,126) = 18.04$, $p < 0.001$], reflecting within-group temporal variation common to all groups. A similar pattern was seen for water bouts (**Fig.14, right**), with no significant group-related effects.

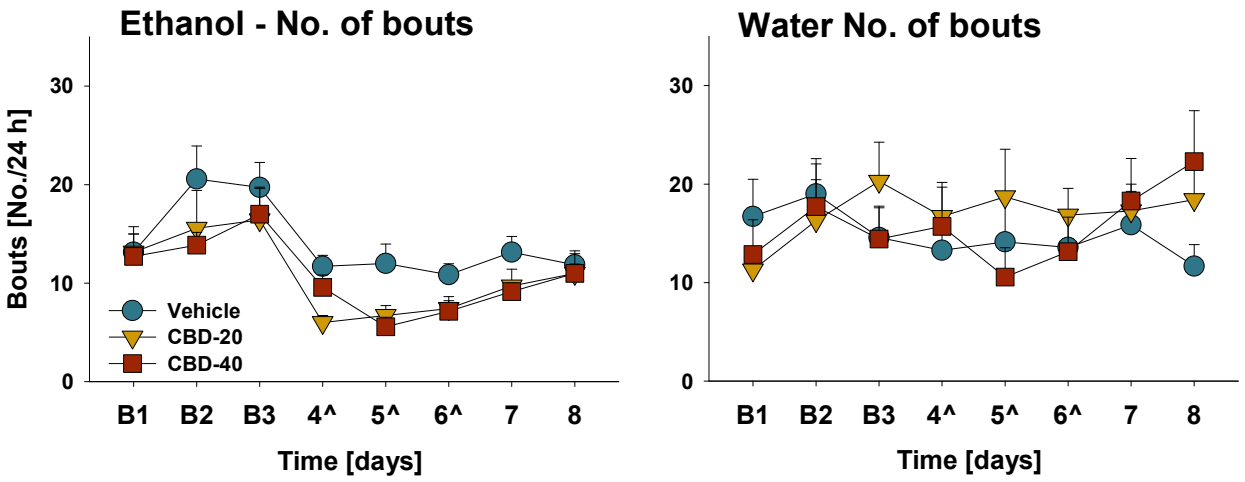


Figure. 14 Effect of CBD (20 and 40 mg/kg, i.p.) on the number of ethanol (left) and water (right) drinking bouts per 24-hour period during baseline (B1–B3), injection (days 4–6), and post-treatment (days 7–8) phases. n = 7 per group. Data are shown as mean ± SEM.

Bout duration, expressed as a percentage of each animal's own pre-treatment baseline, was stable across all three groups for both ethanol and water throughout the injection and post-treatment phases, with no significant Group × Day interaction (ethanol: p = 0.200; water: p = 0.592; **Fig.15**). Individual group means remained close to the 100% reference line across all injection and post-treatment days, indicating that the structural length of individual drinking episodes was not disrupted by CBD at either dose.

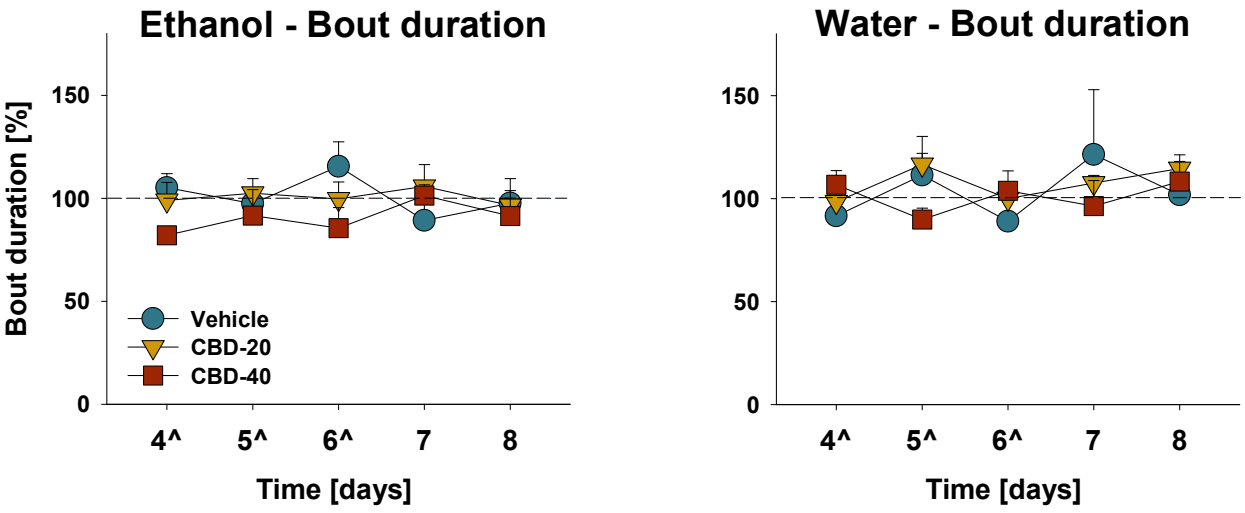


Figure. 15 Effect of CBD (20 and 40 mg/kg, i.p.) on ethanol (left) and water (right) bout duration during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 7 per group. Dashed line at 100% represents baseline. Data are shown as mean ± SEM.

Bout size (licks per bout), similarly expressed as a percentage of baseline, showed no significant Group × Day interaction for ethanol (p = 0.898) or water (p = 0.916; **Fig.16**). All groups maintained bout sizes close to their respective pre-treatment baseline throughout the injection and post-

treatment phases, confirming that the size of individual drinking episodes was unaffected by either CBD dose.

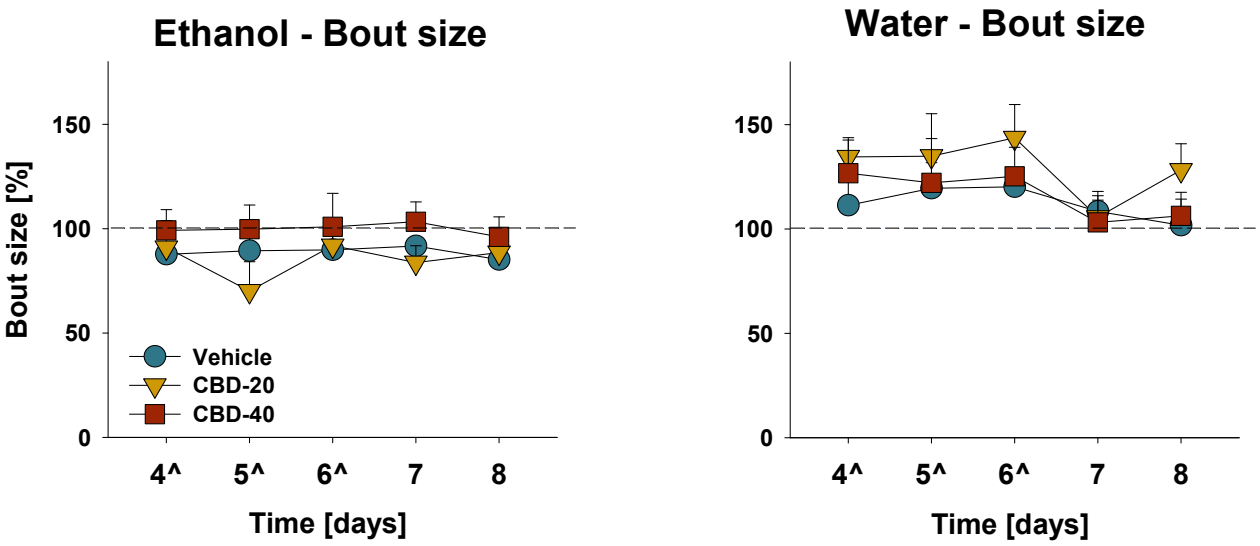


Figure. 16 Effect of CBD (20 and 40 mg/kg, i.p.) on ethanol (left) and water (right) bout size during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 7 per group. Dashed line at 100% represents baseline. Data are shown as mean ± SEM.

Bout lick rate showed a significant Group × Day interaction for both ethanol [$F(8, 72) = 2.35$, $p < 0.05$] and water [$F(8, 72) = 2.33$, $p < 0.05$] drinking (**Fig.17**). For ethanol, this was driven by a transient reduction in the vehicle group on post-treatment day 7 (**Fig.17 left**); the interaction therefore likely reflects between-group variability in post-treatment rebound rather than a direct drug effect on consummatory patterning. For water, the interaction was driven by transiently elevated lick rates in CBD-treated animals on the last two treatment days (**Fig.17 right**).

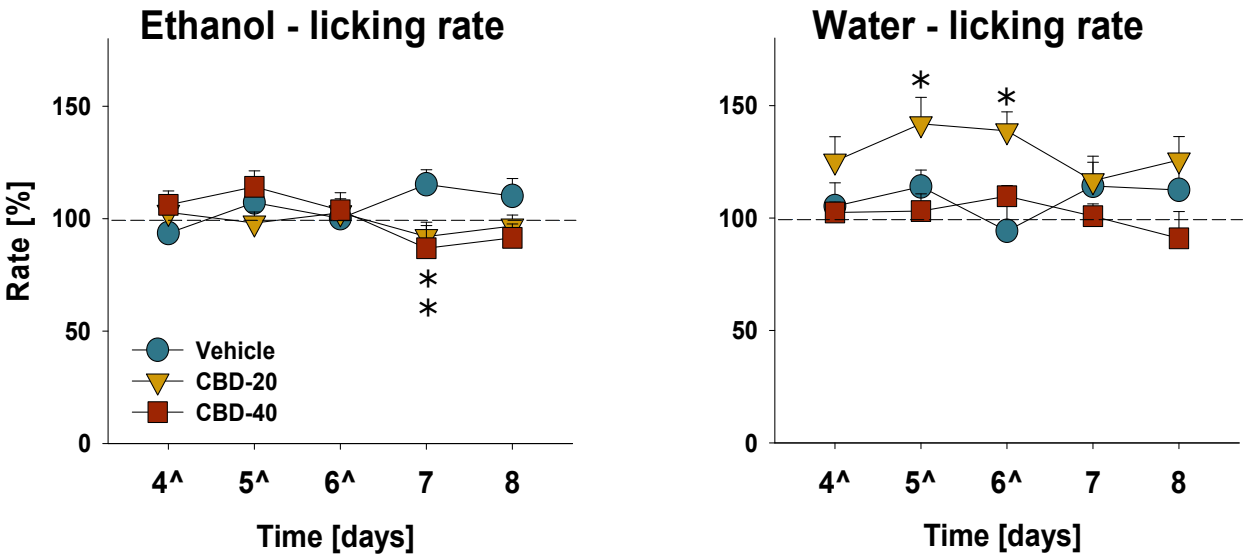


Figure. 17 Effect of CBD (20 and 40 mg/kg, i.p.) on ethanol (left) and water (right) within-bout lick rate (licks/min) during injection (4^–6^) and post-treatment (7–8) days, expressed as percentage over each animal's pre-treatment baseline. n = 7 per group. Dashed line at 100% represents baseline. Data are shown as mean ± SEM. * denotes significant difference from the vehicle group; $p < 0.05$.

7.4. Summary of Results

Across both experiments, acute intraperitoneal administration of 5-20 mg/kg of THCV and 20–40 mg/kg of CBD produced transient reductions in voluntary ethanol intake during the three-day injection phase, reflected in significant Group × Day interactions in repeated-measures ANOVA. These reductions were not accompanied by significant changes in water intake, indicating a degree of selectivity for alcohol-directed consumption. In contrast, lick-based microstructure parameters: number of bouts, bout size, bout duration, and within-bout lick rate did not show consistent, statistically significant treatment-related changes for ethanol drinking in either experiment, though pronounced numerical trends in bout frequency and lick rate were apparent in CBD-treated animals.

8. DISCUSSION

The present study investigated the effects of two phytocannabinoids, THCV and CBD, on voluntary alcohol consumption and its behavioural microstructure in male Wistar rats with a history of long-term ethanol access. The main findings are: (1) both 5-20 mg/kg THCV and 20–40 mg/kg CBD acutely suppressed voluntary ethanol intake during a three-day injection period without significantly affecting water intake; (2) these gravimetric reductions were accompanied by statistically significant changes in several lick-based microstructure parameter for ethanol and water; THCV reduced ethanol bout size and increased water bout duration and size, and CBD reduced ethanol licking rate

and increased water licking rate; and (3) these effects were transient, with changes in drinking microstructure returning toward pre-treatment levels within two post-treatment days. These findings are discussed below in relation to existing pharmacological and mechanistic literature.

8.1. THCV and CBD Reduce Voluntary Ethanol Intake in Rats with Chronic Alcohol Exposure

The observation that THCV significantly reduced ethanol intake during acute administration is consistent with evidence for CB1 receptor antagonism as a mechanism of alcohol suppression. THCV acts as a neutral CB1 antagonist at low-to-moderate doses and as a partial CB1 agonist at higher doses (Pertwee, 2008; Englund et al., 2016). Given that chronic alcohol consumption upregulates endocannabinoid signalling and increases CB1 receptor sensitivity in limbic circuits (Basavarajappa & Hungund, 2002), pharmacological blockade of CB1 receptors would be expected to attenuate the reinforcing properties of alcohol. This mechanism is supported by the large literature on rimonabant which is a selective CB1 inverse agonist that robustly reduces alcohol self-administration across multiple rodent models (Arnone et al., 1997; Cippitelli et al., 2005). THCV's advantage over rimonabant lies in its neutral antagonist profile, which does not suppress the constitutive activity of CB1 and may not induce adverse psychiatric effects that ultimately led to the withdrawal of rimonabant from clinical development (Cahill & Ussher, 2007). The present data provide behavioural evidence consistent with this profile in the context of voluntary drinking.

The modest effect of 5 mg/kg of THCV treatment on ethanol consumption is consistent with a dose-dependent mechanism. This dose-effect relationship has been described for THCV in models of appetite and feeding (Riedel et al., 2009; Wargent et al., 2013), where lower doses were ineffective and doses in the 3–20 mg/kg range suppressed food intake. Whether the effective dose range in alcohol-related behaviour mirrors the feeding literature remains to be systematically characterised in a full dose-response study.

The finding that 20 and 40 mg/kg of CBD similarly reduced ethanol intake during acute injection days extends a modest but growing body of rodent literature on CBD and alcohol use. Liput et al. (2013) reported that transdermal CBD reduced ethanol intake and preference in the two-bottle choice paradigm in mice, and Viudez-Martínez et al. (2018) demonstrated that oral CBD (60 mg/kg) reduced voluntary ethanol consumption and motivation for alcohol in rats. The mechanism underlying CBD's effects on alcohol consumption is less well-defined than that of THCV. Since CBD is a low-affinity CB1 negative allosteric modulator (Laprairie et al., 2015) and exerts actions at TRPV1, GPR55, and 5-HT1A receptors (Russo et al., 2005; Ryberg et al., 2007), all of which are implicated in reward processing and stress-related alcohol motivation. Additionally, CBD has been shown to

reduce alcohol-induced neurodegeneration (Hamelink et al., 2005) and attenuate cue-induced reinstatement (Ren et al., 2009), which may partially account for its effects on consumption under chronic access conditions. The similar magnitude of suppression at 20 and 40 mg/kg in the current study suggests a possible ceiling effect in this dose range, though definitive conclusions require a broader dose-response investigation.

Importantly, both compounds produced selective reductions in ethanol intake without significantly affecting water consumption, and neither drug suppressed voluntary drinking in the vehicle-injected control animals. This argues against a non-specific reduction in motor activity or general fluid intake as the primary explanation and is consistent with selective modulation of ethanol-directed motivation. However, alternative explanations involving mild non-specific effects cannot be completely ruled out given the modest magnitude of the effect and the absence of direct locomotor measurement.

8.2. Microstructure Changes: Implications for Mechanism

Within the framework of the incentive-sensitization model of motivation (Berridge & Robinson, 1998; Baldo & Kelley, 2007), drinking microstructure parameters are proposed to map onto distinct motivational constructs: bout number reflects the motivation to initiate drinking (incentive salience or 'wanting'), while bout size and within-bout lick rate reflect consummatory processes and the hedonic experience ('liking') of the fluid (Davis et al., 2007; Doyle et al., 2023). The dissociation observed here; gravimetric reduction without microstructure change therefore carries mechanistic implications.

One interpretation is that THCV reduced the total amount consumed per session primarily through a reduction in the bout duration. The numerical trends in bout number for CBD-treated groups were of a magnitude consistent with the gravimetric changes, yet high between-subject variability prevented these from reaching statistical significance. However, CBD significantly reduced ethanol licking rate and increased water licking rate. These data suggest that both drugs would primarily modulate the hedonic value of the fluid once a bout has been initiated. Effect on hedonic processing once a bout begins is consistent with evidence that CB1 signalling in the nucleus accumbens regulates initiation of reward-seeking (Mahler et al., 2007; Soria-Gómez et al., 2014). This distinction has practical importance: if cannabinoid-based treatments selectively reduce rewarding quality of the alcohol, they may be more compatible with harm reduction frameworks.

8.3. Transient Nature of Drug Effects and Return to Baseline

Both THCV and CBD effects on ethanol intake were transient, with consumption returning toward pre-treatment baseline within two to three days of the final injection. This time-course is consistent with acute pharmacological actions rather than any persistent neuroadaptation or conditioned suppression induced by treatment. The transient nature of the effect is not unique to cannabinoid compounds: similar rebounds have been reported for acute administration of naltrexone and nalmefene in two-bottle choice paradigms (Hyytia & Sinclair, 1993; Panocka et al., 1993), and for acute CB1 antagonism with rimonabant (Cippitelli et al., 2005). It suggests that repeated daily dosing may be required to maintain clinically meaningful reductions in consumption, and that tolerance to the intake-suppressing effect which has been reported for chronic CB1 antagonist treatment in feeding models (ref) should be investigated in future studies with extended dosing protocols.

The rapid return to baseline could also be interpreted as the absence of any conditioned aversion to the ethanol bottle following treatment, which would have been evidenced by a more prolonged suppression. This is relevant because one concern with any treatment that reduces drinking acutely is whether it may create conditioned taste aversion, which would confound the interpretation of suppressed intake as a motivational effect. The clean return to baseline within the post-treatment phase argues against a strong aversive conditioning component, and is consistent with the reported neutral taste aversion profile of CBD in animal studies (Parker et al., 2002; Parker, 2014)

8.4. Methodological Considerations and Limitations

Several methodological factors warrant consideration when interpreting the present findings. First, all animals were male Wistar rats, and the growing literature on sex differences in alcohol use disorder and in endocannabinoid system function (Fattore et al., 2010; Cooper & Craft, 2018) suggests that findings may not generalise to females without further investigation. Second, the use of intraperitoneal injection as the route of administration introduces a stress component associated with handling and injection, which may itself influence alcohol motivation on injection days; however, the vehicle group, which received the same handling and injection procedure, did not show changes in intake, mitigating this concern. Third, the sample size of n = 7–9 per group, while appropriate for initial characterisation, limits the statistical power for microstructure analyses where within-subject variability is high. Fourth, given that injections were administered approximately one hour before lights-off coinciding with onset of the dark phase, the pharmacological window of both THCV and CBD (estimated half-lives of 1–4 hours for i.p. administration in rodents; Deiana et al., 2012; Patricio-Martínez et al., 2019) may be concentrated in the early part of the dark-phase drinking session, potentially underestimating the full drug effect if intake is distributed across the full 24-hour period.

Finally, it should be noted that the two compounds were tested on separate cohorts of rats with different baseline intake levels. Vehicle animals in the THCV experiment showed higher mean alcohol intakes (~3.0–3.5 g/kg/day) than vehicle animals in the CBD experiment (~2.3–2.7 g/kg/day). While these represent typical outbred Wistar rat consumption levels (Vengeliene et al., 2014), the different baselines complicate direct quantitative comparisons between THCV and CBD efficacy, and future within-cohort crossover designs would be informative.

8.5. Future Directions

The present data demonstrate that both THCV (5-20 mg/kg) and CBD (20–40 mg/kg) acutely and selectively suppress voluntary ethanol intake in rats with chronic alcohol exposure. These results support the potential of phytocannabinoid-based compounds as pharmacological tools for investigating the neurobiological regulation of alcohol motivation and contribute to the growing preclinical evidence base for cannabinoid-directed pharmacotherapy in alcohol use disorder.

9. CONCLUSIONS

1. Using the long-term two-bottle choice paradigm male Wistar rats established stable, high voluntary ethanol intake. Baseline microstructure validated the model: ethanol bouts were significantly larger, longer, and faster-licked than water, while bout number did not differ, confirming ethanol's hedonic salience without increased drinking initiation frequency.

2. THCV (5–20 mg/kg) treatment significantly reduced total ethanol intake in a dose-dependent manner without significantly affecting water consumption. Alcohol bout number remained unchanged, ruling out pure incentive-salience suppression. Instead, THCV treatment reduced alcohol bout size alongside increased water bout size, suggesting selective attenuation of alcohol hedonic reinforcement.

3. Similarly to THCV, CBD (20–40 mg/kg) reduced daily ethanol consumption. In contrast, CBD-treated animals did not exhibit a pronounced reduction in alcohol bout duration or size but reduced alcohol licking rate and increased water licking rate, indicating a potential reduction in hedonic value of alcohol.

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Summary in English

THE EFFECT OF TETRAHYDROCANNABIVARIN (THCV) AND CANNABIDIOL (CBD) ON VOLUNTARILY ALCOHOL DRINKING IN MALE RATS

Ceyda Türkcan, Master's Thesis, 2026

Faculty of Life Sciences, Vilnius University, Supervisor: Dr. Valentina Vengelienė

Alcohol Use Disorder (AUD) is defined by compulsive drinking and loss of control over consumption, affecting approximately 280 million people globally. Current pharmacological treatments provide only modest benefit. The endocannabinoid system, particularly CB1 receptors, modulates both the motivational and hedonic components of alcohol reinforcement. Two phytocannabinoids were evaluated: delta-9-tetrahydrocannabivarin (THCV), a neutral CB1 antagonist, and cannabidiol (CBD), a negative allosteric modulator of CB1.

Sixty male Wistar rats (2-months-old) were given 6-8 months of continuous two-bottle choice access to water and 10% ethanol. The highest-drinking animals were transferred to optical lickometer chambers. Drinking microstructure (bout number, size, duration and lick rate) was analysed separately for alcohol and water using a custom Python script. A bout was defined as at least 20 licks with inter-lick intervals below 60 seconds. Each drug block comprised three baseline days, three treatment days, and two post-treatment days. THCV (vehicle 5% Tween 80, 5 mg/kg, 20 mg/kg) and CBD (vehicle 0.5% methylcellulose, 20 mg/kg, 40 mg/kg) were tested in separate blocks.

Baseline microstructure confirmed that ethanol bout size, duration, and lick rate were significantly higher than water. Administration of THCV (5-20 mg/kg) significantly reduced total 24-hour ethanol intake in a dose-dependent manner without altering water consumption. Microstructure analysis revealed that this reduction was not accompanied by a significant change in alcohol bout number, indicating that the motivational drive to initiate drinking was preserved. Instead, THCV trended toward reduced bout duration and significantly reduced bout size alongside increased water bout size and duration, suggesting a selective attenuation of within-bout hedonic reinforcement. CBD (20–40 mg/kg) similarly reduced daily ethanol consumption. However, in contrast to THCV, CBD-treated animals did not exhibit a pronounced reduction in alcohol bout duration or size but reduced ethanol licking rate. These divergent microstructural trends suggest that THCV and CBD may engage dissociable mechanisms in the regulation of voluntary alcohol consumption, supporting their further investigation as candidate pharmacotherapies for AUD.

Keywords: Alcohol Use Disorder, tetrahydrocannabivarin, cannabidiol, drinking microstructure, incentive-sensitization, CB1 receptor, lickometer.

Santrauka

TETRAHIDROKANABIVAREĮNO (THCV) IR KANABIDIOLIO (CBD) POVEIKIS SAVANORISKAI ALKOHOLIO VARTOJIMUI PATINAMS ŽIURKĖMS

Ceyda Türkcan, Magistro baigiamasis darbas, 2026

Gyvybės mokslų centras, Vilniaus universitetas, Vadovas: Dr. Valentina Vengelienė

Alkoholio vartojimo sutrikimas (AVS) apibrėžiamas kaip gėrimo elgesio kontrolės praradimas, paveikiantis apie 280 milijonų žmonių visame pasaulyje. Deja, esami AVS gydymui skirti vaistai padeda tik mažai daliai pacientų. Endokanabinoidinė sistema, ypač CB1 receptoriai, moduliuoja tiek motyvacinį elgesį, tiek hedonines alkoholio savybes. Šiame darbe buvo įvertinti du fitokanabinoidai: delta-9-tetrahidrokanabivarinas (THCV) – neutralus CB1 antagonistas, ir kanabidiolis (CBD) – neigiamas alosterinis CB1 moduliatorius.

Šešiasdešimt Wistar žiurkių patinų (2 mėnesių amžiaus) gavo 6-8 mėnesių nuolatinę prieigą prie dviejų butelių – vandens ir 10 % etanolio. Daugiausia geriantys gyvūnai buvo perkelti į automatines gėrimo registravimo kameras. Gėrimo mikrostruktūra (gėrimo epizodų skaičius, epizodo dydis, trukmė, lyžtelėjimų dažnis) buvo ištirta atskirai alkoholiui ir vandeniui naudojant tam sukurtą Python kodą. Epizodas buvo apibrėžtas kaip mažiausiai 20 lyžtelėjimų, tarpai tarp atskirų lyžtelėjimų mažiau negu 60 sekundžių. THCV ir CBD gydimas buvo trys bazinių matavimų dienos, trys gydymo dienos ir dvi dienos po gydymo. THCV (kontrolinis tirpalas 5 % Tween 80, 5 mg/kg, 20 mg/kg) ir CBD (kontrolinis tirpalas 0,5 % metilceliuliozė, 20 mg/kg, 40 mg/kg) buvo tiriami atskiruose blokuose.

Bazinio gėrimo mikrostruktūros analizė parodė, kad alkoholio epizodo dydis, trukmė ir lyžtelėjimų dažnis buvo reikšmingai didesni nei vandens. THCV gydymas (5-20 mg/kg) reikšmingai sumažino bendrą 24 valandų etanolio suvartojimą, nekeičiant vandens vartojimo. Mikrostruktūros analizė atskleidė, kad šis sumažėjimas nebuvo lydimas reikšmingo alkoholio epizodų skaičiaus pokyčio, o tai rodo, kad motyvacinė paskata pradėti gėrimą išliko. Vietoj to, THCV rodė tendenciją mažinti priėjimo dydį ir reikšmingai sumažino epizodo trukmę, tuo pat metu padidino vandens epizodo dydį ir trukmę, o tai leidžia manyti apie selektyvų alkoholio hedoninės vertės slopinimą. CBD (20–40 mg/kg) taip pat sumažino 24 val. etanolio vartojimą. Tačiau, skirtingai nei THCV, CBD grupės gyvūnams nebuvo stebimas ryškus alkoholio epizodų trukmės sumažėjimas, bet reikšmingai sumažėjo lyžtelėjimų dažnis. Šios skirtingos mikrostruktūrinės tendencijos leidžia manyti, kad THCV ir CBD gali veikti skirtingais mechanizmais reguliuojant savanorišką alkoholio vartojimą, pagrindžiant jų tolesnį tyrimą kaip kandidatų AVS farmakoterapijų.

Raktiniai žodžiai: alkoholio vartojimo sutrikimas, tetrahidrokanabivarin, kanabidiolas, gėrimo mikrostruktūra, paskatų sensibilizacija, CB1 receptorius, lickometras.