GUT-BRAIN PEPTIDES AND DRUGS OF ABUSE:

Highlighting the role of GLP-1 and amylin

Cajsa Aranäs

Department of Pharmacology
Institute of Neuroscience and Physiology
Sahlgrenska Academy, University of Gothenburg

UNIVERSITY OF GOTHENBURG
Gothenburg 2025

Cover illustration: Maria Nyman

cajsa.aranas@gu.se

ISBN 978-91-8115-095-7 (PRINT)
ISBN 978-91-8115-094-0 (PDF)

Printed in Borås, Sweden 2025
Printed by Stema Specialtryck AB

The more knowledge you get, the more questions you ask
- George St-Pierre

GUT-BRAIN PEPTIDES AND DRUGS OF ABUSE:

Highlighting the role of GLP-1 and amylin

Cajsa Aranäs

Department of Pharmacology
Institute of Neuroscience and Physiology
Sahlgrenska Academy, University of Gothenburg
Gothenburg, Sweden

ABSTRACT

Alcohol use disorder (AUD) and cocaine use disorder (CUD) are complex conditions with diverse neurobiological foundations. Current treatments for AUD and CUD are suboptimal; gaining a deeper understanding of the underlying mechanisms could aid in identifying more effective treatment options. While various factors play a role in AUD and CUD progression, the rewarding effect of the drug of abuse, primarily driven by increased dopamine levels in the nucleus accumbens (NAc), remains a central mechanism. Previous studies have demonstrated that targeting gut-brain peptides, such as glucagon-like peptide-1 receptor (GLP-1R) and the amylin receptor (AMYR), decreases the intake of alcohol and cocaine through modulation of the reward circuitry. However, there are still unexplored opportunities to enhance the efficacy of potential treatment strategies involving GLP-1R and AMYR for AUD and CUD. This thesis, therefore, explores the potential of semaglutide, a highly potent GLP-1R agonist, and the combination of GLP-1R and AMYR agonists to improve outcomes.
Additionally, this thesis investigates a brain region involved in the reward context, the paraventricular nucleus of the thalamus (PVT), and

the unexplored role of AMYR within this area. First, low doses of semaglutide demonstrated a reduction of alcohol- and cocaine-related responses in rodents, likely through its influence on the reward circuitry. Second, a combination of GLP-1R and AMYR agonists revealed a synergistic-like reduction of alcohol intake in male rats. Third, infusion of sCT into the middle part of PVT reduced alcohol intake in males but not in females, potentially due to sex-specific differences in projections to the NAc. Together, the studies presented in this thesis provide novel insight into enhancing the efficacy of GLP-1R and AMYR agonists in modulating alcohol- and cocaine-related responses while also offering new knowledge about the underlying mechanisms involved. These findings provide the advantage of maintaining effective drug intake reduction while enabling the use of lower doses, thereby minimizing potential side effects. Importantly, several studies in this thesis underline differences in responses between males and females, stressing the importance of conducting further research that includes both sexes to optimize treatment strategies for both men and women. In conclusion, this thesis highlights the significant potential of GLP-1R and AMYR agonists as therapeutic options for AUD and CUD.

Keywords: GLP-1, amylin, reward, AUD, CUD, mid-PVT

ISBN 978-91-8069-095-7 (PRINT)
ISBN 978-91-8069-094-0 (PDF)

SAMMANFATTNING PÅ SVENSKA

”Aptitreglerande hormoner och beroendeframkallande substanser: fokus på GLP-1 och amylin”

Beroendesjukdomar är ett omfattande samhällsproblem som påverkar inte bara den drabbade individen utan även deras anhöriga och samhället i stort. Dessa sjukdomar är komplexa och ofta förknippade med följdsjukdomar och en hög dödlighet. Beroende av substanser som alkohol och kokain har flera orsaker, där en central drivkraft är de belönande effekter som dessa substanser utlöser i hjärnan.

I dagsläget är alkohol- och kokainberoende utmanande att behandla, vilket gör att det finns ett stort behov av nya farmakologiska behandlingsstrategier.

Forskning har visat att hormoner som reglerar blodglukosnivåer och mättnad även spelar en avgörande roll i moduleringen av belöning och beroende. Aktiveringen av receptorerna för de aptitreglerande hormonerna glukagonliknande peptid-1 (GLP-1) och amylin har visat sig minska intaget av både alkohol och kokain. Denna effekt tros delvis bero på peptidernas påverkan på hjärnans belöningssystem.

Det finns dock outforskade möjligheter för att förbättra effektiviteten hos behandlingsstrategier som riktar sig mot GLP-1 receptorn (GLP-1R) och amylin receptorn (AMYR). Denna avhandling undersöker därför ifall semaglutid, en substans som aktiverar GLP-1R med hög bindnings- och aktiveringsförmåga, samt aktivering av GLP-1R och AMYR i kombination kan förbättra behandlingsresultaten. Utöver det undersöks ett hjärnområde som nyligen fått uppmärksamhet i sammanhang med belöning, den paraventrikulära thalamuskärnan (PVT), och amylinreceptorns outforskade roll i detta hjärnområde. Sammanfattningsvis syftar avhandlingen till att förbättra effektiviteten hos behandlingsstrategier som involverar dessa receptorer.

Studie 1 och 4 visade att aktiveringen av GLP-1R genom låga doser av semaglutide minskar alkohol- och kokainintag hos gnagare, troligen via påverkan på belöningssystemet. Studie 2 visade att aktivering av GLP-1R och AMYR i kombination gav en synergistisk minskning av alkoholintag vid doser som inte hade någon effekt när de användes enskilt. Vidare visade studie 3 att AMYR finns i mellersta delen av PVT. Lokal aktivering av AMYR i mellersta PVT minskade alkoholintag hos hanar men inte hos honor. Vidare analyser visade att AMYR finns på de

neuron som kopplar mellersta PVT till accumbenskärnan i hanar, men saknas i honor.

Denna avhandling tillför kunskap i hur aktiveringen av GLP-1R och AMYR påverkar alkohol- och kokainintag. Effekten på minskat alkohol- och kokainintag kan bibehållas med lägre doser vilket potentiellt ger fördelar så som lägre risk för biverkningar. Det bör understrykas att flera av mina studier visar på skillnader mellan hanar och honor, vilket betonar vikten av att bedriva vidare forskning som inkluderar båda könen för att optimera behandlingsstrategier för både män och kvinnor. Sammanfattningsvis visar denna avhandling att aktiveringen av GLP-1R och AMYR utgör potentiella mål för behandling av alkohol- och kokainberoende.

LIST OF PAPERS

This thesis is based on the following studies, referred to in the text by their Roman numerals.

I. **Aranäs C**, Edvardsson CE, Shevchouk OT, Zhang Q,Witley S, Sköldheden Blid S, Zentveld L, Vallöf D, Tufvesson-Alm M, Jerlhag E. (2023) Semaglutide reduces alcohol intake and relapse-like drinking in male and female rats. *EBioMedicine*, 93:104642

II. **Aranäs C,** Caffrey A, Edvardsson CE, Vestlund J, Schmidt HD, Jerlhag E. (2024) Synergistic decreases in alcohol intake following the combined GLP-1 and amylin pharmacotherapy. *British Journal of Pharmacology*, Epub ahead of print doi: 10.1111/bph.17406.

III. **Aranäs C**, Caffrey A, Edvardsson CE, Witley S, Zhang Q, Schmidt HD, Jerlhag E. Sex-specific responses on alcohol intake in rodents following sCT infusion in the middle parts of the paraventricular nucleus of thalamus. Submitted to *EBioMedicine*

IV. **Aranäs C**, Caffrey A, Edvardsson CE, Schmidt HD, Jerlhag E. Semaglutide attenuates cocaine taking and seeking and decreases elevated dopamine levels evoked by cocaine in the nucleus accumbens of male rodents. Under revision in *Translational Psychiatry*

LIST OF ADDITIONAL PAPERS

The following is a list of additional publications not included in the thesis

I. Kalafateli AL, **Aranäs C,** Jerlhag E. (2020) Effects of sub-chronic amylin receptor activation on alcohol-induced locomotor stimulation and monoamine levels in mice. *Psychopharmacology,* 237(11):3249-3257

II. Kalafateli AL, **Aranäs C,** Jerlhag E. (2021) Activation of the amylin pathway modulates cocaine-induced activation of the mesolimbic dopamine system in male mice. *Hormones and behaviors***,** 127:104885

III. **Aranäs C**, Vestlund J, Witley S, Edvardsson CE, Kalafateli AL, Jerlhag E. (2021) Salmon calcitonin attenuates some behavioural responses to nicotine in male mice. *Frontiers Pharmacology,* 12:685631

IV. **Aranäs C,** Blid Sköldheden S, Jerlhag E. (2023) Antismoking agents do not contribute synergistically to semaglutide's ability to reduce alcohol intake in rats. *Frontiers Pharmacology,* 14:1180512

V. Tufvesson-Alm M, Zhang Q, **Aranäs C**, Blid Sköldheden S, Edvardsson CE, Jerlhag E. (2024) Decoding the influence of central LEAP2 on food intake and its effect on accumbal dopamine release. *Progress in Neurobiology*, 236:102615

VI. Witley S, Edvardsson CE, **Aranäs C**, Tufvesson-Alm M, Stalberga D, Green H, Vestlund J, Jerlhag E. (2024) Des-acyl ghrelin reduces alcohol intake and alcohol-induced reward in rodents. *Translational Psychiatry,* 14(1):277

VII. Tufvesson-Alm M, **Aranäs C**, Blid Sköldheden S, Vestlund J, Edvardsson CE, Jerlhag E. (2024) LEAP2, a ghrelin receptor inverse agonist, and its effect on alcohol-related responses in rodents. *Translational Psychiatry*, 14(1):401

VIII. **Aranäs C,** Edvardsson CE, Zentveld L, Vallöf D, Witley S, Tufvesson-Alm M, Shevchouk OT, Vestlund J, Jerlhag E. The combination of a glucagon-like peptide-1 and amylin receptor agonists reduces alcohol consumption in both male and female rats. *Acta Neuropsychiatrica*, 6:1-15

TABLE OF CONTENTS

ABBREVIATIONS

AMYR	Amylin receptor
AUD	Alcohol use disorder
CUD	Cocaine use disorder
COMT	Catechol-O-methyltransferase
DAT	Dopamine transporter
DPP-IV	Dipeptidyl peptidase IV
ELISA	Enzyme-linked immunosorbent assay
FG	Fluorogold
GLP-1	Glucagon-like peptide 1
GLP-1R	Glucagon-like peptide 1 receptor
GRAB	G-protein-coupled receptor activation-based
IP	Intraperitoneal
MAOA	Monoamine oxidase A
NAc	Nucleus accumbens
NTS	Nucleus of the solitary tract
SC	Subcutaneous
sCT	Salmon calcitonin
VTA	Ventral tegmental area

RATIONALE

This section explains the rationale behind the four articles included in this thesis, highlighting how they collectively contribute to advancing knowledge in the field.

Alcohol use disorder (AUD) and cocaine use disorder (CUD) are complex, multifaceted conditions influenced by various biological, psychological, and environmental factors (Gerring et al., 2024, Clarke et al., 2012). Central to the development and maintenance of AUD and CUD are the rewarding effects of alcohol and cocaine, particularly the dopamine release in the nucleus accumbens (NAc), a key component of the ventral striatum (Blomqvist et al., 1997, Engel et al., 1988, Chiara and Imperato, 1988). Recent research has demonstrated that gut-brain peptides such as glucagon-like peptide 1 (GLP-1) and amylin reduce alcohol and cocaine-related behaviors in rodent models tentatively by attenuating drug-induced reward (Kalafateli et al., 2021a, Egecioglu et al., 2013a, Egecioglu et al., 2013b, Vallöf et al., 2016a, Schmidt et al., 2016). However, there are still unexplored opportunities to enhance the efficacy of potential treatment strategies involving the GLP-1 receptor (GLP-1R) and the amylin receptor (AMYR) for AUD and CUD. Increased efficacy allows for effective drug intake reduction while enabling the use of lower doses, which could potentially result in fewer side effects. The overall aim of this thesis was to further explore the role of GLP-1R and AMYR in alcohol and cocaine-related responses in rodents and thereby assess their tentative potential as therapeutic targets for AUD and CUD. One GLP-1R agonist, semaglutide, stands out for its high potency, strong receptor affinity, and oral administration capability, offering distinct advantages over other GLP-1R agonists (Holst and Madsbad, 2017, Bucheit et al., 2020, Kalra and Sahay, 2020). In order to provide insight into semaglutide's potential as a treatment against AUD, **Paper I** explored the effects of semaglutide on alcohol-related responses in rodents. Furthermore, given the heterogeneity of AUD, combining agents that independently reduce alcohol consumption may provide more effective treatment strategies. In **Paper II,** dual treatment with GLP-1R and AMYR agonists on alcohol intake was examined to evaluate a potential synergistic effect as a potential therapeutic approach for AUD. The knowledge gap concerning the

presence of AMYR within the paraventricular nucleus of the thalamus (PVT), a region that has recently gained interest in reward-related research (Hamlin et al., 2009), led to the development of a study design for the next project. **Paper III** explored for the first time the presence of AMYR in the middle part of PVT (mid-PVT) and its potential role in mediating alcohol-related responses. Finally, **Paper IV** extended the investigation of semaglutide's therapeutic potential to include other substances of abuse, specifically cocaine, to assess its efficacy as a potential treatment for CUD. This study explored semaglutide's ability to suppress cocaine-related responses, offering a broader perspective on its applicability in substance use disorder (SUD).

In summary, the findings from this thesis highlight the critical role of gut-brain peptides, particularly GLP-1 and amylin, in modulating alcohol- and cocaine-related responses.

INTRODUCTION

This section provides an overview of the foundational concepts that underpin this thesis. It summarizes key research findings on the reward system, the addictive properties of alcohol and cocaine, and the background regarding gut-brain peptides as treatment options for alcohol- and cocaine use disorders.

THE REWARD SYSTEM

The reward system in the brain has deep evolutionary roots, playing a crucial role in survival by driving behaviors essential for the species. Rewards can be categorized as natural or artificial (Kelley and Berridge, 2002). Natural rewards, like food and sex, fulfill basic biological needs and provide inherent pleasure. In contrast, artificial rewards, such as alcohol and cocaine, are external stimuli hijacking the brain's reward system, disrupting its natural functioning (Schultz, 2015). Both types of rewards influence decision-making by activating brain regions involved in reward processing (Skibicka, 2013, Vadnie et al., 2014, Pfaus et al., 1990, Hernandez and Hoebel, 1988). A key component of the reward system is the mesoaccumbal dopamine pathway, comprising dopaminergic neurons projecting from the ventral tegmental area (VTA), a major dopamine-projecting region, to the NAc, which is involved in motivation and reward processing (Figure 1). NAc is divided into two subregions, the NAc core and the NAc shell with anatomical and functional differences (Zahm, 1999). The shell is the outer region of the NAc, while the core is the inner substructure (Zahm and Brog, 1992). Dopamine, a neurotransmitter in this system, is released by VTA neurons in response to rewarding stimuli or their anticipation (Cador et al., 1991, Berridge and Robinson, 1998, Berridge and Kringelbach, 2015). Research has demonstrated that both artificial and natural reward stimuli elevate dopamine levels in the NAc (Blomqvist et al., 1997, Pfaus et al., 1990, Engel et al., 1988, Hernandez and Hoebel, 1988, Chiara and Imperato, 1988), resulting in feelings of pleasure. Beyond the projection from the VTA to the NAc, the VTA also projects to several other brain regions involved in the reward circuitry, including the medial prefrontal cortex, amygdala, and hippocampus, creating the mesocorticolimbic dopamine system (Koob, 1992a). The projection between VTA and medial prefrontal cortex supports cognitive functions

and reward processing (Jo and Mizumori, 2016, Han et al., 2017), the VTA-amygdala projection helps establish associations between environmental cues and rewarding or aversive experiences (Fadok et al., 2010) and the VTA-hippocampus projection plays a role in associating context with reward (Loh et al., 2016). Another brain region that recently gained interest in the reward context is the paraventricular nucleus of the thalamus (PVT). PVT is a heterogeneous structure consisting of distinct neuron types, which predominate in its anterior and posterior regions (Gao et al., 2020). It is important to note that these neuronal subtypes are not strictly confined to their respective regions (Gao et al., 2020). Instead, they exhibit an anterior-posterior gradient, with each subtype also present in the opposite half of the PVT (Gao et al., 2020). Functionally, the anterior PVT is associated with appetitive behaviors, while the posterior PVT is linked to aversive behaviors, with the mid-PVT playing an intermediate role along this behavioral spectrum (Barson et al., 2020). However, the PVT sends dense glutamatergic projections to the NAc (Figure 1), particularly to the shell region, where it innervates medium spiny neurons expressing both dopamine 1 and 2 receptors (McDevitt et al., 2024). The PVT-NAc pathway is involved in regulating reward and goal-directed behaviors (Choi et al., 2019). Specifically, the PVT-NAc pathway plays a critical role in drug-seeking, withdrawal, and relapse (Smith et al., 2020, James et al., 2010), with drugs of abuse inducing both short- and long-term changes at these synapses (De Groote and de Kerchove d'Exaerde, 2021). A fundamental driver of SUD is the modulation of synaptic neurotransmission, including alterations in dopamine signaling (Poisson et al., 2021).

The reward system and SUD pathology are deeply linked, with substances like alcohol and cocaine hijacking the brain’s natural reward circuitry, leading to profound changes that underlie the development and persistence of SUD (Gilman et al., 2008, King et al., 2021).

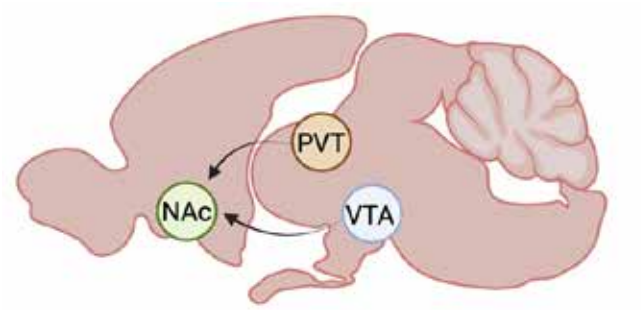

Figure 1. Simplified schematic representation of projections within the reward context. A well-known pathway is the projection from the ventral tegmental area (VTA) to the nucleus accumbens (NAc). Another region that has recently gained attention in the reward context is the paraventricular nucleus of the thalamus (PVT), which projects to the NAc.

ADDICTIVE SUBSTANCES

Artificial reward includes various addictive substances that influence the reward system through different mechanisms; this thesis specifically examines alcohol and cocaine.

ALCOHOL

Alcohol is rapidly distributed throughout the body, crosses the blood-brain barrier, and exerts significant effects on the central nervous system (Lee, 1962). Unlike many psychoactive substances that target specific neurotransmitter receptors or transporters, alcohol demonstrates a unique pharmacological profile (Gilpin and Koob, 2008) and affects many neurochemical pathways. It acts as an allosteric modulator on various ligand-gated ion channels, such as nicotinic acetylcholine, γ-aminobutyric $acid_A$, N-Methyl-D-aspartic acid, glycine, and serotonin type 3 receptor (Lovinger et al., 1989, Lovinger and Zhou, 1998, Yoshida et al., 1982, Söderpalm et al., 2000, Volkow et al., 2012, Burgos et al., 2015). Alcohol influences several critical brain regions, including NAc and VTA (Engel et al., 1988, Di Chiara, 1997, Ericson et al., 2003, Gessa et al., 1985), where dopamine release in the NAc shell is believed to underlie the sensations of pleasure and euphoria often associated with alcohol consumption (Engel and Jerlhag, 2014a, Engel, 1977a, Engel, 1977b, Engel et al., 1988). Early rodent and human studies suggested this connection, demonstrating that inhibiting

dopamine synthesis reduced alcohol's stimulatory properties in rodents (Engel et al., 1974) and diminished its euphoric effects in humans (Ahlenius et al., 1973). Furthermore, research has demonstrated that alcohol elevates extracellular dopamine levels in the NAc shell in rodents (Engel et al., 1988, Imperato and Di Chiara, 1986, Larsson and Engel, 2004, Blomqvist et al., 1993). Studies have confirmed that alcohol elevates dopamine levels in the NAc in humans (Boileau et al., 2003), linked with self-reported feelings of euphoria in clinical settings (Ramchandani et al., 2011, Yoder et al., 2007). Furthermore, alcohol infusion local in the NAc elevates dopamine levels in the same area, an effect attenuated by blocking nicotinic acetylcholine receptors in the anterior VTA in rodents (Ericson et al., 2008), supporting the involvement of the mesoaccumbal dopamine pathway. However, chronic alcohol use introduces profound changes to the brain's reward circuitry and neurotransmitter systems (Gilpin and Koob, 2008). These alterations diminish executive control over decision-making and action selection, reshaping the brain's responses to rewards and its sensitivity to alcohol's effects (Renteria et al., 2018). Further, chronic alcohol use downregulates the dopamine D2 receptor (Jonsson et al., 2014, Feltmann et al., 2018) and diminishes dopamine release in the NAc, potentially due to enhanced dopamine uptake (Carroll et al., 2006). In rats, chronic alcohol intake increases the sensitivity of the VTA to the reinforcing properties of alcohol (Rodd et al., 2005). Rats consuming high levels of alcohol exhibit lower dopamine tone in the NAc compared to those consuming lower amounts (Ericson et al., 2020). One additional consequence of prolonged alcohol exposure is the disruption of communication between the prefrontal cortex and the striatum (Renteria et al., 2018). This disconnection impairs goal-directed behavior while fostering habitual actions, further reinforcing addictive patterns of consumption (Renteria et al., 2018). Moreover, chronic alcohol intake robustly increases the expression of the transcription factor ΔFosB (Li et al., 2010), a molecule that is believed to drive structural changes in neurons, potentially leading to the lifelong persistence of these alterations (Nestler et al., 2001). These complex and multifaceted mechanisms illustrate alcohol's extensive impact on the brain, driving both its acute effects and the long-term consequences associated with chronic use. The pharmacodynamic effects of alcohol are extensive, also influencing reinforcement, sedation, and anxiety modulation (Engel et al., 1992). Emerging research highlights that increased sensitivity to the

euphoric effect of alcohol precedes the onset of AUD (King et al., 2021, Gilman et al., 2008). This observation underscores the critical role of reward mechanisms in both the initiation and progression of AUD.

COCAINE

Cocaine has a more direct role in the dopamine system than alcohol, by binding with high affinity to the dopamine transporter protein (DAT). This binding prevents the reuptake of dopamine from the synaptic cleft which results in an accumulation of dopamine (Huang et al., 2009, Verma, 2015). The increase of dopamine in key brain regions, especially NAc, creates intense pleasure that exceeds natural reward (Nestler, 2005). Previous research has revealed that cocaine administration rapidly increases extracellular dopamine levels in the NAc shell in rodents (Egecioglu et al., 2013a, Aragona et al., 2008). Repeated cocaine administration produces a substantial increase in the basal dopamine release in the NAc (Weiss et al., 1992), and changes the synaptic strength over time (Kourrich et al., 2007, Koya and Hope, 2011). Furthermore, cocaine induces significant structural modifications in NAc neurons, such as increasing the size and complexity of both excitatory and inhibitory synapses (Blazquez-Llorca et al., 2021). Moreover, repeated cocaine exposure decreases the excitability of dopamine receptor-expressing neurons in the NAc (Kourrich and Thomas, 2009, Mu et al., 2010) and dramatically reduces cocaine-induced dopamine signaling and unbalances D1 over D2 receptor signaling (Park et al., 2013). Similar to chronic alcohol intake, prolonged cocaine consumption increases the expression of the transcription factor ΔFosB (Nestler et al., 2001), potentially driving structural changes in neurons and contributing to the lifelong persistence of these alterations.

Furthermore, while direct measurements of dopamine increase in the human NAc shell are challenging due to ethical and technical limitations, the consistent findings across species and the known mechanisms of cocaine action strongly suggest that cocaine increases dopamine in the NAc shell in humans, contributing to its rewarding and addictive properties. Supporting this, imaging studies in both cocaine-exposed rats, non-human primates, and humans have consistently identified hypoactivity within the ventral striatum/NAc (Porrino et al., 2002, Macey et al., 2004, Volkow et al., 1993). Additionally, acute electrophysiology recording studies have shown that repeated cocaine

exposure induced hypoactivity in accumbal neurons (Thomas et al., 2001, White et al., 1995). Altogether this suggests that reduced activity of accumbal dopamine neurons play a role in the development and manifestation of CUD.

Both cocaine and alcohol use are considered highly addictive substances that can lead to SUD (Wagner and Anthony, 2002).

SUBSTANCE USE DISORDER

Various hypotheses have been proposed to explain the foundations of SUD. The SUD process is suggested to consist of distinct stages - preoccupation, intoxication, and negative affect - each stage reflecting an aspect of the disorder's progression and its impact on behavior and neurobiology (Koob and Volkow, 2010). Preoccupation involves an intense focus on obtaining and consuming the drug of interest, often accompanied by cravings and a loss of control over drug-seeking behaviors (Koob and Volkow, 2010). Intoxication refers to the phase during and immediately after drug consumption, characterized by pleasurable effects like euphoria (Koob and Volkow, 2010). Negative affect encompasses the emotional and physiological distress experienced during withdrawal or period of abstinence (Koob and Volkow, 2010). Although individuals consume the drug of interest for various reasons, SUD is a complex condition involving multifaceted neurobiological processes, with reward playing a central role (Boileau et al., 2003, King et al., 2021, Gilman et al., 2008). The rewarding effects of an addictive drug increase the risk of developing SUD and contribute to its persistence (Chavarria et al., 2021, King et al., 2021), highlighting the importance of studying the mechanisms that modulate these reward properties. By exploring this dimension, the work aims to shed light on the neurobiological pathways that sustain SUD to find potential therapeutic interventions.

ALCOHOL USE DISORDER

AUD is a prevalent and complex disorder, considered a socioeconomic burden for both the individual and society at large (Ferrari et al., 2014). Approximately 7 % of the global population, 15 years and older, was 2019 living with AUD (WHO, 2024). AUD poses a serious health risk and is strongly associated with higher rates of mortality and morbidity

(Lim et al., 2012). Harmful alcohol consumption is responsible for approximately 2.6 million deaths annually worldwide while also contributing to disabilities and poor health for millions of individuals (WHO, 2024). Alcohol affects not only the brain but also the rest of the body, with excessive or chronic consumption causing significant organ damage and eventual organ failure (Fogle et al., 2010, Kawano, 2010, Joo et al., 2020, Koning et al., 2015). The liver is particularly vulnerable, as it plays a crucial role in detoxifying alcohol from the body (Gao and Bataller, 2011). Furthermore, research has established links between alcohol consumption and certain forms of cancer (WHO, 2024). Alarmingly, even light alcohol consumption has been suggested to increase the risk of developing specific types of cancer (Jun et al., 2023). Additionally, excessive alcohol consumption is associated with a heightened risk of injuries that can result in death or serious health complications (WHO, 2024).

EXISTING TREATMENTS

Four different pharmacological treatment options are today available for the treatment of AUD: disulfiram, acamprosate, naltrexone, and nalmefene (Wallhed Finn et al., 2021). Disulfiram inhibits the enzyme aldehyde dehydrogenase, which results in an accumulation of acetaldehyde when alcohol is consumed, causing unpleasant symptoms that aim to deter alcohol consumption (Barth, 2010). Acamprosates exact mechanism of action is not fully understood (Witkiewitz et al., 2012). However, acamprosate has been shown to increase dopamine levels, serving as a substitute for the dopamine elevation induced by alcohol (Chau et al., 2018, Ademar et al., 2022). Studies suggest that its ability to reduce alcohol intake may stem from the effects of its calcium and N-acetyl homotaurine components, which influence both dopamine and taurine levels in the brain (Ademar et al., 2023, Ademar et al., 2022). Naltrexone and nalmefene interact with opioid receptors in slightly different ways (Swift, 2013). Naltrexone functions primarily as a competitive antagonist at these receptors, whereas nalmefene acts as an antagonist at μ- and δ-opioid receptors while also serving as a partial agonist at the κ-opioid receptor (Swift, 2013). Despite these differences, both medications reduce the reinforcing effects of alcohol by modulating the reward system, ultimately decreasing dopamine release in NAc (Quelch et al., 2017, Gonzales and Weiss, 1998, Rose et al., 2016). Studies have shown a reduced consumption of alcohol in humans

following treatment with all four pharmacotherapies (Mason and Lehert, 2012, Christensen et al., 1991, Roerecke et al., 2015, Pettinati et al., 2011). However, the efficacy is suboptimal even when combined with psychosocial therapy (Anton, 2008, Nitya Jayaram-Lindström, 2016, Koob, 2010).

POTENTIAL FUTURE TREATMENT STRATEGIES

Moreover, combining different pharmaceuticals may enhance treatment effectiveness for disorders with complex neurobiology such as AUD. Rodent studies have demonstrated that a combination of naltrexone and acamprosate produces significantly greater suppression of alcohol consumption than naltrexone alone (Kim et al., 2004). In contrast, the combination of acamprosate and naltrexone demonstrated no significant advantages in clinical settings (Pettinati et al., 2006). Other studies suggest promising effects of combining naltrexone with varenicline or baclofen on alcohol-related behaviors in rodents (Froehlich et al., 2017, Stromberg, 2004). Additionally, the combination of varenicline and bupropion, two anti-smoking agents, has shown promising results in blocking the alcohol deprivation effect in rodents (Söderpalm et al., 2020). Furthermore, the combination of disulfiram and naltrexone appears to have a stronger impact on alcohol and cocaine abstinence than either medication used individually in a clinical trial (Pettinati et al., 2008). The disease's complexity and severity combined with interindividual variability in treatment response (Heilig and Egli, 2006) makes it important to find new pharmacological treatment options.

SEX DIFFERENCES

Although AUD is currently more prevalent among men, the incidence of AUD in women is rising (Slade et al., 2016), highlighting an emerging public health challenge. Not only the differences in prevalence for AUD between women and men are shrinking but also drinking patterns, hospitalizations, emergency department visits, and deaths (White, 2020). Interestingly, among adolescents and young adults, females are now more likely than their male counterparts to report drinking in the past month (White, 2020). Notably, this shift is not solely due to increased drinking among women; rather, it suggests that drinking patterns between the two genders are becoming more similar (White, 2020). Moreover, treatment responses in AUD are influenced by sex, with women being less likely than men to seek treatment (Greenfield et al., 2007, Harris et al., 2022). Women exhibit greater

vulnerability to the medical consequences of alcohol consumption (Agabio et al., 2017, Chambers et al., 2019). In addition, they face an increased risk of adverse alcohol-medication interactions due to physiological differences in metabolism and pharmacodynamics (Sinclair et al., 2016, Agabio and Sinclair, 2019). Women achieve better outcomes when participating in programs designed to address their specific needs (Agabio et al., 2017, Harris et al., 2022), highlighting that men and women require different treatment approaches. Furthermore, preclinical findings demonstrate that female rats generally consume more alcohol than males when adjusted for body weight, a sex difference in alcohol consumption often emerging at puberty (Li et al., 2019, Scott et al., 2020). Notably, approved medications for AUD have been developed based on research conducted predominantly in male subjects, underscoring the need to include more females in preclinical research and to increase women's representation in clinical trials (Harris et al., 2022). This is crucial for developing treatment options that are effective and tailored to women.

COCAINE USE DISORDER

CUD is a serious public health issue that has widespread negative impacts on both individuals and society and can develop after prolonged cocaine use (Peacock et al., 2018). Cocaine use negatively affects millions of people worldwide, with both cocaine use and CUD on the rise among adults and adolescents (Simpson et al., 2019, Schneider et al., 2018). Those affected by CUD often suffer from dysregulated motivation, characterized by intense cravings, compulsive drug-seeking behavior, and a high likelihood of relapse even after periods of abstinence (Koob and Le Moal, 2001, Dackis and O'Brien, 2001, Leshner, 1997, Meyer et al., 2016). Similar to AUD, CUD affects not only the brain but also various parts of the body, resulting in significant systemic complications and organ damage (Richards and Le, 2025). Chronic cocaine use can severely damage the heart, increasing the risk of thrombus formation and ischemic events (Richards and Le, 2025).

EXISTING TREATMENT

Despite the severity of the disorder, no pharmaceutical treatment currently exists (Buchholz and Saxon, 2019). Psychosocial interventions remain the primary evidence-based treatments for CUD, and while

current treatment can be effective for some, CUD remains challenging to treat (Kampman, 2019).

POTENTIAL FUTURE TREATMENT STRATEGIES

Hence, there is a significant demand for novel pharmacotherapies to treat CUD. Continued research into new pharmacological and combination approaches is needed to improve outcomes for those struggling with CUD. A substantial body of evidence indicates that the brain's dopamine system plays a pivotal role in mediating the rewarding effects of cocaine and sustaining drug-use behaviors (King et al., 2021, Bardo, 1998, Koob, 1992b). Consequently, numerous medications targeting the dopamine system have been investigated with varied results as potential pharmacotherapies for CUD (Sofuoglu and Kosten, 2005). Disulfiram which is used for AUD decreased craving for cocaine and was associated with a greater reduction in cocaine use in a clinical trial (Carroll et al., 1998, McCance-Katz et al., 1998).

SEX DIFFERENCES

Men represent the majority of cocaine users and tend to develop the condition earlier than women (Requena-Ocaña et al., 2021). They are also more likely than women to engage in polydrug use (Requena-Ocaña et al., 2021). Even though men continue to represent the majority of cocaine users, increasing gender equality has led to a rapid rise in the consumption of various addictive substances among young women (Requena-Ocaña et al., 2021). This historical gender disparity in substance use is partly attributed to social and cultural roles that historically granted men easier access (McHugh et al., 2018). However, neurobiological sex differences are also found, estradiol enhances dopamine release in reward-related brain regions and facilitates behavioral sensitization to cocaine (Hu et al., 2004). Preclinical findings reveal that female rats acquire cocaine self-administration more rapidly and self-administer more cocaine at a faster rate than males, even when controlling for circulating hormones (Hu et al., 2004). This underscores the importance of including females in both preclinical and clinical studies, with the potential to develop sex-specific treatments tailored to their unique needs.

Developing a deeper understanding of the complex mechanisms underlying AUD and CUD is crucial for advancing new treatment strategies. Notably, gut-brain peptides have been identified as

modulators of mechanisms involved in AUD and CUD (Jerlhag, 2019, Hernandez and Schmidt, 2019).

GUT-BRAIN PEPTIDES

Gut-brain peptides are important in signaling between the gastrointestinal tract and the central nervous system (Wachsmuth et al., 2022). There are several different peptides, each playing vital roles in numerous physiological processes and being implicated in conditions such as obesity and diabetes (Woodward et al., 2022, Boyle et al., 2018, Jorsal et al., 2016, Eržen et al., 2024, Roth et al., 2012, Meier, 2012).

GLP-1

GLP-1 synthesis occurs mainly in the small intestine (Reimann et al., 2008) with additional production occurring in pancreatic islets (Hugo Mendieta et al., 2013, Tornehave et al., 2008) and the brain, primarily in the brainstem, specifically in the nucleus of the solitary tract (NTS) (Shughrue et al., 1996, Holt et al., 2019). GLP-1 binds to its receptor, GLP-1R, a G-protein-coupled receptor widely expressed in the pancreas, brain, heart, and gastrointestinal tract (Drucker et al., 1987). GLP-1 has a short half-life due to the action of dipeptidyl peptidase IV (DPP-IV), which plays a key role in its inactivation by cleaving the first two amino acids. DPP-IV specifically removes dipeptides from proteins and oligopeptides containing an alanine or proline residue at the second position. GLP-1R agonists have been developed with modifications that replace these amino acids, effectively prolonging their half-life and enhancing their therapeutic potential (Baggio and Drucker, 2007). GLP-1 is primarily recognized for regulating glucose homeostasis (Holst and Gromada, 2004, Komatsu et al., 1989) and controlling gastric emptying (Flint et al., 1998). GLP-1 regulates blood glucose by enhancing insulin production and secretion (Kreymann et al., 1987) while suppressing glucagon release (Orskov et al., 1988). Activation of the GLP-1R slows gastric emptying, which contributes to an increased feeling of satiety. This effect helps suppress appetite, leading to a reduction in food intake and body weight (Ard et al., 2021). Intriguingly, rodent studies reveal that systemic treatment with GLP-1R agonists reduces food reward behavior and the motivation to consume palatable food (Dickson et al., 2012). Notably, its ability to regulate glucose levels, reduce food intake, and promote weight loss has led to the development and widespread use

of GLP-1R agonists as an effective treatment for type II diabetes and obesity (Nuffer and Trujillo, 2015). Multiple GLP-1R agonists are currently available on the market, differing in their duration of action. Exenatide is a relatively short-acting GLP-1R agonist that requires twice-daily administration (McCormack, 2014). In contrast, liraglutide has a slightly longer duration of action and is administered once daily (Peterson and Pollom, 2010). Moreover, the longer-acting GLP-1R agonists, dulaglutide and semaglutide, are administered only once weekly (Lau et al., 2015, Tham et al., 2022).

SEMAGLUTIDE

Semaglutide is a GLP-1R agonist clinically approved for treatment against diabetes type II (Ozempic® and Rybelsus®) and obesity (Wegovy®) to improve blood sugar control and through increased satiety cause weight loss (Overgaard et al., 2019, Bergmann et al., 2023, Chao et al., 2021). Semaglutide is a modified form of natural GLP-1 designed to enhance enzymatic stability, increase albumin binding, prolong absorption from the injection site, reduce renal elimination, and ultimately extend the compound's half-life (Lau et al., 2015, Overgaard et al., 2019). These modifications make semaglutide a long-acting GLP-1 receptor agonist with improved pharmacokinetic properties compared to native GLP-1 (Lau et al., 2015, Overgaard et al., 2019). However, its half-life differs between species, being considerably shorter in rats compared to humans (Lee et al., 2023). Semaglutide produces more significant weight loss and glucose-lowering effects than other GLP-1R agonists, attributed to its higher potency and affinity for the GLP-1R (Bucheit et al., 2020, Holst and Madsbad, 2017, Kalra and Sahay, 2020). Semaglutide is available as a subcutaneous injection and is the first GLP-1R medication that can also be administrated orally (Kommu and Whitfield, 2024).

DULAGLUTIDE

The GLP-1R agonist, dulaglutide, is a clinically approved treatment for diabetes type II (Trulicity) to improve glycaemic control (Fala, 2015). Dulaglutide is a modified analog of the naturally occurring GLP-1, engineered to exhibit enhanced enzymatic stability and reduced renal clearance (Thompson and Trujillo, 2015). These modifications produce a significantly prolonged half-life compared to native GLP-1, making it suitable for once-weekly administration (Vahle et al., 2015). Dulaglutide is available as a subcutaneous injection (Fala, 2015).

AMYLIN

Amylin is a hormone secreted along with insulin in pancreatic β-cells in response to food intake (Zhang et al., 2016). Amylin exerts its effect by binding to the AMYR, a G-protein-coupled receptor closely related to the calcitonin receptor (CTR) family (Poyner, 1995, Poyner et al., 2002), as both share the same CTR core structure (Hay et al., 2015). The CTR core exists in two forms: CTRa and CTRb (Poyner et al., 2002). The AMYR includes a receptor activity modifying protein (RAMP) attached to the core; this exists in three different forms: RAMP1, 2, or 3. RAMPs play a critical role in modulating signalling for both amylin and calcitonin by altering the activity of the CTR core (Hay et al., 2015, Hay and Pioszak, 2016). A peripherally administrated AMYR receptor agonist crosses the blood-brain barrier (Kalafateli. et al., 2020), and AMYR is found in several reward-related brain areas (Reiner et al., 2017, Mietlicki-Baase et al., 2015a, Mietlicki-Baase and Hayes, 2014, Sexton et al., 1994, Kalafateli et al., 2021b). As GLP-1, amylin plays a key role in modulating glucose homeostasis and regulating gastric emptying (Mietlicki-Baase et al., 2015a). Specifically, amylin reduces glucagon secretion (Gedulin et al., 1997) and inhibits the insulin release from pancreatic α cells (Silvestre et al., 1994), leading to the development of AMYR agonists as a treatment for diabetes type I and II (Eržen et al., 2024). Additionally, amylin decreases food intake and body weight in rodents, an effect observed following both peripheral and central administration (Lutz, 2010, Reiner et al., 2017, Roth et al., 2007). The reduction in food intake mediated by amylin affects both homeostatic and hedonic feeding, processes that involve the mesolimbic dopamine system (Mietlicki-Baase et al., 2015b, Boyle et al., 2018). This underscores amylin's multifaceted role in regulating energy balance and metabolism.

Interestingly, combining GLP-1R and AMYR agonists has demonstrated synergistic-like effects in reducing food intake and body weight in rodents (Liberini et al., 2019) and humans (Frias et al., 2023, Wong et al., 2023).

SCT

sCT acts as a dual agonist for both CTR and AMYR (Epand et al., 1986, Lutz et al., 2000). sCT has a crucial C-terminal proline residue that is important for receptor binding, whereas amylin's C-terminal tyrosine

has little influence on binding (Lee et al., 2016). sCT binds and activates receptors for an extended period compared to human calcitonin and amylin (Mathiesen et al., 2020, Hay et al., 2004). Despite differences in the amino acid sequences between amylin and sCT (Bower and Hay, 2016), several studies show that sCT mimics the role of amylin when it comes to food intake suppression (Eiden et al., 2002, Lutz et al., 2000, Reidelberger et al., 2007) and body weight decrease (Lutz et al., 2001, Reidelberger et al., 2007).

However, previous findings have revealed that GLP-1 and amylin have additional physiological functions (Nizari et al., 2021, Holt et al., 2020, Diz-Chaves et al., 2022, Vestlund and Jerlhag, 2020, Suchankova et al., 2015, Kalafateli et al., 2021b) beyond these well-established effects.

GUT-BRAIN PEPTIDES AND DRUGS OF ABUSE

Research has demonstrated that gut-brain peptides play a significant role in both natural and artificial reward processes and have substantial effects on drugs of abuse, including alcohol and cocaine (Jerlhag, 2019, Tufvesson-Alm et al., 2022). This association has been found for both orexigenic and anorexigenic peptides. Ghrelin, an orexigenic peptide, enhances the reward of alcohol and cocaine, as well as the intake (Schuette et al., 2013, Carroll et al., 1979, Wellman et al., 2005, Jerlhag et al., 2009). Moreover, ghrelin receptor antagonists effectively reduce alcohol consumption in rodents (Suchankova et al., 2013). In contrast, the anorexigenic peptides GLP-1, amylin, and Neuromedin U suppress the rewarding effects of alcohol and other drugs of abuse, thereby reducing intake in rodents (Schmidt et al., 2016, Egecioglu et al., 2013b, Egecioglu et al., 2013a, Kalafateli et al., 2021a, Kalafateli et al., 2019a, Kalafateli et al., 2019b, Vallöf et al., 2017, Vallöf et al., 2016b). This thesis focuses on GLP-1 and amylin, both have demonstrated potential as targets for modulating alcohol- and cocaine-related responses in rodents (Kalafateli et al., 2019b, Egecioglu et al., 2013a, Kalafateli et al., 2021a, Sørensen et al., 2016, Sørensen et al., 2015, Vallöf et al., 2020)

GLP-1 AND DRUGS OF ABUSE

PRECLINICAL PERSPECTIVE

Extensive research has shown that GLP-1R agonists effectively decrease voluntary alcohol consumption (Vallöf et al., 2016a, Vallöf et al., 2020, Egecioglu et al., 2013b, Sørensen et al., 2016) relapse-like drinking behaviors (Vallöf et al., 2016a, Thomsen et al., 2017) and the motivation to consume alcohol (Vallöf et al., 2016a, Egecioglu et al., 2013b). Specifically, studies have demonstrated that GLP-1R agonists effectively reduce alcohol intake across various species, including mice (Sørensen et al., 2016), rats (Vallöf et al., 2020), and non-human primates (Thomsen et al., 2020, Fink-Jensen et al., 2024). Additionally, acute activation of GLP-1R suppresses alcohol's well-known effects on the reward system, including alcohol-induced dopamine release in the NAc shell and increased locomotor activity (Egecioglu et al., 2013b, Vallöf et al., 2016a). Importantly, studies highlight that GLP-1R activation within specific reward-related brain regions plays a crucial role in modulating alcohol-related responses (Vallöf et al., 2019a, Colvin et al., 2020, Vallöf et al., 2019b, Dixon et al., 2020). Similarly, research consistently underlines the role of GLP-1R activation in modulating cocaine-related behaviors. Activation of GLP-1Rs has been shown to reduce both cocaine self-administration and cocaine-seeking behaviors in rodent models (Schmidt et al., 2016, Sørensen et al., 2015). Furthermore, systemic administration of GLP-1R agonists attenuated cocaine-induced effects on the reward system, including reduction in elevated dopamine levels within the NAc and locomotor stimulation (Egecioglu et al., 2013a). Additionally, systemic administration of GLP-1R agonists impacts cocaine-induced striatal c-fos expression, further supporting GLP-1's influence on cocaine-related neural activity (Sørensen et al., 2015). Moreover, local activation of GLP-1Rs within reward-related brain regions significantly reduces cocaine self-administration and seeking behaviors (Hernandez et al., 2018, Hernandez et al., 2019, Hernandez et al., 2021, Schmidt et al., 2016, Reddy et al., 2016). Complementary evidence from GLP-1R deficient mice, which exhibit heightened cocaine-induced locomotor responses and conditioned place preference, underscore the critical role of GLP-1R signaling in regulating cocaine's behavioral and neural effects (Harasta et al., 2015). Taken together these findings underline the

therapeutic potential of GLP-1R agonists in addressing SUD by targeting the neural circuits associated with reward and reinforcement.

CLINICAL PERSPECTIVE

While the effect of GLP-1R agonists on alcohol and cocaine intake has been researched pre-clinically, clinical insights are more scarce. However, there have been some promising clinical studies of GLP-1R agonists for treating AUD. Recent clinical trials found that GLP-1R agonists reduced alcohol consumption in obese AUD individuals (Klausen et al., 2022, Quddos et al., 2023). A preliminary report on diabetes type II patients treated with a GLP-1R agonist showed a possible reduction of alcohol intake (Kalra et al., 2011). Furthermore, associations between genetic variation in GLP-1R and AUD in humans have been noted (Suchankova et al., 2015). Recent studies on semaglutide have shown that people in a small case study experienced decreased AUD symptoms (Richards et al., 2023) and that semaglutide was associated with approximately 50 % lower risk of both incidence and recurrence of AUD compared to other anti-obesity medication in a large retrospective cohort study (Wang et al., 2024b). Supporting this, a nationwide cohort study in Sweden found that the use of semaglutide and liraglutide was significantly associated with a reduced risk of hospitalization due to AUD in patients with comorbid obesity or type II diabetes and AUD (Lähteenvuo et al., 2024). Furthermore, a retrospective cohort study conducted in the United States revealed that patients prescribed GIP and/or GLP-1 receptor agonists exhibited significantly lower rates of alcohol intoxication (Qeadan et al., 2024). In contrast, a GLP-1R agonist administered to individuals with CUD did not alter the subjective responses to cocaine or cocaine self-administration (Angarita et al., 2021) likely due to the use of low doses and the timing of the treatment. However, more rigorous clinical trials are needed to establish their efficacy and safety for AUD, and further clinical trials need to investigate the potential of GLP-1R agonists for CUD. Overall, these findings indicate that the GLP-1R represents a promising therapeutic target for treating AUD and potentially CUD as well.

AMYLIN AND DRUGS OF ABUSE

PRECLINICAL PERSPECTIVE

Earlier studies have shown that both acute and repeated activation of AMYR has an impact on alcohol-related responses in rodents (Kalafateli et al., 2019b, Kalafateli et al., 2021c, Kalafateli et al., 2019a, Kalafateli et al., 2020, Kalafateli et al., 2021b). In more detail, acute sCT administration prevents the expression of alcohol's rewarding properties, which is evident as it blocks alcohol-induced locomotor activity and dopamine release in the NAc shell (Kalafateli et al., 2019b). Treatment with sCT attenuates alcohol intake in both low- and high-alcohol-consuming rats - with a more distinct decrease in high-consumers - and prevents relapse drinking (Kalafateli et al., 2019a). In line with this, a single administration with an AMYR antagonist increased levels of alcohol intake (Kalafateli et al., 2019a). Moreover, treatment with a long-acting AMYR agonist decreased alcohol intake in both male and female rats (Kalafateli et al., 2021c). Further, infusion of sCT into reward-related brain regions reduced alcohol intake and attenuated alcohol's rewarding properties by blocking alcohol-induced locomotor activity and dopamine release in the NAc shell (Kalafateli et al., 2021b). Further evidence for AMYR's role in the reward circuit is the expression of the CTR on VTA dopamine neurons (Mietlicki-Baase et al., 2015b). The fact that there is different gene expression of AMYR components in the NAc between low and high-alcohol-consuming rats (Kalafateli et al., 2019a) strengthens the hypothesis that AMYR plays a significant role in the reward system and can be a suitable target for treatment against AUD.

CLINICAL PERSPECTIVE

While the effects of AMYR agonists on alcohol-related responses have been explored preclinically, clinical evidence remains lacking.

Although previous research has demonstrated that gut-brain peptides attenuate drug-induced responses, significant knowledge gaps remain. There are still unexplored opportunities to enhance the efficacy of potential treatment strategies involving GLP-1R and AMYR for AUD and CUD.

AIM

The overall aim of this thesis was to further explore the roles of GLP-1R and AMYR in alcohol and cocaine-related responses in rodents, thereby evaluating their potential as therapeutic targets for AUD and CUD.

SPECIFIC AIMS

Paper I Examine the potential of semaglutide to modulate alcohol-related responses in female and male rodents

Paper II Investigate the effect of the combination of GLP-1R and AMYR agonists on alcohol intake in female and male rats

Paper III Identify whether sCT locally infused in mid-PVT regulates alcohol-related responses in female and male rodents

Paper IV Explore the effect of semaglutide on cocaine taking and reward in male rodents.

MATERIALS AND METHODS

This section provides an overview of the experimental models and procedures employed in this thesis. Detailed information on the methodologies and statistical analyses for each study are available in the accompanying articles and manuscripts.

ANIMALS

RELEVANCE OF ANIMAL MODELS

In the field of addiction research, rodents serve as a suitable animal model because the brain's reward system and its associated structures are highly conserved across species, making them comparable to humans (Mullins and Mullins, 2004). No animal model can mirror SUD fully and therefore, several different animal models are used to include several parts of the SUD process.

CONDITIONS AND ETHICAL CONSIDERATIONS

All experiments performed were either approved by the Ethics Committee for Animal Experiments, Gothenburg, Sweden, or the Institutional Animal Care and Use Committee of the University of Pennsylvania. All experiments were reported in accordance with the ARRIVE guidelines and planned according to PREPARE guidelines. All effort was made to minimize the number of animals used and to reduce their suffering, adhering to the principles of the 3Rs (replacement, reduction, and refinement).

Moreover, the validity of the models has been carefully evaluated,, including face, construct, and predictive validity. Face validity refers to the extent to which the animal model replicates the human condition, encompassing both behavioral similarities and biological markers. Construct validity assesses the degree to which the mechanisms underlying behavior in the model align with those in the human condition being studied. Predictive validity measures how well treatment effects observed in the animal model translate to human subjects. Many experiments included male and female rats, while others included only male rats. All experiments conducted on mice were solely performed in male mice.

MICE

The mice used in locomotor activity tests (**Papers I, III, and IV)**, *in vivo* microdialysis **(Papers I, III, IV),** condition place preference (CPP) (**Papers I and III**), palatable food intake (**Paper I**), and the biochemical analysis (**Paper I and III**) are adult weight-matched outbred male NMRI mice (20-25 g body weight at arrival, Charles River; Susfeldt, Germany). This mouse strain was selected as it previously displayed robust stimulatory response in drug-induced responses with the models used within this thesis (Kalafateli et al., 2019b)

RATS

The rats used in the intermittent alcohol drinking paradigm (**Paper I-III**) the elevated plus maze (EPM) (**Paper I**), the biochemical analysis (**Paper I and III**), the infusion of CY3-semaglutide (**Paper I**), and *in vivo* microdialysis (**Paper II**) were age-matched (8 weeks at arrival) outbred male and female RccHan Wistar rats (weight at arrival: approximately 250 g for males and 190 g for females, Envigo; Horst, Netherlands). This strain displays stable alcohol intake with relevant blood-alcohol concentration levels (Simms et al., 2008, Priddy et al., 2017, Palm et al., 2011). For the self-administration experiments of cocaine (**Paper IV**), the PICA experiments (**Papers II and IV**), and the Fluorogold and RNAscope experiment (**Paper III**) weight-matched Sprague-Dawley rats (225-250 g at arrival, Taconic Laboratories; NY, US) were used. This strain has previously been found to robustly self-administer cocaine and is commonly used for both PICA tests and neurochemical studies (Caffrey et al., 2023, Hernandez et al., 2019).

DRUGS

ALCOHOL

Alcohol (95%, Solveco AB; Stockholm, Sweden) administered through intraperitoneal (IP) injection (**Papers I and III**) was diluted in 0.9 % sodium chloride (vehicle) and at the dose of 1.75 g/kg, 5 minutes prior to initiation of the experiment. This dose activates the mesolimbic dopamine system and has previously been used in alcohol-induced locomotor stimulation, accumbal dopamine release, and CPP experiments with successful results (Vallöf et al., 2019a, Jerlhag et al., 2009). When alcohol was voluntarily ingested (**Paper I-III**), 95 % of

alcohol was diluted to 20 % v/v with tap water and was available for the rats for 24 hours. This alcohol concentration has previously been successfully used in studies with the same experimental model and is preferred over other concentrations (Vallöf et al., 2016a, Kalafateli et al., 2021c, Simms et al., 2008).

COCAINE

Cocaine (National Institutes of Drug Abuse) was used for the self-administration experiments (**Paper IV**). For the extinction experiment, cocaine was dissolved in the vehicle and administered IP at a dose of 10 mg/kg as a priming injection before the experiment. During the self-administration phase, cocaine was delivered intravenously (IV) through a catheter (0.25 mg cocaine/59µl saline, infused over 5 secs). These doses have been successfully employed in previous cocaine self-administration studies (Hernandez et al., 2019). Cocaine hydrochloride (Apoteket AB, Gothenburg, Sweden), used for cocaine-induced locomotor stimulation, and cocaine elevated dopamine levels, (**Paper IV**), was diluted in vehicle and injected IP (10 mg/kg). This dose has previously been used in these experimental models as the dose activated the mesolimbic dopamine system (Kalafateli et al., 2021a).

SEMAGLUTIDE

Semaglutide (Apoteket AB, Gothenburg, Sweden) was dissolved in vehicle and injected subcutaneously (SC). In the alcohol experiments (**Paper I**) two low doses (0.026 mg/kg and 0.052 mg/kg) were evaluated to minimize the dosage. These doses represent reductions of 74% and 48%, respectively, compared to previously tested doses (Marty et al., 2020). In the cocaine experiments (**Paper IV**), three doses (0.013 mg/kg, 0.026 mg/kg, and 0.039 mg/kg) were tested. These doses were selected based on the previous study demonstrating that 0.026 mg/kg effectively decreased alcohol intake. The other two doses were selected to establish a broad dose interval. Semaglutide was administered 1 hour prior to experiment initiation on the experimental day. Semaglutide has a shorter half-life in rats than in humans (Lau et al., 2015), necessitating its administration prior to each test session during repeated treatment protocols.

DULAGLUTIDE

Dulaglutide (Trulicity®; Kronans Apotek, Gothenburg, Sweden) was dissolved in vehicle and injected SC. Dulaglutide was used at a dose range between 0.0125 to 0.4 mg/kg once weekly one hour prior to experiment initiation (**Paper II**). Dulaglutide at the dose of 0.1 mg/kg administered one hour prior to alcohol exposure weekly, effectively decreased alcohol intake in both male and female rats (Vallöf et al., 2020).

SCT

Salmon calcitonin (sCT) (Tocris Bioscience; Bristol, United Kingdom) administered systemically was dissolved in vehicle and injected IP. sCT was used at a dose range between 0.25-7 μg/kg administered 30 minutes prior to experiment initiation at each alcohol-drinking session (**Paper II**). sCT at the dose of 5 μg/kg decreased alcohol intake in male rats in a previous study (Kalafateli et al., 2019b). sCT infused locally into the brain (**Paper III**) was diluted in Ringer solution (NaCl 140 mM, $CaCl_2$ 1.2 mM, KCl 3.0 mM, and $MgCl_2$ 1.0 mM). A volume of 0.5 μl was administered over 60 seconds, and the cannula was left in place for another 60 seconds to allow for complete drug diffusion. A dosage of 0.025 μg was selected for mice since initial experiments showed decreased alcohol-induced locomotor stimulation without affecting locomotor activity *per se*. In rats, a dosage of 0.05 μg was initially chosen as it showed no impact on locomotor activity *per se*. An additional dose of 0.1 μg was tested in a drinking trial with female rats, as the lower dose failed to affect alcohol intake. Previous studies have injected sCT into other reward-related regions using following doses; 0.005 μg bilaterally in the laterdorsal tegmental nuclei (LDTg), 0.4 μg bilaterally in the VTA and 0.02 μg bilaterally in the NAc shell (Kalafateli et al., 2021b).

SURGICAL PROCEDURES

INTRACRANIAL SURGERY

Surgical procedures were used to facilitate *in vivo* collection of monoamine and metabolites (**Paper I-IV**), measure G-protein-coupled activation-based (GRAB) fluorescence in NAc shell (**Paper IV**), and enable local injection into the paraventricular thalamus (PVT) (**Paper III**).

GUIDE AND PROBE

The same surgical process was performed to place the guide (facilitate local drug infusion) and probe (measurement of transmitters) on mice and rats. The rodents were positioned in a stereotaxic frame (David Kopf Instruments, Tujunga, CA, USA) under anesthesia with isoflurane (Isofluran Baxter, Apoteket AB, Gothenburg, Sweden) administered via a pump (Univentor 400 Anesthesia Unit, Univentor Ldt., Zejtun, Malta). The rodents were maintained on a heating pad to prevent hypothermia and pain relief was administered by local application of Xylocaine Adrenaline (5 µg/ml; Pfizer Inc, New York, NY, USA) and SC injection of Caprofen (5 mg/kg; Astra Zeneca, Gothenburg, Sweden). The skull bone was exposed and holes for the guide and/or probe (see table 1 for coordinates) and two anchoring screws were drilled. The guide was implanted 1 mm below the surface of the brain and the probe was immediately placed in NAc shell. Both the guide and/or the probe were anchored to the screw and the skull bone with dental cement (DENTALON® plus; Agntho's AB, Lidingö, Sweden).

VIRUS INJECTION AND OPTIC FIBERS LOCATION

The rats were anesthetized with ketamine (100 mg/kg; Midwest Veterinary Supply, Valley Forge, PA) and Xylazine (10 mg/kg; Akron Animal Health, Lake Forest, IL). Using an 8 mm microinjector, the rat was infused with the virus AAV9- hSyn-GRAB_DA1h (500nL; nL; $1x10^{\wedge 12}$ vg/mL, Addgene) into NAc shell. Optic fibers (9 mm, Doric Lenses) were implanted 0.03 mm dorsal to the virus injection and cemented in place. The rats were allowed 21 days of recovery from surgery and for maximal expression of GRAB_DA1h.

Table 1. Coordinates used for each specific brain region and purpose (Paxinos and Watson, 2007). Anterior/Posterior (AP) relative to bregma, Medial/Lateral (ML) relative to midline, Dorsal/Ventral (DV) relative to the skull. SD (Sprague Dawley). FG (fluorogold)

Coordinates						
	Gender, rodent type	**Motive**	**AP**	**ML**	**DV**	**Paper**
PVT	Male mice, NMRI	Guide	-1.3	0	-3.4	III
	Male rat, Wistar	Guide	-2.5	0	-6.0	III
	Female rat, Wistar	Guide	-2.4	0	-5.5	III
NAc shell	Male mice, NMRI	Probe	+1.3	±0.6	-4.7	I, III, IV
	Male rat, Wistar	Probe	+1.85	±1.0	-7.8	II
	Female/male rat, SD	Vrius	+1.5	±0.6	-7.5	IV
	Female/male rat, SD	FG inj	+1.5	±0.6	-7.5	III

CATHETER SURGERY

A surgical procedure to implant a jugular catheter was performed to allow I.V. self-administration of cocaine (**Paper IV**). Rats were anesthetized with ketamine (100 mg/kg; Midwest Veterinary Supply, Valley Forge, PA) and Xylazine (10 mg/kg; Akron Animal Health, Lake Forest, IL). An indwelling catheter (SAI Infusion Technologies, Lake Villa, IL) was inserted into the right jugular vein and secured with sutures. The catheter was routed to a mesh backmount platform implanted subcutaneously between the shoulder blades. To prevent infection and ensure patency, catheters were flushed daily with Timentin (0.2 ml; 0.93 mg/ml; Fisher, Pittsburg, PA, US) dissolved in heparinized 0.9 % saline.

IN VIVO EXPERIMENTS

The present thesis used a combination of different behavioral and neurochemical experiments as summarized in table 2.

Table 2. *In vivo* experiments used that are represented in the thesis

***In vivo* experiments**			
Experiment	**Species**	**Sex**	**Paper**
Intermittent drinking paradigm	Rat	Male/Female	I, II, III
Locomotor activity	Rat/Mouse	Male/Female	I, III, IV
In vivo microdialysis	Rat/Mouse	Male	I, II, III, IV
Condition place preference	Mouse	Male	I, III
Palatable food intake	Mouse	Male	I
Cocaine self-administration	Rat	Male	IV
Elevated plus maze	Rat	Male/Female	I
PICA	Rat	Male	II, IV

INTERMITTENT DRINKING PARADIGM

A key factor in the development of AUD is prolonged high alcohol intake, to simulate this in a rodent model, we utilized the two-bottle choice intermittent access paradigm with 20% alcohol, an established model (Kalafateli et al., 2019b, Vallöf et al., 2020). The model is considered to demonstrate high face and predictive validity, accurately reflecting the behaviors observed in humans (Nieto et al., 2021). The effect of treatments on alcohol intake in rodents can indicate potential therapeutic effectiveness for AUD patients, as naltrexone reduced alcohol intake in rodent models, validating the predictive value of these preclinical studies for human outcomes (De Oliveira Sergio et al., 2024).

Intermittent drinking paradigm were applied in **Papers I-III**. During three 24-hour sessions (Monday, Wednesday and Friday) each week the

rats were given access to two bottles – one containing alcohol and the other containing water – at the beginning of the dark phase. Rats were exposed to alcohol during a baseline period (5-10 weeks) before initiation of any experiment. To ensure balanced alcohol consumption across treatment groups, we applied a design that generated equally sized groups with comparable baseline alcohol intake. Throughout the drinking experiments, we measured the effects of treatment on alcohol intake, food and water consumption, total fluid intake (water plus alcohol), alcohol preference (the amount of alcohol divided by the total fluid intake), and changes in body weight (calculated as post-treatment weight minus pre-treatment weight) at 24 hours.

ACUTE TREATMENT

Acute treatment was assessed in **Papers I, II, and III** where the rats were given treatment of semaglutide, combination treatment with dulaglutide and sCT or locally infused sCT into mid-PVT on one occasion.

REPEATED TREATMENT

Repeated treatments were conducted in **Papers I and II**, to assess repeated treatment effect and potential tolerance development. Building tolerance to treatment poses a challenge, as it can diminish the efficacy of the intervention over time (Löscher and Schmidt, 2006). The rats received semaglutide or the combination therapy of dulaglutide and sCT on 5-6 alcohol-drinking sessions.

ALCOHOL DEPRIVATION EFFECT

The alcohol deprivation model was used in **Papers I and II**, to model abstinence-induced/relapse-like drinking (Vallöf et al., 2016a, Kalafateli et al., 2019a, Sanchis-Segura and Spanagel, 2006). Patients with AUD frequently experience relapse during attempts to abstain from alcohol, a fundamental characteristic of the AUD diagnosis (Koob, 2014). Rats were deprived from alcohol for 9 days after the baseline drinking. The rats were then given semaglutide or the combination therapy of dulaglutide and sCT or vehicle before alcohol exposure.

LOCOMOTOR EXPERIMENTS

Locomotor activity experiments are quick and easy to conduct, providing an initial indication of pharmacological effects. However, it is important to consider variability, as locomotor activity can be

influenced by external factors such as handling, noise, and light (Klein et al., 2022, Rinwa et al., 2024).

Locomotor experiments are in the thesis used in three different ways, general locomotor activity, drug-induced locomotor stimulation, and exploration/novelty seeking. The rodent was placed in the middle of an open field box and the distance traveled, our primarily outcome, together with other parameters, was registered with infrared laser beams or with a camera.

GENERAL LOCOMOTOR ACTIVITY

Dose-response studies were conducted in **Paper III,** mice and rats were administered various sCT doses locally in PVT to investigate whether sCT affected locomotion *per* se. Locomotor activity tests in cocaine-experienced rats were conducted in **Paper IV**, to determine if the effect of semaglutide on cocaine intake was due to general locomotor suppression.

DRUG-INDUCED LOCOMOTOR STIMULATION

Drugs of abuse stimulate locomotion, an indication of increased dopamine release in NAc (Blomqvist et al., 1992). Acute drug-induced locomotor stimulation experiments were performed in **Papers I, III, and IV,** where the effect of semaglutide or sCT locally administered into mid-PVT was evaluated on drug-induced (alcohol or cocaine) locomotor stimulation.

EXPLORATION AND NOVELTY SEEKING

To further explore the treatment effect on dopaminergic neurotransmission, behaviors driven by dopamine, such as exploration and novelty seeking (Costa et al., 2014), were tested. In **Paper I**, were semaglutide's effect on exploratory behaviors and novelty-seeking investigated. Semaglutide or vehicle was administered, and the rat was placed in the center of the open field box. The rat was allowed to explore the arena for 60 minutes and then removed shortly. A novel object was placed in the center of the arena and the rats were given two minutes to explore the arena and the novel object. Time spent in inner/novel zones and corners, as well as zone entries, were analyzed.

IN VIVO MICRODIALYSIS

In vivo microdialysis enables the collection of samples, in small specific tissue regions, over extended periods in freely moving animals (Bazzu et al., 2012). This method enables the monitoring of dynamic neurochemical changes in the brain and makes it possible to measure a wide range of substances, including neurotransmitters and metabolites, without the need for genetic modifications (Bazzu et al., 2012). However, it is important to consider that probe implantation can cause tissue damage (Dykstra et al., 1992), which may influence the interpretation of the results.

In vivo microdialysis was performed in freely moving rodents in **Papers I-IV**, to investigate dopamine levels in the NAc shell as this area shows a strong dopamine release in response to alcohol and cocaine (Nitya Jayaram-Lindström, 2016). In **Papers I, III, and IV,** alcohol or cocaine was administered following treatment of semaglutide or sCT to evaluate drug-induced dopamine release. In contrast, **Paper II** focused on the effect of combined therapy with dulaglutide and sCT on dopamine release *per se*. The animals were a couple of days after surgery connected to the microdialysis set-up and samples were after 2 hours of habituation collected every 20 minutes. The collected samples were analyzed through an electrochemical detection in two HPLC apparatuses, according to a modified protocol (Lindgren et al., 2010).

IN VIVO FIBER PHOTOMETRY

In vivo fiber photometry enables real-time imaging of neural activity and neurotransmitter dynamics with high temporal resolution (Simpson et al., 2024). This capability allows research to capture rapid changes in neural signaling, making it particularly valuable for studying dynamic processes such as reward responses (Simpson et al., 2024). However, there are some limitations to consider, related to tethering and tissue damage, that need to be considered in data interpretation.

Rats were catheterized and injected with AAV9-hSyn-GRAB_DA1h (500nL; nL; 1x10^12 vg/mL, Addgene) into the medial shell of the NAc using an 8 mm microinjector (Protech International Inc.). Optic fibers (9 mm, Doric Lenses) were stereotaxically implanted 0.03 mm dorsal to the virus injection site in the NAc shell and cemented in place. GRAB fluorescence was recorded for 10 min. Each session began with a 5 min

block in which a baseline GRAB signal was recorded followed by an i.v. infusion of vehicle or cocaine and a subsequent 5-minute block in which GRAB signal was recorded.

CONDITION PLACE PREFERENCE

Condition place preference of alcohol reward-dependent memory retrieval (mCPP) is a valuable model for studying drug-associated behaviors and underlying mechanisms (Nieto et al., 2021). It provides a quantifiable measure of context-specific drug-seeking behavior that is widely used in addiction research (Prus et al., 2009). While CPP effectively models how environmental context reinforces drug-seeking, the complexity of memory formation can complicate result interpretation (Bisaz et al., 2014). Variability from the interplay of reward, memory, and environmental factors highlights the need for careful experimental design (Rinwa et al., 2024).

mCPP was employed in **Papers I and III.** These studies evaluated the effects of systemic semaglutide treatment or sCT locally infused into the mid-PVT, on memory of alcohol reward. This method was conducted in a box with distinct visual and tactile cues on each side (custom made), which the mice learned to associate with the presence or absence of reward in 20-minute-long sessions. The paradigm includes three phases: pre-conditioning (day 1), conditioning (day 2-5), and post-conditioning (day 6). The preference for the post-conditioning day was scored and the CPP expression was calculated as the percentage difference in time spent in the alcohol-paired (least preferred) compartment between post- and pre-conditioning.

PALATABLE FOOD INTAKE

Palatable food consumption offers valuable insight into the potential influence of treatment on non-drug-related forms of reward (Berridge, 1996).

The effect of semaglutide on palatable food intake was investigated in **Paper I.** Mice were pre-exposed to rewarding food such as, peanut butter or Nutella, when group housed. On the day of the experiment, they received an injection of semaglutide or vehicle and were then placed in an individual cage with free access to chow and the rewarding

food. Consumption of chow, rewarding food, preference, and caloric intake were measured after 2- and 4 hours.

COCAINE SELF-ADMINISTRATION

Cocaine self-administration serves as a valuable model for exploring the neurobiological mechanisms underlying reward and addiction associated with cocaine use (Panlilio and Goldberg, 2007). It enables the study of various aspects of addiction, including the acquisition, maintenance, extinction, and reinstatement of drug-seeking behavior. However, this model has limitation, as studies typically span relatively short durations, potentially restricting its utility for long-term addiction modeling. Additionally, the need for catheterization in animals may introduce factors that could influence their behavior and physiological responses.

The rats recovered for 7 days after surgery before cocaine-self-administration started. Rats were then placed in the self-administration boxes for 2 hours; they were allowed to lever press for intravenous infusions of cocaine in a fixed ratio 1 (FR1) schedule. A stable response on FR1 resulted in a switch to a fixed-ratio 5 (FR5) schedule. In all different experiments, each rat served as its own control, using a counterbalanced, within-subject design, as previously described (Hernandez et al., 2019).

COCAINE SELF-ADMINISTRATION

In **Paper IV**, once the rats demonstrated stable responses at an FR5 schedule, the effects of semaglutide or vehicle on cocaine self-administration were evaluated to investigate semaglutide's impact on cocaine-seeking behavior.

PROGRESSIVE RATIO

The effect of semaglutide on the progressive ratio was investigated in **Paper IV** to evaluate the motivation to self-administer cocaine. After treatment with semaglutide or vehicle was the rat placed in the operant box, and the response requirement for each subsequent infusion increased until the rat failed to meet a requirement.

REINSTATEMENT

The effects of semaglutide on the reinstatement of cocaine-seeking behavior were investigated in **Paper IV**, a defining feature of CUD is

the high rate of relapse during periods of abstinence (Koob and Le Moal, 2001, Dackis and O'Brien, 2001). Rats were maintained without access to cocaine until the behavior had been extinguished. Subsequently, the rats were pretreated with vehicle or semaglutide before receiving an acute priming dose of cocaine. Rats were placed into the operant boxes for a two-hour reinstatement session.

EPM

Anxiety may confound the treatment's effect on alcohol-related responses. The elevated plus maze (EPM) assesses anxiety-related behaviors, such as open-arm avoidance and risk assessment (Rodgers and Dalvi, 1997, Campos et al., 2013). Testing conditions, including the rodent's starting position and orientation, can influence results. Proper handling and acclimation of animals are essential for reliable outcomes (Rinwa et al., 2024).

The effects of semaglutide on anxiety-like behavior were investigated in **Paper I.** After administration with semaglutide or vehicle the rat was placed in the center of the EPM, which consists of two closed and two open arms. Time spent in open/closed arms and the central zones was analyzed by Observer XT (Noldus, Wageningen, Netherlands).

PICA

Another confounding factor in assessing the effects of gut-brain peptides on drug-induced responses is malaise (Filippatos et al., 2014, Lean et al., 2014, Boccia et al., 2022). The PICA method evaluates emetic potential in rodents, which lack a vomiting reflex, by providing access to kaolin clay (Takeda et al., 1993). While this approach does not fully replicate the complex physiological mechanisms of emesis in species capable of vomiting, it offers valuable indications of nausea-like behavior.

The effect of semaglutide or the combination treatment of dulaglutide and sCT on kaolin intake was conducted in **Papers II and IV.** Rats were habituated to kaolin clay in their hanging wire cages where they were single housed. Intake of food, kaolin, and water, as well as body weight, were measured 24h after treatment.

EX VIVO EXPERIMENTS

QPCR

Quantitative polymerase chain reaction (qPCR) a method that measures gene expression, and offers high sensitivity and specificity, minimizing false-positive results (Zhang et al., 2021). This method also has high-throughput capability, enabling the simultaneous analysis of numerous samples (Zhang et al., 2021). However, it is expensive and highly dependent on the quality and quantity of the samples, emphasizing the importance of meticulous sample preparation (Zhang et al., 2021).

Gene expression was evaluated in **Papers I and III** by quantitative polymerase chain reaction (qPCR)**.** In **Paper I,** the expression of dopamine-metabolizing enzymes - monoamine oxidase A (MAOA) and B and catechol-O-methyltransferase (COMT) - as well as DAT in the NAc and VTA, was investigated in mice treated with semaglutide + alcohol or vehicle + alcohol. In **Paper III** the expression of CTR and RAMP1 was analyzed from PVT brain punches. Low- and high-alcohol-consuming rats were compared, to evaluate the impact of alcohol consumption levels on the expression of CTR and RAMP1.

ELISA

Enzyme-linked immunosorbent assay (ELISA) is a widely used technique for detecting and quantifying specific antigens or antibodies (Alhajj et al., 2025). It is straightforward, easy to perform, and allows for the simultaneous analysis of multiple samples. However, insufficient blocking or cross-reactivity can lead to potential false-positive or false-negative results, emphasizing the need for careful optimization and validation (Terato et al., 2014, Aydin, 2015).

The effect of semaglutide on cyclic adenosine monophosphate was measured in the NAc, amygdala, VTA, and NTS in **Paper I**, as it is a key downstream signaling molecule of GLP-1R. Brain punches from semaglutide or vehicle-treated male mice were analyzed with ELISA (Catalog number: ADI-900-066A) according to the manufacturer's instructions.

IMMUNOFLUORESCENCE

Immunofluorescence is a powerful technique for detecting and localizing specific antigens in tissue samples (Im et al., 2019). It offers high sensitivity and specificity, capable of identifying even low levels of target proteins, and supports multi-color imaging for the simultaneous visualization of multiple targets (Im et al., 2019). However, the method is susceptible to photobleaching, which can diminish signal intensity over time, highlighting the need for careful handling and imaging conditions (Im et al., 2019).

A fluorescently labeled version of semaglutide (CY3-semaglutide) was in **Paper I** administered systemically to evaluate the possibility of it reaching NAc shell. In **Paper III** a fluorescently labeled version of sCT (FAM-sCT) was injected ICV to investigate whether sCT binds to the AMYR in mid-PVT. Following injections in both experiments, the rats were perfused, their brains removed and post-fixed, and subsequently sectioned into coronal slices. In **Paper I** the slices were washed and directly mounted, whereas in **Paper III** the slices were stained for microtubule, neurons and cell nuclei and then mounted. The slices were visualized using a ZEISS Axio observer microscope.

RNASCOPE

RNAscope is an advanced technique the detecting and visualizing specific RNA molecules within intact cells and tissues (Wang et al., 2012). This method offers high sensitivity and specificity, allowing for the detection of single RNA molecules with precise spatial resolution (Wang et al., 2012). It also supports multiplexing, allowing simultaneous visualization of multiple RNA targets (Wang et al., 2012). However, RNAscope requires thin tissue sections, specialized equipment, and reagents making it a costly approach.

In **Paper III**, retrograde trace fluorogold (FG) was injected into the NAc shell to investigate the projection from mid-PVT to the NAc shell. RNAscope was then used to detect CTR, GABAergic neurons, glutamatergic neurons, and cell nuclei to identify which neurons that project to the NAc shell and whether these neurons are co-localized with the receptor of interest. The dissected brains were postfixed overnight, and coronal sections of PVT were sliced using a sliding microtome (Leica SM2000R, Leica Microsystems; Nuss Loch, Germany)

WESTERN BLOT

Western blot is a widely used analytical technique for detecting and analyzing specific proteins (Gavini and Parameshwaran, 2025). Western blot is time-consuming and generally less sensitive than ELISA, making it more difficult to detect low-abundance proteins (Gavini and Parameshwaran, 2025).

In **Paper III,** western blot was used to measure protein levels of CTRa and CTRb in the thalamus, aiming to investigate the presence of CTR in this region. Punches of the thalamus were homogenized, and protein concentrations were measured. The electrophoresis was performed, and the membrane was incubated with primary antibodies for CTRa and CTRb (1:1000), overnight. The reference protein used was Anti-COX IV. The secondary antibodies (1:5000) were then incubated for 1 hour. Following washing, the membrane was dried and visualized using a SpectraMax i3v Platform.

STATISTICS AND VISUALISATION

All analyses were performed in GraphPad Prism (GraphPad Software Inc; CA, USA). The western Blot membrane bands were quantified using the ImageJ software (public domain software, NIH; MD, USA) before statistical analysis. The most appropriate analyses were selected based on experimental setup and outcome variables. With two groups included, a t-test (paired or unpaired) was performed. For comparisons involving three or more groups, one-way ANOVA was used. When repeated measures were involved, a repeated-measures two-way ANOVA was applied. The most appropriate post-hoc tests were then selected. Figures included in this thesis were created with BioRender.

RESULTS

This section summarizes the key findings from each study included in this thesis. Detailed descriptions of results, graphs, and statistical analyses are available in the corresponding articles and manuscripts.

PAPER I

This study aimed to evaluate the potential of the GLP-1R agonist semaglutide to influence alcohol-related responses in rodents and to investigate the underlying mechanisms driving these effects. We could reveal that semaglutide, both acutely and repetitively administrated, decreased alcohol consumption and prevented relapse-like drinking in male and female rats (Figure 2). Furthermore, fluorescently labeled semaglutide was detected in the NAc shell of alcohol-drinking rats of both sexes, and semaglutide reduced alcohol-induced locomotor activity and accumbal dopamine release in male mice. These findings indicate that semaglutide influences the mesolimbic dopamine system.

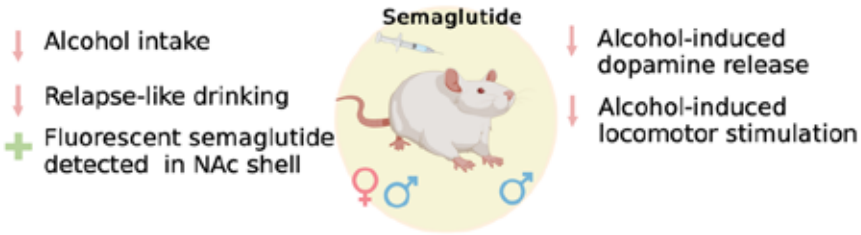

Figure 2. Semaglutide decreases alcohol intake and relapse-like drinking in male and female rats. Additionally, fluorescent semaglutide is detected in the nucleus accumbens (NAc) shell in male and female rats. Furthermore, semaglutide decreases alcohol-induced locomotor stimulation and dopamine release in NAc shell in male mice.

Additionally, in male mice, semaglutide pretreatment before alcohol administration increased the levels of the dopamine metabolites DOPAC and HVA in the NAc shell. Semaglutide upregulates expression of the genes COMT and MAOA - which encode enzymes involved in dopamine metabolism, in mice when alcohol was onboard. These findings suggest that semaglutide suppresses dopamine release by enhancing dopamine metabolism. In contrast, semaglutide did not alter

the memory consolidation of alcohol reward in male mice. This study also revealed that acute and repeated semaglutide treatment decreased food intake and body weight in rats of both sexes. To further demonstrate semaglutide's capacity to suppress reward-associated behaviors, we found that it reduced the consumption of highly rewarding foods such as peanut butter and Nutella, and increased dopamine-driven behaviors such as exploration and novelty-seeking. In the study with rewarding foods, semaglutide increased chow intake as a compensatory response. Overall, semaglutide decreased total caloric intake and reduced the preference for rewarding food. Activity and anxiety are potential confounding factors in the observed reduction in alcohol intake. Semaglutide increased locomotor activity in males and extended time spent in the inner zone in females. While semaglutide did not affect anxiety-like behaviors in male rats, it caused a slight increase in such behaviors in female rats.

PAPER II

This paper investigated the potential synergy of a GLP-1R and AMYR agonist combination in reducing alcohol intake. An initial test revealed that combination treatment of dulaglutide and sCT (0.1 mg/kg + 5 µg/kg) decreased alcohol intake in male rats but not in females. We, therefore, further evaluated a potential synergistic effect in male rats. We first defined doses of sCT and dulaglutide that did not alter alcohol intake *per se* (Figure 3). Thereafter, three dulaglutide and sCT dose combinations were evaluated (**A:** 0.05 mg/kg + 1 µg/kg, **B:** 0.05 mg/kg + 2 µg/kg, and **C:** 0.075 mg/kg + 2 µg/kg). Combinations A and B did not alter alcohol intake, while combination C decreased alcohol intake and prevented relapse-like drinking in male rats, suggesting a synergistic-like effect. However, during repeated treatment over two weeks, combination C decreased alcohol intake initially, but the effect subsided during later sessions. Importantly, combination C did not increase kaolin intake, a marker of malaise.

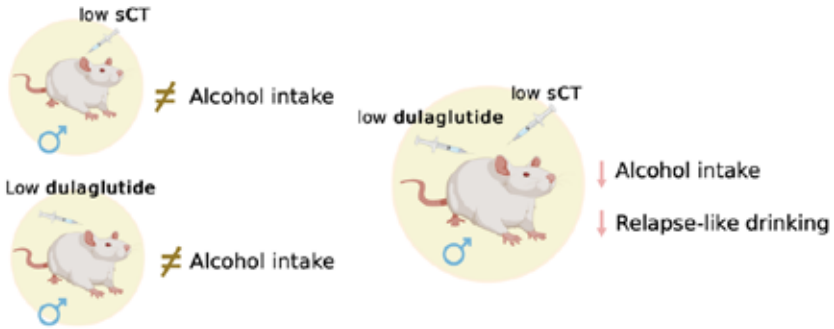

Figure 3. Low doses sCT and dulaglutide monotherapy do not affect alcohol intake, but their combination reduces consumption and relapse-like drinking in male rats.

Additionally, combinations A and C reduced food intake, whereas combination B did not affect this parameter. All three combinations led to a reduction in body weight. Treatment with the higher dose combination (0.1 mg/kg + 5 µg/kg) decreased both food intake and body weight in both sexes. Higher doses (0.2 mg/kg + 6 µg/kg and 0.4 mg/kg + 7 µg/kg) were tested in female rats to determine whether the lack of response on alcohol intake in females was dose dependent. Both combinations effectively reduced alcohol intake in female rats. Similarly, these doses significantly decreased food consumption and led to reductions in body weight.

PAPER III

This paper aimed to identify whether AMYR in mid-PVT contributes to the effects of alcohol-related behaviors in male and female rodents. CTR, the main component of the AMYR, was, for the first time, identified in the thalamus and, more specifically, in the mid-PVT in both male and female rats (Figure 4). Furthermore, locally administrated sCT in mid-PVT decreased alcohol intake in male rats, but not in females despite testing a higher dose in females. Further experiments revealed that CTR was identified on glutamatergic projections from mid-PVT to NAc in male rats, whereas this was not evident in females, suggesting a sex-specific difference in the mechanism. Additionally, in male mice, local infusion of sCT into the mid-PVT attenuated alcohol-induced locomotor stimulation and dopamine release in the NAc shell. These findings further support the role of AMYR in the mid-PVT in mediating alcohol-related responses. Lastly, sCT locally infused in mid-PVT did not alter the memory consolidation of alcohol reward.

Figure 4. Salmon calcitonin (sCT) locally infused into the middle part of the paraventricular nucleus of the thalamus (mid-PVT) decreased alcohol intake in male rats but not in females. A marker for the calcitonin receptor (CTR) was found in mid-PVT in both sexes. However, CTR was found on glutamatergic neurons projecting to the nucleus accumbens (NAc) in male rats but not in females. Additionally, sCT locally infused in mid-PVT decreased alcohol-induced locomotor stimulation and dopamine release in NAc shell in male mice.

Further sex differences in responses to sCT were found as locally infused sCT into mid-PVT decreased food intake in both sexes with different doses acquired (0.05 µg in males and 0.1 µg in females). sCT reduced body weight in male rats, while body weight in female rats was unchanged with sCT treatment locally infused into mid-PVT.

PAPER IV

This paper aimed to explore the effect of semaglutide on various cocaine-taking behaviors and the elevation of dopamine induced by cocaine in male rodents to determine if its promising impact on alcohol-related responses could also extend to cocaine. Semaglutide dose-dependently reduced cocaine self-administration, the motivation to consume cocaine, and cocaine-seeking in male rats (Figure 5). Furthermore, semaglutide attenuated cocaine-induced locomotor stimulation in male mice. *In vivo,* microdialysis in male mice revealed that semaglutide treatment attenuated the ability of cocaine to elevate dopamine levels in the NAc shell. Similarly, fiber photometry in rats demonstrated reduced cocaine-induced elevation of dopamine levels following semaglutide treatment, providing further support for semaglutide's effect on reward-related responses.

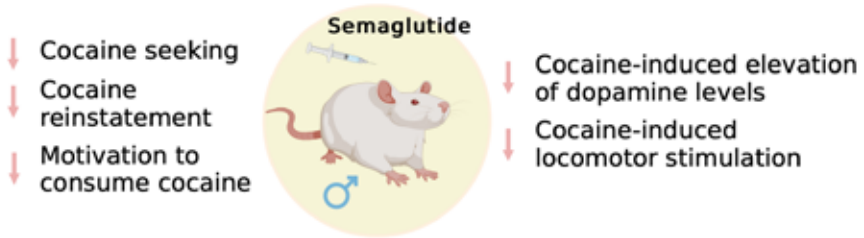

Figure 5. Semaglutide reduced cocaine self-administration, cocaine-seeking behavior, and the motivation to consume cocaine in male rats. Additionally, semaglutide attenuated cocaine-induced locomotor stimulation and decreased dopamine levels in the nucleus accumbens shell.

Moreover, in cocaine-experienced rats, semaglutide decreased food intake, body weight, and water intake without altering kaolin intake, a measurement of malaise to exclude that as a confounding factor. Another confounding factor is the presence of changes in locomotion, semaglutide did not affect this parameter.

DISCUSSION

This section summarizes and discusses key findings from the four studies, aiming to support the overarching conclusions outlined earlier. It addresses the implications and limitations of these findings, focusing on critical aspects such as drug intake, reward-related responses, food intake, and body weight change. Additional topics discussed include tolerance effects, synergism, sex differences, and the overlapping and distinct mechanisms underlying drug and food intake.

EVIDENCE SUPPORTING GLP-1R AND AMYR AGONISTS AS TREATMENT FOR SUD

Recent studies have demonstrated that gut-brain peptides play a role in modulating alcohol- and cocaine-related responses. However, further research is needed to develop more effective treatment approaches and to better understand the underlying mechanisms of action. **Paper I, II,** and **IV** in this thesis contribute to understanding how the promising effects on drug-related responses can be maintained at treatment with lower doses to minimize side effects. This was achieved either by utilizing a more potent GLP-1R agonist (semaglutide; **Papers I and IV**) or by combining two gut-brain peptides (GLP-R agonist dulaglutide + AMYR agonist, sCT; **Paper II**). Further, **Paper III** contributed to new insights into AMYR in PVT, an area that had previously not been studied.

VOLUNTARY INTAKE OF ALCOHOL- AND COCAINE

Semaglutide, a compound with several advantages over other GLP-1R agonists, (Bucheit et al., 2020) shows promising results for treating AUD and CUD (**Papers I and IV)**. Semaglutide decreases voluntary intake of both alcohol and cocaine. These results align with previous studies on GLP-1R activation, both systemically and within reward-related brain regions, where various GLP-1R agonists have been shown to reduce alcohol and cocaine intake (Schmidt et al., 2016, Vallöf et al., 2020, Vallöf et al., 2016a, Vallöf et al., 2019a, Hernandez et al., 2019, Sørensen et al., 2015). Recent clinical evidence supports our findings as semaglutide reduces alcohol consumption in individuals with type II diabetes and/or obesity (Quddos et al., 2023, Richards et al., 2023,

Qeadan et al., 2024, Wang et al., 2024b). However, semaglutide exhibits higher affinity and potency for the GLP-1R compared to other agonists (Bucheit et al., 2020), suggesting that it may have a greater effect on reducing alcohol and cocaine intake. Our study revealed promising reductions in voluntary intake of alcohol and cocaine with low doses, which can offer the advantage of less side effects.

Furthermore, the complexity of AUD's underlying mechanisms indicates that combining agents could enhance treatment efficacy. In **Paper II,** the study revealed that combining a GLP-1R agonist, dulaglutide, and an AMYR agonist, sCT synergistic-like decreased alcohol intake in male rats. Previous studies have shown that dulaglutide and sCT as monotherapies suppress alcohol intake (Vallöf et al., 2020, Kalafateli et al., 2019b) and that combining GLP-1R and AMYR agonists produces synergistic effects on food intake and body weight in rodents and non-human primates (Liberini et al., 2019, Bello et al., 2010). In line with this, combined GLP-1R agonist and amylin analog therapy has shown promising results in patients with obesity and diabetes, highlighting the clinical relevance of this combination therapy (Wong et al., 2023, Frias et al., 2023). However, in the present study, this was the first time their potential synergism was evaluated on alcohol intake.

Understanding the complex mechanisms underlying AUD would aid in the development of novel treatment options. Previous research on the PVT has demonstrated its involvement in reward-related processes. In **Paper III**, we revealed for the first time that CTR, a key component of AMYR, is present in mid-PVT. Additionally, we found that local infusion of sCT into the mid-PVT suppresses alcohol intake in male rats but not in females, even when a higher dose was tested. Previous research has demonstrated that AMYR is present in other reward-related areas such as the LDTg, VTA, and NAc shell in male rats (Kalafateli et al., 2021b). Studies have shown that activation of AMYR in these regions modulates alcohol-related responses in male rodents, together indicating that AMYR expressed in various brain regions contributes to the regulation of alcohol-related responses.

RELAPSE-LIKE BEHAVIORS WITH ALCOHOL AND COCAINE

Relapse is a severe risk for individuals with SUD, as studies consistently show high relapse rates across various substances and populations (Mhaidat et al., 2024, Domino et al., 2005, Connor et al., 2016). Semaglutide and the combining treatment of dulaglutide and sCT not only reduces intake of the drug but also helps maintain abstinence by reducing relapse behaviors. Specifically, semaglutide (**Paper I**) and the combining treatment of dulaglutide and sCT (**Paper II**) decreased relapse-like drinking in both male and female rats when alcohol was reintroduced after a 10-day deprivation period. Similarly, semaglutide (**Paper IV**) lowers cocaine reinstatement, a well-established model of relapse (Shaham et al., 2003), in male rats. Previous studies support these findings, demonstrating that GLP-1R agonists effectively reduce relapse behaviors associated with both alcohol (Vallöf et al., 2016a, Thomsen et al., 2017, Thomsen et al., 2020) and cocaine (Hernandez et al., 2019, Hernandez et al., 2021, Hernandez et al., 2018). Indeed, studies with sCT have previously been shown to prevent relapse-like drinking in rats (Kalafateli et al., 2019a).

These findings presented in **Papers I, II, and IV** suggest that lower doses of GLP-1R and AMYR agonists could serve as effective treatment options for individuals with SUD, potentially minimizing the risk of side effects.

GLP-1R AGONISTS POTENTIAL AS TREATMENT OPTIONS FOR OTHER TYPES OF SUD

Previous research has highlighted the potential of GLP-1R agonists as treatment options for other types of SUD as well, such as opioid use disorder and cannabis use disorder. Preclinical studies have demonstrated that GLP-1R agonists can effectively reduce opioid intake and seeking behaviors (Douton et al., 2022, Douton et al., 2021, Zhang et al., 2020). Additionally, a retrospective cohort study revealed that patients prescribed GIP and/or GLP-1R agonists exhibited a lower rate of opioid overdose among those with opioid use disorder (Qeadan et al., 2024). Moreover, a large retrospective cohort study found that semaglutide was associated with a reduced incidence and relapse of cannabis use disorder in patients with obesity or diabetes type II (Wang et al., 2024a).

These data suggest that semaglutide is not only a promising candidate for treating AUD and CUD but may also be effective for other types of SUD.

REWARD-RELATED RESPONSES

Both alcohol and cocaine increase dopamine levels in NAc reflecting their rewarding properties, which are linked to hyperlocomotion in rodents (Blomqvist et al., 1992, Di Michele et al., 1998, Imperato and Di Chiara, 1986, Ito et al., 2000). Human studies highlight the clinical relevance of this mechanism, as imaging research demonstrates that drugs of abuse increase dopamine levels in the striatum, where NAc is located (Drevets et al., 2001) linked with self-reported feelings of euphoria in clinical settings (Ramchandani et al., 2011, Yoder et al., 2007).

DRUG-INDUCED LOCOMOTOR STIMULATION

Papers I and IV reveal that semaglutide attenuates drug-induced (alcohol or cocaine) locomotor stimulation in male mice. Likewise, locally infused sCT into mid-PVT shows decreased alcohol-induced locomotor stimulation in **Paper III**. This is consistent with previous research showing that activation of GLP-1R and AMYR attenuates alcohol and cocaine-induced locomotor stimulation in male rodents (Egecioglu et al., 2013b, Egecioglu et al., 2013a, Kalafateli et al., 2019b, Kalafateli et al., 2021a, Vallöf et al., 2016a, Sørensen et al., 2015, Vallöf et al., 2019a, Vallöf et al., 2019b). Further evidence highlights the role of AMYR in modulating drug-induced reward, as sCT reduces both alcohol-induced locomotor stimulation (Kalafateli et al., 2019b), as well as similar responses induced by cocaine (Kalafateli et al., 2021a)

DOPAMINE LEVELS IN NAC SHELL

In line with the results in drug-induced locomotor stimulation, a reflection of increased dopamine levels in NAc, **Papers I and IV** demonstrated that semaglutide attenuated drug-induced elevation of accumbal dopamine levels in NAc shell. Likewise, locally infused sCT into mid-PVT shows similar results in **Paper III**. This is consistent with previous research showing that activation of GLP-1R and AMYR attenuates drug-induced elevation of accumbal dopamine levels in the NAc shell (Egecioglu et al., 2013b, Egecioglu et al., 2013a, Kalafateli

et al., 2019b, Kalafateli et al., 2021a, Vallöf et al., 2016a, Sørensen et al., 2015, Vallöf et al., 2019a, Vallöf et al., 2019b). Further evidence highlights the role of AMYR in modulating drug-induced elevation of dopamine levels, as sCT reduces alcohol-induced dopamine release in the NAc shell (Kalafateli et al., 2019b).

Taken together, the findings found on elevated locomotor stimulation and dopamine levels in NAc shell by drugs of abuse suggests that the effects on alcohol and cocaine taking is modulated by reward-related responses.

MEMORY CONSOLIDATION OF ALCOHOL REWARD

Furthermore, humans and animals quickly learn to associate cues and context with addictive drugs (Hyman et al., 2006). Once established, these cues strongly drive drug-seeking behaviors (Hyman et al., 2006). In **Paper I,** we found that semaglutide does not alter the memory consolidation of alcohol reward, consistent with previous studies involving liraglutide (Vallöf et al., 2016a). These findings suggest that these treatments primarily target the acute rewarding effects of alcohol rather than the memory processes associated with alcohol reward. In contrast, shorter-acting agonists such as GLP-1 and Ex4 seem to reduce the memory consolidation of alcohol reward (Egecioglu et al., 2013b, Shirazi et al., 2013). Different GLP-1R agonists differ in their affinity and binding for the GLP-1R, contributing to varied efficacy, duration of action, and pharmacological profiles (Garber, 2011), a possible explanation for different responses on the same behavior. Furthermore, previous studies show that local activation of GLP-1R by Ex4 in the NAc shell and NTS attenuates the memory of alcohol reward, whereas activation in the VTA and LDTg has no such effect (Vallöf et al., 2019a, Vallöf et al., 2019b), suggesting that the NAc shell and NTS play a more critical role in modulating memory processes associated with alcohol reward.

Moreover, results from **Paper III** reveal that local activation of AMYR in mid-PVT does not alter the memory consolidation of alcohol reward, in line with previous studies showing local activation of AMYR in LDTg, VTA, and NAc shell. However, systemic treatment with sCT attenuated the memory consolidation of alcohol reward (Kalafateli et al., 2019b), suggesting that other brain regions than PVT and neurotransmitter systems are involved in the processing of reward-

dependent memory expression (Berke and Hyman, 2000, Hyman et al., 2006).

POTENTIAL MECHANISMS BEHIND THE OBSERVED EFFECTS

SUD is a complex condition, with the reasons for consuming a particular substance varying between individuals. The mechanism through which GLP-1R and AMYR agonists affect SUD is unlikely to involve a single pathway; instead, a combination of different mechanisms is probably at play. Notably, addictive drugs share the ability to influence the reward system and increase dopamine levels in the NAc (Cheer et al., 2004, Gooding et al., 2024, Engel and Jerlhag, 2014b, Aragona et al., 2008), though via distinct pathways. Importantly, GLP-1R and AMYR agonists are known to modulate drug-induced elevations of dopamine in the NAc (Egecioglu et al., 2013b, Egecioglu et al., 2013a, Kalafateli et al., 2019b), which may represent a key mechanism underlying their effect on substances of abuse, a finding also observed in this thesis. Additionally, substance use disorder is influenced by multiple pathways, suggesting that the impact of AMYR and GLP-1R on various substances of abuse may involve distinct mechanisms. For instance, GLP-1R modulation may alter stress-induced drug use, as the central GLP-1 system is activated in response to stress (Holt and Trapp, 2016). Furthermore, growing evidence highlights the role of neuroinflammatory processes in SUD. Substance use over a prolonged period induces neuroinflammation, which can lead to functional and structural neuroadaptations that perpetuate SUD (Agarwal et al., 2022). Therefore, the anti-inflammatory effects of GLP-1R agonists may contribute to their efficacy in treating SUD (Alharbi, 2024). Pharmacological interventions that induce sedation or impair motor function may reduce alcohol consumption simply by limiting the animal's ability to access or consume alcohol. However, motor function studies conducted in the various experiments do not support the presence of such effects. Further, nausea is a known side effect of both GLP-1R and amylin agonists (Filippatos et al., 2014, Boccia et al., 2022, Lean et al., 2014), which could potentially influence alcohol intake. However, the studies presented in this thesis suggest that the observed effects are not attributed to nausea or malaise, as kaolin intake, a marker for such conditions, remains unchanged. Moreover, pharmacological

interventions may induce behavioral changes, such as increased anxiety, which could indirectly affect alcohol consumption. While anxiety has not been extensively assessed in this thesis, the anxiety test conducted in **Paper I** revealed no signs of anxiety in males, while semaglutide slightly enhanced anxiety-like behaviors in female rats, potentially influencing treatment outcomes. These studies were performed in alcohol-naïve rats, and future research should examine these effects in alcohol-experienced rats.

SEMAGLUTIDE TENTATIVELY REDUCES DOPAMINE LEVELS IN NAC SHELL THROUGH ENHANCED DOPAMINE METABOLISM

Findings from **Paper I** suggest that alcohol-induced dopamine release is reduced via semaglutide's ability to enhance dopamine metabolism. Specifically, *in vivo* microdialysis revealed that semaglutide enhanced the levels of DOPAC and HVA in the NAc when alcohol was onboard. Furthermore, gene expression analysis showed that semaglutide increased the expression of COMT and MAOA, when alcohol was present. These enzymes play a crucial role in the metabolism of dopamine (Graves et al., 2020, Finberg, 2019), suggesting that semaglutide may modulate alcohol effects by altering dopamine metabolism in NAc shell. This mechanism may also influence cocaine-induced dopamine levels; however, alcohol and cocaine increase dopamine in the NAc shell through different mechanisms. Cocaine binds to the DAT and modulates dopamine levels via this interaction (Verma, 2015), while alcohol increases the dopamine release in NAc shell (Boileau et al., 2003). In **Paper I**, we found no evidence that semaglutide modulates the expression of DAT in the NAc or VTA when administered prior to alcohol exposure. This suggests that semaglutide does not alter DAT expression in these regions. It remains possible that semaglutide affects DAT in function or through other mechanisms within these areas. Previous research has shown that GLP-1R activation enhances DAT surface expression and function in the lateral septum, promoting more efficient dopamine reuptake and potentially reducing synaptic dopamine levels (Reddy et al., 2016). Further studies are needed to explore the mechanisms underlying semaglutide's effect on cocaine-related responses and to further examine its potential mechanisms of action in alcohol-related responses.

AMYR IN MID-PVT PLAYS A ROLE IN SUPPRESSING ALCOHOL-RELATED RESPONSES IN MALES

For the first time, we identified the presence of CTR, a main component of AMYR, in the PVT (**Paper III**), a brain region of interest in the reward context. We initially identified CTR in the thalamus by measuring CTR protein levels. Further analysis, including gene expression profiling, labeled sCT binding to CTR, and RNA detection of CTR, demonstrated that CTR is present in the PVT, specifically within the mid-PVT. It is well known that PVT integrates signals related to appetite, motivation, and energy states from various brain regions (Millan et al., 2017, Petrovich, 2021). Furthermore, PVT neurons respond to hunger signals and drive food-seeking behaviors through connections with the NAc (Penzo and Gao, 2021). Previous research shows that the PVT is involved in regulating food intake and reward processing, functions that are heavily influenced by gut-brain peptides (Ong et al., 2017, Hamlin et al., 2009, Christoffel et al., 2021). Furthermore, prior evidence suggests the involvement of gut-brain peptides in the PVT, with previous research identifying GLP-1R on PVT neurons projecting to the NAc (Ong et al., 2017). The projection from PVT to NAc is confirmed in **Paper III**. Moreover, **Paper III** further revealed that CTR in the mid-PVT is colocalized with markers for glutamatergic neurons rather than GABAergic neurons. These findings align with previous studies showing that PVT-to-NAc projections are predominantly glutamatergic (Barson et al., 2020, Zhu et al., 2016).

TOLERANCE EFFECT

Repeated treatments have been assessed in **Papers I** and **II**. We learned that repeated treatment with semaglutide decreased alcohol intake on several occasions in both male and female rats, consistent with other GLP-1R agonists (Vallöf et al., 2020, Vallöf et al., 2016a). These findings indicate that no tolerance is developed for the alcohol-suppressing properties. However, the dual treatment with dulaglutide and sCT decreased alcohol intake initially but did not persist throughout the treatment period. Earlier studies with dulaglutide do not indicate any tolerance effects and a prolonged decrease of ethanol intake is observed in males following treatment discontinuation (Vallöf et al., 2020). On the contrary repeated treatment with sCT has revealed tolerance effects when it comes to both alcohol- and food-suppressing properties

(Kalafateli et al., 2019a, Chelikani et al., 2007). This suggests that the observed tolerance may result from the tolerance effect of sCT. Tolerance development often involves changes in receptor expression or function in response to repeated drug exposure. This principle could apply to sCT and its effect on AMYR or CTR in reward-related brain areas. It is known that repeated calcitonin exposure downregulated the CTR in osteoclasts (Samura et al., 2000, Wada et al., 1996). Another possible explanation is behavioral adaptation. Organisms may adapt behaviorally to the presence of sCT, due to compensatory mechanisms within the reward pathway that diminish the suppressive effects of sCT on alcohol intake. However, recent studies on other dual amylin and calcitonin receptor agonists have shown promising results in maintaining their efficacy over time, suggesting a potential resistance to tolerance effects (Larsen et al., 2021, Gydesen et al., 2017, Andreassen et al., 2014). Studies have shown that KBP-042, a dual amylin and calcitonin receptor agonist demonstrates a more potent activation of the amylin and calcitonin receptor than sCT (Andreassen et al., 2014). This enhances receptor activation and thus likely contributes to the prolonged effects observed. While these findings are promising for obesity and type II diabetes treatment, the effects of KBP-042 on alcohol intake have not been examined. However, another study with dulaglutide and sCT demonstrated that adding sCT to an ongoing dulaglutide treatment reduced alcohol intake in both male and female rats on several alcohol-drinking sessions in contrast to when sCT and dulaglutide were initiated simultaneously (Aranäs et al., 2024). These findings suggest that altering the treatment paradigm could potentially overcome the observed tolerance effect.

In summary, while semaglutide appears to have long-term effects on drug-related behaviors, the combined treatment exhibits a tolerance effect that requires further investigation and optimization before it can be developed as a potential treatment option for SUD.

SYNERGISM

Given the complexity of the mechanisms underlying AUD, combining therapeutic agents may enhance treatment efficacy. An advantage of a potential synergistic effect is that it allows for the use of lower doses in treatment, which may help minimize adverse effects. Previous research has shown that a combination of naltrexone and/or antismoking agents

such as varenicline or bupropion synergistically reduces alcohol intake in rodents (Söderpalm et al., 2020, Zhou et al., 2019, Ray et al., 2021, Nicholson et al., 2018). Moreover, treatment with tirzepatide, a compound that targets both GLP-1R and glucose-dependent insulinotropic polypeptide receptors (GIPR), has shown promising results in reducing alcohol intake (Quddos et al., 2023). In contrast, the combination of semaglutide with the antismoking agents varenicline or bupropion did not demonstrate any superior effect on alcohol intake compared to semaglutide alone (Aranäs et al., 2023). In **Paper II**, we investigated the combined effects of dulaglutide and sCT, both of which have been shown to reduce alcohol intake as monotherapies (Vallöf et al., 2020, Kalafateli et al., 2019a). Previous research demonstrated that combining a GLP-1R agonist and an AMYR agonist produces a synergistic-like reduction in food intake and body weight (Liberini et al., 2019). Similarly, we found that subthreshold doses of dulaglutide and sCT, which do not independently affect alcohol intake, produced a synergistic-like reduction in alcohol consumption when combined (combination C, 0.075 mg/kg dulaglutide + 2 μg/kg sCT). The same dose combination also demonstrated an effect on relapse-like drinking, significantly reducing alcohol intake upon reintroducing alcohol after a period of abstinence. However, repeated treatment revealed a tolerance effect on alcohol intake, as discussed above, likely attributed to sCT, which has been previously shown to induce tolerance (Kalafateli et al., 2019a). The combined treatment loses efficacy when tolerance to sCT develops, reinforcing the evidence that dulaglutide and sCT work synergistically to reduce alcohol consumption. This further highlights the critical role of their synergy at these doses in achieving the observed reduction in alcohol intake.

GLP-1 and amylin receptor agonists activate distinct yet interconnected neural circuits. Previous research has shown that dual activation of these receptors enhances c-Foc activation in the dorsal vagal complex, indicating a more robust neural response (Liberini et al., 2019). While GLP-1 and amylin exert complementary peripheral actions – GLP-1 receptor agonists stimulate insulin secretion from pancreatic β-cells and amylin analogs reduce insulin requirement (Wong et al., 2023, Roth et al., 2012) – it remains unclear whether these mechanisms influence alcohol intake. Given that both amylin and GLP-1 target reward-related brain areas (Vallöf et al., 2019a, Kalafateli et al., 2021b) combination therapy may more effectively modulate dopaminergic

signaling in these regions, potentially contributing to reduced alcohol consumption.

In conclusion, the insights gained from the synergistic-like effects observed in this study could serve as a foundation for further research into developing effective treatment options for SUD.

SEX DIFFERENCES

The discovery of neurobiological sex differences in mechanisms associated with SUD and evidence showing that women achieve better outcomes in programs tailored to their specific needs underscore the importance of gender-specific treatment approaches (Harris et al., 2022, Agabio et al., 2017). These findings highlight that men and women may benefit from distinct strategies in addressing SUD, reflecting the necessity of personalized care to optimize treatment effectiveness for each group.

Several studies in this thesis include both male and female rats, revealing potential sex-dependent effects. In **Paper I**, semaglutide reduced alcohol intake both acutely, repeatedly, and after a period of abstinence in both sexes, with similar results. However, a slight difference in sensitivity and response magnitude was observed between the sexes. Semaglutide significantly decreased alcohol intake during five alcohol-drinking sessions in females compared to three in males. **Paper II** revealed that higher doses of the dulaglutide and sCT combination are required to reduce alcohol intake in female rats compared to male rats. A previous study found that dulaglutide treatment affected alcohol intake differently between the sexes, with males exhibiting a more pronounced and longer-lasting reduction in alcohol intake post-treatment compared to females (Vallöf et al., 2020). sCT treatment as monotherapy has solely been examined in male rats; however, AM1213, a selective long-acting amylin analog, has shown an initial decrease in alcohol intake in both sexes. Alcohol consumption was increased at later sessions in males, while consumption in females returned to baseline drinking (Kalafateli et al., 2021c). While semaglutide appeared to have similar results on alcohol intake between the sexes, dulaglutide showed a greater impact in males, underscoring the importance of conducting sex-specific research to advance understanding and the development of tailored treatment.

In **Paper III**, a sex difference was observed on alcohol intake and the associated neurochemical pathway in the mid-PVT. sCT locally administered into mid-PVT decreased alcohol intake in male rats but not in female rats, even though a higher dose was examined. CTR was found in mid-PVT of both sexes and no significant difference was observed between the sexes in the expression of the receptor. However, when analyzing the co-localization of CTR on glutamatergic neurons projection to NAc shell was a significant difference found between the sexes as a possible explanation for the different responses to alcohol intake. **Paper IV** primarily focused on male subjects in the majority of experiments; however, cocaine-induced dopamine levels were found to decrease in both sexes following semaglutide pretreatment. Previous research has found that males show a greater mesolimbic dopamine response than females when consuming alcohol, demonstrating a higher sensitivity to alcohol-rewarding effects (Dir et al., 2017, Becker and Koob, 2016). Since the effects of gut-brain peptides on alcohol intake appear to arise from their modulation of the reward circuitry, this may provide a possible explanation for the observed sex-dependent effects of dulaglutide and sCT having a greater impact in males than in females. Earlier findings have demonstrated sex differences with other types of treatment; for example, acute administration of bupropion and naltrexone reduced alcohol intake in male mice but not in female mice (Zhou et al., 2019). These findings are supported by some clinical evidence showing that naltrexone is effective in men but not in women with AUD (Garbutt et al., 2005, O'Malley et al., 2007). In contrast, other studies have shown that women experienced significantly greater reductions in craving scores compared to men in a study involving extended-release naltrexone for alcohol use (Herbeck et al., 2016). This underscores the need for further investigation into sex differences in treatment against AUD.

Furthermore, sex-specific variations in AMYR expression have been observed, with notable differences in CTR expression in the striatum of zebra finches (Zachar et al., 2019). Different expression and expression patterns of GLP-1R and/or AMYR could potentially contribute to sex-based differences in alcohol intake responses. Another possible factor is the pharmacokinetic properties of the drugs. A pharmacokinetic analysis of liraglutide revealed that its exposure was 32% higher in women than in men of comparable weight (Overgaard et al., 2016), indicating potential differences in drug absorption or elimination between sexes.

Additionally, sex hormones may influence the gut-brain axis, which can impact the central nervous system and endocrine system. However, further research is needed to fully elucidate this mechanism.

Taken together, the sex-specific effects observed in this thesis emphasize the need to incorporate both males and females into preclinical research for maximize benefits for both sexes.

FOOD INTAKE AND BODY WEIGHT

Gut-brain peptides are well-recognized for their role in regulating food intake by enhancing feelings of satiety, thereby contributing to reduced caloric consumption and subsequent decreases in body weight. In **Paper I**, semaglutide reduced food intake and body weight in both male and female rats, with a more prominent decrease in body weight in male rats than in females, consistent with the results on the alcohol intake. These findings are further supported by the results in **Paper IV**, where semaglutide, at all doses, decreased both food intake and body weight in male rats. Likewise, prior studies have shown that semaglutide reduces body weight and food intake in rodents (Gabery et al., 2020). In contrast, in clinical studies, sex differences have been consistently observed, with women generally experiencing greater weight loss than men (Jensterle et al., 2023). Possible explanations for the differences observed can be that women have higher fat mass relative to lean mass, which may contribute to a more pronounced response to treatment, as it targets pathways involved in appetite regulation and fat metabolism (Aldhoon-Hainerová et al., 2014). Differences in hormonal regulation and metabolic responses between sexes may also play a role (Trevaskis et al., 2010, Koceva et al., 2024). In **Paper II,** the combined treatment of dulaglutide and sCT decreased food intake and body weight in both sexes, demonstrating a synergistic-like effect consistent with previous findings (Liberini et al., 2019). In **Paper III**, local administration of sCT into the mid-PVT reduced body weight in males but not in female rats. This aligns with findings using the selective long-acting AMYR agonist AM1213, which reduced body weight in both sexes but showed a more pronounced effect in males (Kalafateli et al., 2021c). In summary, these findings highlight sex-specific differences in body weight loss, underscoring the need for further investigation to optimize dosing strategies to maximize benefits for both sexes.

OVERLAPPING AND DISTINCT MECHANISMS

Gut-brain peptides play a role in both homeostatic needs (hunger) and hedonic (pleasure-driven) aspects of feeding. Gut-brain peptides convey information about the body's nutritional state to the brain, particularly the hypothalamus (Li et al., 2023, Timper and Brüning, 2017). Gut-brain peptides also influence neural systems involved in reward and motivation, thereby modulating hedonic feeding and reward of alcohol and cocaine (Woodward et al., 2022). In **Paper I**, semaglutide was shown to reduce the intake of palatable food, suggesting that it modulates hedonic feeding. This is further supported by previous studies reporting similar findings, such as a decrease in chocolate consumption in mice (Gabery et al., 2020). Additionally, our results revealed an increase in chow intake as compensation for decreased intake of palatable food intake, aligning with prior research that observed a nonsignificant rise in chow consumption following semaglutide treatment (Gabery et al., 2020). These findings suggest that the reduction in palatable food consumption is likely mediated through reward-related mechanisms, rather than solely through GLP-1R's effects on satiety. This dual effect – decreasing hedonic eating while increasing homeostatic feeding – points to a complex role of GLP-1R in regulating both reward-driven and energy-driven food intake. In **Papers I, II, III,** and **IV**, treatment with various gut-brain peptides decreased chow intake, suggesting an effect on homeostatic feeding. Lower doses of the combination treatment with dulaglutide and sCT (**Paper II**) reduced food intake but not alcohol intake, and local administration of sCT into the mid-PVT (**Paper III**) decreased food intake in females without affecting alcohol intake. Moreover, the lowest dose of semaglutide examined on cocaine intake indeed decreased food intake without altering the self-administration of cocaine intake (**Paper IV**). This suggests that the anorexigenic effects of gut-brain peptides on food consumption may involve more sensitive or distinct neural circuits than those regulating alcohol consumption. It has been concluded that central and peripheral GLP-1 systems independently regulate food consumption via distinct gut-brain circuits (Brierley et al., 2021). Previous research has demonstrated that central GLP-1 systems are involved in suppressing alcohol and cocaine intake (Hernandez et al., 2019, Hernandez et al., 2018, Vallöf et al., 2019a). However, further studies

are needed to determine whether both central and peripheral GLP-1 systems contribute to this effect. Moreover, with repeated treatment using the combination of dulaglutide and sCT (**Paper II**), we observed a consistent decrease in food intake and body weight across all time points, whereas alcohol intake was only reduced initially, likely due to a tolerance effect of sCT (Kalafateli et al., 2019a).

These findings support our hypothesis that food and alcohol intake share some common mechanisms while engaging distinct action pathways.

LIMITATIONS

A limitation of this thesis is the absence of female subjects in some experiments, particularly in **Paper IV**. Our findings, especially in **Papers II and III,** reveal several sex-dependent effects, emphasizing the importance of investigating both sexes in future studies. Further, the inclusion of different rat strains in **Papers II and III** could be considered as a potential limitation. Similarly, the use of both mice and rats in **Papers I, III and IV** may also present limitations. Another limitation is that repeated treatment with sCT shows a tolerance effect in **Paper II**, particularly in alcohol intake suppression. This limits the long-term effectiveness of this treatment and necessitates further exploration of strategies to mitigate tolerance development. Moreover, **Paper II** suggests a potential synergistic effect from the combination of a GLP-1R and an AMYR agonist. However, a limitation is the absence of advanced mathematical modeling to rigorously confirm this synergistic effect. An additional limitation in **Paper II** is that only the combination treatment of Combination C was evaluated in the abstinence-induced drinking test, while monotherapy at these doses was not assessed for its effect on this behavior. Although semaglutide (**Papers I and IV**) appears more effective than other GLP-1R agonists, direct comparative studies between semaglutide and other GLP-1R agonists are lacking. Furthermore, while GLP-1R and AMYR agonists modulate alcohol and cocaine intake (**Papers I and IV**), their impact on poly-substance use remains unexplored, limiting their application in more complex SUD scenarios. Most studies in this thesis (**Papers I-IV**) focus on acute or short-term outcomes, with a limited investigation into the long-term impact of GLP-1R and AMYR agonists on alcohol or cocaine use. Additionally, rodent models do not fully replicate the

complexity of human SUD, including psychological, social, and environmental factors. The predictive validity of these models for clinical outcomes remains a limitation, highlighting the need to examine these substances in a clinical setting. In **Paper III**, mid-PVT is investigated in behavioral studies; however, a limitation is the lack of exploration of the anterior and posterior parts of PVT. In **Papers II and III,** sCT is used as an agonist for amylin and calcitonin receptors; its tolerance-inducing effects pose limitations for long-term studies.

CONCLUSION

In summary, the findings from this thesis highlight the critical role of gut-brain peptides, particularly GLP-1 and amylin, in modulating alcohol- and cocaine-related responses.

Semaglutide at low doses demonstrated significant potential in reducing both voluntary intake and relapse behaviors associated with alcohol and cocaine. Further, the combination of GLP-1R and amylin agonists showed synergistic-like effects in suppressing alcohol intake and relapse in male rats, suggesting a novel approach to enhancing treatment efficacy. An advantage of using lower doses while maintaining efficacy in reducing drug intake is the potential to minimize side effects. Furthermore, new evidence revealed the presence of CTR in the mid-PVT with its activation playing a role in reducing alcohol-related responses in males. The involvement of these peptides in reward-related responses, along with their ability to modulate dopamine levels, provides insights into potential therapeutic strategies for SUD. Notably the sex-specific effects observed within this thesis highlight the importance of including both sexes in future research. Overall, the research underscores the potential of gut-brain peptides as key players in the development of effective treatments for AUD and CUD.

FUTURE PERSPECTIVES

Gaining knowledge often raises new questions. Reflecting on the findings of this thesis, there are several ways in which this work could be further advanced.

It would be valuable to investigate further the neurocircuitry underlying semaglutide's effects on alcohol-related behaviors. Our findings in **Paper I** suggest that reduced alcohol intake is linked to a decrease in alcohol-induced dopamine release via increased dopamine metabolism. This could be explored further in female rodents and possibly in alcohol-experienced rodents of both sexes to determine whether prior alcohol exposure influences the outcome. Additionally, as evidenced by elevated levels of DOPAC and HVA, along with upregulated expression of the dopamine-metabolizing enzymes MAOA and COMT, future studies could use MAOA or COMT inhibitors to assess whether this metabolic mechanism is critical to the observed effects. Furthermore, we demonstrated in **Paper I** that a fluorescently labeled semaglutide compound binds in the NAc. However, further studies are necessary to determine the precise localization of semaglutide binding, such as whether it is positioned on neurons, astrocytes, or other cell types and whether semaglutide crosses the blood-brain barrier in alcohol-experienced rats to reach NAc. Previous studies have indicated that semaglutide does not cross the blood-brain barrier but can still interact with the brain through alternative mechanisms in alcohol-naïve rodents (Gabery et al., 2020). Alcohol consumption, however, has been shown to impair the integrity of the blood-brain barrier by disrupting tight junction proteins, which are essential for maintaining its structure (Carrino et al., 2021). Therefore, further research is needed in alcohol-experienced animals to determine whether semaglutide might cross the compromised blood-brain barrier in these cases and reach the brain. Given semaglutide's higher potency and affinity for GLP-1R, it would be interesting to compare its efficacy against other GLP-1R agonists and semaglutide administered orally to determine whether it offers a superior reduction in alcohol intake and cocaine self-administration. Furthermore, in **Paper I**, semaglutide did not alter anxiety-like behavior in males but slightly increased anxiety-like behavior in females. This outcome warrants further investigation in alcohol-experienced rats. In **Paper II,** further exploration of optimal dosing in female rats would be

beneficial, as higher doses were required to reduce alcohol intake in this group. Further research into the tolerance effect observed with sCT is also warranted, including potential strategies to mitigate or overcome this limitation. Additionally, evaluating other combinations of GLP-1R and AMYR agonists, such as semaglutide paired with a more long-acting AMYR agonist like cagrilinitide, could yield promising results. In **Paper III** we found that CTR is present in PVT and through all three different subregions (anterior, mid, and posterior) in both sexes. Despite this, the infusion of sCT, an CTR and AMYR agonist, locally in mid-PVT decreased alcohol intake in males but not in females probably due to differences in the amylinergic pathway between the sexes. It would be valuable to investigate this further, specifically examining sCT locally administrated in anterior and posterior PVT on alcohol-related responses in both sexes. It would further be interesting to explore the effect of infusions of sCT in the different subregions on dopamine release in NAc. While previous studies have established that activation of both GLP-1R and AMYR in the NAc, and other reward-related areas decreases alcohol intake in male rats, it would be valuable to examine these effects in both sexes and evaluate potential sex differences. Investigating sex-dependent differences through measures of sex hormones in male and female subjects, could provide deeper insights into their influence on gut-brain peptide signaling and alcohol intake, as sex-hormones is known to impact AUD (Erol et al., 2019). Additionally, selecting a more selective AMYR agonist for the experiments described above, while excluding the involvement of CTR, could provide deeper insights into the role of AMYR in alcohol-related responses. In **Paper IV** we showed that semaglutide decreases cocaine self-administration, motivation to consume cocaine, and cocaine seeking in male rats. Future studies should investigate the effect of semaglutide on cocaine-related responses in female rodents. Furthermore, it would be interesting to discover more about the neurocircuitry underpinning the results observed in male rats in **Paper IV**. We demonstrated that semaglutide decreased cocaine-induced dopamine release in both cocaine-naïve male mice and cocaine-experienced male and female rats. However, the underlying mechanism behind the reduced dopamine levels remains unclear. Future studies investigating semaglutide's effect on DAT would be valuable, as previous research has shown that GLP-1R activation enhances DAT surface expression and function in the lateral septum (Reddy et al., 2016). Finally, it would be intriguing to investigate

the effects of GLP-1R and AMYR on poly-substance use. The comorbidity between AUD and CUD can be attributed to both biological and social factors, as pathological alcohol use enhances cocaine use, or reverse cocaine use intensifies alcohol use (Griffin et al., 2017). Additionally, it would be interesting to explore the role of gut-brain peptides in other SUDs and behavioral addictions, such as gambling disorder and gaming disorder.

ACKNOWLEDGEMENT

First, I will express my gratitude to my supervisor **Elisabet**. Thank you for always being supportive and for being the best mentor I could have hoped for. Your trust in me and the freedom you've given me in our work together have meant so much to me. You are truly an inspiration with your drive and passion for science. Your positive feedback has been invaluable, helping me grow and gain confidence. I have genuinely enjoyed every single day of this journey and am deeply thankful for the years I've spent in your lab.

Also, thanks to my co-supervisors **Mia** and **Lars** for your support and encouragement. Thank you to my off-label co-supervisor **Jörgen**. It has been a privilege to have you participate in the meetings and to receive your encouragement along the way. I am deeply grateful for your kind words and for generously sharing your never-ending knowledge of science.

I have been lucky to have the opportunity to gain valuable expertise from my co-workers in the lab. Thank you, **Christian** and **Lindsay**, for your support in both experimental work and writing. Your expertise and guidance have meant so much to me. A special thanks to **Daniel**, **Lydia,** and **Jesper**, who warmly welcomed me to the lab. I truly appreciate your helpfulness and generosity in sharing your scientific knowledge, as well as the experimental support you've provided. Your encouragement during the moments when my confidence wavered has been invaluable. I am also deeply grateful to my post-doc colleagues, **Olesya** and **Max**, with whom I had the privilege of spending a couple of years. Thank you for sharing your scientific insights and for being such wonderful additions to the lab. Thank you, **Qian,** for sharing your expertise in molecular techniques. It was amazing having you in the lab! To **Sarah,** thank you for being an incredible support, as well as a dear friend and colleague. You've managed to trick me a couple of times, but I'm still looking forward to working with you for a longer period ;). Your sincerity, positive energy, and thoughtfulness mean so much to me. Thank you, **Yasmine**, for your company at all the conferences and for your encouragement and listening – it has meant a lot to me. Thank you

to **Sebastian**, **Gustav**, **Emilia**, **Sirinthra**, and all the other master students I've had the privilege to meet over the years. Lastly, a big thank you to all my co-workers at Farmen for creating a nice work environment.

A heartfelt thank you to my US lab group, the Schmidt lab. **Heath,** I'm so grateful for the months I spent in your lab with you and your amazing team. You all made me feel so welcome, and those months were incredibly meaningful to me, both scientifically and personally. It's a time I often reflect on as a great memory for both me and my family. A special thank you to **Antonia** for guiding me through all the experiments. It was such a pleasure to spend time with you and get to know you – you truly made the experience unforgettable. A big thank you as well to **Riley** and **Rae**, who were always there with a helping hand and ready to support whenever needed.

I would also like to extend my gratitude to our exceptional animal caretakers. A special and heartfelt thank you to **Kate, Teres, Rolf** and **Hanna,** who have taken care of most of "my" animals over the years. Thank you for doing an outstanding job and for always being kind, approachable, and open to discussions.

I also want to express my warm appreciation to my dearest friends for all the support and encouragement. First, thanks to my closest childhood friends, **Max, Axel, Mia,** and **Hanna,** for always being there for me. The support and thoughtfulness you've shown me from our teenage years to today have been incredibly important. A special thank you to Axel – not only for being a wonderful friend but also for introducing me to one of my best friends, your amazing partner. **Tora** thank you for being such a warm and kind friend and for always keeping your door open to me. **Lisa,** where do I even begin? You are my closest friend, who is always there for me, whether I need to share bad news or celebrate the good moments. Thank you for being my sounding board, always ready to listen and offer your support. Thank you for welcoming me so warmly into your family; your family has been a true source of support for me, and I cherish the bond we've built. Also, a heartfelt thank you to all the other friends in my life who continue to support me and bring so much joy into my days.

A big thank you to my partners in crime during the pharmacy program – **Frida, Anas,** and **Reab**. Your unwavering support and friendship throughout both my pharmacy education and PhD studies have meant so much to me. I'm deeply grateful for all the great times we shared, whether it was studying together or creating wonderful memories outside of academia.

Many thanks to my family for all the love and support. **Mum**, thank you for always being there and for putting me and my brother first, no matter what. Your constant care and dedication have meant the world to me. **Dad**, thank you for sparking my passion for science and for supporting me throughout this journey. I wish you could be here today to see me finish, but I'm grateful that you were a part of this path. I miss you. **Olle**, my little brother, and my best friend, I appreciate you and your family more than words can express. Having you by my side through both the happy and difficult times means so much to me. Your pride in my work truly touches my heart. My dear aunt **Eva,** thank you for your support and care and for all the thoughtful advice and encouragement you've given me over the years. To my family-in-law: **Malin**, **Stefan**, **Wilma**, **Albin**, and mormor **Marianne**, thank you for being the most wonderful bonus family I could ever have hoped for when I met Anton. Your kindness and love have made my life all the richer.

Last but certainly not least, a huge thank you to my amazing family. **Anton**, my better half, thank you for always standing by my side and offering your unwavering support. Your love and calming presence, especially when I'm stressed about deadlines, mean the world to me. I'm so grateful for your help with the technical details and for making our home a warm and inviting place. Thank you for supporting my dream of working in the US by coming with me together with Erwin and making it a reality. And above all, thank you for being the most incredible dad to our sons. To my two sons, **Erwin** and ***little brother* –** little brother, you'll have to wait until the dissertation to join us ;) – thank you for giving my life meaning and perspective. I could never have imagined the depth of love I'd experienced until you came into my life. **Jag älskar er!**

REFERENCES

ADEMAR, K., ADERMARK, L., SÖDERPALM, B. & ERICSON, M. 2022. Sodium acamprosate and calcium exert additive effects on nucleus accumbens dopamine in the rat. *Addict Biol,* 27**,** e13224.

ADEMAR, K., LOFTÉN, A., NILSSON, M., DOMI, A., ADERMARK, L., SÖDERPALM, B. & ERICSON, M. 2023. Acamprosate reduces ethanol intake in the rat by a combined action of different drug components. *Sci Rep,* 13**,** 17863.

AGABIO, R., PISANU, C., GESSA, G. L. & FRANCONI, F. 2017. Sex Differences in Alcohol Use Disorder. *Curr Med Chem,* 24**,** 2661-2670.

AGABIO, R. & SINCLAIR, J. M. A. 2019. 'Mother's ruin'-Why sex and gender differences in the field of alcohol research need consideration. *Alcohol and alcoholism (Oxford),* 54**,** 342-344.

AGARWAL, K., MANZA, P., CHAPMAN, M., NAWAL, N., BIESECKER, E., MCPHERSON, K., DENNIS, E., JOHNSON, A., VOLKOW, N. D. & JOSEPH, P. V. 2022. Inflammatory Markers in Substance Use and Mood Disorders: A Neuroimaging Perspective. *Front Psychiatry,* 13**,** 863734.

AHLENIUS, S., CARLSSON, A., ENGEL, J., SVENSSON, T. & SÖDERSTEN, P. 1973. Antagonism by alpha methyltyrosine of the ethanol-induced stimulation and euphoria in man. *Clin Pharmacol Ther,* 14**,** 586-91.

ALDHOON-HAINEROVÁ, I., ZAMRAZILOVÁ, H., DUŠÁTKOVÁ, L., SEDLÁČKOVÁ, B., HLAVATÝ, P., HILL, M., HAMPL, R., KUNEŠOVÁ, M. & HAINER, V. 2014. Glucose homeostasis and insulin resistance: prevalence, gender differences and predictors in adolescents. *Diabetol Metab Syndr,* 6**,** 100.

ALHAJJ, M., ZUBAIR, M. & FARHANA, A. 2025. Enzyme Linked Immunosorbent Assay. *StatPearls.* Treasure Island (FL) ineligible companies. Disclosure: Muhammad Zubair declares no relevant financial relationships with ineligible companies. Disclosure: Aisha Farhana declares no relevant financial relationships with ineligible companies.: StatPearls Publishing

Copyright © 2025, StatPearls Publishing LLC.

ALHARBI, S. H. 2024. Anti-inflammatory role of glucagon-like peptide 1 receptor agonists and its clinical implications. *Ther Adv Endocrinol Metab,* 15**,** 20420188231222367.

ANDREASSEN, K. V., FEIGH, M., HJULER, S. T., GYDESEN, S., HENRIKSEN, J. E., BECK-NIELSEN, H., CHRISTIANSEN, C., KARSDAL, M. A. & HENRIKSEN, K. 2014. A novel oral dual amylin and calcitonin receptor agonist (KBP-042) exerts antiobesity and antidiabetic effects in rats. *Am J Physiol Endocrinol Metab,* 307**,** E24-33.

ANGARITA, G. A., MATUSKEY, D., PITTMAN, B., COSTEINES, J. L., POTENZA, M. N., JASTREBOFF, A. M., SCHMIDT, H. D. & MALISON, R. T. 2021. Testing the effects of the GLP-1 receptor agonist exenatide on cocaine self-administration and subjective responses in humans with cocaine use disorder. *Drug and alcohol dependence,* 221.

ANTON, R. F. 2008. Naltrexone for the Management of Alcohol Dependence. *New England Journal of Medicine,* 359**,** 715-721.

ARAGONA, B. J., CLEAVELAND, N. A., STUBER, G. D., DAY, J. J., CARELLI, R. M. & WIGHTMAN, R. M. 2008. Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. *J Neurosci,* 28**,** 8821-31.

ARANÄS, C., BLID SKÖLDHEDEN, S. & JERLHAG, E. 2023. Antismoking agents do not contribute synergistically to semaglutide's ability to reduce alcohol intake in rats. *Front Pharmacol,* 14**,** 1180512.

ARANÄS, C., EDVARDSSON, C. E., ZENTVELD, L., VALLÖF, D., WITLEY, S., TUFVESSON-ALM, M., SHEVCHOUK, O. T., VESTLUND, J. & JERLHAG, E. 2024. The combination of a glucagon-like peptide-1 and amylin receptor agonists reduces alcohol consumption in both male and female rats. *Acta Neuropsychiatr***,** 1-15.

ARD, J., FITCH, A., FRUH, S. & HERMAN, L. 2021. Weight Loss and Maintenance Related to the Mechanism of Action of Glucagon-Like Peptide 1 Receptor Agonists. *Adv Ther,* 38**,** 2821-2839.

AYDIN, S. 2015. A short history, principles, and types of ELISA, and our laboratory experience with peptide/protein analyses using ELISA. *Peptides,* 72**,** 4-15.

BAGGIO, L. L. & DRUCKER, D. J. 2007. Biology of Incretins: GLP-1 and GIP. *Gastroenterology,* 132**,** 2131-2157.

BARDO, M. T. 1998. Neuropharmacological mechanisms of drug reward: beyond dopamine in the nucleus accumbens. *Crit Rev Neurobiol,* 12**,** 37-67.

BARSON, J. R., MACK, N. R. & GAO, W. J. 2020. The Paraventricular Nucleus of the Thalamus Is an Important Node in the Emotional Processing Network. *Front Behav Neurosci,* 14**,** 598469.

BARTH, K. S. A. M., R. J. 2010. Disulfiram: an old therapeutic with new applications *CNS & Neurological Disorders - Drug Targets,* 9**,** 5-12.

BAZZU, G., BIOSA, A., FARINA, D., SPISSU, Y., CALIA, G., DEDOLA, S., ROCCHITTA, G., MIGHELI, R., SERRA, P. A. & DESOLE, M. S. 2012. Brain microdialysis in freely moving animals. *Methods Mol Biol,* 846**,** 365-81.

BECKER, J. B. & KOOB, G. F. 2016. Sex Differences in Animal Models: Focus on Addiction. *Pharmacol Rev,* 68**,** 242-63.

BELLO, N. T., KEMM, M. H., OFELDT, E. M. & MORAN, T. H. 2010. Dose combinations of exendin-4 and salmon calcitonin produce additive and synergistic reductions in food intake in nonhuman primates. *American journal of physiology. Regulatory, integrative and comparative physiology,* 299**,** R945-R952.

BERGMANN, N. C., DAVIES, M. J., LINGVAY, I. & KNOP, F. K. 2023. Semaglutide for the treatment of overweight and obesity: A review. *Diabetes Obes Metab,* 25**,** 18-35.

BERKE, J. D. & HYMAN, S. E. 2000. Addiction, Dopamine, and the Molecular Mechanisms of Memory. *Neuron,* 25**,** 515-532.

BERRIDGE, K. C. 1996. Food reward: brain substrates of wanting and liking. *Neurosci Biobehav Rev,* 20**,** 1-25.

BERRIDGE, KENT C. & KRINGELBACH, MORTEN L. 2015. Pleasure Systems in the Brain. *Neuron,* 86**,** 646-664.

BERRIDGE, K. C. & ROBINSON, T. E. 1998. What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience?

BISAZ, R., TRAVAGLIA, A. & ALBERINI, C. M. 2014. The neurobiological bases of memory formation: from physiological conditions to psychopathology. *Psychopathology,* 47**,** 347-56.

BLAZQUEZ-LLORCA, L., MIGUÉNS, M., MONTERO-CRESPO, M., SELVAS, A., GONZALEZ-SORIANO, J., AMBROSIO, E. & DEFELIPE, J. 2021. 3D Synaptic Organization of the Rat CA1 and Alterations Induced by Cocaine Self-Administration. *Cereb Cortex,* 31**,** 1927-1952.

BLOMQVIST, O., ENGEL, J. A., NISSBRANDT, H. & SÖDERPALM, B. 1993. The mesolimbic dopamine-activating properties of ethanol are antagonized by mecamylamine. *Eur J Pharmacol,* 249**,** 207-13.

BLOMQVIST, O., ERICSON, M., ENGEL, J. A. & SÖDERPALM, B. 1997. Accumbal dopamine overflow after ethanol: Localization of the antagonizing effect of mecamylamine. *European Journal of Pharmacology,* 334**,** 149-156.

BLOMQVIST, O., SÖDERPALM, B. & ENGEL, J. A. 1992. Ethanol-induced locomotor activity: involvement of central nicotinic acetylcholine receptors? *Brain Res Bull,* 29**,** 173-8.

BOCCIA, L., BORNER, T., GHIDEWON, M. Y., KULKA, P., PIFFARETTI, C., DOEBLEY, S. A., DE JONGHE, B. C., GRILL, H. J., LUTZ, T. A. & LE FOLL, C. 2022. Hypophagia induced by salmon calcitonin, but not by amylin, is partially driven by malaise and is mediated by CGRP neurons. *Mol Metab,* 58**,** 101444.

BOILEAU, I., ASSAAD, J. M., PIHL, R. O., BENKELFAT, C., LEYTON, M., DIKSIC, M., TREMBLAY, R. E. & DAGHER, A. 2003. Alcohol promotes dopamine release in the human nucleus accumbens. *Synapse,* 49**,** 226-31.

BOWER, R. L. & HAY, D. L. 2016. Amylin structure–function relationships and receptor pharmacology: implications for amylin mimetic drug development.

BOYLE, C. N., LUTZ, T. A. & LE FOLL, C. 2018. Amylin – Its role in the homeostatic and hedonic control of eating and recent developments of amylin analogs to treat obesity. *Molecular Metabolism,* 8**,** 203-210.

BRIERLEY, D. I., HOLT, M. K., SINGH, A., DE ARAUJO, A., MCDOUGLE, M., VERGARA, M., AFAGHANI, M. H., LEE, S. J., SCOTT, K., MASKE, C., LANGHANS, W., KRAUSE, E., DE KLOET, A., GRIBBLE, F. M., REIMANN, F., RINAMAN, L., DE LARTIGUE, G. & TRAPP, S. 2021. Central and peripheral GLP-1 systems independently suppress eating. *Nat Metab,* 3**,** 258-273.

BUCHEIT, J. D., PAMULAPATI, L. G., CARTER, N., MALLOY, K., DIXON, D. L. & SISSON, E. M. 2020. Oral Semaglutide: A Review of the First Oral Glucagon-Like Peptide 1 Receptor Agonist. *Diabetes Technol Ther,* 22**,** 10-18.

BUCHHOLZ, J. & SAXON, A. J. 2019. Medications to treat cocaine use disorders: current options. *Curr Opin Psychiatry,* 32**,** 275-281.

BURGOS, C. F., MUÑOZ, B., GUZMAN, L. & AGUAYO, L. G. 2015. Ethanol effects on glycinergic transmission: From molecular pharmacology to behavior responses. *Pharmacol Res,* 101**,** 18-29.

CADOR, M., TAYLOR, J. & ROBBINS, T. 1991. Potentiation of the effects of reward-related stimuli by dopaminergic-dependent mechanisms in the nucleus accumbens. *Psychopharmacology,* 104**,** 377-385.

CAFFREY, A., LAVECCHIA, E., MERKEL, R., ZHANG, Y., CHICHURA, K. S., HAYES, M. R., DOYLE, R. P. & SCHMIDT, H. D. 2023. PYY(3-36) infused systemically or directly into the VTA attenuates fentanyl seeking in male rats. *Neuropharmacology,* 239**,** 109686.

CAMPOS, A. C., FOGAÇA, M. V., AGUIAR, D. C. & GUIMARÃES, F. S. 2013. Animal models of anxiety disorders and stress. *Revista brasileira de psiquiatria,* 35**,** S101-S111.

CARRINO, D., BRANCA, J. J. V., BECATTI, M., PATERNOSTRO, F., MORUCCI, G., GULISANO, M., DI CESARE MANNELLI, L. & PACINI, A. 2021. Alcohol-Induced Blood-Brain Barrier Impairment: An In Vitro Study. *Int J Environ Res Public Health,* 18.

CARROLL, K. M., NICH, C., BALL, S. A., MCCANCE, E. & ROUNSAVILE, B. J. 1998. Treatment of cocaine and alcohol dependence with psychotherapy and disulfiram. *Addiction,* 93**,** 713-27.

CARROLL, M. E., FRANCE, C. P. & MEISCH, R. A. 1979. Food deprivation increases oral and intravenous drug intake in rats. *Science,* 205**,** 319-21.

CARROLL, M. R., RODD, Z. A., MURPHY, J. M. & SIMON, J. R. 2006. Chronic ethanol consumption increases dopamine uptake in the nucleus accumbens of high alcohol drinking rats. *Alcohol,* 40**,** 103-9.

CHAMBERS, S. E., COPSON, E. R., DUTEY-MAGNI, P. F., PRIEST, C., ANDERSON, A. S. & SINCLAIR, J. M. A. 2019. Alcohol use and breast cancer risk: A qualitative study of women's perspectives to inform the development of a preventative intervention in breast clinics. *Eur J Cancer Care (Engl),* 28**,** e13075.

CHAO, A. M., TRONIERI, J. S., AMARO, A. & WADDEN, T. A. 2021. Semaglutide for the treatment of obesity. *Trends Cardiovasc Med*.

CHAU, P., LIDÖ, H. H., SÖDERPALM, B. & ERICSON, M. 2018. Acamprosate's ethanol intake-reducing effect is associated with its ability to increase dopamine. *Pharmacol Biochem Behav,* 175**,** 101-107.

CHAVARRIA, J., FRIDBERG, D. J., OBST, E., ZIMMERMANN, U. S. & KING, A. C. 2021. Subjective alcohol responses in high- and low-risk adolescents: results from the Dresden Longitudinal Study on Alcohol Use in Young Adults. *Addiction,* 116**,** 1716-1724.

CHEER, J. F., WASSUM, K. M., HEIEN, M. L., PHILLIPS, P. E. & WIGHTMAN, R. M. 2004. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J Neurosci,* 24**,** 4393-400.

CHELIKANI, P. K., HAVER, A. C. & REIDELBERGER, R. D. 2007. Effects of intermittent intraperitoneal infusion of salmon calcitonin on food intake and adiposity in obese rats. *Am J Physiol Regul Integr Comp Physiol,* 293**,** R1798-808.

CHIARA, G. D. & IMPERATO, A. 1988. Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. *Proceedings of the National Academy of Sciences of the United States of America,* 85**,** 5274.

CHOI, E. A., JEAN-RICHARD-DIT-BRESSEL, P., CLIFFORD, C. W. G. & MCNALLY, G. P. 2019. Paraventricular Thalamus Controls Behavior during Motivational Conflict. *J Neurosci,* 39**,** 4945-4958.

CHRISTENSEN, J. K., MØLLER, I. W., RØNSTED, P., ANGELO, H. R. & JOHANSSON, B. 1991. Dose-Effect Relationship of Disulfiram in Human Volunteers. I: Clinical Studies. *Pharmacology & Toxicology,* 68**,** 163-165.

CHRISTOFFEL, D. J., WALSH, J. J., HEIFETS, B. D., HOERBELT, P., NEUNER, S., SUN, G., RAVIKUMAR, V. K., WU, H., HALPERN, C. H. & MALENKA, R. C. 2021. Input-specific modulation of murine nucleus accumbens differentially regulates hedonic feeding. *Nat Commun,* 12**,** 2135.

CLARKE, T. K., NYMBERG, C. & SCHUMANN, G. 2012. Genetic and environmental determinants of stress responding. *Alcohol Res,* 34**,** 484-94.

COLVIN, K. J., KILLEN, H. S., KANTER, M. J., HALPERIN, M. C., CURRIE, P. J. & ENGEL, L. 2020. Brain site-specific inhibitory effects of the GLP-1 analogue exendin-4 on alcohol intake and operant responding for palatable food. *International journal of molecular sciences,* 21**,** 1-15.

CONNOR, J. P., HABER, P. S. & HALL, W. D. 2016. Alcohol use disorders. *Lancet,* 387**,** 988-998.

COSTA, V. D., TRAN, V. L., TURCHI, J. & AVERBECK, B. B. 2014. Dopamine modulates novelty seeking behavior during decision making. *Behav Neurosci,* 128**,** 556-66.

DACKIS, C. A. & O'BRIEN, C. P. 2001. Cocaine dependence: a disease of the brain's reward centers. *J Subst Abuse Treat,* 21**,** 111-7.

DE GROOTE, A. & DE KERCHOVE D'EXAERDE, A. 2021. Thalamo-Nucleus Accumbens Projections in Motivated Behaviors and Addiction. *Front Syst Neurosci,* 15**,** 711350.

DE OLIVEIRA SERGIO, T., JANE SMITH, R., WEAN, S. E., ENGLEMAN, E. A. & HOPF, F. W. 2024. Greater inhibition of female rat binge alcohol intake by adrenergic receptor blockers using a novel Two-Shot rat binge drinking model. *Sci Rep,* 14**,** 14029.

DI CHIARA, G. 1997. Alcohol and dopamine. *Alcohol Health Res World,* 21**,** 108-14.

DI MICHELE, S., ERICSON, M., SILLÉN, U., ENGEL, J. A. & SÖDERPALM, B. 1998. The role of catecholamines in desmopressin induced locomotor stimulation. *J Neural Transm (Vienna),* 105**,** 1103-15.

DICKSON, S. L., SHIRAZI, R. H., HANSSON, C., BERGQUIST, F., NISSBRANDT, H. & SKIBICKA, K. P. 2012. The glucagon-like peptide 1 (GLP-1) analogue, exendin-4, decreases the rewarding value of food: a new role for mesolimbic GLP-1 receptors. *Journal Of Neuroscience : The Official Journal Of The Society For Neuroscience,* 32**,** 4812-20.

DIR, A. L., BELL, R. L., ADAMS, Z. W. & HULVERSHORN, L. A. 2017. Gender Differences in Risk Factors for Adolescent Binge Drinking and Implications for Intervention and Prevention. *Front Psychiatry,* 8**,** 289.

DIXON, T. N., MCNALLY, G. P. & ONG, Z. Y. 2020. Glucagon-Like Peptide-1 Receptor Signaling in the Ventral Tegmental Area Reduces Alcohol Self-Administration in Male Rats. *Alcohol Clin Exp Res,* 44**,** 2118-2129.

DIZ-CHAVES, Y., MASTOOR, Z., SPUCH, C., GONZÁLEZ-MATÍAS, L. C. & MALLO, F. 2022. Anti-Inflammatory Effects of GLP-1 Receptor Activation in the Brain in Neurodegenerative Diseases. *Int J Mol Sci,* 23.

DOMINO, K. B., HORNBEIN, T. F., POLISSAR, N. L., RENNER, G., JOHNSON, J., ALBERTI, S. & HANKES, L. 2005. Risk factors for relapse in health care professionals with substance use disorders. *Jama,* 293**,** 1453-60.

DOUTON, J. E., AUGUSTO, C., STOLTZFUS, B., CARKACI-SALLI, N., VRANA, K. E. & GRIGSON, P. S. 2021. Glucagon-like peptide-1 receptor agonist, exendin-4, reduces reinstatement of heroin-seeking behavior in rats. *Behav Pharmacol,* 32**,** 265-277.

DOUTON, J. E., HORVATH, N., MILLS-HUFFNAGLE, S., NYLAND, J. E., HAJNAL, A. & GRIGSON, P. S. 2022. Glucagon-like peptide-1 receptor agonist, liraglutide, reduces heroin self-administration and drug-induced reinstatement of heroin-seeking behaviour in rats. *Addict Biol,* 27**,** e13117.

DREVETS, W. C., GAUTIER, C., PRICE, J. C., KUPFER, D. J., KINAHAN, P. E., GRACE, A. A., PRICE, J. L. & MATHIS, C. A. 2001. Amphetamine-induced dopamine release in human ventral striatum correlates with euphoria. *Biol Psychiatry,* 49**,** 81-96.

DRUCKER, D. J., PHILIPPE, J., MOJSOV, S., CHICK, W. L. & HABENER, J. F. 1987. Glucagon-like peptide I stimulates insulin gene expression and increases cyclic AMP levels in a rat islet cell line. *Proc Natl Acad Sci U S A,* 84**,** 3434-8.

DYKSTRA, K. H., HSIAO, J. K., MORRISON, P. F., BUNGAY, P. M., MEFFORD, I. N., SCULLY, M. M. & DEDRICK, R. L. 1992. Quantitative examination of tissue concentration profiles associated with microdialysis. *J Neurochem,* 58**,** 931-40.

EGECIOGLU, E., ENGEL, J. A. & JERLHAG, E. 2013a. The glucagon-like peptide 1 analogue, exendin-4, attenuates the rewarding properties of psychostimulant drugs in mice. *PLoS One,* 8**,** e69010.

EGECIOGLU, E., STEENSLAND, P., FREDRIKSSON, I., FELTMANN, K., ENGEL, J. & JERLHAG, E. 2013b. The glucagon-like peptide 1 analogue Exendin-4 attenuates alcohol mediated behaviors in rodents. *Psychoneuroendocrinology, 2013, Vol. 38, Iss. 8, pp. 1259-.1270,* 38**,** 1259-1270.

EIDEN, S., DANIEL, C., STEINBRUECK, A., SCHMIDT, I. & SIMON, E. 2002. Salmon calcitonin - a potent inhibitor of food intake in states of impaired leptin signalling in laboratory rodents. *J Physiol,* 541**,** 1041-8.

ENGEL, J. 1977a. Neurochemical aspects of the euphoria induced by dependence-producing drugs. . *Recent Advances in the Study of Alcoholism***,** 16-22.

ENGEL, J., CARLSSON A 1977b. Catecholamines and behavior. *In Current development in psychopharmacology***,** 4:1-32.

ENGEL, J., ENERBÄCK, C. & FAHLKE, C. 1992. Serotonergic and dopaminergic involvement in ethanol intake. Springers Verlag: New York.

ENGEL, J., FAHLKE, C., HULTHE, P., HÅRD, E., JOHANNESSEN, K., SNAPE, B. & SVENSSON, L. 1988. Biochemical and behavioral evidence for an interaction between ethanol and calcium channel antagonists. *Journal of Neural Transmission,* 74**,** 181-193.

ENGEL, J. & JERLHAG, E. 2014a. Role of Appetite-Regulating Peptides in the Pathophysiology of Addiction: Implications for Pharmacotherapy. *Cns Drugs, 2014, Vol. 28, Iss. 10, pp. 875-.886,* 28**,** 875-886.

ENGEL, J., STRÖMBOM, U., SVENSSON, T. H. & WALDECK, B. 1974. Suppression by alpha-methyltyrosine of ethanol-induced locomotor stimulation: partial reversal by L-dopa. *Psychopharmacologia,* 37**,** 275-9.

ENGEL, J. A. & JERLHAG, E. 2014b. Alcohol: mechanisms along the mesolimbic dopamine system. *Prog Brain Res,* 211**,** 201-33.

EPAND, R. M., EPAND, R. F., ORLOWSKI, R. C., SEYLER, J. K. & COLESCOTT, R. L. 1986. Conformational flexibility and biological activity of salmon calcitonin. *Biochemistry,* 25**,** 1964-8.

ERICSON, M., LÖF, E., STOMBERG, R., CHAU, P. & SÖDERPALM, B. 2008. Nicotinic acetylcholine receptors in the anterior, but not posterior, ventral tegmental area mediate ethanol-induced elevation of accumbal dopamine levels. *J Pharmacol Exp Ther,* 326**,** 76-82.

ERICSON, M., MOLANDER, A., LÖF, E., ENGEL, J. A. & SÖDERPALM, B. 2003. Ethanol elevates accumbal dopamine levels via indirect activation of ventral tegmental nicotinic acetylcholine receptors. *Eur J Pharmacol,* 467**,** 85-93.

ERICSON, M., ULENIUS, L., ANDRÉN, A., JONSSON, S., ADERMARK, L. & SÖDERPALM, B. 2020. Different dopamine tone in ethanol high- and low-consuming Wistar rats. *Addict Biol,* 25**,** e12761.

EROL, A., HO, A. M., WINHAM, S. J. & KARPYAK, V. M. 2019. Sex hormones in alcohol consumption: a systematic review of evidence. *Addict Biol,* 24**,** 157-169.

ERŽEN, S., TONIN, G., JURIŠIĆ ERŽEN, D. & KLEN, J. 2024. Amylin, Another Important Neuroendocrine Hormone for the Treatment of Diabesity. *Int J Mol Sci,* 25.

FADOK, J. P., DARVAS, M., DICKERSON, T. M. & PALMITER, R. D. 2010. Long-term memory for pavlovian fear conditioning requires dopamine in the nucleus accumbens and basolateral amygdala. *PLoS One,* 5**,** e12751.

FALA, L. 2015. Trulicity (Dulaglutide): A New GLP-1 Receptor Agonist Once-Weekly Subcutaneous Injection Approved for the Treatment of Patients with Type 2 Diabetes. *American health & drug benefits,* 8**,** 131.

FELTMANN, K., BORROTO-ESCUELA, D. O., RÜEGG, J., PINTON, L., DE OLIVEIRA SERGIO, T., NARVÁEZ, M., JIMENEZ-BERISTAIN, A., EKSTRÖM, T. J., FUXE, K. & STEENSLAND, P. 2018. Effects of Long-Term Alcohol Drinking on the Dopamine D2 Receptor: Gene Expression and Heteroreceptor Complexes in the Striatum in Rats. *Alcohol Clin Exp Res,* 42**,** 338-351.

FERRARI, A. J., NORMAN, R. E., FREEDMAN, G., BAXTER, A. J., PIRKIS, J. E., HARRIS, M. G., PAGE, A., CARNAHAN, E., DEGENHARDT, L., VOS, T., WHITEFORD, H. A. & BAUNE, B. T. 2014. The Burden Attributable to

Mental and Substance Use Disorders as Risk Factors for Suicide: Findings from the Global Burden of Disease Study 2010. *PLoS ONE,* 9.

FILIPPATOS, T. D., PANAGIOTOPOULOU, T. V. & ELISAF, M. S. 2014. Adverse Effects of GLP-1 Receptor Agonists. *Rev Diabet Stud,* 11**,** 202-30.

FINBERG, J. P. M. 2019. Inhibitors of MAO-B and COMT: their effects on brain dopamine levels and uses in Parkinson's disease. *J Neural Transm (Vienna),* 126**,** 433-448.

FINK-JENSEN, A., WÖRTWEIN, G., KLAUSEN, M. K., HOLST, J. J., HARTMANN, B., THOMSEN, M., PTITO, M., BEIERSCHMITT, A. & PALMOUR, R. M. 2024. Effect of the glucagon-like peptide-1 (GLP-1) receptor agonist semaglutide on alcohol consumption in alcohol-preferring male vervet monkeys. *Psychopharmacology (Berl)*.

FLINT, A., RABEN, A., ASTRUP, A. & HOLST, J. J. 1998. Glucagon-like peptide 1 promotes satiety and suppresses energy intake in humans. *The Journal of clinical investigation,* 101**,** 515.

FOGLE, R. L., LYNCH, C. J., PALOPOLI, M., DEITER, G., STANLEY, B. A. & VARY, T. C. 2010. Impact of chronic alcohol ingestion on cardiac muscle protein expression. *Alcohol Clin Exp Res,* 34**,** 1226-34.

FRIAS, J. P., DEENADAYALAN, S., ERICHSEN, L., KNOP, F. K., LINGVAY, I., MACURA, S., MATHIEU, C., PEDERSEN, S. D. & DAVIES, M. 2023. Efficacy and safety of co-administered once-weekly cagrilintide 2·4 mg with once-weekly semaglutide 2·4 mg in type 2 diabetes: a multicentre, randomised, double-blind, active-controlled, phase 2 trial. *Lancet,* 402**,** 720-730.

FROEHLICH, J. C., FISCHER, S. M., NICHOLSON, E. R., DILLEY, J. E., FILOSA, N. J., SMITH, T. N. & RADEMACHER, L. C. 2017. A Combination of Naltrexone + Varenicline Retards the Expression of a Genetic Predisposition Toward High Alcohol Drinking. *Alcohol Clin Exp Res,* 41**,** 644-652.

GABERY, S., SALINAS, C. G., PAULSEN, S. J., AHNFELT-RØNNE, J., ALANENTALO, T., BAQUERO, A. F., BUCKLEY, S. T., FARKAS, E., FEKETE, C., FREDERIKSEN, K. S., HELMS, H. C. C., JEPPESEN, J. F., JOHN, L. M., PYKE, C., NØHR, J., LU, T. T., POLEX-WOLF, J., PREVOT, V., RAUN, K., SIMONSEN, L., SUN, G., SZILVÁSY-SZABÓ, A., WILLENBROCK, H., SECHER, A., KNUDSEN, L. B. & HOGENDORF, W. F. J. 2020. Semaglutide lowers body weight in rodents via distributed neural pathways. *JCI Insight,* 5.

GAO, B. & BATALLER, R. 2011. Alcoholic liver disease: pathogenesis and new therapeutic targets. *Gastroenterology,* 141**,** 1572-85.

GAO, C., LENG, Y., MA, J., ROOKE, V., RODRIGUEZ-GONZALEZ, S., RAMAKRISHNAN, C., DEISSEROTH, K. & PENZO, M. A. 2020. Two genetically, anatomically and functionally distinct cell types segregate across anteroposterior axis of paraventricular thalamus. *Nat Neurosci,* 23**,** 217-228.

GARBER, A. J. 2011. Long-acting glucagon-like peptide 1 receptor agonists: a review of their efficacy and tolerability. *Diabetes Care,* 34 Suppl 2**,** S279-84.

GARBUTT, J. C., KRANZLER, H. R., O'MALLEY, S. S., GASTFRIEND, D. R., PETTINATI, H. M., SILVERMAN, B. L., LOEWY, J. W. & EHRICH, E.

W. 2005. Efficacy and tolerability of long-acting injectable naltrexone for alcohol dependence: a randomized controlled trial. *Jama,* 293**,** 1617-25.
GAVINI, K. & PARAMESHWARAN, K. 2025. Western Blot. *StatPearls.* Treasure Island (FL) ineligible companies. Disclosure: Kodeeswaran Parameshwaran declares no relevant financial relationships with ineligible companies.: StatPearls Publishing

GEDULIN, B. R., RINK, T. J. & YOUNG, A. A. 1997. Dose-response for glucagonostatic effect of amylin in rats. *Metabolism,* 46**,** 67-70.
GERRING, Z. F., THORP, J. G., TREUR, J. L., VERWEIJ, K. J. H. & DERKS, E. M. 2024. The genetic landscape of substance use disorders. *Mol Psychiatry,* 29**,** 3694-3705.
GESSA, G. L., MUNTONI, F., COLLU, M., VARGIU, L. & MEREU, G. 1985. Low doses of ethanol activate dopaminergic neurons in the ventral tegmental area. *Brain Res,* 348**,** 201-3.
GILMAN, J. M., RAMCHANDANI, V. A., DAVIS, M. B., BJORK, J. M. & HOMMER, D. W. 2008. Why we like to drink: a functional magnetic resonance imaging study of the rewarding and anxiolytic effects of alcohol. *J Neurosci,* 28**,** 4583-91.
GILPIN, N. W. & KOOB, G. F. 2008. Neurobiology of alcohol dependence: focus on motivational mechanisms. *Alcohol Research & Health,* 31**,** 185.
GONZALES, R. A. & WEISS, F. 1998. Suppression of ethanol-reinforced behavior by naltrexone is associated with attenuation of the ethanol-induced increase in dialysate dopamine levels in the nucleus accumbens. *J Neurosci,* 18**,** 10663-71.
GOODING, S. W., LEWIS, E., CHAU, C., SANDHU, S., GLIENKE, J. & WHISTLER, J. L. 2024. Nucleus accumbens sub-regions experience distinct dopamine release responses following acute and chronic morphine exposure. *bioRxiv*.
GRAVES, S. M., XIE, Z., STOUT, K. A., ZAMPESE, E., BURBULLA, L. F., SHIH, J. C., KONDAPALLI, J., PATRIARCHI, T., TIAN, L., BRICHTA, L., GREENGARD, P., KRAINC, D., SCHUMACKER, P. T. & SURMEIER, D. J. 2020. Author Correction: Dopamine metabolism by a monoamine oxidase mitochondrial shuttle activates the electron transport chain. *Nat Neurosci,* 23**,** 293.
GREENFIELD, S. F., BROOKS, A. J., GORDON, S. M., GREEN, C. A., KROPP, F., MCHUGH, R. K., LINCOLN, M., HIEN, D. & MIELE, G. M. 2007. Substance abuse treatment entry, retention, and outcome in women: a review of the literature. *Drug Alcohol Depend,* 86**,** 1-21.
GRIFFIN, E. A., JR., MELAS, P. A., ZHOU, R., LI, Y., MERCADO, P., KEMPADOO, K. A., STEPHENSON, S., COLNAGHI, L., TAYLOR, K., HU, M. C., KANDEL, E. R. & KANDEL, D. B. 2017. Prior alcohol use enhances vulnerability to compulsive cocaine self-administration by promoting degradation of HDAC4 and HDAC5. *Sci Adv,* 3**,** e1701682.
GYDESEN, S., HJULER, S. T., FREVING, Z., ANDREASSEN, K. V., SONNE, N., HELLGREN, L. I., KARSDAL, M. A. & HENRIKSEN, K. 2017. A novel

dual amylin and calcitonin receptor agonist, KBP-089, induces weight loss through a reduction in fat, but not lean mass, while improving food preference. *Br J Pharmacol,* 174**,** 591-602.

HAMLIN, A. S., CLEMENS, K. J., CHOI, E. A. & MCNALLY, G. P. 2009. Paraventricular thalamus mediates context-induced reinstatement (renewal) of extinguished reward seeking. *Eur J Neurosci,* 29**,** 802-12.

HAN, X., JING, M. Y., ZHAO, T. Y., WU, N., SONG, R. & LI, J. 2017. Role of dopamine projections from ventral tegmental area to nucleus accumbens and medial prefrontal cortex in reinforcement behaviors assessed using optogenetic manipulation. *Metab Brain Dis,* 32**,** 1491-1502.

HARASTA, A. E., POWER, J. M., VON JONQUIERES, G., KARL, T., DRUCKER, D. J., HOUSLEY, G. D., SCHNEIDER, M. & KLUGMANN, M. 2015. Septal Glucagon-Like Peptide 1 Receptor Expression Determines Suppression of Cocaine-Induced Behavior. *Neuropsychopharmacology,* 40**,** 1969-78.

HARRIS, M. T. H., LAKS, J., STAHL, N., BAGLEY, S. M., SAIA, K. & WECHSBERG, W. M. 2022. Gender Dynamics in Substance Use and Treatment: A Women's Focused Approach. *Med Clin North Am,* 106**,** 219-234.

HAY, D. L., CHEN, S., LUTZ, T. A., PARKES, D. G. & ROTH, J. D. 2015. Amylin: Pharmacology, Physiology, and Clinical Potential. *Pharmacological Reviews,* 67**,** 564-600.

HAY, D. L., CHRISTOPOULOS G, CHRISTOPOULOS A & PM, S. 2004. Amylin receptors: molecular composition and pharmacology. *Biochem Soc Trans,* 32.

HAY, D. L. & PIOSZAK, A. A. 2016. Receptor Activity-Modifying Proteins (RAMPs): New Insights and Roles. *Annual Review of Pharmacology and Toxicology,* 56**,** 469-487.

HEILIG, M. & EGLI, M. 2006. Pharmacological treatment of alcohol dependence: Target symptoms and target mechanisms. *Pharmacology and Therapeutics,* 111**,** 855-876.

HERBECK, D. M., JETER, K. E., COUSINS, S. J., ABDELMAKSOUD, R. & CRÈVECOEUR-MACPHAIL, D. 2016. Gender differences in treatment and clinical characteristics among patients receiving extended release naltrexone. *J Addict Dis,* 35**,** 305-314.

HERNANDEZ, L. & HOEBEL, B. G. 1988. Food reward and cocaine increase extracellular dopamine in the nucleus accumbens as measured by microdialysis. *Life Sciences,* 42**,** 1705-1712.

HERNANDEZ, N. S., IGE, K. Y., MIETLICKI-BAASE, E. G., MOLINA-CASTRO, G. C., TURNER, C. A., HAYES, M. R. & SCHMIDT, H. D. 2018. Glucagon-like peptide-1 receptor activation in the ventral tegmental area attenuates cocaine seeking in rats. *Neuropsychopharmacology,* 43**,** 2000-2008.

HERNANDEZ, N. S., O'DONOVAN, B., ORTINSKI, P. I. & SCHMIDT, H. D. 2019. Activation of glucagon-like peptide-1 receptors in the nucleus accumbens attenuates cocaine seeking in rats. *Addiction biology,* 24**,** 170-181.

HERNANDEZ, N. S. & SCHMIDT, H. D. 2019. Central GLP-1 receptors: Novel molecular targets for cocaine use disorder. *Physiol Behav,* 206**,** 93-105.

HERNANDEZ, N. S., WEIR, V. R., RAGNINI, K., MERKEL, R., ZHANG, Y., MACE, K., RICH, M. T., CHRISTOPHER PIERCE, R. & SCHMIDT, H. D. 2021. GLP-1 receptor signaling in the laterodorsal tegmental nucleus attenuates cocaine seeking by activating GABAergic circuits that project to the VTA. *Mol Psychiatry,* 26**,** 4394-4408.

HOLST, J. J. & GROMADA, J. 2004. Role of incretin hormones in the regulation of insulin secretion in diabetic and nondiabetic humans. *American journal of physiology. Endocrinology and metabolism,* 287**,** E199.

HOLST, J. J. & MADSBAD, S. 2017. Semaglutide seems to be more effective the other GLP-1Ras. *Ann Transl Med,* 5**,** 505.

HOLT, M. K., COOK, D. R., BRIERLEY, D. I., RICHARDS, J. E., REIMANN, F., GOURINE, A. V., MARINA, N. & TRAPP, S. 2020. PPG neurons in the nucleus of the solitary tract modulate heart rate but do not mediate GLP-1 receptor agonist-induced tachycardia in mice. *Mol Metab,* 39**,** 101024.

HOLT, M. K., RICHARDS, J. E., COOK, D. R., BRIERLEY, D. I., WILLIAMS, D. L., REIMANN, F., GRIBBLE, F. M. & TRAPP, S. 2019. Preproglucagon Neurons in the Nucleus of the Solitary Tract Are the Main Source of Brain GLP-1, Mediate Stress-Induced Hypophagia, and Limit Unusually Large Intakes of Food. *Diabetes,* 68**,** 21-33.

HOLT, M. K. & TRAPP, S. 2016. The physiological role of the brain GLP-1 system in stress. *Cogent Biol,* 2**,** 1229086.

HU, M., CROMBAG, H. S., ROBINSON, T. E. & BECKER, J. B. 2004. Biological basis of sex differences in the propensity to self-administer cocaine. *Neuropsychopharmacology,* 29**,** 81-5.

HUANG, X., GU, H. H. & ZHAN, C. G. 2009. Mechanism for cocaine blocking the transport of dopamine: insights from molecular modeling and dynamics simulations. *J Phys Chem B,* 113**,** 15057-66.

HUGO MENDIETA, Z., MA. VICTORIA DOMINGUEZ, G., MARIA DEL SOCORRO CAMARILLO, R. & MIRIAM, V. F.-M. 2013. Peripheral Pathways in the Food-Intake Control towards the Adipose-Intestinal Missing Link. *International Journal of Endocrinology,* 2013**,** 171-182.

HYMAN, S. E., MALENKA, R. C. & NESTLER, E. J. 2006. Neural mechanisms of addiction: the role of reward-related learning and memory. *Annu Rev Neurosci,* 29**,** 565-98.

IM, K., MARENINOV, S., DIAZ, M. F. P. & YONG, W. H. 2019. An Introduction to Performing Immunofluorescence Staining. *Methods Mol Biol,* 1897**,** 299-311.

IMPERATO, A. & DI CHIARA, G. 1986. Preferential stimulation of dopamine release in the nucleus accumbens of freely moving rats by ethanol. *J Pharmacol Exp Ther,* 239**,** 219-28.

ITO, R., DALLEY, J. W., HOWES, S. R., ROBBINS, T. W. & EVERITT, B. J. 2000. Dissociation in conditioned dopamine release in the nucleus accumbens core and shell in response to cocaine cues and during cocaine-seeking behavior in rats. *J Neurosci,* 20**,** 7489-95.

JAMES, M. H., CHARNLEY, J. L., JONES, E., LEVI, E. M., YEOH, J. W., FLYNN, J. R., SMITH, D. W. & DAYAS, C. V. 2010. Cocaine- and amphetamine-regulated transcript (CART) signaling within the paraventricular thalamus modulates cocaine-seeking behaviour. *PLoS One,* 5**,** e12980.

JENSTERLE, M., RIZZO, M. & JANEŽ, A. 2023. Semaglutide in Obesity: Unmet Needs in Men. *Diabetes Ther,* 14**,** 461-465.

JERLHAG, E. 2019. Gut-brain axis and addictive disorders: A review with focus on alcohol and drugs of abuse. *Pharmacol Ther,* 196**,** 1-14.

JERLHAG, E., EGECIOGLU, E., LANDGREN, S., SALOMÉ, N., HEILIG, M., MOECHARS, D., DATTA, R., PERRISSOUD, D., DICKSON, S. L. & ENGEL, J. A. 2009. Requirement of Central Ghrelin Signaling for Alcohol Reward. *Proceedings of the National Academy of Sciences - PNAS,* 106**,** 11318-11323.

JO, Y. S. & MIZUMORI, S. J. Y. 2016. Prefrontal Regulation of Neuronal Activity in the Ventral Tegmental Area. *Cereb Cortex,* 26**,** 4057-4068.

JONSSON, S., ERICSON, M. & SÖDERPALM, B. 2014. Modest long-term ethanol consumption affects expression of neurotransmitter receptor genes in the rat nucleus accumbens. *Alcohol Clin Exp Res,* 38**,** 722-9.

JOO, Y. S., KOH, H., NAM, K. H., LEE, S., KIM, J., LEE, C., YUN, H. R., PARK, J. T., KANG, E. W., CHANG, T. I., YOO, T. H., OH, K. H., CHAE, D. W., LEE, K. B., KIM, S. W., LEE, J., KANG, S. W., CHOI, K. H., AHN, C. & HAN, S. H. 2020. Alcohol Consumption and Progression of Chronic Kidney Disease: Results From the Korean Cohort Study for Outcome in Patients with Chronic Kidney Disease. *Mayo Clin Proc,* 95**,** 293-305.

JORSAL, T., RUNGBY, J., KNOP, F. & VILSBØLL, T. 2016. GLP-1 and Amylin in the Treatment of Obesity. *Current Diabetes Reports,* 16**,** 1-7.

JUN, S., PARK, H., KIM, U. J., CHOI, E. J., LEE, H. A., PARK, B., LEE, S. Y., JEE, S. H. & PARK, H. 2023. Cancer risk based on alcohol consumption levels: a comprehensive systematic review and meta-analysis. *Epidemiol Health,* 45**,** e2023092.

KALAFATELI, A. L., ARANÄS, C. & JERLHAG, E. 2020. Effects of sub-chronic amylin receptor activation on alcohol-induced locomotor stimulation and monoamine levels in mice. *Psychopharmacology (Berl),* 237**,** 3249-3257.

KALAFATELI, A. L., ARANÄS, C. & JERLHAG, E. 2021a. Activation of the amylin pathway modulates cocaine-induced activation of the mesolimbic dopamine system in male mice. *Horm Behav,* 127**,** 104885.

KALAFATELI, A. L., SATIR, T. M., VALLÖF, D., ZETTERBERG, H. & JERLHAG, E. 2021b. An amylin and calcitonin receptor agonist modulates alcohol behaviors by acting on reward-related areas in the brain. *Progress In Neurobiology, 2021, Vol. 200,* 200.

KALAFATELI, A. L., VALLÖF, D., COLOMBO, G., LORRAI, I., MACCIONI, P. & JERLHAG, E. 2019a. An amylin analogue attenuates alcohol-related behaviours in various animal models of alcohol use disorder. *Neuropsychopharmacology (New York, N.Y.),* 44**,** 1093-1102.

KALAFATELI, A. L., VALLÖF, D. & JERLHAG, E. 2019b. Activation of amylin receptors attenuates alcohol-mediated behaviours in rodents. *Addiction biology,* 24**,** 388-402.

KALAFATELI, A. L., VESTLUND, J., RAUN, K., EGECIOGLU, E. & JERLHAG, E. 2021c. Effects of a selective long-acting amylin receptor agonist on alcohol consumption, food intake and body weight in male and female rats. *Addiction biology,* 26**,** e12910-n/a.

KALAFATELI., A. L., SATIR, T., VALLÖF, D., ZETTERBERG, H. & JERLHAG, E. 2020. Behavioural responses to alcohol involve amylin receptor signalling within brain areas processing reward.

KALRA, S., KALRA, B. & SHARMA, A. 2011. Change in Alcohol Consumption Following Liraglutide Initiation: A Real Life Experience. *Diabetes,* 60**,** A282-A282.

KALRA, S. & SAHAY, R. 2020. A Review on Semaglutide: An Oral Glucagon-Like Peptide 1 Receptor Agonist in Management of Type 2 Diabetes Mellitus. *Diabetes Ther,* 11**,** 1965-1982.

KAMPMAN, K. M. 2019. The treatment of cocaine use disorder. *Sci Adv,* 5**,** eaax1532.

KAWANO, Y. 2010. Physio-pathological effects of alcohol on the cardiovascular system: its role in hypertension and cardiovascular disease. *Hypertens Res,* 33**,** 181-91.

KELLEY, A. E. & BERRIDGE, K. C. 2002. The neuroscience of natural rewards: relevance to addictive drugs. *J Neurosci,* 22**,** 3306-11.

KIM, S. G., HAN, B. D., PARK, J. M., KIM, M. J. & STROMBERG, M. F. 2004. Effect of the combination of naltrexone and acamprosate on alcohol intake in mice. *Psychiatry Clin Neurosci,* 58**,** 30-6.

KING, A., VENA, A., HASIN, D. S., DEWIT, H., O'CONNOR, S. J. & CAO, D. 2021. Subjective Responses to Alcohol in the Development and Maintenance of Alcohol Use Disorder. *Am J Psychiatry,* 178**,** 560-571.

KLAUSEN, M. K., JENSEN, M. E., MØLLER, M., LE DOUS, N., JENSEN, A., ZEEMAN, V. A., JOHANNSEN, C. F., LEE, A., THOMSEN, G. K., MACOVEANU, J., FISHER, P. M., GILLUM, M. P., JØRGENSEN, N. R., BERGMANN, M. L., ENGHUSEN POULSEN, H., BECKER, U., HOLST, J. J., BENVENISTE, H., VOLKOW, N. D., VOLLSTÄDT-KLEIN, S., MISKOWIAK, K. W., EKSTRØM, C. T., KNUDSEN, G. M., VILSBØLL, T. & FINK-JENSEN, A. 2022. Exenatide once weekly for alcohol use disorder investigated in a randomized, placebo-controlled clinical trial. *JCI Insight,* 7.

KLEIN, C., BUDIMAN, T., HOMBERG, J. R., VERMA, D., KEIJER, J. & VAN SCHOTHORST, E. M. 2022. Corrigendum: Measuring Locomotor Activity and Behavioral Aspects of Rodents Living in the Home-Cage. *Front Behav Neurosci,* 16**,** 943307.

KOCEVA, A., HERMAN, R., JANEZ, A., RAKUSA, M. & JENSTERLE, M. 2024. Sex- and Gender-Related Differences in Obesity: From Pathophysiological Mechanisms to Clinical Implications. *Int J Mol Sci,* 25.

KOMATSU, R., MATSUYAMA, T., NAMBA, M., WATANABE, N., ITOH, H., KONO, N. & TARUI, S. 1989. GLUCAGONOSTATIC AND INSULINOTROPIC ACTION OF GLUCAGON-LIKE PEPTIDE I-(7-36)-AMIDE. *Diabetes,* 38**,** 902-905.

KOMMU, S. & WHITFIELD, P. 2024. Semaglutide. *StatPearls.* Treasure Island (FL) ineligible companies. Disclosure: Philip Whitfield declares no relevant financial relationships with ineligible companies.: StatPearls Publishing

KONING, S. H., GANSEVOORT, R. T., MUKAMAL, K. J., RIMM, E. B., BAKKER, S. J. & JOOSTEN, M. M. 2015. Alcohol consumption is inversely associated with the risk of developing chronic kidney disease. *Kidney Int,* 87**,** 1009-16.

KOOB, G. 2010. THE POTENTIAL OF NEUROSCIENCE TO INFORM TREATMENT. *Alcohol Research and Health,* 33**,** 144-51.

KOOB, G. F. 1992a. Drugs of abuse: anatomy, pharmacology and function of reward pathways. *Trends Pharmacol Sci,* 13**,** 177-84.

KOOB, G. F. 1992b. Neural mechanisms of drug reinforcement. *Ann N Y Acad Sci,* 654**,** 171-91.

KOOB, G. F. 2014. Neurocircuitry of alcohol addiction: synthesis from animal models. *Handb Clin Neurol,* 125**,** 33-54.

KOOB, G. F. & LE MOAL, M. 2001. Drug addiction, dysregulation of reward, and allostasis. *Neuropsychopharmacology,* 24**,** 97-129.

KOOB, G. F. & VOLKOW, N. D. 2010. Neurocircuitry of addiction. *Neuropsychopharmacology,* 35**,** 217-38.

KOURRICH, S., ROTHWELL, P. E., KLUG, J. R. & THOMAS, M. J. 2007. Cocaine experience controls bidirectional synaptic plasticity in the nucleus accumbens. *J Neurosci,* 27**,** 7921-8.

KOURRICH, S. & THOMAS, M. J. 2009. Similar neurons, opposite adaptations: psychostimulant experience differentially alters firing properties in accumbens core versus shell. *J Neurosci,* 29**,** 12275-83.

KOYA, E. & HOPE, B. T. 2011. Cocaine and synaptic alterations in the nucleus accumbens. *Biol Psychiatry,* 69**,** 1013-4.

KREYMANN, B., WILLIAMS, G., GHATEI, M. A. & BLOOM, S. R. 1987. Glucagon-like peptide-1 7-36: a physiological incretin in man. *Lancet,* 2**,** 1300-4.

LÄHTEENVUO, M., TIIHONEN, J., SOLISMAA, A., TANSKANEN, A., MITTENDORFER-RUTZ, E. & TAIPALE, H. 2024. Repurposing Semaglutide and Liraglutide for Alcohol Use Disorder. *JAMA Psychiatry*.

LARSEN, A. T., GYDESEN, S., SONNE, N., KARSDAL, M. A. & HENRIKSEN, K. 2021. The dual amylin and calcitonin receptor agonist KBP-089 and the GLP-1 receptor agonist liraglutide act complimentarily on body weight reduction and metabolic profile. *BMC Endocr Disord,* 21**,** 10.

LARSSON, A. & ENGEL, J. A. 2004. Neurochemical and behavioral studies on ethanol and nicotine interactions. *Neurosci Biobehav Rev,* 27**,** 713-20.

LAU, J., BLOCH, P., SCHÄFFER, L., PETTERSSON, I., SPETZLER, J., KOFOED, J., MADSEN, K., KNUDSEN, L. B., MCGUIRE, J., STEENSGAARD, D. B., STRAUSS, H. M., GRAM, D. X., KNUDSEN, S. M., NIELSEN, F. S., THYGESEN, P., REEDTZ-RUNGE, S. & KRUSE, T. 2015. Discovery of the Once-Weekly Glucagon-Like Peptide-1 (GLP-1) Analogue Semaglutide. *J Med Chem,* 58**,** 7370-80.

LEAN, M. E., CARRARO, R., FINER, N., HARTVIG, H., LINDEGAARD, M. L., RÖSSNER, S., VAN GAAL, L. & ASTRUP, A. 2014. Tolerability of nausea and vomiting and associations with weight loss in a randomized trial of liraglutide in obese, non-diabetic adults. *Int J Obes (Lond),* 38**,** 689-97.

LEE, J. C. 1962. Effect of alcohol injections on the blood brain barrier *QJ Stud Alcohol,* 23**,** 4-16.

LEE, S. M., HAY, D. L. & PIOSZAK, A. A. 2016. Calcitonin and amylin receptor peptide interaction mechanisms. INSIGHTS INTO PEPTIDE-BINDING MODES AND ALLOSTERIC MODULATION OF THE CALCITONIN RECEPTOR BY RECEPTOR ACTIVITY-MODIFYING PROTEINS. *J Biol Chem,* 291**,** 16416.

LEE, T. S., PARK, E. J., CHOI, M., OH, H. S., AN, Y., KIM, T., KIM, T. H., SHIN, B. S. & SHIN, S. 2023. Novel LC-MS/MS analysis of the GLP-1 analog semaglutide with its application to pharmacokinetics and brain distribution studies in rats. *J Chromatogr B Analyt Technol Biomed Life Sci,* 1221**,** 123688.

LESHNER, A. I. 1997. Addiction is a brain disease, and it matters. *Science,* 278**,** 45-7.

LI, J., CHEN, P., HAN, X., ZUO, W., MEI, Q., BIAN, E. Y., UMEUGO, J. & YE, J. 2019. Differences between male and female rats in alcohol drinking, negative affects and neuronal activity after acute and prolonged abstinence. *Int J Physiol Pathophysiol Pharmacol,* 11**,** 163-176.

LI, J., CHENG, Y., BIAN, W., LIU, X., ZHANG, C. & YE, J. H. 2010. Region-specific induction of FosB/ΔFosB by voluntary alcohol intake: effects of naltrexone. *Alcohol Clin Exp Res,* 34**,** 1742-50.

LI, S., LIU, M., CAO, S., LIU, B., LI, D., WANG, Z., SUN, H., CUI, Y. & SHI, Y. 2023. The Mechanism of the Gut-Brain Axis in Regulating Food Intake. *Nutrients,* 15.

LIBERINI, C. G., KOCH-LASKOWSKI, K., SHAULSON, E., MCGRATH, L. E., LIPSKY, R. K., LHAMO, R., GHIDEWON, M., LING, T., STEIN, L. M. & HAYES, M. R. 2019. Combined Amylin/GLP-1 pharmacotherapy to promote and sustain long-lasting weight loss. *Scientific reports,* 9**,** 8447-8447.

LIM, S. S., VOS, T., FLAXMAN, A. D., DANAEI, G., SHIBUYA, K., ADAIR-ROHANI, H., ALMAZROA, M. A., AMANN, M., ANDERSON, H. R., ANDREWS, K. G., ARYEE, M., ATKINSON, C., BACCHUS, L. J., BAHALIM, A. N., BALAKRISHNAN, K., BALMES, J., BARKER-COLLO, S., BAXTER, A., BELL, M. L., BLORE, J. D., BLYTH, F., BONNER, C., BORGES, G., BOURNE, R., BOUSSINESQ, M., BRAUER, M., BROOKS, P., BRUCE, N. G., BRUNEKREEF, B., BRYAN-HANCOCK, C., BUCELLO, C., BUCHBINDER, R., BULL, F., BURNETT, R. T., BYERS, T. E., CALABRIA, B., CARAPETIS, J., CARNAHAN, E., CHAFE, Z., CHARLSON, F., CHEN, H., CHEN, J. S., CHENG, A. T.-A., CHILD, J. C., COHEN, A., COLSON, K. E., COWIE, B. C., DARBY, S., DARLING, S., DAVIS, A., DEGENHARDT, L., DENTENER, F., DES JARLAIS, D. C., DEVRIES, K., DHERANI, M., DING, E. L., DORSEY, E. R., DRISCOLL, T., EDMOND, K., ALI, S. E., ENGELL, R. E., ERWIN, P. J., FAHIMI, S., FALDER, G., FARZADFAR, F., FERRARI, A., FINUCANE, M. M., FLAXMAN, S., FOWKES, F. G. R., FREEDMAN, G., FREEMAN, M. K., GAKIDOU, E., GHOSH, S., GIOVANNUCCI, E., GMEL, G., GRAHAM, K., GRAINGER, R., GRANT, B., GUNNELL, D., GUTIERREZ, H. R., HALL, W., HOEK, H. W., HOGAN, A., HOSGOOD,

H. D., HOY, D., HU, H., HUBBELL, B. J., HUTCHINGS, S. J., IBEANUSI, S. E., JACKLYN, G. L., JASRASARIA, R., JONAS, J. B., KAN, H., KANIS, J. A., KASSEBAUM, N., KAWAKAMI, N., KHANG, Y.-H., KHATIBZADEH, S., KHOO, J.-P. & KOK, C. 2012. A comparative risk assessment of burden of disease and injury attributable to 67 risk factors and risk factor clusters in 21 regions, 1990–2010: a systematic analysis for the Global Burden of Disease Study 2010. *The Lancet,* 380**,** 2224-2260.

LINDGREN, H. S., ANDERSSON, D. R., LAGERKVIST, S., NISSBRANDT, H. & CENCI, M. A. 2010. L-DOPA-induced dopamine efflux in the striatum and the substantia nigra in a rat model of Parkinson's disease: temporal and quantitative relationship to the expression of dyskinesia. *J Neurochem,* 112**,** 1465-76.

LOH, E., KUMARAN, D., KOSTER, R., BERRON, D., DOLAN, R. & DUZEL, E. 2016. Context-specific activation of hippocampus and SN/VTA by reward is related to enhanced long-term memory for embedded objects. *Neurobiol Learn Mem,* 134 Pt A**,** 65-77.

LÖSCHER, W. & SCHMIDT, D. 2006. Experimental and clinical evidence for loss of effect (tolerance) during prolonged treatment with antiepileptic drugs. *Epilepsia,* 47**,** 1253-84.

LOVINGER, D. M., WHITE, G. & WEIGHT, F. F. 1989. Ethanol Inhibits NMDA-Activated Ion Current in Hippocampal Neurons. *Science,* 243**,** 1721-1724.

LOVINGER, D. M. & ZHOU, Q. 1998. Alcohol effects on the 5-HT3 ligand-gated ion channel. *Toxicology Letters,* 101**,** 239-246.

LUTZ, T. A. 2010. The role of amylin in the control of energy homeostasis. *American Journal Of Physiology-Regulatory Integrative And Comparative Physi,* 298**,** R1475-R1484.

LUTZ, T. A., RUSHING, P. A. & RIEDIGER, T. 2001. Repeated Salmon Calcitonin Injection Lowers Body Weight and Body Fat. *The Scientific World Journal,* 1**,** 25-25.

LUTZ, T. A., TSCHUDY, S., RUSHING, P. A. & SCHARRER, E. 2000. Amylin receptors mediate the anorectic action of salmon calcitonin (sCT). *Peptides,* 21**,** 233-8.

MACEY, D. J., RICE, W. N., FREEDLAND, C. S., WHITLOW, C. T. & PORRINO, L. J. 2004. Patterns of functional activity associated with cocaine self-administration in the rat change over time. *Psychopharmacology (Berl),* 172**,** 384-92.

MARTY, V. N., FAROKHNIA, M., MUNIER, J. J., MULPURI, Y., LEGGIO, L. & SPIGELMAN, I. 2020. Long-Acting Glucagon-Like Peptide-1 Receptor Agonists Suppress Voluntary Alcohol Intake in Male Wistar Rats. *Frontiers in neuroscience,* 14**,** 599646-599646.

MASON, B. J. & LEHERT, P. 2012. Acamprosate for Alcohol Dependence: A Sex-Specific Meta-Analysis Based on Individual Patient Data. *Alcoholism: Clinical and Experimental Research,* 36**,** 497-508.

MATHIESEN, D. S., LUND, A., VILSBØLL, T., KNOP, F. K. & BAGGER, J. I. 2020. Amylin and Calcitonin: Potential Therapeutic Strategies to Reduce Body Weight and Liver Fat. *Front Endocrinol (Lausanne),* 11**,** 617400.

MCCANCE-KATZ, E. F., KOSTEN, T. R. & JATLOW, P. 1998. Disulfiram effects on acute cocaine administration. *Drug Alcohol Depend,* 52**,** 27-39.

MCCORMACK, P. L. 2014. Exenatide twice daily: a review of its use in the management of patients with type 2 diabetes mellitus. *Drugs,* 74**,** 325-51.

MCDEVITT, D. S., WADE, Q. W., MCKENDRICK, G. E., NELSEN, J., STAROSTINA, M., TRAN, N., BLENDY, J. A. & GRAZIANE, N. M. 2024. The Paraventricular Thalamic Nucleus and Its Projections in Regulating Reward and Context Associations. *eNeuro,* 11.

MCHUGH, R. K., VOTAW, V. R., SUGARMAN, D. E. & GREENFIELD, S. F. 2018. Sex and gender differences in substance use disorders. *Clin Psychol Rev,* 66**,** 12-23.

MEIER, J. J. 2012. GLP-1 receptor agonists for individualized treatment of type 2 diabetes mellitus. *Nat Rev Endocrinol,* 8**,** 728-42.

MEYER, P. J., KING, C. P. & FERRARIO, C. R. 2016. Motivational Processes Underlying Substance Abuse Disorder. *Curr Top Behav Neurosci,* 27**,** 473-506.

MHAIDAT, I., AL-YATEEM, N., AL-MAMARI, S. & AL-SUWAIDI, F. 2024. Resilience and relapse risk in Emirate adult patients with substance use disorder: a national cross-sectional study from the United Arab Emirates. *Front Psychiatry,* 15**,** 1444233.

MIETLICKI-BAASE, E., OLIVOS, D., JEFFREY, B. & HAYES, M. 2015a. Cooperative interaction between leptin and amylin signaling in the ventral tegmental area for the control of food intake. *American Journal of Physiology,* 308**,** E1116.

MIETLICKI-BAASE, E. G. & HAYES, M. R. 2014. Amylin activates distributed CNS nuclei to control energy balance. *Physiology & Behavior,* 136**,** 39-46.

MIETLICKI-BAASE, E. G., REINER, D. J., CONE, J. J., OLIVOS, D. R., MCGRATH, L. E., ZIMMER, D. J., ROITMAN, M. F. & HAYES, M. R. 2015b. Amylin modulates the mesolimbic dopamine system to control energy balance. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology,* 40**,** 372.

MILLAN, E. Z., ONG, Z. & MCNALLY, G. P. 2017. Paraventricular thalamus: Gateway to feeding, appetitive motivation, and drug addiction. *Prog Brain Res,* 235**,** 113-137.

MU, P., MOYER, J. T., ISHIKAWA, M., ZHANG, Y., PANKSEPP, J., SORG, B. A., SCHLÜTER, O. M. & DONG, Y. 2010. Exposure to cocaine dynamically regulates the intrinsic membrane excitability of nucleus accumbens neurons. *J Neurosci,* 30**,** 3689-99.

MULLINS, L. J. & MULLINS, J. J. 2004. Insights from the rat genome sequence. *Genome Biol,* 5**,** 221.

NESTLER, E. J. 2005. The neurobiology of cocaine addiction. *Sci Pract Perspect,* 3**,** 4-10.

NESTLER, E. J., BARROT, M. & SELF, D. W. 2001. DeltaFosB: a sustained molecular switch for addiction. *Proc Natl Acad Sci U S A,* 98**,** 11042-6.

NICHOLSON, E. R., DILLEY, J. E. & FROEHLICH, J. C. 2018. Co-Administration of Low-Dose Naltrexone and Bupropion Reduces Alcohol Drinking in Alcohol-Preferring (P) Rats. *Alcohol Clin Exp Res,* 42**,** 571-577.

NIETO, S. J., GRODIN, E. N., AGUIRRE, C. G., IZQUIERDO, A. & RAY, L. A. 2021. Translational opportunities in animal and human models to study alcohol use disorder. *Transl Psychiatry,* 11**,** 496.

NITYA JAYARAM-LINDSTRÖM, M. E., PIA SEENSLAND, ELISABET JERLHAG 2016. *Recent Advances in Drug Addiction Research and Clinical Applications*, Intech.

NIZARI, S., BASALAY, M., CHAPMAN, P., KORTE, N., KORSAK, A., CHRISTIE, I. N., THEPARAMBIL, S. M., DAVIDSON, S. M., REIMANN, F., TRAPP, S., YELLON, D. M. & GOURINE, A. V. 2021. Glucagon-like peptide-1 (GLP-1) receptor activation dilates cerebral arterioles, increases cerebral blood flow, and mediates remote (pre)conditioning neuroprotection against ischaemic stroke. *Basic Res Cardiol,* 116**,** 32.

NUFFER, W. A. & TRUJILLO, J. M. 2015. Liraglutide: A New Option for the Treatment of Obesity.

O'MALLEY, S. S., SINHA, R., GRILO, C. M., CAPONE, C., FARREN, C. K., MCKEE, S. A., ROUNSAVILLE, B. J. & WU, R. 2007. Naltrexone and cognitive behavioral coping skills therapy for the treatment of alcohol drinking and eating disorder features in alcohol-dependent women: a randomized controlled trial. *Alcohol Clin Exp Res,* 31**,** 625-34.

ONG, Z. Y., LIU, J. J., PANG, Z. P. & GRILL, H. J. 2017. Paraventricular Thalamic Control of Food Intake and Reward: Role of Glucagon-Like Peptide-1 Receptor Signaling. *Neuropsychopharmacology,* 42**,** 2387-2397.

ORSKOV, C., HOLST, J. J. & NIELSEN, O. V. 1988. Effect of truncated glucagon-like peptide-1 [proglucagon-(78-107) amide] on endocrine secretion from pig pancreas, antrum, and nonantral stomach. *Endocrinology,* 123**,** 2009-13.

OVERGAARD, R. V., DELFF, P. H., PETRI, K. C. C., ANDERSON, T. W., FLINT, A. & INGWERSEN, S. H. 2019. Population Pharmacokinetics of Semaglutide for Type 2 Diabetes. *Diabetes Ther,* 10**,** 649-662.

OVERGAARD, R. V., PETRI, K. C., JACOBSEN, L. V. & JENSEN, C. B. 2016. Liraglutide 3.0 mg for Weight Management: A Population Pharmacokinetic Analysis. *Clin Pharmacokinet,* 55**,** 1413-1422.

PALM, S., ROMAN, E. & NYLANDER, I. 2011. Differences in voluntary ethanol consumption in Wistar rats from five different suppliers. *Alcohol,* 45**,** 607-14.

PANLILIO, L. V. & GOLDBERG, S. R. 2007. Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction,* 102**,** 1863-70.

PARK, K., VOLKOW, N. D., PAN, Y. & DU, C. 2013. Chronic cocaine dampens dopamine signaling during cocaine intoxication and unbalances D1 over D2 receptor signaling. *J Neurosci,* 33**,** 15827-36.

PEACOCK, A., LEUNG, J., LARNEY, S., COLLEDGE, S., HICKMAN, M., REHM, J., GIOVINO, G. A., WEST, R., HALL, W., GRIFFITHS, P., ALI, R., GOWING, L., MARSDEN, J., FERRARI, A. J., GREBELY, J., FARRELL, M. & DEGENHARDT, L. 2018. Global statistics on alcohol, tobacco and illicit drug use: 2017 status report. *Addiction,* 113**,** 1905-1926.

PENZO, M. A. & GAO, C. 2021. The paraventricular nucleus of the thalamus: an integrative node underlying homeostatic behavior. *Trends Neurosci,* 44**,** 538-549.

PETERSON, G. E. & POLLOM, R. D. 2010. Liraglutide in clinical practice: dosing, safety and efficacy. *Int J Clin Pract Suppl***,** 35-43.

PETROVICH, G. D. 2021. The Function of Paraventricular Thalamic Circuitry in Adaptive Control of Feeding Behavior. *Front Behav Neurosci,* 15**,** 671096.

PETTINATI, H. M., ANTON, R. F. & WILLENBRING, M. L. 2006. The COMBINE Study-: An Overview of the Largest Pharmacotherapy Study to Date for Treating Alcohol Dependence. *Psychiatry (Edgmont),* 3**,** 36-9.

PETTINATI, H. M., KAMPMAN, K. M., LYNCH, K. G., XIE, H., DACKIS, C., RABINOWITZ, A. R. & O'BRIEN, C. P. 2008. A double blind, placebo-controlled trial that combines disulfiram and naltrexone for treating co-occurring cocaine and alcohol dependence. *Addict Behav,* 33**,** 651-67.

PETTINATI, H. M., SILVERMAN, B. L., BATTISTI, J. J., FORMAN, R., SCHWEIZER, E. & GASTFRIEND, D. R. 2011. Efficacy of extended-release naltrexone in patients with relatively higher severity of alcohol dependence. *Alcohol Clin Exp Res,* 35**,** 1804-11.

PFAUS, J. G., DAMSMA, G., NOMIKOS, G. G., WENKSTERN, D. G., BLAHA, C. D., PHILLIPS, A. G. & FIBIGER, H. C. 1990. Sexual behavior enhances central dopamine transmission in the male rat. *Brain Research,* 530**,** 345-348.

POISSON, C. L., ENGEL, L. & SAUNDERS, B. T. 2021. Dopamine Circuit Mechanisms of Addiction-Like Behaviors. *Front Neural Circuits,* 15**,** 752420.

PORRINO, L. J., LYONS, D., MILLER, M. D., SMITH, H. R., FRIEDMAN, D. P., DAUNAIS, J. B. & NADER, M. A. 2002. Metabolic mapping of the effects of cocaine during the initial phases of self-administration in the nonhuman primate. *J Neurosci,* 22**,** 7687-94.

POYNER, D. 1995. Pharmacology of receptors for calcitonin gene-related peptide and amylin.

POYNER, D., SEXTON, P., MARSHALL, I., SMITH, D. M., QUIRION, R., BORN, W., MUFF, R., FISCHER, J. & FOORD, S. M. 2002. International Union of Pharmacology. XXXII. The mammalian calcitonin gene-related peptides, adrenomedullin, amylin, and calcitonin receptors. *Pharmacological Reviews,* 54**,** 233-246.

PRIDDY, B. M., CARMACK, S. A., THOMAS, L. C., VENDRUSCOLO, J. C., KOOB, G. F. & VENDRUSCOLO, L. F. 2017. Sex, strain, and estrous cycle influences on alcohol drinking in rats. *Pharmacol Biochem Behav,* 152**,** 61-67.

PRUS, A. J., JAMES, J. R. & ROSECRANS, J. A. 2009. Frontiers in Neuroscience

Conditioned Place Preference. *In:* BUCCAFUSCO, J. J. (ed.) *Methods of Behavior Analysis in Neuroscience.* Boca Raton (FL): CRC Press/Taylor & Francis

QEADAN, F., MCCUNN, A. & TINGEY, B. 2024. The association between glucose-dependent insulinotropic polypeptide and/or glucagon-like peptide-1 receptor agonist prescriptions and substance-related outcomes in patients with opioid and alcohol use disorders: A real-world data analysis. *Addiction*.

QUDDOS, F., HUBSHMAN, Z., TEGGE, A., SANE, D., MARTI, E., KABLINGER, A. S., GATCHALIAN, K. M., KELLY, A. L., DIFELICEANTONIO, A. G. & BICKEL, W. K. 2023. Semaglutide and Tirzepatide reduce alcohol consumption in individuals with obesity. *Sci Rep,* 13**,** 20998.

QUELCH, D. R., MICK, I., MCGONIGLE, J., RAMOS, A. C., FLECHAIS, R. S. A., BOLSTRIDGE, M., RABINER, E., WALL, M. B., NEWBOULD, R. D., STEINIGER-BRACH, B., VAN DEN BERG, F., BOYCE, M., ØSTERGAARD NILAUSEN, D., BREUNING SLUTH, L., MEULIEN, D., VON DER GOLTZ, C., NUTT, D. & LINGFORD-HUGHES, A. 2017. Nalmefene Reduces Reward Anticipation in Alcohol Dependence: An Experimental Functional Magnetic Resonance Imaging Study. *Biol Psychiatry,* 81**,** 941-948.

RAMCHANDANI, V. A., UMHAU, J., PAVON, F. J., RUIZ-VELASCO, V., MARGAS, W., SUN, H., DAMADZIC, R., ESKAY, R., SCHOOR, M., THORSELL, A., SCHWANDT, M. L., SOMMER, W. H., GEORGE, D. T., PARSONS, L. H., HERSCOVITCH, P., HOMMER, D. & HEILIG, M. 2011. A genetic determinant of the striatal dopamine response to alcohol in men. *Mol Psychiatry,* 16**,** 809-17.

RAY, L. A., GREEN, R., ENDERS, C., LEVENTHAL, A. M., GRODIN, E. N., LI, G., LIM, A., HARTWELL, E., VENEGAS, A., MEREDITH, L., NIETO, S. J., SHOPTAW, S., HO, D. & MIOTTO, K. 2021. Efficacy of Combining Varenicline and Naltrexone for Smoking Cessation and Drinking Reduction: A Randomized Clinical Trial. *Am J Psychiatry,* 178**,** 818-828.

REDDY, I. A., PINO, J. A., WEIKOP, P., OSSES, N., SØRENSEN, G., BERING, T., VALLE, C., BLUETT, R. J., ERREGER, K., WORTWEIN, G., REYES, J. G., GRAHAM, D., STANWOOD, G. D., HACKETT, T. A., PATEL, S., FINK-JENSEN, A., TORRES, G. E. & GALLI, A. 2016. Glucagon-like peptide 1 receptor activation regulates cocaine actions and dopamine homeostasis in the lateral septum by decreasing arachidonic acid levels. *Transl Psychiatry,* 6**,** e809.

REIDELBERGER, R. D., CHELIKANI, P. K. & HAVER, A. C. 2007. Effects of chronic intermittent intraperitoneal infusion of salmon calcitonin on food intake and adiposity in diet-induced obese rats. *Appetite,* 49**,** 323-323.

REIMANN, F., HABIB, A. M., TOLHURST, G., PARKER, H. E., ROGERS, G. J. & GRIBBLE, F. M. 2008. Glucose Sensing in L Cells: A Primary Cell Study. *Cell Metabolism,* 8**,** 532-539.

REINER, D. J., MIETLICKI-BAASE, E. G., OLIVOS, D. R., MCGRATH, L. E., ZIMMER, D. J., KOCH-LASKOWSKI, K., KRAWCZYK, J., TURNER, C. A., NOBLE, E. E., HAHN, J. D., SCHMIDT, H. D., KANOSKI, S. E. & HAYES, M. R. 2017. Amylin Acts in the Lateral Dorsal Tegmental Nucleus to Regulate Energy Balance Through Gamma-Aminobutyric Acid Signaling. *Biological Psychiatry,* 82**,** 828-838.

RENTERIA, R., BALTZ, E. T. & GREMEL, C. M. 2018. Chronic alcohol exposure disrupts top-down control over basal ganglia action selection to produce habits. *Nat Commun,* 9**,** 211.

REQUENA-OCAÑA, N., FLORES-LOPEZ, M., MARTÍN, A. S., GARCÍA-MARCHENA, N., PEDRAZ, M., RUIZ, J. J., SERRANO, A., SUAREZ, J.,

PAVÓN, F. J., DE FONSECA, F. R. & ARAOS, P. 2021. Influence of gender and education on cocaine users in an outpatient cohort in Spain. *Sci Rep,* 11**,** 20928.

RICHARDS, J. R., DORAND, M. F., ROYAL, K., MNAJJED, L., PASZKOWIAK, M. & SIMMONS, W. K. 2023. Significant Decrease in Alcohol Use Disorder Symptoms Secondary to Semaglutide Therapy for Weight Loss: A Case Series. *J Clin Psychiatry,* 85.

RICHARDS, J. R. & LE, J. K. 2025. Cocaine Toxicity. *StatPearls.* Treasure Island (FL) ineligible companies. Disclosure: Jacqueline Le declares no relevant financial relationships with ineligible companies.: StatPearls Publishing

RINWA, P., ERIKSSON, M., COTGREAVE, I. & BÄCKBERG, M. 2024. 3R-Refinement principles: elevating rodent well-being and research quality. *Lab Anim Res,* 40**,** 11.

RODD, Z. A., BELL, R. L., MCQUEEN, V. K., DAVIDS, M. R., HSU, C. C., MURPHY, J. M., LI, T. K., LUMENG, L. & MCBRIDE, W. J. 2005. Chronic ethanol drinking by alcohol-preferring rats increases the sensitivity of the posterior ventral tegmental area to the reinforcing effects of ethanol. *Alcohol Clin Exp Res,* 29**,** 358-66.

RODGERS, R. J. & DALVI, A. 1997. Anxiety, defence and the elevated plus-maze. *Neurosci Biobehav Rev,* 21**,** 801-10.

ROERECKE, M., SØRENSEN, P., LARAMÉE, P., RAHHALI, N. & REHM, J. 2015. Clinical relevance of nalmefene versus placebo in alcohol treatment: Reduction in mortality risk. *Journal of Psychopharmacology,* 29**,** 1152-1158.

ROSE, J. H., KARKHANIS, A. N., STEINIGER-BRACH, B. & JONES, S. R. 2016. Distinct Effects of Nalmefene on Dopamine Uptake Rates and Kappa Opioid Receptor Activity in the Nucleus Accumbens Following Chronic Intermittent Ethanol Exposure. *Int J Mol Sci,* 17.

ROTH, J. D., ERICKSON, M. R., CHEN, S. & PARKES, D. G. 2012. GLP-1R and amylin agonism in metabolic disease: complementary mechanisms and future opportunities. *Br J Pharmacol,* 166**,** 121-36.

ROTH, J. D., HUGHES, H., COFFEY, T., MAIER, H., TREVASKIS, J. L. & ANDERSON, C. M. 2007. Effects of prior or concurrent food restriction on amylin-induced changes in body weight and body composition in high-fat-fed female rats. *American journal of physiology. Endocrinology and metabolism,* 293**,** E1112.

SAMURA, A., WADA, S., SUDA, S., IITAKA, M. & KATAYAMA, S. 2000. Calcitonin receptor regulation and responsiveness to calcitonin in human osteoclast-like cells prepared in vitro using receptor activator of nuclear factor-kappaB ligand and macrophage colony-stimulating factor. *Endocrinology,* 141**,** 3774-82.

SANCHIS-SEGURA, C. & SPANAGEL, R. 2006. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. *Addict Biol,* 11**,** 2-38.

SCHMIDT, H. D., MIETLICKI-BAASE, E. G., IGE, K. Y., MAURER, J. J., REINER, D. J., ZIMMER, D. J., VAN NEST, D. S., GUERCIO, L. A., WIMMER, M.

E., OLIVOS, D. R., DE JONGHE, B. C. & HAYES, M. R. 2016. Glucagon-Like Peptide-1 Receptor Activation in the Ventral Tegmental Area Decreases the Reinforcing Efficacy of Cocaine. *Neuropsychopharmacology,* 41**,** 1917-28.

SCHNEIDER, K. E., KRAWCZYK, N., XUAN, Z. & JOHNSON, R. M. 2018. Past 15-year trends in lifetime cocaine use among US high school students. *Drug Alcohol Depend,* 183**,** 69-72.

SCHUETTE, L. M., GRAY, C. C. & CURRIE, P. J. 2013. Microinjection of Ghrelin into the Ventral Tegmental Area Potentiates Cocaine-Induced Conditioned Place Preference. *J Behav Brain Sci,* 3**,** 276-580.

SCHULTZ, W. 2015. NEURONAL REWARD AND DECISION SIGNALS: FROM THEORIES TO DATA. *Physiological Reviews,* 95**,** 853-951.

SCOTT, H., TJERNSTRÖM, N. & ROMAN, E. 2020. Effects of pair housing on voluntary alcohol intake in male and female Wistar rats. *Alcohol,* 86**,** 121-128.

SEXTON, P. M., PAXINOS, G., KENNEY, M. A., WOOKEY, P. J. & BEAUMONT, K. 1994. In vitro autoradiographic localization of amylin binding sites in rat brain. *Neuroscience,* 62**,** 553-567.

SHAHAM, Y., SHALEV, U., LU, L., DE WIT, H. & STEWART, J. 2003. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology (Berl),* 168**,** 3-20.

SHIRAZI, R. H., DICKSON, S. L. & SKIBICKA, K. P. 2013. Gut Peptide GLP-1 and Its Analogue, Exendin-4, Decrease Alcohol Intake and Reward. *PloS one,* 8**,** e61965.

SHUGHRUE, P. J., LANE, M. V. & MERCHENTHALER, I. 1996. Glucagon-like peptide-1 receptor (GLP1-R) mRNA in the rat hypothalamus. *Endocrinology,* 137**,** 5159-62.

SILVESTRE, R. A., SALAS, M., GARCÍA-HERMIDA, O., FONTELA, T., DÉGANO, P. & MARCO, J. 1994. Amylin (islet amyloid polypeptide) inhibition of insulin release in the perfused rat pancreas: implication of the adenylate cyclase/cAMP system. *Regulatory Peptides,* 50**,** 193-199.

SIMMS, J. A., STEENSLAND, P., MEDINA, B., ABERNATHY, K. E., CHANDLER, L. J., WISE, R. & BARTLETT, S. E. 2008. Intermittent Access to 20% Ethanol Induces High Ethanol Consumption in Long-Evans and Wistar Rats. *Alcoholism, clinical and experimental research,* 32**,** 1816-1823.

SIMPSON, E. H., AKAM, T., PATRIARCHI, T., BLANCO-POZO, M., BURGENO, L. M., MOHEBI, A., CRAGG, S. J. & WALTON, M. E. 2024. Lights, fiber, action! A primer on in vivo fiber photometry. *Neuron,* 112**,** 718-739.

SIMPSON, K. J., MORAN, M. T., MCCALL, K. L., HERBERT, J., FOSTER, M. L., SIMOYAN, O. M., SHAH, D. T., DESROSIERS, C., NICHOLS, S. D. & PIPER, B. J. 2019. Increasing heroin, cocaine, and buprenorphine arrests reported to the Maine Diversion Alert Program. *Forensic Sci Int,* 303**,** 109924.

SINCLAIR, J. M., CHAMBERS, S. E., SHILES, C. J. & BALDWIN, D. S. 2016. Safety and Tolerability of Pharmacological Treatment of Alcohol Dependence: Comprehensive Review of Evidence. *Drug Saf,* 39**,** 627-45.

SKIBICKA, K. P. 2013. The central GLP-1: Implications for food and drug reward. *Frontiers In Neuroscience,* 7.

SLADE, T., CHAPMAN, C., SWIFT, W., KEYES, K., TONKS, Z. & TEESSON, M. 2016. Birth cohort trends in the global epidemiology of alcohol use and alcohol-related harms in men and women: systematic review and metaregression. *BMJ Open,* 6**,** e011827.

SMITH, R. J., ANDERSON, R. I., HAUN, H. L., MULHOLLAND, P. J., GRIFFIN, W. C., 3RD, LOPEZ, M. F. & BECKER, H. C. 2020. Dynamic c-Fos changes in mouse brain during acute and protracted withdrawal from chronic intermittent ethanol exposure and relapse drinking. *Addict Biol,* 25**,** e12804.

SÖDERPALM, B., DANIELSSON, K., BEJCZY, A., ADERMARK, L. & ERICSON, M. 2020. Combined administration of varenicline and bupropion produces additive effects on accumbal dopamine and abolishes the alcohol deprivation effect in rats. *Addiction biology,* 25**,** e12807-n/a.

SÖDERPALM, B., ERICSON, M., OLAUSSON, P., BLOMQVIST, O. & ENGEL, J. A. 2000. Nicotinic mechanisms involved in the dopamine activating and reinforcing properties of ethanol. *Behavioural Brain Research,* 113**,** 85-96.

SOFUOGLU, M. & KOSTEN, T. R. 2005. Novel approaches to the treatment of cocaine addiction. *CNS Drugs,* 19**,** 13-25.

SØRENSEN, G., CAINE, S. B. & THOMSEN, M. 2016. Effects of the GLP-1 Agonist Exendin-4 on Intravenous Ethanol Self-Administration in Mice. *Alcoholism, clinical and experimental research,* 40**,** 2247-2252.

SØRENSEN, G., REDDY, I. A., WEIKOP, P., GRAHAM, D. L., STANWOOD, G. D., WORTWEIN, G., GALLI, A. & FINK-JENSEN, A. 2015. The glucagon-like peptide 1 (GLP-1) receptor agonist exendin-4 reduces cocaine self-administration in mice. *Physiol Behav,* 149**,** 262-8.

STROMBERG, M. F. 2004. The effect of baclofen alone and in combination with naltrexone on ethanol consumption in the rat. *Pharmacol Biochem Behav,* 78**,** 743-50.

SUCHANKOVA, P., STEENSLAND, P., FREDRIKSSON, I., ENGEL, J. A. & JERLHAG, E. 2013. Ghrelin receptor (GHS-R1A) antagonism suppresses both alcohol consumption and the alcohol deprivation effect in rats following long-term voluntary alcohol consumption. *PLoS One,* 8**,** e71284.

SUCHANKOVA, P., YAN, J., SCHWANDT, M. L., STANGL, B. L., CAPARELLI, E. C., MOMENAN, R., JERLHAG, E., ENGEL, J. A., HODGKINSON, C. A., EGLI, M., LOPEZ, M. F., BECKER, H. C., GOLDMAN, D., HEILIG, M., RAMCHANDANI, V. A. & LEGGIO, L. 2015. The glucagon-like peptide-1 receptor as a potential treatment target in alcohol use disorder: evidence from human genetic association studies and a mouse model of alcohol dependence. *Transl Psychiatry,* 5**,** e583.

SWIFT, R. M. 2013. Naltrexone and nalmefene: any meaningful difference? *Biol Psychiatry,* 73**,** 700-1.

TAKEDA, N., HASEGAWA, S., MORITA, M. & MATSUNAGA, T. 1993. Pica in rats is analogous to emesis: an animal model in emesis research. *Pharmacol Biochem Behav,* 45**,** 817-21.

TERATO, K., DO, C. T., CUTLER, D., WARITANI, T. & SHIONOYA, H. 2014. Preventing intense false positive and negative reactions attributed to the

principle of ELISA to re-investigate antibody studies in autoimmune diseases. *J Immunol Methods,* 407**,** 15-25.

THAM, L. S., PANTALONE, K. M., DUNGAN, K., MUNIR, K., TANG, C. C., KONIG, M. & KWAN, A. Y. M. 2022. A model-based simulation of glycaemic control and body weight when switching from semaglutide to 3.0- and 4.5-mg doses of once-weekly dulaglutide. *Diabetes Obes Metab,* 24**,** 302-311.

THOMAS, M. J., BEURRIER, C., BONCI, A. & MALENKA, R. C. 2001. Long-term depression in the nucleus accumbens: a neural correlate of behavioral sensitization to cocaine. *Nat Neurosci,* 4**,** 1217-23.

THOMPSON, A. M. & TRUJILLO, J. M. 2015. Dulaglutide: The Newest GLP-1 Receptor Agonist for the Management of Type 2 Diabetes. Los Angeles, CA.

THOMSEN, M., DENCKER, D., WÖRTWEIN, G., WEIKOP, P., EGECIOGLU, E., JERLHAG, E., FINK-JENSEN, A. & MOLANDER, A. 2017. The glucagon-like peptide 1 receptor agonist Exendin-4 decreases relapse-like drinking in socially housed mice. *Pharmacology Biochemistry And Behavior, 2017, Vol. 160, pp. 14-.20,* 160**,** 14-20.

THOMSEN, M., HOLST, J. J., MOLANDER, A., LINNET, K., PTITO, M. & FINK-JENSEN, A. 2020. Correction to: Effects of glucagon-like peptide 1 analogs on alcohol intake in alcohol-preferring vervet monkeys. *Psychopharmacology (Berl),* 237**,** 287.

TIMPER, K. & BRÜNING, J. C. 2017. Hypothalamic circuits regulating appetite and energy homeostasis: pathways to obesity. *Dis Model Mech,* 10**,** 679-689.

TORNEHAVE, D., KRISTENSEN, P., RØMER, J., KNUDSEN, L. B. & HELLER, R. S. 2008. Expression of the GLP-1 Receptor in Mouse, Rat, and Human Pancreas. *Journal of Histochemistry & Cytochemistry,* 56**,** 841-851.

TREVASKIS, J. L., TUREK, V. F., WITTMER, C., GRIFFIN, P. S., WILSON, J. K., REYNOLDS, J. M., ZHAO, Y., MACK, C. M., PARKES, D. G. & ROTH, J. D. 2010. Enhanced amylin-mediated body weight loss in estradiol-deficient diet-induced obese rats. *Endocrinology,* 151**,** 5657-68.

TUFVESSON-ALM, M., SHEVCHOUK, O. T. & JERLHAG, E. 2022. Insight into the role of the gut-brain axis in alcohol-related responses: Emphasis on GLP-1, amylin, and ghrelin. *Front Psychiatry,* 13**,** 1092828.

VADNIE, C. A., PARK, J. H., ABDEL GAWAD, N., HO, A. M. C., HINTON, D. J. & CHOI, D.-S. 2014. Gut-brain peptides in corticostriatal-limbic circuitry and alcohol use disorders. *Frontiers in Neuroscience,* 8.

VAHLE, J. L., BYRD, R. A., BLACKBOURNE, J. L., MARTIN, J. A., SORDEN, S. D., RYAN, T., PIENKOWSKI, T., WIJSMAN, J. A., SMITH, H. W. & ROSOL, T. J. 2015. Effects of Dulaglutide on Thyroid C Cells and Serum Calcitonin in Male Monkeys. *Endocrinology,* 156**,** 2409-2416.

VALLÖF, D., KALAFATELI, A. L. & JERLHAG, E. 2019a. Brain region specific glucagon-like peptide-1 receptors regulate alcohol-induced behaviors in rodents. *Psychoneuroendocrinology,* 103**,** 284-295.

VALLÖF, D., KALAFATELI, A. L. & JERLHAG, E. 2020. Long-term treatment with a glucagon-like peptide-1 receptor agonist reduces ethanol intake in male and female rats. *Translational Psychiatry, 2020, Vol. 10, Iss. 1,* 10.

VALLÖF, D., MACCIONI, P., COLOMBO, G., MANDRAPA, M., JÖRNULF, J. W., EGECIOGLU, E., ENGEL, J. A. & JERLHAG, E. 2016a. The glucagon-like peptide 1 receptor agonist liraglutide attenuates the reinforcing properties of alcohol in rodents. *Addiction biology,* 21**,** 422-437.

VALLÖF, D., ULENIUS, L., EGECIOGLU, E., ENGEL, J. A. & JERLHAG, E. 2017. Central administration of the anorexigenic peptide neuromedin U decreases alcohol intake and attenuates alcohol-induced reward in rodents. *Addict Biol,* 22**,** 640-651.

VALLÖF, D., VESTLUND, J., ENGEL, J. A. & JERLHAG, E. 2016b. The Anorexigenic Peptide Neuromedin U (NMU) Attenuates Amphetamine-Induced Locomotor Stimulation, Accumbal Dopamine Release and Expression of Conditioned Place Preference in Mice. *PLoS One,* 11**,** e0154477.

VALLÖF, D., VESTLUND, J. & JERLHAG, E. 2019b. Glucagon-like peptide-1 receptors within the nucleus of the solitary tract regulate alcohol-mediated behaviors in rodents. *Neuropharmacology,* 149**,** 124-132.

VERMA, V. 2015. Classic Studies on the Interaction of Cocaine and the Dopamine Transporter. *Clin Psychopharmacol Neurosci,* 13**,** 227-38.

VESTLUND, J. & JERLHAG, E. 2020. Glucagon-like peptide-1 receptors and sexual behaviors in male mice. *Psychoneuroendocrinology,* 117**,** 104687.

VOLKOW, N. D., FOWLER, J. S., WANG, G. J., HITZEMANN, R., LOGAN, J., SCHLYER, D. J., DEWEY, S. L. & WOLF, A. P. 1993. Decreased dopamine D2 receptor availability is associated with reduced frontal metabolism in cocaine abusers. *Synapse,* 14**,** 169-77.

VOLKOW, N. D., WANG, G.-J., FOWLER, J. S. & TOMASI, D. 2012. Addiction Circuitry in the Human Brain *. *Annual Review of Pharmacology and Toxicology,* 52**,** 321-336.

WACHSMUTH, H. R., WENINGER, S. N. & DUCA, F. A. 2022. Role of the gut-brain axis in energy and glucose metabolism. *Exp Mol Med,* 54**,** 377-392.

WADA, S., UDAGAWA, N., NAGATA, N., MARTIN, T. J. & FINDLAY, D. M. 1996. Calcitonin receptor down-regulation relates to calcitonin resistance in mature mouse osteoclasts. *Endocrinology,* 137**,** 1042-8.

WAGNER, F. A. & ANTHONY, J. C. 2002. From first drug use to drug dependence; developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology,* 26**,** 479-88.

WALLHED FINN, S., LUNDIN, A., SJÖQVIST, H. & DANIELSSON, A. K. 2021. Pharmacotherapy for alcohol use disorders - Unequal provision across sociodemographic factors and co-morbid conditions. A cohort study of the total population in Sweden. *Drug Alcohol Depend,* 227**,** 108964.

WANG, F., FLANAGAN, J., SU, N., WANG, L. C., BUI, S., NIELSON, A., WU, X., VO, H. T., MA, X. J. & LUO, Y. 2012. RNAscope: a novel in situ RNA analysis platform for formalin-fixed, paraffin-embedded tissues. *J Mol Diagn,* 14**,** 22-9.

WANG, W., VOLKOW, N. D., BERGER, N. A., DAVIS, P. B., KAELBER, D. C. & XU, R. 2024a. Association of semaglutide with reduced incidence and relapse of cannabis use disorder in real-world populations: a retrospective cohort study. *Mol Psychiatry,* 29**,** 2587-2598.

WANG, W., VOLKOW, N. D., BERGER, N. A., DAVIS, P. B., KAELBER, D. C. & XU, R. 2024b. Associations of semaglutide with incidence and recurrence of alcohol use disorder in real-world population. *Nat Commun,* 15**,** 4548.

WEISS, F., PAULUS, M. P., LORANG, M. T. & KOOB, G. F. 1992. Increases in extracellular dopamine in the nucleus accumbens by cocaine are inversely related to basal levels: effects of acute and repeated administration. *J Neurosci,* 12**,** 4372-80.

WELLMAN, P. J., DAVIS, K. W. & NATION, J. R. 2005. Augmentation of cocaine hyperactivity in rats by systemic ghrelin. *Regul Pept,* 125**,** 151-4.

WHITE, A. M. 2020. Gender Differences in the Epidemiology of Alcohol Use and Related Harms in the United States. *Alcohol Res,* 40**,** 01.

WHITE, F. J., HU, X. T., ZHANG, X. F. & WOLF, M. E. 1995. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. *J Pharmacol Exp Ther,* 273**,** 445-54.

WHO. 2024. *Alcohol,* https://www.who.int/health-topics/alcohol#tab=tab_1 [Online]. https://www.who.int/health-topics/alcohol#tab=tab_1: World Health Organization. [Accessed 2025].

WITKIEWITZ, K., SAVILLE, K. & HAMREUS, K. 2012. Acamprosate for treatment of alcohol dependence: mechanisms, efficacy, and clinical utility. *Ther Clin Risk Manag,* 8**,** 45-53.

WONG, G., GARNER, E. M. & SRIVASTAVA, G. 2023. Combined GLP-1 Receptor Agonist and Amylin Analogue Pharmacotherapy to Treat Obesity Comorbid With Type 1 Diabetes. *JCEM Case Rep,* 1**,** luad040.

WOODWARD, O. R. M., GRIBBLE, F. M., REIMANN, F. & LEWIS, J. E. 2022. Gut peptide regulation of food intake - evidence for the modulation of hedonic feeding. *J Physiol,* 600**,** 1053-1078.

YODER, K. K., CONSTANTINESCU, C. C., KAREKEN, D. A., NORMANDIN, M. D., CHENG, T. E., O'CONNOR, S. J. & MORRIS, E. D. 2007. Heterogeneous effects of alcohol on dopamine release in the striatum: a PET study. *Alcohol Clin Exp Res,* 31**,** 965-73.

YOSHIDA, K., ENGEL, J. & LILJEQUIST, S. 1982. The effect of chronic ethanol administration on high affinity 3 H-nicotinic binding in rat brain. *Naunyn-Schmiedeberg's Archives of Pharmacology,* 321**,** 74-76.

ZACHAR, G., MONTAGNESE, C., FAZEKAS, E. A., KEMECSEI, R. G., PAPP, S. M., DÓRA, F., RENNER, É., CSILLAG, A., POGÁNY, Á. & DOBOLYI, A. 2019. Brain Distribution and Sexually Dimorphic Expression of Amylin in Different Reproductive Stages of the Zebra Finch (Taeniopygia guttata) Suggest Roles of the Neuropeptide in Song Learning and Social Behaviour. *Front Neurosci,* 13**,** 1401.

ZAHM, D. S. 1999. Functional-anatomical implications of the nucleus accumbens core and shell subterritories. *Ann N Y Acad Sci,* 877**,** 113-28.

ZAHM, D. S. & BROG, J. S. 1992. On the significance of subterritories in the "accumbens" part of the rat ventral striatum. *Neuroscience,* 50**,** 751-67.

ZHANG, H., YAN, Z., WANG, X., GAŇOVÁ, M., CHANG, H., LAŠŠÁKOVÁ, S., KORABECNA, M. & NEUZIL, P. 2021. Determination of Advantages and

Limitations of qPCR Duplexing in a Single Fluorescent Channel. *ACS Omega,* 6**,** 22292-22300.

ZHANG, X. X., PAN, Y. H., HUANG, Y. M. & ZHAO, H. L. 2016. Neuroendocrine hormone amylin in diabetes. *World J Diabetes,* 7**,** 189-97.

ZHANG, Y., KAHNG, M. W., ELKIND, J. A., WEIR, V. R., HERNANDEZ, N. S., STEIN, L. M. & SCHMIDT, H. D. 2020. Activation of GLP-1 receptors attenuates oxycodone taking and seeking without compromising the antinociceptive effects of oxycodone in rats. *Neuropsychopharmacology,* 45**,** 451-461.

ZHOU, Y., LERI, F., LOW, M. J. & KREEK, M. J. 2019. Sex differences in the effect of bupropion and naltrexone combination on alcohol drinking in mice. *Pharmacol Biochem Behav,* 181**,** 28-36.

ZHU, Y., WIENECKE, C. F., NACHTRAB, G. & CHEN, X. 2016. A thalamic input to the nucleus accumbens mediates opiate dependence. *Nature,* 530**,** 219-22.