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Behavioral and Molecular Mechanisms of Cocaine Addiction Vulnerability:

Functional Evidence from Rodent Models

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Abstract

Cocaine addiction is a complex, chronically relapsing disorder shaped by individual vulnerability, neuroadaptive changes, and limited treatment options. Although substantial progress has been made in understanding the reinforcing properties of psychostimulants, the neurobehavioral mechanisms distinguishing recreational use from compulsive, addiction-like behavior remain incompletely understood. This thesis aimed to (i) identify behavioral phenotypes predictive of addiction vulnerability, (ii) examine molecular adaptations underlying compulsive drug use, and (iii) evaluate the therapeutic potential of pharmacological interventions using translationally-relevant rodent models.

The first objective focused on phenotypic predictors of addiction-like behavior. In Study 1A, the role of incentive salience attribution, measured through Pavlovian Conditioned Approach behavior, was evaluated within the 3-CRIT model of cocaine addiction. Contrary to the expectations of incentive sensitization theory, sign- and goal-tracking phenotypes failed to predict the development of addiction-like behavior. However, sign-trackers exhibited greater resistance to punishment. Study 1B examined sex differences in the 3-CRIT model. Although fewer females met the addiction-like criteria, those that did (i.e., "3crit" rats) exhibited more severe behavioral profiles despite lower overall drug intake. These findings highlight the importance of both sex and individual phenotype in modeling addiction vulnerability.

The second aim addressed the molecular mechanisms of addiction. In Study 2A, cocaine self-administration dynamically regulated the expression of metabotropic glutamate receptor 2 ($mGluR_2$, gene: *Grm2*) across cortico-striatal pathways. Extinction training, but not abstinence, robustly upregulated *Grm2* expression, and pharmacological activation of $mGluR_{2/3}$ with LY379268 suppressed cue-induced cocaine seeking. Changes in $mGluR_2$ expression followed a region- and experience-specific pattern: initial downregulation was observed in the prelimbic cortex after short-term exposure, shifting to the infralimbic cortex with prolonged cocaine use. Interestingly, addiction-vulnerable animals (3crit), despite comparable drug intake to 0crit animals, showed elevated *Grm2* levels in the infralimbic cortex and dorsal striatum, likely reflecting compensatory responses. In Study 2B, a ge-

netic dopamine transporter (DAT) impairment (*Slc6a3*_N157K) prevented the acquisition and maintenance of cocaine self-administration, emphasizing the necessity of intact DAT function for cocaine reinforcement.

The final part evaluated novel pharmacological interventions and methodological refinements. In Study 3A, the effects of psilocybin on extinction learning and cue-induced reinstatement in cocaine-experienced rats and mice were examined. Although behavioral outcomes were not significantly altered, the study provided important insight into the feasibility and limitations of applying psychedelic-based interventions in preclinical addiction models. In Study 3B, a rat adaptation of a mouse-based paradigm intended to assess reality testing was developed. While mediated aversion failed to emerge, direct aversion was consistently observed, underscoring both the challenges of cross-species translation and the importance of refining behavioral tools to probe drug-induced changes in cognition.

In summary, this thesis advances understanding of the behavioral, molecular, pharmacological, and methodological factors underlying cocaine addiction. By integrating phenotypic variability, sex differences, and molecular adaptations in glutamatergic and dopaminergic systems, together with exploratory evaluations of serotonergic psychedelics and refined behavioral paradigms, the work underscores the multifaceted nature of addiction vulnerability and the limitations of oversimplified models. Region- and experience-specific regulation of *Grm2* expression highlights dynamic glutamatergic plasticity associated with different stages of cocaine exposure and withdrawal. Additionally, the demonstration that impaired DAT function disrupts the acquisition and maintenance of cocaine self-administration emphasizes the necessity of intact dopaminergic signaling for cocaine reinforcement. Finally, the evaluation of psilocybin, together with efforts to refine cognitive assays, illustrates both therapeutic opportunities and methodological challenges. Together, these findings reinforce the need for refined preclinical approaches in addiction research.

Zusammenfassung

Kokainabhängigkeit ist eine komplexe, chronisch rezidivierende Störung, die durch individuelle Vulnerabilität, neuroadaptive Veränderungen und begrenzte Behandlungsmöglichkeiten geprägt ist. Obwohl erhebliche Fortschritte im Verständnis der verstärkenden Eigenschaften von Psychostimulanzien erzielt wurden, bleiben die neurobehavioralen Mechanismen, die den Übergang vom Freizeitkonsum zum zwanghaften, suchtähnlichen Verhalten bestimmen, unvollständig verstanden. Ziel dieser Arbeit war es, (i) Verhaltensphänotypen zu identifizieren, die eine Suchtvulnerabilität vorhersagen, (ii) molekulare Anpassungen zu untersuchen, die dem zwanghaften Drogenkonsum zugrunde liegen, und (iii) das therapeutische Potenzial pharmakologischer Interventionen in translational relevanten Nagermodellen zu evaluieren.

Das erste Ziel konzentrierte sich auf phänotypische Prädiktoren suchtähnlichen Verhaltens. In Studie 1A wurde die Rolle der Attribution von Anreizsalienz, gemessen durch das Pavlovsche Konditionierungsansatz-Verhalten (Pavlovian Conditioned Approach), im 3-CRIT-Modell der Kokainabhängigkeit untersucht. Entgegen den Erwartungen der Anreiz-Sensitivierungs-Theorie sagten Sign- und Goal-Tracking-Phänotypen die Entwicklung suchtähnlichen Verhaltens nicht vorher. Sign-Tracker zeigten jedoch eine größere Resistenz gegenüber Bestrafung. Studie 1B untersuchte Geschlechtsunterschiede im 3-CRIT-Modell. Obwohl weniger Weibchen die suchtähnlichen Kriterien erfüllten, wiesen diejenigen, die dies taten (d. h. “3crit“-Ratten), trotz geringerer Gesamtdrogenaufnahme ausgeprägtere Verhaltensprofile auf. Diese Ergebnisse unterstreichen die Bedeutung sowohl des Geschlechts als auch individueller Phänotypen bei der Modellierung von Suchtvulnerabilität.

Das zweite Ziel befasste sich mit den molekularen Mechanismen der Abhängigkeit. In Studie 2A regulierte Kokain-Selbstverabreichung dynamisch die Expression des metabotropen Glutamatrezeptors 2 ($mGluR_2$, Gen: *Grm2*) in kortiko-striatalen Netzwerken. Extinktionstraining, nicht jedoch Abstinenz, führte zu einer deutlichen Hochregulation der *Grm2*-Expression, und die pharmakologische Aktivierung von $mGluR_{2/3}$ mit LY379268 unterdrückte cue-induziertes Kokain-Suchverhalten. Veränderungen der $mGluR_2$-Expression

folgten einem regions- und erfahrungsspezifischen Muster: Eine initiale Herunterregulation wurde im prälimbischen Kortex nach kurzfristiger Exposition beobachtet, die sich mit längerer Kokainexposition in den infralimbischen Kortex verlagerte. Interessanterweise zeigten suchtanfällige Tiere (3crit), trotz vergleichbarer Drogenaufnahme mit 0crit-Tieren, erhöhte *Grm2*-Spiegel im infralimbischen Kortex und im dorsalen Striatum, was wahrscheinlich kompensatorische Reaktionen widerspiegelt. In Studie 2B verhinderte eine genetische Beeinträchtigung des Dopamintransporters (DAT; $Slc6a3_{N157K}$) den Erwerb und die Aufrechterhaltung der Kokain-Selbstverabreichung und betonte damit die Notwendigkeit einer intakten DAT-Funktion für die Verstärkungswirkung von Kokain.

Der letzte Teil evaluierte neuartige pharmakologische Interventionen und methodische Weiterentwicklungen. In Studie 3A wurden die Effekte von Psilocybin auf Extinktionslernen und cue-induzierte Reinstatement-Prozesse bei kokain-erfahrenen Ratten und Mäusen untersucht. Obwohl die Verhaltensausgänge nicht signifikant verändert wurden, lieferte die Studie wichtige Einblicke in die Machbarkeit und Grenzen des Einsatzes psychedelischer Interventionen in präklinischen Suchtmodellen. In Studie 3B wurde eine an Ratten adaptierte Version eines ursprünglich für Mäuse entwickelten Paradigmas zur Erfassung von "Reality Testing" etabliert. Während eine vermittelte Aversion nicht nachweisbar war, wurde eine direkte Aversion konsistent beobachtet. Dies unterstreicht sowohl die Herausforderungen der Kreuz-Spezies-Translation als auch die Bedeutung der Weiterentwicklung behavioraler Werkzeuge zur Untersuchung drogeninduzierter Veränderungen kognitiver Prozesse.

Zusammenfassend leistet diese Arbeit einen Beitrag zum Verständnis der behavioral, molekularen, pharmakologischen und methodischen Faktoren, die der Kokainabhängigkeit zugrunde liegen. Durch die Integration von phänotypischer Variabilität, Geschlechtsunterschieden und molekularen Anpassungen in glutamatergen und dopaminergen Systemen sowie durch explorative Untersuchungen zu serotonergen Psychedelika und weiterentwickelten Verhaltensparadigmen wird die multifaktorielle Natur der Suchtvulnerabilität und die Grenzen vereinfachter Modelle hervorgehoben. Die regions- und erfahrungsspezifische Regulation der *Grm2*-Expression verdeutlicht die dynamische glutamaterge Plastizität, die mit verschiedenen Stadien der Kokainexposition und des Entzugs assoziiert ist. Darüber hinaus zeigt der Nachweis, dass eine beeinträchtigte DAT-Funktion den Erwerb und die Aufrechterhaltung der Kokain-Selbstverabreichung verhindert, die Notwendigkeit einer intakten dopaminergen Signalübertragung für die Verstärkungswirkung von Kokain. Schließlich illustrieren die Evaluation von Psilocybin sowie Bemühungen zur Weiterentwicklung kognitiver Testverfahren sowohl therapeutische Chancen als auch methodische Herausforderungen. Zusammengenommen unterstreichen diese Befunde die Notwendigkeit verfeinerter präklinischer Ansätze in der Suchtforschung.

Table of contents

List of Figures

Abbreviations

ABST	Abstinence
ADE	Alcohol deprivation effect
ADHD	Attention deficit hyperactivity disorder
AIC	Akaike Information Criterion
Amy	Amygdala
APA	American Psychiatric Association
ASI	Addiction severity index
BIC	Bayesian Information Criterion
BP	Break point
cAMP	Cyclic adenosine monophosphate
CBT	Cognitive behavioral therapy
cDNA	complementary DNA
CNS	Central nervous system
COMT	Catechol-O-methyltransferase
CPP	Conditioned place preference
CR	Conditioned response
CS	Conditioned stimulus
CSA	Cocaine self-administration
CUD	Cocaine use disorder
DA	Dopamine
DAT	Dopamine transporter
DMT	N,N-dimethyltryptamine
DNA	Deoxyribonucleic acid
DRI	Dopamine reuptake inhibitor
DS	Dorsal striatum
DSM	Diagnostic and Statistical Manual of Mental Disorders
EMA	European Medicines Agency
EXT	Extinction
FDA	Food and Drug Administration

FR	Fixed-ratio
FSCV	fast-scan cyclic voltammetry
FSCAV	fast-scan controlled-adsorption voltammetry
Grm2	Glutamate metabotropic receptor 2 gene
GT	Goal-tracker
Hipp	Hippocampus
HTR	Head-twitch response
i.p.	intraperitoneal
i.v.	intravenous
iGluRs	Ionotropic glutamate receptors
INT	Intermediate
ITI	Intertrial interval
IVSA	Intravenous self-administration
KO	Knockout
LH	Lateral hypothalamus
LiCl	Lithium chloride
LMM	Linear mixed-effects model
LSD	Lysergic acid diethylamide
LTD	Long-term depression
MAO	Monoamine oxidase
mCS	mediated Conditioned stimulus
$mGluR_2$	Metabotropic glutamate receptor 2
mGluRs	Metabotropic glutamate receptors
mPFC	Medial prefrontal cortex
NAc	Nucleus accumbens
NET	Norepinephrine transporter
NP	Nose-poke
PCA	Pavlovian conditioned approach
pERK	phosphorylation of Extracellular signal-regulated kinase
PFC	Prefrontal cortex
PPI	Pre-pulse inhibition
PR	Progressive-ratio
PrLC	Prelimbic cortex
qPCR	quantitative Polymerase Chain Reaction
RMTA	Representation-mediated taste aversion
RNA	Ribonucleic acid
RT	Reality testing

s.c.	subcutaneous
SA	Self-administration
SERT	Serotonin transporter
SN	Substantia nigra
snRNA-seq	single-nucleus RNA sequencing
ST	Sign-tracker
SUD	Substance use disorder
TH	Tyrosine hydroxylase
TMS	Transcranial magnetic stimulation
TO	Time-out
tRNA	total RNA
US	Unconditioned stimulus
VMAT2	Vesicular monoamine transporter 2
VS	Ventral striatum
VTA	Ventral tegmental area
WHO	World Health Organization
WT	Wild-type

Full list of publications

Thesis-related publications

Psilocybin administered following extinction sessions does not affect subsequent cocaine cue reinstatement in male and female rats and mice.

Pohořalá, V., Kuchař, M., Spanagel, R., and Bernardi, R. E. (2024). *Neuroscience*, 559:156–165.

Comment on Flagel et al.: Sign-tracking as a predictor of addiction vulnerability

Pohořalá, V.*, Enkel, T.*, Bartsch, D., Spanagel, R., and Bernardi, R. E. (2021). *Psychopharmacology*, 238(9), 2665–2666.

Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration.

Pohořalá, V.*, Enkel, T.*, Bartsch, D., Spanagel, R., and Bernardi, R. E. (2021). *Psychopharmacology*, 238(8):2335–2346.

Non thesis-related publications

Cell type-specific multi-omics analysis of cocaine use disorder in the human caudate nucleus.

Zillich, L., Artioli, A., **Pohořalá, V.**, Zillich, E., Stertz, L., Belschner, H., Jabali, A., Frank, J., Streit, F., Avetyan, D., Völker, M. P., Müller, S., Hansson, A. C., Meyer, T. D., Rietschel, M., Ladewig, J., Spanagel, R., Oliveira, A. M. M., Walss-Bass, C., Bernardi, R. E., Koch, P., Witt, S. H. (2025). *Nature communications*, 16(1), 3381.

Nicotine self-administration and ERK signaling are altered in RasGRF2 knockout mice.

Morella, I.*, **Pohořalá, V.***, Calpe-López, C., Brambilla, R., Spanagel, R., and Bernardi, R. E. (2022). *Frontiers in pharmacology*, 13, 986566.

(*shared 1st authorship)

Chapter 1

Introduction

1.1 Cocaine use disorder

1.1.1 Definition and prevalence

Substance use disorder (SUD), commonly referred to as drug addiction, is a multifaceted and chronic condition characterized by impaired control over drug acquisition and consumption, persistent use despite adverse consequences, and a heightened vulnerability to relapse even after prolonged periods of abstinence. Focusing specifically on cocaine, global estimates from the United Nations Office on Drugs and Crime for 2020 indicate that approximately 21 million individuals worldwide engaged in cocaine use within the past year, representing 0.4 % of the global population aged 15 to 64. Notably, North America and Europe have emerged as the primary consumer markets for this substance (United Nations: Office on Drugs and Crime, 2022) In Europe, cocaine ranks as the most commonly tried illicit psychostimulant, with roughly 5.8 % of adults aged 15 to 64 reporting lifetime use (European drug report, 2021) However, it is important to note that only a minority, approximately 15–17 %, of users eventually progress to addiction (Anthony et al., 1994; Koob and Volkow, 2010; Lopez-Quintero et al., 2011; O'Brien, 2005; O'Brien et al., 1998). Cocaine use is also associated with numerous health risks, including an increased likelihood of stroke, seizures, movement disorders, and various cognitive impairments (Maraj et al., 2010; Riezzo et al., 2012; Spronk et al., 2013). Consequently, cocaine addiction imposes a substantial burden not only on individuals but also on public health systems and society as a whole.

Two internationally recognized classification systems are used for diagnosing diseases, including SUDs: the International Classification of Diseases, 11th Revision (ICD-11), published by the World Health Organization (WHO, 2019), and the Diagnostic and Statistical

Manual of Mental Disorders (DSM-5), published by the American Psychiatric Association (APA, 2013; latest revision 2022), which is specific to mental disorders. For the purposes of this dissertation, I will refer to the DSM definition of cocaine use disorder, as the 3-CRIT model of addiction used in Studies 1A and 1B is based on DSM criteria.

According to the DSM-5, cocaine use disorder is a multifaceted neuropsychiatric condition defined by significant impairment or distress, evidenced by the presence of at least 2 out of 11 criteria occurring within a 12-month period prior to diagnosis (American Psychiatric Association, 2013). These criteria can be divided into 4 categories—(1) physiological symptoms such as craving, tolerance, and withdrawal; (2) impaired control over cocaine use; (3) prioritization of drug use over other responsibilities or interests; and (4) continued use despite adverse consequences. The DSM-5 further categorizes severity into three levels: mild (2–3 criteria met), moderate (4–5 criteria), and severe (6 or more criteria). The diagnostic framework for SUD, including CUD, underwent significant changes between the DSM-IV (1994; revised 2000) and DSM-5 (2013) to better reflect emerging evidence on addiction mechanisms. The DSM-IV distinguished between two separate conditions—*Substance Abuse* and *Substance Dependence*. *Substance Abuse* was defined as a maladaptive pattern of substance use leading to significant impairment or distress, while *Substance Dependence* was considered more severe, marked by compulsive use, tolerance, and withdrawal (American Psychiatric Association, 2000). In contrast, the DSM-5 integrates these two categories into a single diagnosis, *Substance Use Disorder*, emphasizing a continuum of problematic use and reflecting the complex biopsychosocial nature of addiction (American Psychiatric Association, 2013).

Throughout this thesis, the terms substance use disorder and addiction will be used interchangeably.

1.1.2 Cocaine

1.1.2.1 History

Cocaine, methyl ester of benzoylecgonine (IUPAC name: [1R-(exo,exo)] -3-(benzoyloxy)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylic acid methyl ester), is a tropane alkaloid commonly found in the leaves of the *Erythroxylum coca* plant, which is native to the Andean region of South America, as well as parts of Mexico, Indonesia, and the West Indies. The concentration of cocaine typically ranges between 0.3–1 % of the dry leaf mass (Moore and Casale, 1994; Plowman and Rivier, 1983). Coca leaves have been used since ancient times, primarily for religious and ceremonial purposes. Archaeological findings in Peru

show evidence of communal coca leaf chewing dating back over 8,000 years (Dillehay et al., 2010). Traces of cocaine have also been identified in approximately 2,000-year-old Peruvian mummies (Altman et al., 1985), and it is believed that coca leaves mixed with saliva were used locally during ritual trephination procedures due to their anaesthetic properties (Gay et al., 1975).

Although coca was first introduced to Europe in the 16th century, its popularity rose significantly only in the mid-19th century, when Paolo Mantegazza described its psychostimulant effects in his paper *Sulle Virtù Igieniche e Medicinali della Coca* (Mantegazza, 1859). Around the same time, Albert Niemann, a doctoral student of Friedrich Wöhler, successfully isolated cocaine and reported its local anaesthetic properties (Niemann, 1860), followed by the first synthesis from tropinone by Richard Willstätter (Willstätter and Bode, 1901). Sigmund Freud, a regular user and early advocate of cocaine, published the influential paper *Über Coca* (Freud, 1885), in which he promoted its mood-elevating and cardiostimulatory effects and proposed its use in treating morphine addiction. However, it was Freud's colleague Carl Koller who first demonstrated its medical potential as a local anaesthetic, presenting the use of a cocaine solution in ophthalmic surgery at the 1884 International Ophthalmological Congress in Heidelberg (Markel, 2011). By the late 1800s, cocaine was widely adopted in medicine for its anaesthetic properties, e.g., nerve block anaesthesia (1885, Halsted) and epidural anaesthesia (1885, Corning) (Gorelick and Zych, 1985; López-Valverde et al., 2011).

Due to its cognitive-enhancing effects, both coca and purified cocaine gained popularity in Western consumer markets. Coca wines such as *Vin Mariani*, various patent medicines, and over-the-counter powdered cocaine products were widely sold. Notably, the original formulation of Coca-Cola included coca leaf extract as one of its active ingredients. However, the addictive potential of cocaine soon became evident. In response, the United States passed the Harrison Narcotics Tax Act in 1914, restricting access to cocaine to prescription use only. Its popularity declined during the 1920s, largely replaced by amphetamines, but resurged in the 1970s as a recreational drug. In the 1980s, the emergence of crack cocaine, a cheap and smokable form, sparked a public health crisis in the United States. The "crack epidemic" led to a surge in hospitalizations, violence, and incarceration, disproportionately affecting low-income and minority communities (Cornish and O'Brien, 1996; Hamid, 1992; Hart and Ksir, 2012). This period underscored the highly addictive nature of cocaine and reinforced its classification as a high-risk substance under public health and legal frameworks.

1.1.2.2 Pharmacology

Cocaine can be found in various forms, the most prominent are cocaine hydrochloride, a highly water soluble white powder substance, and cocaine free base ("crack"), essentially a water-insoluble compound in the form of white hard lumps created by neutralization of cocaine hydrochloride with a weak base (e.g baking soda) and water, both shown in figure 1.1. Cocaine can be administered via several routes—topically, intravenous injection, insufflation (snorting), smoking, ingestion, or absorption via mucosal surfaces (oral, urogenital, rectal, etc.). These routes significantly influence the onset, intensity, and duration of cocaine's effects (Gossop et al., 1994; Roque Bravo et al., 2022). In the traditional practice of coca chewing, sublingual absorption is the primary route of uptake; however, the dose of cocaine obtained from coca leaves is substantially lower than that from purified cocaine powder (Biondich and Joslin, 2016).

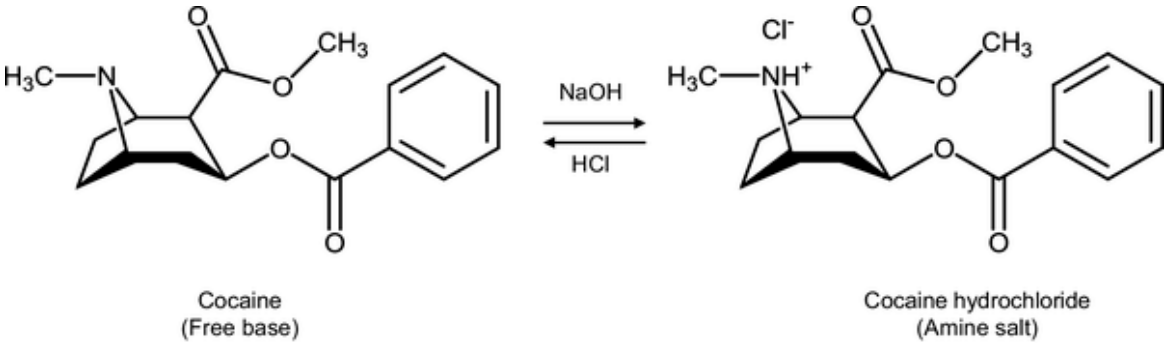

Figure 1.1. Chemical structure and conversion of cocaine free base and cocaine hydrochloride. Adapted from Oliveira and Dinis-Oliveira (2018)

Cocaine is generally rapidly absorbed, with immediate systemic availability following intravenous administration and absorption completed within 1–5 minutes when smoked (Cone, 1995). After intranasal administration, absorption occurs over approximately 15–45 minutes, with peak plasma concentrations typically reached within 30–60 minutes (Barnett et al., 1981; Cone, 1995; Javaid et al., 1983). Exceptions from rapid absorption include topical administration, where absorption is delayed due to local vasoconstriction, and oral ingestion, which is slowed by gastric and intestinal metabolism. Bioavailability ranges from nearly 100 % with intravenous and smoked routes to approximately 30 % for oral use (Benowitz, 1993; Coe et al., 2018). Generally, faster absorption results in more intense effects, although these are typically shorter in duration (Goldstein et al., 2009).

Once absorbed, cocaine is rapidly distributed to highly perfused tissues including the brain, spleen, kidneys, lungs, heart, and skeletal muscles. It exhibits strong plasma protein binding, primarily to albumin. The elimination half-life varies depending on the route of administration but typically ranges from 40 to 90 minutes, with intravenous use showing the shortest

half-life (Roque Bravo et al., 2022). Cocaine is primarily metabolized in the liver into ecgonine methyl ester and benzoylecgonine (Roque Bravo et al., 2022). Another clinically relevant metabolite is cocaethylene, which forms in the presence of ethanol and serves as a biomarker for concurrent cocaine and alcohol use (Andrews, 1997; Natekar et al., 2012; Tamargo et al., 2022). Most metabolites, along with a small fraction of unchanged cocaine, are excreted renally. Unmodified cocaine can be detected in urine for up to 36 hours, while metabolites such as benzoylecgonine may remain detectable for 2 to 4 days in occasional users, and up to 10 days in chronic users (Cunha-Oliveira et al., 2013).

Pharmacodynamically, cocaine acts as both a local anaesthetic and a sympathomimetic stimulant of the central and peripheral nervous systems. Its local anaesthetic properties arise from the blockade of voltage-gated sodium channels (Drake and Scott, 2018; Roque Bravo et al., 2022). Despite these effects, its clinical use as an anaesthetic is now rare and largely restricted to specific otorhinolaryngological procedures (e.g., *Solutio Bonain*, *Solutio Hirsch*), as safer alternatives such as procaine, lidocaine, and bupivacaine, non-tropane alkaloids with improved pharmacological profiles, are available (Petrželová, 2018).

As the primary focus of this thesis lies in the effects of cocaine on the central nervous system, this section emphasizes its central pharmacological action. Cocaine exerts its stimulant effects by inhibiting presynaptic monoamine transporters, most notably the dopamine transporter (DAT), but also the norepinephrine transporter (NET) and serotonin transporter (SERT). This inhibition prevents the reuptake of dopamine, norepinephrine, and serotonin into the presynaptic neuron, thereby elevating their concentrations in the synaptic cleft and prolonging the activation of postsynaptic receptors; see figure 1.2 (Roque Bravo et al., 2022). At low to moderate doses, cocaine typically produces euphoric effects, heightened arousal, increased sociability, and elevated locomotor activity. However, adverse effects such as anxiety, restlessness, and paranoia are also frequently observed, particularly at higher doses or following repeated administration (Benowitz, 1993).

1.1.2.3 Toxicity and public health concerns

Cocaine use exerts harmful effects on various physiological systems, especially cardiovascular and central nervous system, and is associated with life-threatening complications, with overdoses often resulting in fatal outcomes (Drake and Scott, 2018; Riezzo et al., 2012). The acute toxicity of cocaine primarily arises from activation of the sympathetic nervous system and the renin-angiotensin system, both centrally and peripherally. This results in increased myocardial oxygen demand, due to elevated heart rate, blood pressure, and contractility,

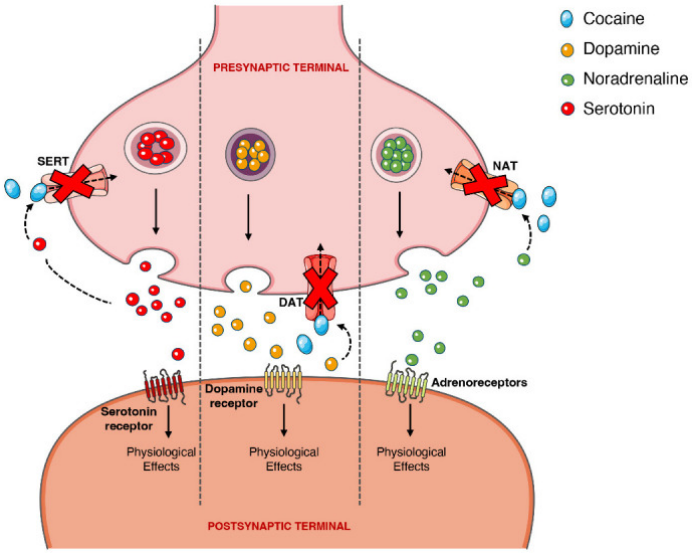

Figure 1.2. Schematic representation of cocaine´s mechanism of action at the site of the dopaminergic, serotonergic and noradrenergic synapse. Cocaine causes the blockade of presynaptic monoamine transporters, therefore obstructing the reuptake of dopamine, serotonin and noradrenaline (norepinephrine) back into the presynaptic terminal, which leads to an increased level of these neurotransmitters in the synaptic cleft resulting in intensified and prolonged activation of their respective postsynaptic receptors. DAT = dopamine transporter; NAT = noradrenaline (norepinephrine) transporter; SERT = serotonine transporter. Adapted from Roque Bravo et al. (2022)

coupled with reduced coronary perfusion as a consequence of vasoconstriction. These effects can cause a range of dysrhythmias (e.g., sinus tachycardia, atrial fibrillation, ventricular arrhythmias) and may progress to myocardial ischemia or infarction, which are among the most common cardiovascular complications associated with cocaine abuse (Mladěnka et al., 2018; Riezzo et al., 2012; Zimmerman, 2012). Although rare, aortic dissection should also be considered in patients presenting with cocaine-associated chest pain (Steinhauer and Caulfield, 2001; Zimmerman, 2012). Chronic cocaine use has been linked to structural myocardial damage and accelerated atherosclerosis (Riezzo et al., 2012).

Cocaine is frequently co-used with alcohol. As previously mentioned, this results in the *in vivo* formation of cocaethylene (ethylcocaine), a psychoactive metabolite. Interestingly, this is thought to be the only known instance in which an entire new and potent psychoactive compound is formed within the body (Andrews, 1997). The pharmacological properties of cocaethylene are similar to those of cocaine; however, the pharmacokinetics differ considerably, with cocaethylene having prolonged clearance, a larger volume of distribution and a subsequently longer elimination half-life (Hart et al., 2000; Pergolizzi et al., 2022). Co-administration of cocaine and ethanol is reported to enhance the desired euphoric effects

while masking some of the adverse symptoms, such as agitation from cocaine and feeling drunk from alcohol (Andrews, 1997; Harris et al., 2003). However, this combination significantly increases toxicity, especially cardiotoxicity—cocaethylene is estimated to be up to ten times more cardiotoxic than cocaine alone, likely due to its stronger sodium channel-blocking capacity (Pergolizzi et al., 2022; Wilson et al., 2001).

Cocaine affects the central nervous system (CNS) on the cerebrovascular, neurological and psychological levels. The most common CNS manifestation of cocaine toxicity resulting in immediate medical intervention is acute agitation (Zimmerman, 2012), which in extreme cases can escalate to cocaine-associated agitated delirium (Bell´s mania or excited delirium). This syndrome typically progresses through four stages: hyperthermia → delirium with agitation → respiratory arrest → death. Hyperthermia, a common effect of cocaine use that occurs as a result of extensive muscular activity and abnormal thermoregulation in the hypothalamus, imposes a high risk on the development of fatal excited delirium (Zimmerman, 2012). Many patients experiencing excited delirium die while restrained, and it has been suggested that a contributing factor may be the rush of catecholamines released as a stress response that further compromises a myocardium already weakened by chronic cocaine use (Karch et al., 1995; Mirchandani et al., 1994). Cocaine-induced seizures are more frequently observed in chronic users; however, they may also occur following a single exposure (Zimmerman, 2012). Furthermore, cocaine use has been linked to an elevated risk of hemorrhagic and ischemic strokes, various types of cerebral hemorrhages, movement disorders and cognitive impairments (Cardoso and Jankovic, 1993; Daras et al., 1994; Potvin et al., 2014; Spronk et al., 2013; Treadwell and Robinson, 2007). Additionally, frequent cocaine use has been associated with the worsening or new onset of psychiatric symptoms and disorders, such as psychosis, schizophrenia or depression (Alexander Morton, 1999; Brady et al., 1991; Ellison, 1994). Although the cardiovascular and CNS effects are most prominent, other organ systems are also affected. Pulmonary toxicities such as barotrauma, bronchospasm resulting in the exacerbation of asthma or hemoptysis, are common following inhalation. Other toxic effects include gastrointestinal ischemia, gastroduodenal perforation, renal infarction, glomerulonephritis, rhabdomyolysis, and hepatotoxicity (Riezzo et al., 2012; Zimmerman, 2012).

Taken together, the toxicity associated with cocaine use presents a major public health challenge. Its widespread availability and serious health consequences contribute to considerable social and economic burdens, affecting not only individuals but also families, communities, and healthcare systems.

1.1.3 Risk factors for CUD

Cocaine use disorder is a multifaceted progressive condition driven by a complex interplay of various factors, including genetic, epigenetic, psychosocial, environmental and developmental influences associated with chronic drug use (Anderson and Hearing, 2019). Nevertheless, not all individuals who use cocaine will progress to a fully developed addiction. In fact, the majority will not, as it is estimated that only 15-17 % of cocaine users ultimately develop CUD (Anthony et al., 1994; Wagner and Anthony, 2002). Those who do are likely to have an increased vulnerability due to a combination of biological predispositions and environmental influences.

Despite years of bringing awareness to the topic, society oftentimes still sees addiction as a problem of a weak willpower and lack of desire to "just quit". However, a substantial body of research, including twin and adoption studies, has demonstrated that addiction has a strong heritable component, emphasizing the importance of genetic risk factors. CUD is considered one of the most heritable psychiatric disorders, with heritability estimates ranging from 65 to 79 % (Ducci and Goldman, 2012; Fernàndez-Castillo et al., 2022; Schwartz et al., 2022). For example, first-degree relatives of individuals with CUD have more than a four-fold increased risk of developing the disorder (Merikangas et al., 1998), and siblings of individuals with cocaine dependence face a 1.3–3 times greater likelihood of receiving an SUD diagnosis (Bierut et al., 2008). Furthermore, comorbidity with other psychiatric conditions further increases vulnerability. Various studies have reported the frequent co-occurrence of CUD with schizophrenia, depression, attention deficit hyperactivity disorder (ADHD) (Currie et al., 2005; Piñeiro-Dieguez et al., 2016; Westermeyer, 2006), and associated behavioral traits, such as impulsivity and risk-taking behavior (Cabana-Domínguez et al., 2019; Fernàndez-Castillo et al., 2022).

Environmental risk factors also play a key role. These include early age of first use, peer group dynamics, socioeconomic status, and employment in high-stress or high-risk occupations (Badiani and Spagnolo, 2013; Pierce et al., 2018; Ystrom et al., 2014). Pharmacokinetic factors further influence addiction vulnerability: higher frequency and dose of use, as well as the route of administration, especially intravenous injection or smoking crack cocaine, are associated with an elevated risk of CUD (Gossop et al., 1994; Liu et al., 2020).

Finally, a growing body of evidence shows that sex and gender play a crucial role in the prevalence, progression and treatment outcome of CUD. For example, although men are more likely to use cocaine and subsequently develop CUD, a phenomenon known as telescoping has been described in women, indicating that their progression from initial use to addiction may be accelerated (Becker et al., 2017; Fattore et al., 2014; Gallop et al., 2007).

Once addicted, women report more difficulties to quit (Back et al., 2005), shorter periods of abstinence (Kosten et al., 1993), and are more likely to relapse in the context of stressful life events and interpersonal conflicts (Back et al., 2005; Terry-McElrath et al., 2009). In addition, women exhibit greater cue-induced craving compared to men (Robbins et al., 1999).

1.1.4 Treatment options

Although the first epidemic of cocaine use was documented in the late 19th century, and addiction was recognized as a clinical condition in the original DSM published by the APA in 1952, there are still no approved pharmacological treatments specifically indicated for managing CUD. As a result, psychosocial interventions remain the current "gold standard". While several approaches have demonstrated clinical efficacy, a significant portion of patients with CUD do not respond adequately, and psychosocial treatments are often associated with high dropout rates. Additionally, these interventions place substantial demands on treatment infrastructure, including the availability of trained professionals, and are further complicated by societal and provider-related stigma (Kampman, 2019; Schwartz et al., 2022).

Although a comprehensive discussion of psychosocial therapies falls outside the scope of this thesis, several core approaches deserve brief mention. Intensive outpatient therapy (typically involving a combination of group, individual and sometimes family sessions) is one of the most widely used strategies. Another highly effective approach, especially in promoting initial abstinence, is contingency management, utilizing the idea that the behavior that is rewarded is likely to be repeated, essentially stemming from operant conditioning principles that will be discussed later. During this approach, patients receive escalating voucher-based incentives (monetary or goods/services) for each drug-negative urine toxicology result, effectively reinforcing abstinence and promoting continued engagement (Schwartz et al., 2022). Cognitive-Behavioral Therapy (CBT) is another widely used approach aimed at helping individuals identify, understand, and modify maladaptive patterns of cocaine use. CBT promotes alternative coping strategies to reduce drug craving and avoid relapse, including distraction techniques, cognitive reframing, and positive behavior substitution (Schwartz et al., 2022). In recent years, digital adaptations of CBT have been explored to enhance accessibility and reduce treatment costs (Aharonovich et al., 2018; Carroll et al., 2008, 2014; Kiluk, 2019). These psychosocial modalities can be used sequentially or in combination to enhance treatment outcomes. Additionally, non-invasive methods such as transcranial magnetic stimulation (TMS) have shown promising potential in reducing cocaine use and

craving (Politi et al. 2008; Rapinesi et al. 2016; for reviews, see Amerio et al. 2023; Edinoff et al. 2023).

Over the past few decades, significant research has been devoted to pharmacotherapeutic strategies for CUD. Despite these efforts, no medication has yet received regulatory approval, and the need for targeted pharmacotherapies remains pressing. Although no single drug class has emerged as definitively effective, several compounds have been investigated off-label, with varying degrees of success. A growing number of meta-analyses and systematic reviews have addressed these efforts (Brandt et al., 2021; Buchholz and Saxon, 2019; Chan et al., 2019; Kampman, 2019; Schwartz et al., 2022). Broadly, pharmacological strategies can be divided into four categories: agonists (e.g., dextroamphetamine, modafinil), antagonists or blockers (e.g., aripiprazole, vigabatrin, topiramate, doxazosin, disulfiram, and cocaine vaccines), novel mechanisms (e.g., galantamine, ketamine), and combination therapies (e.g., disulfiram/naltrexone, extended-release mixed amphetamine salts/topiramate). However, clinical trials and studies have often yielded inconsistent results, partly due to the considerable variance in the population of CUD patients and common comorbidities, such as preexisting mental disorders and polydrug use (Brandt et al., 2021; Chan et al., 2019). Among these categories, agonist-based treatments have attracted particular interest due to their conceptual grounding in the substitution principle—they generally share pharmacodynamic properties with cocaine but differ in pharmacokinetics, resulting in lower abuse potential (e.g., prolonged onset of action, longer duration, mitigated peak in concentration). Although agonist substitution has proven effective in the treatment of other SUDs (e.g., methadone or buprenorphine for opioids; varenicline or nicotine patches for tobacco), finding a suitable agonist for CUD has proven difficult due to cocaine's complex mechanism of action (Brandt et al., 2021; Kampman, 2019). The assessment of pharmacological efficacy typically relies on qualitative urine toxicology screening. However, sustained and complete abstinence is difficult to achieve, and clinical trials often suffer from high dropout rates and poor medication adherence (Brandt et al., 2021), complicating the interpretation of outcomes. Therefore, continued research into both novel compounds and improved delivery strategies is necessary.

In recent years, the renaissance of psychedelic research has introduced new possibilities for the treatment of various psychiatric conditions (Hadar et al., 2023; Murnane, 2018). In controlled clinical trial settings, serotonergic psychedelics, such as psilocybin, N,N-dimethyltryptamine (DMT), and lysergic acid diethylamide (LSD), administered alongside psychotherapy, have shown promise in managing chronic psychiatric disorders including major depression (Carhart-Harris et al., 2021; Davis et al., 2021; Goodwin et al., 2022), anxiety (Griffiths et al., 2016), and substance use disorders (Bogenschutz and Johnson, 2016;

Garcia-Romeu et al., 2019, 2015). However, much of the research in this area has focused on alcohol and tobacco use disorders, and this trend is mirrored in preclinical studies. Furthermore, a major challenge in translating psychedelic therapies to preclinical models of addiction involves the integration of psychotherapeutic elements, which are considered essential to the therapeutic efficacy observed in humans.

1.2 Reward-associated learning and addiction

Rewards are essential for sustaining and enhancing life—they promote behaviors that increase access to food, mating opportunities, and other survival-relevant resources, thereby improving the organism's chances of reproduction and survival. To achieve this, individuals must learn to recognize the stimuli, objects, actions, and contexts necessary for obtaining these rewards. This process involves learning, approach behaviors, and the experience of positive emotions. Therefore, rewards are not defined solely by their physical properties but by the behavioral responses they elicit and their role as positive reinforcers (Schultz, 2015). Reward-associated learning is a cognitive process that allows individuals to learn to make the connection between certain behaviors and positive outcomes, subsequently resulting in the reinforcement of those behaviors.

Classical, also known as Pavlovian, conditioning is a form of associative learning that occurs when a neutral stimulus (e.g., the sound of a bell) is paired with an unconditioned stimulus (US, e.g., food display), resulting in an unconditioned response (e.g., salivation). Through repeated pairings, this originally neutral stimulus is then able to evoke a conditioned response (CR), similar to the unconditioned response, even in the absence of the US, and therefore becomes a conditioned stimulus (CS) (Pavlov, 1927). Operant conditioning, as formulated by Burrhus F. Skinner, represents another form of associative learning that is based on the principles of reward and punishment: behaviors followed by positive outcomes are more likely to be repeated, whereas those followed by punishment tend to decrease in frequency (Skinner, 1938). Unlike classical conditioning, operant conditioning centers on voluntary behaviors and their consequences. Importantly, the stimuli involved in operant conditioning often gain predictive value through concurrent Pavlovian stimulus-response learning. Although distinct in their mechanisms, these two learning processes frequently occur in parallel, underscoring the complexity of associative learning.

The Rescorla-Wagner model offers a theoretical framework for understanding how learning occurs during classical conditioning, centering on the concept of *prediction error*—the difference between expected and actual outcomes (Rescorla and Wagner, 1972). When the

received reward is greater than anticipated (positive prediction error), the model predicts an increase in associative strength and behavioral responding. Conversely, when the reward is smaller than expected or absent (negative prediction error), responding decreases, leading to extinction. According to this model, learning is most effective when the US is unexpected, and as the stimulus becomes more predictable through experience, the capacity for further learning declines.

These foundational concepts have contributed to the development of the incentive-sensitization theory of addiction, introduced by Robinson and Berridge (Berridge and Robinson, 1998, 2016; Robinson and Berridge, 1993, 2008). Repeated exposure to drugs of abuse results in enhanced sensitivity of the brain´s reward system ("sensitization") to drug-associated cues, stimuli previously paired with drugs (e.g., places, people or drug paraphrenalia – syringe, pipe, aluminium foil, etc.). These cues then acquire amplified incentive-motivational properties, making them more desirable or "wanted", which is independent of the "liking" or actual pleasurable effects of the drug, oftentimes reduced by tolerance. This dissociation between wanting and liking results in heightened incentive salience of drug cues, which can elicit strong cravings and compulsive drug-seeking behaviors, and potentially trigger relapse, further exacerbating the cyclical nature of addiction (Kalivas and O'Brien, 2008; Robinson and Berridge, 1993, 2003).

Over the past three decades, both clinical and preclinical research has proposed other theories of addiction on the premise that drugs of abuse "hijack" the brain's reward system, leading to long-lasting changes in neuroplasticity and motivational processes normally involved in obtaining natural rewards. In addition to the incentive-sensitization theory, several other influential frameworks have shaped addiction research. For instance, aberrant learning theory lies in a premise that compulsive drug consumption is a result of overlearned, automatic habits that become resistant to change (Everitt and Robbins, 2005, 2016; Tiffany, 1990). The hedonic allostasis hypothesis, derived from the opponent-process theory of motivation (Solomon and Corbit, 1973), introduces the concept of an anti-reward system. It suggests that as the hedonic impact of the drug diminishes, continued use is driven less by pleasure and more by the need to avoid discomfort and achieve a normal hedonic state (Koob, 2001; Koob and Le Moal, 2008). Lastly, the frontostriatal dysfunction theory emphasizes impairments in top-down executive control, proposing that addiction results from disrupted regulatory processes that facilitate a shift from controlled use to compulsive drug-taking behavior (Jentsch and Taylor, 1999; Kalivas and Volkow, 2005; Volkow and Fowler, 2000).

1.3 Dopamine and reward pathways

Dopamine (DA), a member of catecholamine family, was first synthesized in 1910 by George Barger and James Ewens. Initially, it was assumed to be solely a precursor of norepinephrine, and little research followed for several decades. However, DA gained scientific attention in the 1950s, when it was first identified in the brains of various animals (Montagu, 1957). A series of subsequent studies by Arvid Carlsson and colleagues established dopamine´s function as an important neurotransmitter (Carlsson, 1959; Carlsson et al., 1958), involved in motor control—a discovery for which he was awarded the Nobel Prize in Physiology and Medicine in 2000. Since these early findings, interest in DA and its physiological functions has expanded significantly. Today, DA is recognized as a crucial neurotransmitter involved in motor control, motivation, reward processing, and learning.

Dopamine is enclosed into vesicles via vesicular monoamine transporter 2 (VMAT2), transported to their release site, and ejected into the synaptic cleft, where it interacts with metabotropic, G-protein-coupled receptors. Based on pharmacological and structural properties, dopamine receptors can be divided into two groups (Kebabian and Calne, 1979; Marsden, 2006): the D_1-like, consisting of D_1 (Dearry et al., 1990; Zhou et al., 1990) and D_5 (Grandy et al., 1991; Sunahara et al., 1991) that activate adenylyl cyclase, and subsequently increase cyclic adenosine monophosphate (cAMP) production, and D_2-like, including D_2 (Bunzow et al., 1988), D_3 (Sokoloff et al., 1990) and D_4 (Van Tol et al., 1991) receptors that inhibit adenylyl cyclase (and subsequently decrease cAMP production). The most predominantly expressed DA receptors in the brain are D_1 and D_2. Both have low and high DA affinity states; however, the latter's *in vivo* affinity for dopamine is much higher – estimated up to 100-fold higher than that of D_1 (Costa and Schoenbaum, 2022; Tritsch and Sabatini, 2012). This means that baseline DA levels are sufficient enough to keep D_2 occupied, consequently resulting in their activation, and deactivation arises only from temporary drops in dopamine, whereas D_1 can be activated only by higher concentrations of extracellular dopamine ("burst firing") (Wise, 2008). This difference in sensitivity to DA concentrations leading to the activation of either D_1 or D_2 have led to a differentiation between so-called tonic (sustained, "background") and phasic (transient, "burst-like") DA release, linked to the reaction to behaviorally relevant stimuli (Grace, 1991). Following receptor binding, dopamine is quickly released back into the synaptic cleft, where it is cleared by either diffusion or via uptake by the DAT back into presynaptic terminals (Torres et al., 2003). This reuptake mechanism is a key regulatory step that determines the duration and intensity of dopamine signaling.

As touched on above, cocaine, a competitive inhibitor of DAT, increases extracellular DA levels (Di Chiara and Imperato, 1988) by "locking" the DAT in its inactive form and there-

fore blocking the re-uptake of DA back into presynaptic terminals (Kahlig and Galli, 2003; Ritz et al., 1987). Although inhibition of DAT is considered the primary mechanism underlying cocaine's addictive properties, cocaine also affects other monoamine transporters, including the serotonin and norepinephrine transporters (Kuhar et al., 1991; Pierce and Kumaresan, 2006; Torres et al., 2003).

As discussed above, rewards play a fundamental role in survival and behavior by reinforcing actions that promote access to essential resources or help avoid harm. To support such adaptive behavior, evolution has shaped specific neural circuits and molecular mechanisms driven by reward and aversion (Hu, 2016). A pivotal early study by Olds and Milner (1954) demonstrated that rats would repeatedly press a lever to receive direct electrical stimulation of certain brain regions, thereby identifying areas critical for reinforcement (Olds and Milner, 1954).

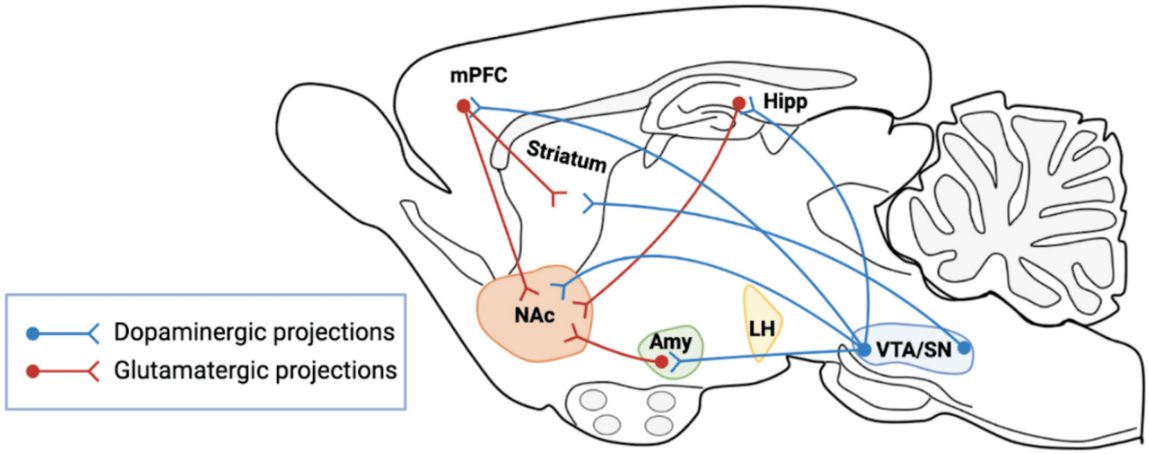

Figure 1.3. Schematic representation of reward pathways in rodent brain. Reward and reward-associated stimuli trigger the release of dopamine, resulting in activation of dopaminergic circuitry from the ventral tegmental (VTA) area to the nucleus accumbens (NAc) which is heavily innervated by glutamatergic neurons originating from several brain areas, including the medial prefrontal cortex (mPFC), hippocampus (Hipp), amygdala (Amy), and lateral hypothalamus (LH). Additionally, the striatum is densely innervated from the zona compacta of substantia nigra (SN) and the prefrontal cortex (PFC). Modified from Speranza et al. (2021).

Subsequent research led to the identification of three major dopaminergic pathways: the nigrostriatal, mesolimbic, and mesocortical pathways (see Fig. 1.3). The latter two are often grouped together as the mesocorticolimbic system due to their anatomical and functional overlap. The nucleus accumbens (NAc) is functionally divided into two distinct subregions: the core, linked to cue-triggered, goal-directed behavior and motor output, and the shell, associated with the affective and motivational aspects of reward (Ikemoto, 2007; Kelley,

2004; Zahm, 2000). This distinction reflects their differing connectivity—while the shell is more interconnected with limbic structures such as the amygdala and hypothalamus, the core aligns more with the dorsal striatum (DS) and motor circuits (Heimer and Van Hoesen, 2006). Notably, drugs of abuse such as cocaine preferentially elevate dopamine in the shell during early use, with this activity shifting toward the core as behavior becomes more habitual and compulsive (Di Chiara, 1999; Everitt and Robbins, 2005). The mesocortical pathway also arises from the ventral tegmental area (VTA) and innervates prefrontal regions involved in executive function and decision-making. Together, these circuits are central to both natural and drug reward processing and play a key role in addiction progression (Lüscher and Malenka, 2011; Wise, 2004). Chronic drug exposure induces long-term neuroplastic adaptations in this system, including potentiated glutamatergic input from the prefrontal cortex (PFC) to the NAc and increased dendritic spine density on medium spiny neurons (Kalivas and Volkow, 2005; Nestler, 2005b; Robinson and Kolb, 2004). These changes contribute to persistent craving and relapse vulnerability. Neuroimaging studies in humans confirm that drug-related cues activate overlapping regions, such as the ventral striatum (VS) and medial prefrontal cortex (mPFC), highlighting the translational relevance of these findings (Volkow et al., 2003).

1.4 The role of glutamate in addiction

Glutamate, the main excitatory neurotransmitter in the mammalian brain, is pivotal for normal cognitive and emotional processing, as it is highly involved in synaptic plasticity, cognition, learning and memory (McEntee and Crook, 1993; Zhou and Danbolt, 2014). However, excessive extracellular glutamate ("hyperglutamatergic state") can be neurotoxic and has been implicated in both acute and chronic neurodegenerative conditions, such as cerebral ischemia, traumatic brain injury, amyotrophic lateral sclerosis, and Huntington's disease (Lau and Tymianski, 2010; McEntee and Crook, 1993). Therefore, strict regulation of extracellular glutamate concentrations is vital. This regulation is sustained by a robust system of glutamate transporters that rapidly remove excess glutamate from the synaptic cleft and prevent excitotoxic overstimulation of glutamate receptors (Zhou and Danbolt, 2014).

In addition to its physiological functions, glutamate is also highly involved in addiction-related neuroplasticity and drug-seeking behavior (Kalivas, 2004, 2009). Microdialysis studies have shown that during drug- or stress-primed reinstatement of cocaine-seeking, extracellular glutamate levels in the NAc increase in rats with prior cocaine self-administration (CSA) experience (McFarland et al., 2004, 2003), whereas only dopamine was elevated in yoked cocaine and saline controls (McFarland et al., 2003), suggesting impaired regulation

in glutamate transmission following CSA (Uys and LaLumiere, 2008). In fact, basal levels of glutamate in the NAc are elevated during CSA, but not altered by acute intravenous (i.v.) cocaine administration (Miguéns et al., 2008), and attenuated in rats after repeated cocaine experience followed by a period of abstinence (withdrawal period or extinction) from cocaine (Baker et al., 2003; Miguéns et al., 2008; Trantham-Davidson et al., 2012). This reduction might result in reduced tonic activation of presynaptic group II metabotropic glutamate receptors ($mGluR_2$), which normally act as autoreceptors inhibiting glutamate release (Uys and LaLumiere, 2008).

Two main groups of glutamatergic receptors have been identified in the mammalian brain: ionotropic (iGluRs) and metabotropic (mGluRs). The first group (iGluRs) mediate fast excitatory synaptic transmission. iGluRs are tetramers with a central ion channel pore which is - when activated by glutamate - permeable for cations, in particular Na^+, K^+ and Ca^{2+}, and can be classified into 3 subtypes based on their pharmacological properties: AMPA receptors, NMDA receptors and kainate receptors (Hansen et al., 2021; Reiner and Levitz, 2018; Traynelis et al., 2010). In contrast, mGluRs are G protein–coupled receptors that act through a signal transduction cascade and mediate slow, modulatory, glutamate transmission. Based on the structure and physiological activity, 8 types of mGluRs ($mGluR_1$ - $mGluR_8$) can be divided into 3 groups - I, II and III. Group I ($mGluR_1$, $mGluR_5$) receptors, predominantly located postsynaptically, are coupled with G_q, resulting in activation of cAMP-dependent protein kinase, whereas group II ($mGluR_2$, $mGluR_3$) and group III ($mGluR_4$ - $mGluR_8$), predominantly located presynaptically, are coupled with $G_{i/o}$, resulting in inhibition of cAMP-dependent protein kinase (Niswender and Conn, 2010; Pinheiro and Mulle, 2008; Reiner and Levitz, 2018). Activation of Group II and III receptors reduces synaptic glutamate release, making them key regulators of glutamate homeostasis and potential targets for relapse prevention (D'Souza, 2015).

Among these, $mGluR_2$ has sparked great interest in addiction research due to its prominent expression in relapse-relevant brain regions, including the prefrontal cortex, nucleus accumbens, and ventral tegmental area, and its role in regulating excitatory input and synaptic strength (Moussawi and Kalivas, 2010; Niedzielska-Andres et al., 2021; Olive, 2009; Schmidt and Pierce, 2010). $mGluR_{2/3}$ have been shown to mediate dopamine release within NAc: the administration of direct (e.g., LY379268) or indirect (e.g., 2-PMPA) agonists decreased basal DA levels, whereas the infusion of antagonists (e.g., MGS0039) results in enhanced DA levels (Greenslade and Mitchell, 2004; Karasawa et al., 2006; Xi et al., 2010). Furthermore, the $mGluR_{2/3}$ agonist LY379268 reduces cocaine-, cue-, and stress-induced reinstatement of drug-seeking in preclinical models (Baptista et al., 2004; Cannella et al., 2013; Martin-Fardon and Weiss, 2012).

Despite extensive research, the role of $mGluR_2$ in addiction is not completely understood. Yang et al. (2017) showed that $mGluR_2$-knockout (KO) rats increased self-administration (SA) of cocaine and nicotine, but not sucrose, suggesting a drug-specific effect. Paradoxically, these KO rats also showed reduced motivation for cocaine, indicated by lower break points on a PR schedule, and decreased drug-seeking during extinction and reinstatement. While these outcomes resemble those observed with $mGluR_{2/3}$ agonists like LY379268, the authors argue that the underlying mechanisms differ—KO rats exhibit elevated baseline dopamine and glutamate levels, diminishing the impact of cocaine, whereas LY379268 suppresses the cocaine-induced release of these neurotransmitters (Yang et al., 2017).

To better understand how $mGluR_2$ function is regulated in addiction, it is essential to consider the molecular mechanisms that control its expression and synaptic availability. $mGluR_2$ is encoded by the glutamate metabotropic receptor 2 gene (*Grm2*). *Grm2* mRNA reflects transcriptional activity and is typically quantified via quantitative polymerase chain reaction (qPCR) or *in situ* hybridization. However, increased mRNA levels do not necessarily result in more functional receptor protein due to post-transcriptional regulation (Vogel and Marcotte, 2012). Total $mGluR_2$ protein, often measured by Western blot or ELISA, includes both intracellular and membrane-localized receptors; however, only receptors present on the cell surface, assessable through biotinylation or ligand-binding assays, are capable of modulating synaptic activity (Pomierny-Chamiolo et al., 2017; Schwendt et al., 2012). Moreover, $mGluR_2$ functions as a homodimer, and improper dimerization can impair signaling even in the presence of normal protein levels (Levitz et al., 2016). Therefore, a comprehensive characterization of $mGluR_2$-related plasticity in addiction ideally requires integration of transcriptional, translational, and post-translational analyses. However, such multifaceted approaches are often constrained by methodological limitations, analytical complexity, or resource availability.

1.5 Modeling addiction-related behaviors in rodents

Although addiction in all its breadth and complexity is unique to humans, it is still possible to model some of the behavioral aspects convincingly in a laboratory setting in animals, including rodents (Kuhn et al., 2019; Sanchis-Segura and Spanagel, 2006). Over the years, several protocols have been developed in order to investigate the short- and long-term effects of a certain drug on an organism and to mimic various features of addiction, which have proven to be immensely helpful in advancing the frontiers of knowledge about mechanisms, risk factors and underlying processes behind this disease. However, much remains unknown.

In order to assess the validity of a certain model of neuropsychiatric disease, like addiction, a system evaluating 3 sets of criteria—face, construct and predictive validity—has been put in place to ensure that the model properly reflects the human condition it aims to simulate (McKinney, 1969; Sams-Dodd, 1999; Willner et al., 1992). Face validity refers to the extent to which the model mimics the condition in humans, e.g., behavior and physiological signs. Construct validity describes whether the underlying mechanisms in the animal model are the same as in the clinical condition, e.g., neurobiological pathways. Predictive validity refers to the ability of the model to correctly predict outcome relevant to clinical condition, e.g., the effectiveness of potential treatments.

In general, animal models of addiction can be divided into 2 categories: non-contingent and contingent (Kuhn et al., 2019). In non-contingent models, such as conditioned place preference or behavioral sensitization, the drug is administered by the experimenter, independent of the animals´ motivation. This approach is particularly convenient when investigating the effects of drug exposure in a standardized manner, because it allows for the precise regulation of dose, timing and frequency while limiting the variability introduced by self-administration in other models. These types of models are often used to measure the reinforcing properties of a given drug. However, their face validity in replicating the voluntary aspect of human addiction is limited. Therefore, it is common to use these models in combination with other approaches. Contingent models, by contrast, rely on the animals' motivation to self-administer the drug, typically either perorally or intravenously. The paradigms used in the experiments performed for this thesis will be discussed in more detail below.

1.5.1 Intravenous self-administration (IVSA)

Intravenous self-administration is an essential paradigm for investigating the mechanisms of cocaine addiction and testing potential therapeutic interventions, as it closely mimics the volitional nature of human drug-taking behavior, resulting in a considerable degree of face validity. Both humans and animals will voluntarily self-administer cocaine, as well as other drugs of abuse, because of its reinforcing properties, and they will do so even when not in a state of dependence (Deneau et al., 1969; Koob and Le Moal, 2006; Schuster and Thompson, 1969; Sughondhabirom et al., 2005). Furthermore, substances readily self-administered by animals have been shown to have high potential for abuse in humans, which implies substantial predictive validity of IVSA models in preclinical addiction research (Lile and Nader, 2005; Lynch et al., 2010; Panlilio and Goldberg, 2007).

The basic principles of IVSA are rooted in operant conditioning, a learning process in which

the behavior is influenced or "controlled" by its consequences (Skinner, 1938; Thorndike, 1898); hence, behavior that is reinforced (rewarded) tends to be repeated whereas punished behavior will decrease. In IVSA, drug delivery occurs as a consequence of an instrumental action, such as a lever press or nose poke (NP), performed by an animal, thus acting as a positive reinforcer. However, this is true only for responses on the active lever/NP, as responses on the inactive lever/NP do not result in drug infusion. Therefore, the degree of learning can be later assessed by the active vs. inactive lever press/NP ratio. Additionally, stimuli present in the environment (cues) will be associated with the drug and can later become predictive of the reward, thus acting as conditioned reinforcers via classical, or Pavlovian, conditioning. This mechanism seems to be especially important for craving and relapse-like behavior in both animals and humans (O'Brien et al., 1992; Panlilio and Goldberg, 2007; Perry et al., 2014).

In natural environments, it is considerably rare for a response to be consistently rewarded upon each occurrence; typically, a degree of effort must be applied, or a certain time must pass, a principle that is reflected by ratio schedules, in which a certain pre-programmed number of responses is required, or interval schedules when certain time must pass before the subject will receive the reinforcer. Both of the schedules can be set to constant (fixed-ratio, fixed-interval) or intermittent (variable-ratio, variable-interval) (Panlilio and Goldberg, 2007; Sanchis-Segura and Spanagel, 2006). Progressive ratio (PR) schedules utilize an increasing ratio of responses necessary to receive the reinforcer on subsequent trials, providing a measure of the rising cost of reward and thus assessing motivation. The maximum number of responses an animal is willing to make in order to get the reward is referred as the "break point" (BP) (Hodos, 1961; Roberts et al., 2007, 1989b).

1.5.2 Extinction and reinstatement

High craving and relapse rates, especially in response to environmental stimuli previously associated with drug-taking behaviors (O'Brien et al., 1992), but also to re-exposure to the drug itself (de Wit, 1996) or stress (Sinha, 2007), are the main difficulties in successful SUD management (Hunt et al., 1971; O'Brien, 2005; O'Brien et al., 1998). Therefore, the ability to model relapse-like behavior in laboratory conditions has proven to be very valuable, with the reinstatement model emerging as one of the most widely used paradigms. As with human addicts, reinstatement of drug-seeking behavior in animals can be triggered by stress (Shaham and Stewart, 1995), drugs (Stewart and de Wit, 1987) and discrete, discriminative and contextual cues (Crombag and Shaham, 2002; Meil and See, 1996; Weiss et al., 2000); furthermore, these triggers can potentiate each other when combined (Liu and Weiss,

2002).

Subsequent to SA training and the acquisition of stable SA behavior, an animal is subjected to a period of extinction training, during which the previously rewarded operant response is no longer reinforced. Extinction training then results in a gradual decline in an animal´s operant responses, and when a pre-defined criteria is met (e.g., a certain number of extinction days or certain percentage of responses relative to baseline SA responding), an animal is tested for drug-seeking behavior in the reinstatement test using one of the aforementioned triggers (Panlilio and Goldberg, 2007; Sanchis-Segura and Spanagel, 2006).

In the current thesis I focused primarily on cue-induced reinstatement, the most powerful of the triggers associated with a return to drug-seeking following extinction in animal models (Bossert et al., 2013; Perry et al., 2014). Cue-induced reinstatement is performed under drug-free conditions and more indicative of drug craving and cue reactivity rather than an actual relapse as seen in human addicts (Sanchis-Segura and Spanagel, 2006; Spanagel, 2017). Moreover, the extinction of operant responding also does not accurately reflect the human condition, subsequently impacting the paradigm´s face validity, but has nevertheless provided a wealth of knowledge on the behavioral and molecular underpinnings of drug-seeking (Epstein et al., 2006; Shaham et al., 2003). A schematic representation of each phase of a cue reinstatement paradigm can be found below in Fig. 1.4.

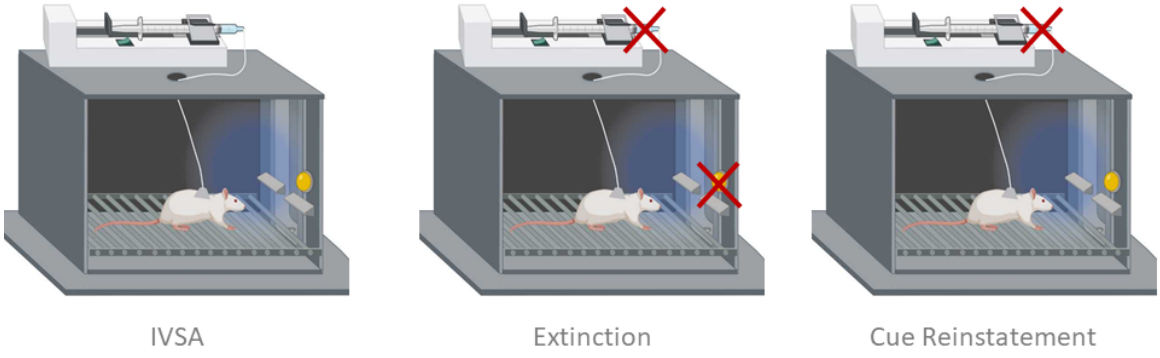

Figure 1.4. Schematic representation of intravenous self-administration *(left)*, extinction *(middle)* and cue-induced reinstatement *(right)*; *Figure created with BioRender.com*

1.5.3 3-CRIT model of cocaine addiction

The 3-CRIT model of cocaine addiction, developed in 2004 by Deroche-Gamonet et al. (2004), is a multisymptomatic DSM-IV/5–based model incorporating criteria from the DSM-IV definition of CUD and modeled in rats: the inability to refrain from drug seeking (measured as active hole/lever responding during the “no-drug” period), high motivation

for drug-taking (BP during a PR test), and continued use despite negative consequences (cocaine intake in the presence of punishment). Additionally, an overall addiction score is calculated, which correlates with the Addiction Severity Index (ASI) in humans (Belin and Deroche-Gamonet, 2012; Belin et al., 2008).

Based on the number of criteria met, animals are classified into four groups: 0crit, 1crit, 2crit, and 3crit. Rats in the 0crit group are considered addiction-resilient (nonaddict-like), whereas those in the 3crit group are considered addiction-vulnerable (addict-like). In addition to these diagnostic criteria, the 3-CRIT protocol is extended in duration (more than 45 CSA sessions across ~ 3 months) compared with standard CSA experiments (typically 10–15 sessions). This longer timeline allows examination of behavioral adaptations following chronic cocaine exposure, as addiction typically develops only after prolonged use (Deroche-Gamonet and Piazza, 2014). Although not formally part of the three criteria, additional comparisons further support the model's validity. For example, 3crit rats escalate cocaine intake to a much greater extent than 0crit during extended sessions (5 h), thereby fulfilling another DSM criterion—loss of control over intake (Deroche-Gamonet et al., 2004). Moreover, 3crit animals display heightened cocaine-induced (Belin et al., 2009) and cue-induced (Cannella et al., 2013) reinstatement, even after a prolonged period of abstinence. Importantly, these extreme subgroups emerge despite comparable genetic backgrounds, training conditions, and overall cocaine intake, distinguishing the 3-CRIT model from more conventional approaches to studying addiction-like behavior. Beyond these features, the 3-CRIT also captures variability in susceptibility within a population, a hallmark of addiction in humans. Only a minority ($\sim$ 15–17 %) of people who use cocaine develop addiction (Anthony et al., 1994; Nutt et al., 2007), a proportion closely mirrored by the fraction of animals identified as 3crit (Belin et al., 2008; Cannella et al., 2013; Deroche-Gamonet et al., 2004; Pohořalá et al., 2021). This close resemblance to human relapse provides considerable face validity to the model.

Taken together, the 3-CRIT model enables examination of several human-relevant features of addiction in a preclinical setting, making it a powerful and valuable tool for investigating the development of addiction and for identifying addiction-resilient and addiction-vulnerable subpopulations.

1.6 Hypothesis and aims

Cocaine addiction is a chronically relapsing disorder characterized by compulsive drug use, high relapse rates, and limited treatment efficacy. Despite extensive research into the reinforcing properties of psychostimulants, the neurobehavioral mechanisms underlying addiction vulnerability and the emergence of addiction-like behavior remain incompletely understood. In particular, the role of individual vulnerability factors, such as phenotypic traits and sex, as well as the underlying molecular and neuropharmacological mechanisms contributing to compulsive cocaine use, require further clarification. Alterations in glutamatergic and dopaminergic signaling pathways have been implicated in the development and maintenance of addictive behaviors, yet region-specific changes and their behavioral correlates remain to be fully characterized. In parallel, interest in emerging pharmacotherapeutic approaches, including serotonergic psychedelics, highlights the necessity for systematic, preclinical investigation into their potential benefits and liabilities, as well as the refinement of behavioral tools to probe drug-induced changes in cognition.

Thus, the following hypotheses are tested within the framework of this thesis:

Hypothesis 1: It is hypothesized that individual phenotypic traits, specifically sign- and goal-tracking behaviors, and biological sex influence vulnerability to developing addiction-like behavior as defined by the 3-CRIT model.

Hypothesis 2: It is hypothesized that cocaine exposure dynamically alters the expression of $mGluR_2$ (*Grm2*) in a region-specific and experience-dependent manner, and that pharmacological activation of $mGluR_{2/3}$ can suppress relapse-relevant behavior.

Hypothesis 3: It is hypothesized that genetic or pharmacological disruption of dopamine signaling alters the acquisition and expression of cocaine-seeking behavior, reflecting the necessity of dopamine transporter function in psychostimulant reinforcement.

Hypothesis 4: It is hypothesized that novel compounds targeting the serotonergic system, such as psilocybin, will influence relapse-related behaviors and cognitive processes relevant to addiction, and that the refinement of behavioral paradigms will provide tools to assess such drug-induced cognitive alterations.

1.6.1 Specific aims

To identify behavioral, molecular, and pharmacological mechanisms contributing to cocaine addiction, with a particular focus on relapse vulnerability, therapeutic prospects, and the refinement of behavioral paradigms to assess drug-induced cognitive alterations.

Aim 1: Phenotypic predictors of addiction
The first aim focuses on identifying behavioral phenotypes that influence addiction-like behavior. Specifically, differences in sign- and goal-tracking behavior, as well as sex-specific patterns, are examined using the 3-CRIT model.

Aim 2: Molecular mechanisms of addiction
The second aim addresses the molecular basis of addiction. It investigates glutamatergic and dopaminergic mechanisms relevant to addiction-like behavior, with a focus on $mGluR_2$ expression and dopamine transporter function. These studies involve molecular profiling, pharmacological interventions, and the use of transgenic rat models.

Aim 3: Preclinical evaluation of novel therapeutic strategies and behavioral paradigms
The third aim evaluates pharmacotherapeutic strategies for cocaine addiction, focusing on serotonergic psychedelics, and incorporates the refinement of behavioral paradigms to probe drug-induced alterations in cognition.

1.6.2 List of studies

Aim 1: Phenotypic predictors of addiction

- **Study 1A** Sign- vs. goal-tracking in the 3-CRIT model
- **Study 1B** Sex differences in the 3-CRIT model

Aim 2: Molecular mechanisms of addiction

- **Study 2A** $mGluR_2$: Stage-specific regulation during cocaine SA
- **Study 2B** *Slc6a3*$_{N157K}$ mutation: Effects on cocaine self-administration

Aim 3: Preclinical evaluation of novel therapeutic strategies and behavioral paradigms

- **Study 3A** Psilocybin and cocaine-seeking in rodents
- **Study 3B** Reality testing in rats: Adaptation of representation-mediated aversion protocol

1.6.3 Publication statement

This thesis incorporates material from two previously published peer-reviewed articles, in both of which the author of this thesis is the first author. Studies 1A and 3A include content from these publications (Introduction, Methods, Results, and Discussion). The Introduction and Discussion sections have been adapted to align with the overall narrative and structure of the thesis, with adjustments in phrasing, emphasis, and integration into the broader thesis context where appropriate. The Methods and Results sections have been incorporated with only minor editorial modifications. All adaptations were undertaken solely to meet the formatting and stylistic requirements of the thesis and were carried out in full compliance with academic integrity policies.

Study 1A: Sign- and goal-tracking in the 3-CRIT model

> **Pohořalá, V.***, Enkel, T.*, Bartsch, D., Spanagel, R., and Bernardi, R. E. (2021). *Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration.* Psychopharmacology, 238(8):2335–2346. (*shared 1st authorship)

Study 3A: Psilocybin and cocaine-seeking in rodents

> **Pohořalá, V.**, Kuchař, M., Spanagel, R., and Bernardi, R. E. (2024). *Psilocybin administered following extinction sessions does not affect subsequent cocaine cue reinstatement in male and female rats and mice.* Neuroscience, 559:156–165.

1.6.4 Statement on the Use of AI-Based Tools

During the preparation of this thesis, I used ChatGPT (GPT–4o model (OpenAI, 2024), 2024–2025) via a ChatGPT Plus subscription. The tool was used to assist with the refinement of language and style in the final manuscript. All scientific content, data interpretation, and conceptual contributions are entirely my own. No parts of the research design, analysis, or scientific argumentation were generated by or delegated to AI tools. All AI-assisted content was manually reviewed and edited. The author assumes full responsibility for the final content.

Chapter 2

Materials and methods

2.1 Subjects

All experiments were carried out at the Central Institute of Mental Health in Mannheim (Germany). Experimental procedures were conducted according to the NIH ethical guidelines for the care and use of laboratory animals, in compliance with the German Animal Welfare Act, and approved by the local animal care committee (Regierungspräsidium Karlsruhe, Germany).

2.1.1 Rats

Sprague Dawley Subjects were male (Study 1A, 1B, 2A, 2B, 3A, 3B) and female (Study 1B, 3A) Sprague Dawley rats obtained from Charles River, Germany, 6–8 weeks old at the time of arrival at the CIMH animal housing facility. In Study 1A, the animals were housed on a reversed 12 h light-dark cycle (lights on: 7 pm – 7 am) in 3 UniProtect airflow cabinets (Bioscape, Germany), located in a temperature (22 $\pm$ 1 °C) and humidity (40 $\pm$ 5 %) controlled room. For all other studies, the UniProtect cabinets were replaced with standard racks and the animals were housed on a reversed 12h light-dark cycle (lights on: 6pm – 6am) in a temperature (22 $\pm$ 1 °C) and humidity (40 $\pm$ 5 %) controlled room. From their arrival to the catheterization surgery, all rats were housed in groups of four (Makrolon Type IV cages). Following the surgery until the end of the experiment, all rats were housed individually (Makrolon Type III cages). During training and tests, rats received 20 g/day of standard chow food, with water provided *ad libitum*. All experiments were performed during the dark phase.

***Slc6a3*_N157K (Study 2B)** Subjects were male homozygous *Slc6a3*_N157K mutant rats bred on the Fischer 344 (F344) genetic background and their wild-type littermates (WT) (Vengeliene et al., 2017). The rats were bred in the on-site breeding facility of the CIMH Mannheim. The animals were housed on a reversed 12h light-dark cycle (lights on: 6 pm – 6 am) in a temperature (22 $\pm$1 °C) and humidity (40 $\pm$ 5 %) controlled room. From their arrival to the catheterization surgery, all rats were housed in groups of four (Makrolon Type IV cages). Following surgery until the end of the experiment, all rats were housed individually (Makrolon Type III cages). During training and tests, rats received 20 g/day of standard chow food, with water provided *ad libitum*. All experiments were performed during the dark phase.

2.1.2 Mice (Study 3B)

C57Bl/6N Subjects were male and female C57Bl/6N mice obtained from Charles River, Germany, aged 8-10 weeks at the time of arrival at the CIMH animal housing facility. Mice were individually housed in a temperature (22 $\pm$ 1 °C) and humidity (40 $\pm$ 5 %) controlled room on a 12-hr light–dark cycle (lights on: 6am – 6pm). Food was provided *ad libitum*, except prior to the food training experiment, during which body weights were measured and mice were maintained at $\sim$85 % of *ad libitum* feeding weight. All experiments were performed during the light phase.

2.2 Drugs

Cocaine hydrochloride (Sigma-Aldrich, Germany or VŠCHT, Czech republic) was dissolved in sterile 0.9 % NaCl for i.v. administration of 0.8 mg/kg/36µl infusion for rats (Study 1A, 1B, 2A, 2B, 3A) and 0,5 mg/kg/14 µl for mice (Study 3A). Psilocybin (VŠCHT, Czech Republic) was dissolved in sterile 0,9 NaCl for i.p. administration of 1 mg/kg (10 ml/kg) in mice and subcutaneous (s.c.) administration of 1.0 mg/kg and 2.5 mg/kg (1 ml/kg) in rats (Study 3A). The $mGluR_{2/3}$ agonist LY379268 (Tocris, UK) was dissolved in sterile 0.9 % NaCl for s.c. administration of 3 mg/kg in rats (Study 2A).

2.3 Apparatus

2.3.1 Rats

2.3.1.1 Cocaine Self-Administration apparatus

As previously described (Pohořalá et al., 2021), all CSA training and testing was performed in 24 boxes for SA (40 x 30 x 52 cm; Imetronic, France) located in cabinets with fans that ensured air exchange and masked external sounds. Two nose poke (NP) holes were located on opposite walls of the chambers, 5 cm above the grid floor. When rats poked their snout in the holes, breaking an infrared beam, an instrumental response was recorded. One hole was associated with cocaine delivery and designated as the active hole, while the other was designated as the inactive hole and served as a control. A white house light located at the top allowed for the illumination of the entire chamber; a white cue light was located 9.5 cm above the active hole, a green cue light was 10 cm to the right of the white one, and a blue cue light was on the left side of the opposite wall 33 cm above the grid floor. PC-Windows compatible software (Imetronic, France) controlled all experiments.

2.3.1.2 Pavlovian Conditioned Approach apparatus (Study 1A)

Pavlovian conditioned approach (PCA) training and testing was performed in 4 chambers (21 x 24 x 29 cm; MED Associates, St. Albans, VT, USA) located in cabinets with fans that ensured air exchange and masked external sounds, as previously described (Pohořalá et al., 2021). The chambers contained a liquid dispenser, a pellet dispenser, a receptacle with head entry detectors and two retractable levers (on each side of the receptacle) that required a downward force of $\sim$ 12–15 g to record a press. All procedures were controlled by a PC running Med-PC IV (MED Associates, St. Albans, VT, USA).

2.3.2 Mice (Study 3A)

2.3.2.1 Cocaine self-administration apparatus

CSA training and testing was performed in 10 operant chambers (Med Associates, St. Albans, VT, USA) housed in light- and sound-attenuating cubicles, as previously described (Bernardi et al., 2019). Each chamber (24.1 x 20.3 x 18.4 cm) was equipped with

two levers (left and right), a food dispenser, and a drug delivery system connected via infusion pump (PHM-100, Med Associates) located outside the cubicle. Operant chambers were controlled using Med-PC IV (Med Associates) software.

2.4 Experimental procedures

2.4.1 Study 1A: Sign-tracking vs. goal-tracking in the 3-CRIT model

Methods in the following section are adapted from Pohořalá et al. (2021), with slight editorial modifications.

Subjects were forty-six male Sprague-Dawley rats that first underwent a Pavlovian conditioned approach procedure resulting in their characterization into 3 groups—sign-trackers (ST), intermediates (INT), and goal-trackers (GT). Rats were then implanted with catheters and subsequently trained to self-administer cocaine in the 3-CRIT model of cocaine addiction. At the end of the training, the rats were then tested and scored based on their performance on the 3 criteria measured by this model (persistence of drug-seeking, motivation for cocaine-taking, resistance to punishment). The relationship between sign- or goal-tracking phenotype and addiction severity measured by the 3 criteria was then investigated by performing correlational analyses.

2.4.1.1 Pavlovian Conditioned Approach

The general procedure followed earlier descriptions (Saunders and Robinson, 2012) with minor adaptations (Enkel et al., 2019; Scülfort et al., 2016). Briefly, PCA assessment started with 20 free deliveries of the US (80 µl of a 20 % sweetened condensed milk solution) on two successive days. This was followed by 7 d of acquisition; every session consisted of 20 trials: the lever (CS) was presented for 8 s, and after its retraction the liquid dispenser provided the US. Trials were separated by intertrial intervals (ITIs; 30–115 s) ; however, trial-onset was postponed by 8 s if a head entry occurred immediately prior to trial start. This avoided a confounding recording bias towards GT behavior due to non-CS triggered ITI activity. During a given trial, a CR was scored if any form of responding (lever deflection or food-cup entry) occurred within the 8 s of CS presentation. The pattern of responding was quantified using a PCA score, consisting of the mean of three measures: the probability of lever deflection or food-cup entry, the response bias for lever/food-cup responses, and the

latency to make lever/food-cup responses (Saunders and Robinson, 2012). Rats were then grouped into GTs (-0.5 to -1.0) INTs (-0.5 to $+0.5$) and STs ($+0.5$ to $+1.0$).

2.4.1.2 Intravenous catheter implantation

Catheter preparation The catheters were made in-house by connecting Micro-Renathane® tubing (internal diameter: 0.58 mm; external diameter: 0.94 mm) to a back-mount (Plastic One Inc., USA; 313-000BM, 22 gauge). The connection was then secured with superglue (UHU) and an anchor point was made from silicone (Loctite 5980, Henkel) towards the end of the tubing (cca 4 cm from the end) to help secure the tubing in place after insertion into the vein. Additionally, the end of the tubing was cut beveled in order to allow easier insertion into the vein.

Catheter implantation surgery Catheterization surgeries were performed after the end of PCA. In order to create and maintain as sterile an environment for the surgery as possible, all surfaces were cleaned with Antifect® N liquid (Schülke & Mayr, Germany) every day, all surgical tools were manually cleaned and then autoclaved at 121 °C for 20 minutes at 15 psig, and a hot bead sterilizer was used between surgeries. Prior to start of the surgery, the rat was weighed in order to determine the correct dose of analgesia (carprofen). Next, the rat was placed into a small Plexiglas chamber (26 x 12 x 12 cm) which was then slowly filled with an air-isoflurane ($\sim$ 4,5-5 %) mixture from the veterinary anesthesia machine (EZ-108SA, E-Z Anesthesia, USA) and the rat was left there until general anaesthesia was induced. Then the unconscious rat was placed onto a sterile drape-covered heating pad (37 °C), injected with caprofen (5 mg/kg, s.c.) and a lack of reflexes (e.g. tail and toe pinch reflex) was checked. To keep the rat under general anesthesia during the surgical procedure, the air-isoflurane mixture at a flow rate of 405 ml/min containing $\sim$ 2 % of isoflurane was delivered via a breathing mask. The ophthalmic ointment (Bepanthen®, Bayer) was applied to protect the animal´s eyes to prevent dryness. Next, the surgical areas—on the back: between the shoulder blades and in front: above the jugular vein—were shaved and cleaned with iodine solution (Vet-sept, 50 mg/ml, Livisto) and Alcohol Pads (Softa® Swabs, B.Braun). The surgery itself started with making a $\sim$ 2 cm long diagonal incision on the rat´s cervical region and in proximity to the rat´s scapulae, then another small incision was made $\sim$ 7 cm caudally from the first incision, close to the middle of the rat´s back. The skin between both incisions was then carefully separated from the underlying tissue, creating a space for the catheter´s back mount to be later inserted. The rat was then turned ventrally and a small incision ($\sim$ 0.5 cm) was made above the right jugular vein. In order to have the catheter body

implanted from the dorsal side, a small surgical conduit was created, linking the cervical and ventral incisions and the catheter tubing was carefully threaded through it. Then the tissue below the ventral incision was carefully pushed aside and the jugular vein was found and carefully cleaned (~ 1.5 cm in length) and separated from surrounding tissue. A small puncture into the vein was created using a sterile 21G needle, followed by gently inserting the tubing inside the jugular vein until the anchor point. The correct position of the tubing was checked by gently flushing the tubing with saline containing heparin (100 I.U./ml; Heparin-Natrium-25000, Ratiopharm®, Ratiopharm) and enrofloxacin (5 mg/kg; Baytril®, Bayer). Then two knots from silk surgical thread (18020-50, Fine Science Tools, USA) were made below and two above the anchor point in order to prevent leakage from the vein and secure the tubing in place by connecting it to surrounding fat tissue. When this step was finished, the secured connection was gently covered with surrounding fat tissue using one simple interrupted stitch (Ethilon 5/0, Ethicon, USA). Then the skin incision was closed by 2–4 simple interrupted sutures (Ethilon 4/0, Ethicon, USA) based on the size of the wound. The catheter´s body was then inserted from the dorsal side into the pre-prepared incision on the cervical/scapulae region with the cannula exiting from the second pre-prepared incision in the rat´s back and then all remaining skin incisions were closed using the simple interrupted sutures. The catheter was flushed with heparinized saline/Baytril solution once again before closing the cannula with a small silicone cap (made from tubing) and securing it with bigger, metal, cap. After the end of the surgery, the rat was transferred to a home cage with fresh bedding and carefully monitored until fully conscious.

Catheter maintenance All post-surgery animals were closely monitored on a daily basis and checked for possible infections, distress and pain. The rats were given ~ 5 days to recover from the surgery before the beginning of cocaine SA training. The catheter was treated every day until the end of the experiment (before and after each session) with 0.1 ml of heparinized saline/Baytril solution.

2.4.1.3 3-CRIT

The timeline and organization of the daily sessions are shown in Fig. 2.1. All procedures used during 3-CRIT training were adapted from previously published work (Belin et al., 2009; Cannella et al., 2017, 2013; Deroche-Gamonet et al., 2004; Kasanetz et al., 2013). Animals were trained to self-administer cocaine during daily sessions. Each session was 2.5 h long and consisted of three drug periods (40 min each) alternating with two non-drug periods (15 min each). The “Drug” period was signaled by illumination of the chamber by

the blue light (discriminative stimulus, SD), while the "no-Drug" periods were signaled by the illumination of the chamber by the house light. The required amount of nose-poking in the active hole during "Drug" periods resulted in illumination of the white cue light (4 s in total) that was followed 1 s later by activation of the infusion pump (36 µl/infusion over 2 s, containing 0.8 mg/kg of cocaine). Cocaine infusion was followed by a 40 s time out (TO) period. After 10 sessions of initial training under a fixed-ratio schedule 3 (FR3), the program was switched to FR5 for the remainder of the experiment. During each CSA session, except those during which motivation for cocaine taking and resistance to punishment were tested (described below), a maximum of 35 infusions was allowed. If an animal reached 35 infusions, the session ended. NPs in the inactive hole was recorded but had no programmed consequences. During the "no-Drug" periods, NPs in both holes was recorded but had no programmed consequences. When rats achieved 43 CSA sessions, two criteria for addiction behavior were evaluated: persistence of drug-seeking and motivation for cocaine taking, and, following 2 days of additional cocaine SA sessions, resistance to punishment.

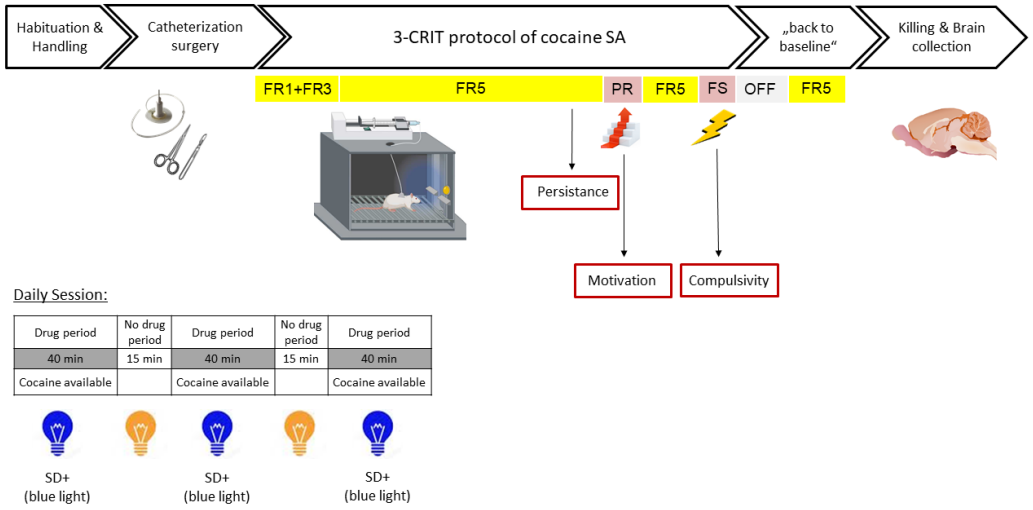

Figure 2.1. Timeline of the 3-CRIT protocol, illustrating the overall experimental design and daily session structure. Following acquisition (FR1, FR3) and extended CSA training (FR5), rats were tested on three addiction-like criteria: persistence (responding during no-drug periods), motivation (PR test), and compulsivity (FS test). FR = fixed ratio; PR = progressive ratio; FS = footshock; SD^+ (blue light) = drug-paired discriminative stimulus; CSA = cocaine self-administration. *Figure created with BioRender.com.*

Persistence of Drug-Seeking This addiction criterion was evaluated daily during SA training by measuring the number of NPs in the active hole during the "no-Drug" periods, during which cocaine was unsignaled and unavailable. The last three SA training sessions

prior to the motivation test (days 41 – 43) were averaged for each animal to determine the persistence of drug-seeking.

Motivation for Cocaine-Taking This criterion was assessed on session No. 44 using a PR schedule. The PR session used identical features as the "Drug" periods above, but with the ratio of responses to achieve cocaine increasing with every infusion according to the following NP progression: 10, 20, 30, 45, 65, 85, 115, 145, 185, 225, 275, 325, 385, 445, 515, 585, 665, 745, 835, 925, 1025, 1125, 1235, 1345, 1465, 1585, 1715, 1845, 1985, 2125, 2275, 2425, 2585, 2745, 2915, 3085, 3265, 3445, 3635, 3825, 4025, 4225. The last ratio completed is referred to as the break point (BP) and used to score the criterion. The session ceased after 6 h or a 60 min limited hold (i.e., 1 h passed without completion of the next response ratio).

Resistance to Punishment Following PR testing, rats were subjected to 2 days of normal SA training (days 45 – 46) to ensure normal responding and cocaine intake. The resistance to punishment criterion was assessed on session No. 47 by pairing cocaine seeking and taking with electric foot shocks. The session lasted 40 min and was a modified version of the standard "Drug" period. As usual, rats were required to achieve an FR5 ratio of responses to receive a cocaine injection. However, here a single NP led to the illumination of the green cue-light, signaling the presence of foot-shock. When 4 NPs were achieved, rats received an electric foot-shock (FS; 0.2 mA, 1 s). Completion of the FR5 ratio then resulted in simultaneous delivery of the cocaine infusion and exposure to a 2nd FS (0.2 mA, 1 s). From illumination of the green light, rats had 1 min to complete the 4th NP and then another minute to complete the FR5 ratio; if these requirements were not met, the green cue light was extinguished and the sequence reinitiated. The blue and white cue light schedules functioned as in the standard "Drug" period. This criterion was expressed as percentage of cocaine infusions earned relative to the number of infusions earned during the 1st 40 min "Drug" period on day 46.

Characterization of Addiction-Like Behavior A subject was considered positive for one criterion if its score was above the 60th percentile of the population distribution, and negative if its score was below the 60th percentile (Cannella et al., 2017, 2013). Thus, depending on the number of positive criteria met, a subject was assigned to one of the following groups: 0crit, 1crit, 2crit, and 3crit. Rats negative for all criteria (0crit) were characterized as non-addict-like or addiction-resilient, whereas rats positive for all the criteria (3crit) were characterized as addict-like or addiction-vulnerable.

Addiction score An addiction score was also calculated for each animal as the sum of the normalized scores of the three addiction-like criteria. To calculate the normalized score of a criterion, the average across the whole population of that criterion was subtracted from the subject score, and the result of this subtraction was divided by the standard deviation of the whole population. Thus, each subject had three normalized scores, one for each addiction criterion (motivation, persistence, resistance to punishment). In addition to the categorical classification (number of positive criteria met), this score is a dimensional index of cocaine use severity that has been shown to be linearly related to the number of criteria met, supporting the hypothesis that the model identifies a pathologic-like continuum, from controlled to compulsive-like drug use (Belin et al., 2009, 2008; Rikoon et al., 2006).

2.4.1.4 Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were conducted using SPSS software (StatSoft, USA). Group scores for PCA (GTs, INTs, and STs) were analyzed using repeated measures ANOVA (repeated measure: day), followed by one-way ANOVA to detect between group differences. CSA data were analyzed using repeated measures ANOVA [repeated measures: day and NP (active, inactive)]. The 3-CRIT model criteria (motivation for cocaine-taking, persistence of cocaine-seeking, resistance to punishment, as well as addiction score) were analyzed using descriptive statistics (mean, standard error, and population distribution), with independent-samples *t*-tests to indicate differences between 0 and 3 crit scores. Correlations between PCA scores and addiction criteria were performed using Pearson's correlation coefficient (r). Comparisons of GTs and STs on addiction-like criteria were analyzed using independent-samples *t*-tests. Significance was set at $p = 0.05$.

2.4.2 Study 1B: Sex differences in the 3-CRIT model

Subjects were 46 male and 47 female Sprague-Dawley rats trained to self-administer cocaine using the 3-CRIT protocol. Behavioral data were analyzed by sex and addiction phenotype (0crit, 3crit, and CTRL).

Following completion of behavioral testing, brains were collected for subsequent molecular analyses:

- Study 2A (part of this thesis): $mGluR_2$ expression was assessed in brain tissue from male rats (0crit: n = 6; 3crit: n = 3–5; CTRL: n = 4–6, depending on brain region), across multiple brain regions (see section 2.4.3 for details).

- snRNA-seq study (ongoing): Brain regions of interest (ILC, PrLC, CgC, INS) from male (n = 12; 0crit, 3crit, CTRL; each n = 4) and female (n = 11; 0crit and CTRL n = 4, 3crit n = 3) rats are included in an ongoing single-nucleus RNA sequencing (snRNA-seq) study aimed at investigating transcriptional differences based on crit score and sex to complement the behavioral findings presented here.
- Zillich et al., 2025 (*Nat. Commun.*): DS samples from male rats only (0crit n = 6; 3crit n = 5; CTRL n = 6) were also included in a separate, independent publication not covered in this thesis (Zillich et al., 2025).

2.4.2.1 Intravenous catheter implantation

Described in detail in section 2.4.1.2.

2.4.2.2 3-CRIT

Described in detail in section 2.4.1.3; however, the initial training phase of the CSA protocol was slightly adjusted—all rats were trained under combination of FR1 and FR3 for the first 6 sessions of the CSA training and from 7th session on, the program was switched to FR5 for the rest of the experiment (except PR test).

2.4.2.3 Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were performed using SPSS software (StatSoft, USA). CSA data (number of infusions) were analyzed using three-way repeated measures ANOVA (session × sex × group), followed by Bonferroni-adjusted post hoc comparisons for group-level differences. Operant responding during drug and no-drug periods was evaluated using four-way repeated measures ANOVA (session × NP × group × sex). Group-level distributions (e.g., 3-CRIT prevalence by sex) were summarized using descriptive statistics. Differences in addiction-like criteria (persistence, motivation, compulsivity, and addiction score) were analyzed using one-way ANOVA and independent-samples t-tests. Assumptions of normality and homogeneity of variances were assessed using Shapiro-Wilk and Levene's tests, respectively. In cases of assumption violation, standard parametric tests were retained due to adequate sample size and consistency with established practices in similar research. Results were interpreted with caution where these violations occurred. Statistical significance was set at $p = 0.05$.

2.4.3 Study 2A: Metabotropic glutamate receptor 2 – Stage-specific regulation

Levels of *Grm2* (the gene encoding $mGluR_2$) were measured across seven brain regions in multiple experimental groups. These included rats subjected to short-term cocaine SA (15 sessions; CSA), short-term CSA followed by extinction training (10 sessions; EXT), and short-term CSA followed by a 10-day forced abstinence period (ABST). Each group was compared to its respective cocaine-naïve, operant chamber-experienced control group. Additionally, *Grm2* expression was assessed in both short-term (15 sessions) and long-term (> 50 sessions) self-administration cohorts relative to cocaine-naïve home-caged controls (HC, naïve) . Comparisons were also made between addiction-resilient (0crit) and addiction-vulnerable (3crit) phenotypes as defined by the 3-CRIT model. For better visual representation, see the timeline of this experiment 2.2.

2.4.3.1 Intravenous catheter implantation

Described in detail in section 2.4.1.2.

2.4.3.2 Short-term CSA protocol

All cocaine groups of Sprague-Dawley rats (CSA, EXT, ABST) learned to self-administer cocaine hydrochloride (0.8 mg/kg/infusion) under FR1 for 15 consecutive days. Each session was started by illumination of the blue light signaling the cocaine availability. During daily 1h session, responses in the active nose hole resulted in the delivery of cocaine infusion paired with 2 s presentation of a blinking white light stimulus (CS). Each infusion was followed by a 40 s TO period during which NPs into the active hole were recorded but not reinforced. A limit of 40 infusions per session was imposed in order to prevent the overdosing of the animals. The first group of animals (CSA, n = 7) finished the experiment after the end of CSA. Another group (EXT, n = 10) continued training with 10 consecutive sessions of extinction—the animals were put in their boxes, the light signaling cocaine availability was not on, all responses in both holes were recorded but not reinforced. The last group of cocaine experienced animals (ABST, n = 8) were given 10 days of abstinence following CSA, during which the rats were handled as usual but not put in the SA boxes and were kept in their home cages.

For each respective SA group, a cocaine-naïve control group was used (SA CTRL, n = 8, Ext CTRL, n = 10, Abst CTRL, n = 8)—the animals went through the same instrumental training

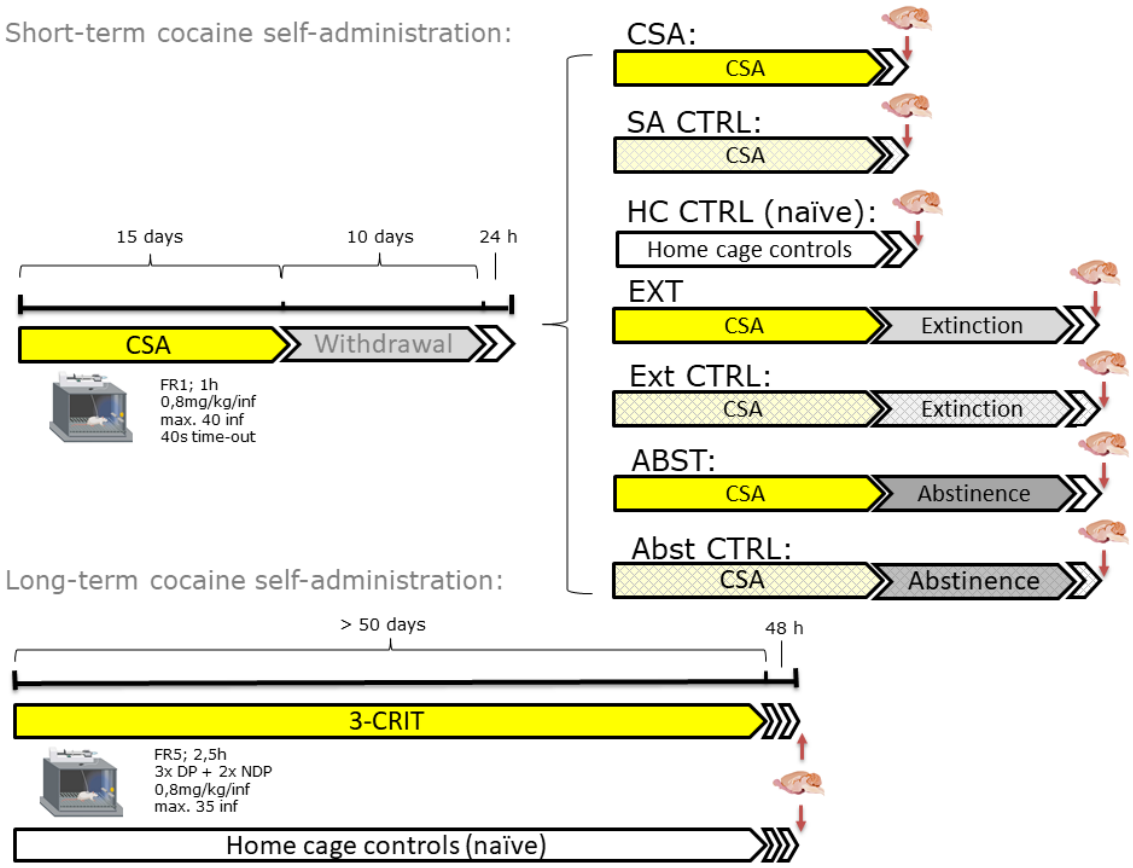

Figure 2.2. Schematic timeline of mGluR$_2$ experiments, illustrating short- and long-term self-administration protocols. **(Top)** Short-term self-administration consisted of 15 daily 1-hour sessions, followed by one of three post-training conditions: no further procedure (CSA group), extinction training (EXT group; 10 daily sessions), or forced abstinence (ABST group; 10 days in home cage). Each group had a protocol-matched cocaine-naïve control sacrificed at the same timepoint (SA CTRL, Ext CTRL, Abst CTRL). A separate, age-matched, cocaine-naïve home-caged group (HC CTRL, naïve) served as control for the short-term cohort used in the short- vs. long-term comparison. **(Bottom)** Long-term self-administration was performed in rats phenotyped using the 3-CRIT model of addiction-like behavior. An independent, age-matched, cocaine-naïve home-caged group served as control for the long-term cohort. CSA = cocaine self-administration; EXT = extinction; ABST = abstinence; CTRL = control; HC = home-caged. *Figure created with BioRender.com.*

but did not receive any cocaine. Another control group (HC CTRL/naïve, n = 6) consisted of cocaine-naïve animals kept in their home cages during the duration of the experiment and was sacrificed 24 h later).

Note: The orbitofrontal cortex (OFC) was added to the study at a later stage, resulting in a smaller sample size for this region in the extinction groups (EXT and Ext CTRL; n = 4 each)

compared to other brain regions. Similarly, some experiments comparing short- and long-term CSA were conducted prior to the inclusion of the OFC, and thus data for this region are not available for those comparisons.

2.4.3.3 Effect of LY379268 on Reinstatement

The subjects were six Sprague-Dawley male rats that underwent the short-term CSA protocol (15 CSA sessions) followed by 10 extinction sessions as described in detail above. After extinction, rats were divided into two groups (n = 3 per group) for drug testing: one group received the selective $mGluR_{2/3}$ agonist LY379268 (3 mg/kg, i.p.), and the other received vehicle (VEH). Group assignment was based on matching for operant responding during CSA and extinction to ensure behavioral equivalence prior to treatment. Cue-induced reinstatement testing occurred 24 hours after the final extinction session and consisted of a single 1-hour session in which active NP responding resulted in presentation of the previously drug-associated cue (blue light), but no cocaine delivery. Inactive NP responding had no programmed consequence.

2.4.3.4 Long-term CSA protocol

In order to evaluate *Grm2* expression in selected brain regions of rats exposed to chronic cocaine (> 50 sessions), the brains of 0crit (n = 6), 3crit (n = 3) and age-matched cocaine-naïve control (n = 4) animals from male cohort of Study 1B: Sex comparison in 3-CRIT (Section 2.4.2) were used.

Note: To increase statistical power for analyses of the ILC and DS, additional samples were obtained from an independent 3-CRIT study conducted by the same research group (AG Bernardi, unpublished), with overlapping personnel and consistent methodology. Sample sizes for these regions were increased due to preliminary analyses revealing trends toward group differences that required validation with a larger dataset. For the ILC, the final group sizes were: naïve (n = 8), long-access cocaine (n = 21), 0crit (n = 12), and 3crit (n = 9). For the DS: naïve (n = 6), long-access cocaine (n = 11), 0crit (n = 6), and 3crit (n = 5).

2.4.3.5 Brain samples preparation

To quantify *Grm2* expression levels following CSA, all male Sprague-Dawley rats were euthanized by decapitation approximately 24 hours (short-term cohort) or 48 hours (long-term cohort) after their final session, or at identical time points for the respective control

groups. Immediately after decapitation, the brains were extracted, snap-frozen in isopentane at -50 °C, and stored at -80 °C until further processing. For sectioning, the brains were brought to -20 °C overnight and then mounted in a Leica cm3000 cryostat (Leica, Nußloch, Germany). Coronal sections (120 µm thick) were collected, and specific brain regions were extracted via micropunching using needles ranging in diameter from 0.5 to 1.0 mm (FMI, Seeheim, Germany). Micropunching was conducted by an experienced technician (Elisabeth Röbel) to ensure consistency across samples. The targeted brain regions (with approximate coordinates)—the cingulate cortex (CgC; AP: $+1.6$, ML: ± 0.5, DV: -2.0), prelimbic cortex (PrlC; AP: $+3.2$, ML: ± 0.6, DV: -3.2), infralimbic cortex (ILC; AP: $+3.0$, ML: ± 0.5, DV: -5.0), orbitofrontal cortex (OFC; AP: $+3.7$, ML: ± 2.0, DV: -5.0), dorsal striatum (DS; AP: $+1.2$, ML: ± 2.5, DV: -4.5), ventral striatum (VS; AP: $+1.6$, ML: ± 1.6, DV: -5.5), and insular cortex (INS; AP: $+1.0$, ML: ± 5.0, DV: -5.0)—were identified based on landmarks from the stereotaxic descriptions in Paxinos and Watson's Rat Brain Atlas (Paxinos and Watson, 2006). The micropunched samples were then stored at -80 °C until RNA extraction.

2.4.3.6 TaqMan quantitative real-time PCR

The total RNA (tRNA) extraction tRNA extraction and isolation was done from micropunched tissue using the RNeasy Mini Kit (Qiagen, Germany) in accordance with the manufacturer´s instructions. Residual DNA was removed by treatment with the RNase-Free DNase set (Qiagen, Germany). The concentration and quality of tRNA in each sample was then assessed by measuring the optical density at 260 and 280 nm in a NanoDrop 1000 (peqlab, Germany) spectrophotometer.

cDNA synthesis Complementary DNA (cDNA) was produced by using the High-Capacity cDNA Reverse Transcription Kit (ThermoFisher). Briefly, each reaction was performed with a minimum of 600 ng of tRNA per sample in total volume of 20 or 50 µl (based on the quality of individual sample) containing also: 3.7 µl of DNase/RNase-free water, 2 µl of 10x RT buffer, 0.8 µl of 25x dNTP mix (100nM), 2 µl of 10x Random primers, 0.5 µl of RNase inhibitor and 1 µl of Reverse transcriptase. Additionally, two controls were created—one lacking reverse transcriptase, one lacking template tRNA. Then the samples were placed into the Thermocycler machine with following program—step 1: 25 °C for 10 min, step 2: 37 °C for 120 min followed by step 3, brief heat inactivation: 85 °C for 5 s and finally the samples were cooled down to 4 °C.

TaqMan qPCR Quantitative real-time PCR (qRT-PCR) was conducted using an ABI QuantStudio 7 Flex RT-PCR system and QuantStudio™ Real-Time PCR software. Reactions were performed in a 20 µL volume containing 9 µL of cDNA solution, and cycling conditions consisted of 40 cycles at 95 °C for 15 s followed by 60 °C for 1 min. TaqMan® Gene Expression Assays were used to quantify *Grm2* expression (Assay ID: Rn00566655_m1; Life Technologies), with GusB (Assay ID: Rn01447672_m1; Life Technologies) serving as the endogenous control. All reactions were performed in technical duplicates. Relative expression levels of *Grm2* were normalized to GusB.

2.4.3.7 Statistical analysis

Data are presented as mean ± SEM. Statistical analyses were conducted using SPSS software (StatSoft, USA). Behavioral data from self-administration and extinction sessions were analyzed using repeated measures ANOVA (session x group). Operant responding—active and inactive NP—was analyzed using two- or three-way repeated measures ANOVAs (factors: session × NP × group), with Bonferroni-corrected post hoc comparisons applied where appropriate. Welch's t-tests were used for planned between-group comparisons (e.g., LY379268 experiment, 0crit vs. 3crit) due to small or unequal sample sizes and potential variance heterogeneity, offering a more robust alternative to the standard t-test. Student's t-tests were used when group sizes were more balanced and variance equality was assumed.

For gene expression data, linear mixed-effects models (LMMs) were employed to analyze *Grm2* expression across seven brain regions. Brain Region and Group were included as fixed effects, with Subject ID modeled as a random intercept to account for repeated measures within subjects. Residual diagnostics were performed to assess normality; where assumptions were violated, *Grm2* values were log- or square root-transformed to improve model fit. Model fit was evaluated using Akaike Information Criterion (AIC) and Bayesian Information Criterion (BIC). Bonferroni-corrected post hoc comparisons were conducted to assess significant main effects and interactions. All *Grm2* expression values were normalized to their respective control group (control group = 1), and are graphically presented in their original (non-transformed) normalized form for clarity. Pairwise differences are reported as $\Delta M \pm SE$, where SE denotes the standard error of the mean difference estimated from the mixed model. Identical SE values across some brain regions reflect the pooled variance estimation inherent in the model.

2.4.4 Study 2B: *Slc6a3*_N157K mutation – Effects on CSA

Male *Slc6a3*_N157K mutant rats (n = 8) and their wild-type (WT) littermates (n = 5) were trained to self-administer cocaine for 13 days and subsequently tested for motivation during a PR test. The behavioral parameters were then compared. The timeline of the experiment is shown in 2.3

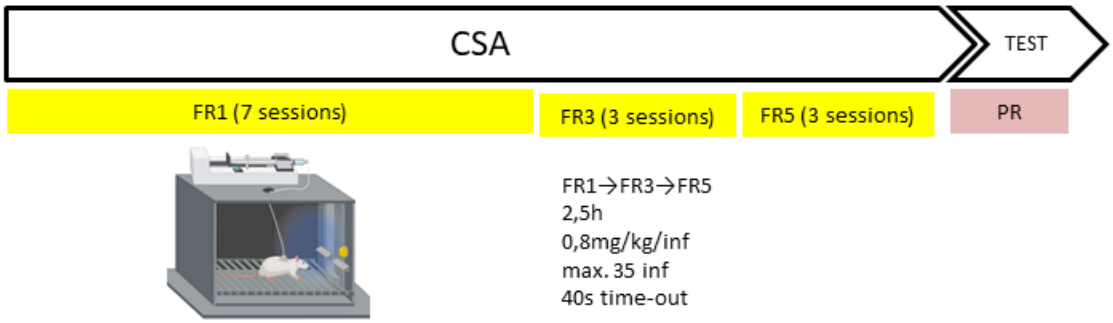

Figure 2.3. Experimental timeline of CSA in DAT mutant (*Slc6a3*_N157K) and WT rats. Rats were trained under escalating fixed-ratio schedules (FR1→FR3→FR5) followed by a PR test to assess motivation for cocaine. CSA = cocaine self-administration; FR = fixed ratio; PR = progressive ratio. *Figure created with BioRender.com.*

2.4.4.1 Intravenous catheter implantation

Described in detail in section 2.4.1.2.

2.4.4.2 Cocaine self-administration protocol

Daily CSA training Animals underwent daily CSA sessions, structurally identical to those described in section 2.4.1.3, but following a shorter and modified protocol. The rats received 13 CSA training sessions in total—7 sessions under FR1 schedule, followed by 3 sessions under FR3, and the final 3 sessions under FR5.

Progressive ratio test The motivation for cocaine-taking was assessed a day after the last CSA session (Day 14) using a PR schedule, with session parameters identical to those described in section 2.4.1.3 (Motivation for Cocaine-Taking).

2.4.4.3 Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were conducted using SPSS software (StatSoft, USA). CSA data during drug periods (active and inactive NP responses, and infusions obtained) were analyzed using three-way repeated measures ANOVA (session x NP x genotype), separately for each FR schedule (FR1, FR3, FR5). Where appropriate, follow-up independent-samples *t*-tests were performed to examine genotype differences in active and inactive responses. BP scores from the PR test were analyzed using independent-samples *t*-tests to compare motivational strength between WT and *Slc6a3*_N157K rats. Statistical significance was set at $p = 0.05$.

2.4.5 Study 3A: Psilocybin and cocaine-seeking in rodents

Methods in this section are adapted from Pohořalá et al. (2024), with slight editorial modifications.

Subjects were female and male rats (n = 48) and mice (n = 37) trained to self-administer cocaine followed by extinction training. Animals received psilocybin treatment following extinction sessions. The effect of these psilocybin injections was assessed on subsequent cue-induced reinstatement. For better visual representation, see Fig. 2.4.

2.4.5.1 Intravenous catheter implantation

Mice

Catheter preparation The catheters were made in-house by connecting a Silastic tube (internal diameter: 0.51 mm; external diameter: 0.94 mm) with a back-mount made in-house (24 gauge). The connection was then secured with a superglue (UHU) and an anchor point was made from silicone (Loctite 5980, Henkel) towards the end of the tubing (cca 1,4 cm from the end) to help secure the tubing in place after insertion into the vein. Additionally, the end of the tubing was cut beveled in order to allow easier insertion into the vein.

Catheter implantation surgery The catheretization surgeries were performed after the end of food training (described below). In order to create and maintain as sterile environment for the surgery as possible, all surfaces were cleaned with Antifect® N liquid (Schülke &

Figure 2.4. Experimental timelines of psilocybin administration during extinction learning and subsequent cue-induced reinstatement. **(A)** In mice, PSI (1 mg/kg, i.p.) or vehicle (VEH) was administered immediately after extinction sessions 1 and 2 following 8 CSA sessions. **(B)** In rats, PSI (1 or 2.5 mg/kg, s.c.) or VEH was administered after each of 7 extinction sessions following 15 CSA sessions. In both species, cue-induced reinstatement was assessed after extinction. CSA = cocaine self-administration; PSI = psilocybin; VEH = vehicle; CR = cue-induced reinstatement; i.p. = intraperitoneal; s.c. = subcutaneous. *Figure created with BioRender.com.*

Mayr, Germany) every day, all surgical tools were manually cleaned and then autoclaved at 121 °C for 20 min at 15 psig. A hot bead sterilizer was used between surgeries. Prior to start of the surgery, the mouse was weighed in order to determine the correct dose of analgesia (carprofen). Next, the mouse was placed into a small Plexiglas chamber (12 x 12 x 12 cm) which was then slowly filled with air-isoflurane (∼ 3–3.5 %) mixture from the veterinary anesthesia machine (EZ-108SA, E-Z Anestesia, USA) and the mouse was left there until the general anaesthesia was induced. Then the unconscious mouse was placed onto a sterile drape covered heating pad (37 °C), injected with caprofen (5 mg/kg, s.c.) and the lack of reflexes (e.g. tail and toe pinch reflex) was checked. To keep the mouse under general anaesthesia during the whole surgical procedure, the air-isoflurane mixture at a flow rate of 405 ml/min containing ∼ 1.5–2 % of isoflurane was delivered via a breathing mask.

Ophthalmic ointment (Bepanthen®, Bayer) was applied to protect the animal´s eyes and prevent dryness. Next, the surgical areas—on the back: between the shoulder blades and in front: above the jugular vein—were shaved and cleaned several times with iodine solution (Vet-sept, 50 mg/ml, Livisto) and Alcohol Pads (Softa® Swabs, B.Braun). The surgery itself started with making an $\sim$ 1 cm long diagonal incision in mouse´s cervical region and in proximity to the scapulae. The skin in close proximity of the incision was carefully separated from the underlying tissue, creating a space for the catheter´s back mount to be later inserted. The mouse was then turn ventrally and a small incision (cca 0.5 cm) was made above the right jugular vein. In order to have the catheter body implanted from the dorsal side, a small surgical conduit was created, linking the cervical and ventral incisions and the catheter tubing was carefully threaded through it. Then the tissue below ventral incision was carefully pushed aside and the jugular vein was found and carefully cleaned ($\sim$ 0.5 cm in length) from surrounding tissue. A small puncture into the vein was created using a sterile 22G needle, followed by gently inserting the tubing inside the jugular vein until the anchor point. The correct position of the tubing was checked by gently flushing the tubing with saline containing heparin (100 I.U./ml; Heparin-natrium-25000, Ratiopharm®, Ratiopharm) and enrofloxacin (5 mg/kg; Baytril®, Bayer). Then two knots from silk surgical thread (18020-60, Fine Science Tools, USA) were made below and two above the anchor point in order to prevent leakage from the vein and secure the tubing in place by connecting it to surrounding fat tissue. Then the skin incision was closed using 2–3 simple interrupted sutures (Ethilon 6/0, Ethicon, USA) based on the size of the wound. The catheter´s body was then inserted from the dorsal side into the pre-prepared incision which was then closed using simple interrupted sutures. The catheter was flushed with heparinized saline/Baytril solution once again before closing the cannula with a small silicone cap (made in house from the tubing). After the end of the surgery, the mouse was transferred to a home cage with fresh bedding and carefully monitored until fully conscious.

Catheter maintenance All post-surgery animals were closely monitored on a daily basis and checked for possible infections, distress and pain. The mice were given a minimum of 72 h to recover from the surgery before the beginning of cocaine SA training. The catheter was treated every day until the end of the experiment (before and after each session) with 0.05 ml of heparinized saline/Baytril solution.

Rats

Described in detail in section 2.4.1.2.

2.4.5.2 Cocaine self-administration protocol

Mice

Mice first underwent lever training with 14 mg sweetened food pellets (TestDiet, USA), during which food pellet delivery was contingent upon pressing on the active lever under an FR1 schedule of reinforcement during a 60 min session. The active lever alternated between left and right daily, and changed within a session when 10 food reinforcers were achieved (1 cycle). Lever training was completed when 2 cycles were achieved on each lever on a minimum of two separate days. Following lever training, mice were implanted with an indwelling intravenous catheter (made in-house) into the jugular vein (described above). After 3 d recovery, mice underwent daily 1 h CSA session for 8 consecutive days. Sessions were run without illumination in the operant chamber and began with the extension of the levers. Cocaine (0.5 mg/kg/14 µl infusion) delivery was contingent upon pressing on the active lever under an FR2 schedule of reinforcement and paired with the 20 s presentation of a blinking light stimulus (CS), which also served as a TO period, during which lever presses were recorded but not reinforced. For all sessions, presses on the inactive lever were recorded but had no scheduled consequence. The active lever was counterbalanced across subjects (left vs. right) and remained constant throughout the experiment. CSA training was followed by daily 1 h extinction of cocaine seeking sessions 24 h after the completion of CSA. During extinction sessions presses on the active lever were not reinforced, and the CS was omitted. Immediately following the 1st 2 extinction sessions, mice received i.p. injection of vehicle (VEH) or 1 mg/kg psilocybin (PSI 1.0). This 2-injection regimen was based on previous studies in which limited administration of drugs during non-reinforced trials following learning has been shown to result in enhanced extinction and/or reduced drug responses (Bernardi and Lattal, 2012; Bernardi and Spanagel, 2014). Extinction concluded when each mouse reached a criterion level of responding on the active lever (less than 50 % responding over two days relative to the mean active lever presses during the last two days of self-administration; two mice that failed to reach this extinction criterion by the 24th extinction session were removed from the study). Once the extinction criterion was reached, cue reinstatement was tested 24 h later during a single 1 h session that began with a 20 s blinking of the light CS, and during which active lever presses resulted in the presentation of the 20 s blinking light CS, but no cocaine.

Rats

The rats underwent daily 1 h CSA sessions for 15 consecutive days. Each session began with the illumination of a blue light, which served as a contextual cue indicating cocaine availability. Cocaine (0.8 mg/kg/36µl infusion) delivery was contingent upon a NP into the

active hole under an FR1 schedule of reinforcement and paired with the 4 s presentation of a white light CS. Cocaine infusion was followed by a 40 s TO period, during which NPs were not reinforced. NPs in the active hole were recorded but had no consequences. Twenty-four hours following the completion of CSA, rats underwent 7 daily 1 h extinction sessions, during which active NPs were not reinforced, and both the blue light and the white light cue were omitted. Immediately following each of the 7 extinction sessions, rats were injected with vehicle (VEH), 1 mg/kg (PSI 1.0), or 2.5 mg/kg (PSI 2.5) psilocybin. Compared to the mouse studies, the number of post-extinction psilocybin injections was increased, the extinction criterion was removed in favor of a fixed number of sessions, and a higher psilocybin dose (2.5 mg/kg) previously shown to affect relapse-like behavior (Meinhardt et al., 2021) was included. One rat from the PSI 2.5 group was excluded due to abnormally low responding during CSA, indicating insufficient drug-seeking behavior. Twenty-four hours after the final extinction session, cue-induced reinstatement was assessed in a single 1-hour session. The session began with the onset of the blue light, and active NPs once again triggered the white light cue, but no cocaine was delivered.

2.4.5.3 Statistical analysis

Data are expressed as mean ± SEM. Statistical analyses were conducted using SPSS software (StatSoft, USA). Some deviations from normality were identified, predominantly among the SA data (Shapiro-Wilk test); however, ANOVA has been demonstrated to be robust to non-normality (Blanca et al., 2017, 2023). SA, extinction, and reinstatement data were analyzed using 3-way repeated measures ANOVA (treatment: vehicle vs. psilocybin; lever or NP: active vs. inactive, repeated measure; day, repeated measure). Sphericity violations during repeated measures ANOVA, as indicated by Mauchly's test, were corrected using the Greenhouse-Geisser correction. Post hoc analysis was performed using Bonferroni-corrected paired samples t-tests, where indicated. Extinction criteria data in mice were measured using independent samples t-tests. Reinstatement ratios in male rats were measured using one-way ANOVA. Statistical significance was set at $p = 0.05$.

2.4.6 Study 3B: Reality testing in rats

Rats were trained to consume a flavored solution with distinctive odor and taste during a various number of pairing sessions. Then the odor was devaluated by lithium chloride (LiCl) injection. The direct and mediated aversion were subsequently tested via the Two bottle choice test. The timeline of this set of experiments is shown in Fig. 2.5.

2.4.6.1 Reality testing protocol

All rats were single-housed (5 days prior the start of the experiment), water restricted and kept in the room where the protocol was performed during the entire duration of the experiment. The rat´s weight was also periodically measured to ensure the minimum 85-90 % of initial weight. The bottle position was alternated (left or right side) every day while maintaining the height and angle during the entire protocol in order to avoid any bias. To verify no pre-existing preference for any of the odor/taste stimuli used, the basal consumption of each odor and taste was measured in naïve rats.

The protocol started with the habituation period during which the rats were given 60 min access to water during 3 consecutive days. The precise water intake was measured every day by weighing the bottle before and after the session. Following day, the pre-conditioning period started during which the rats underwent a varying number of odor-taste pairings (1 pairing or 3 pairings or 6 pairings; see 2.5). Each pairing was done over two days – on the first day rats received 60 min access to a flavored solution containing an aqueous mixture of a taste (T1; **S**ucrose 2 %) paired with an odor (O1; **B**anana 0,05 %) and on the second, the aqueous solution contained the remaining taste (T2; **M**altodextrin 2 %) paired with odor (O2; **A**lmond 0,01 %). After the last odor-taste pairing, one of the odors (O1; **B**anana 0,05 %) was devaluated in order to become the CS^+. During this conditioning phase, the rats had 60 min access to a solution containing only the odor – **A**lmond on odd days and **B**anana on even days. Immediately after the 1h access to solution, the rats received an i.p. injection of either saline following access to the **A**lmond odored solution (CS^-) or LiCl (dose: 65 mg/kg), an emetic agent, following access to the **B**anana odored solution (CS^+). The number of odor-devaluation pairings differed (1 pairing or 3 pairings); see 2.5. Following the conditioning phase, the rats were given 1 day to recover, during which water was accessible for 60 min. Over the next two days, the mediated aversion and then the direct aversion were tested using a 60 min two bottle-choice test. At the beginning of both tests, forced sampling was performed in order to ensure that rats tasted both fluids—both bottles were presented at the same time and each was removed immediately after initial sampling. Both bottles were then simultaneously reintroduced to the rat and the test started. Mediated aversion was evaluated by the choice between the bottles containing **M**altodextrin (mCS^-) and **S**ucrose (mCS^+) previously paired with the devaluated odor – **B**anana (CS^+). The position of the bottles was counterbalanced to avoid any position bias. The aversion was observed when the intake of **S**ucrose solution (mCS^+) was lower than the consumption of **M**altodextrin solution (mCS^-). The last day of the protocol, the direct aversion was tested—the rats were given the choice between **A**lmond- (CS^-) and **B**anana-odored (CS^+) solutions and consumption was measured. Direct aversion was manifested by a lower consumption of

Banana-odored (CS^+) relative to **A**lmond-odored solution.

2.4.6.2 Statistical analysis

Data are expressed as mean ± SEM total liquid consumption (in grams). Direct and mediated aversion were assessed by comparing consumption of CS^+ vs. CS^- and mCS^+ vs. mCS^-, respectively. Consumption differences during the final test were analyzed using independent-samples *t*-tests. Aversion indices were calculated as follows:

$$\text{Mediated aversion} = \frac{mCS^- - mCS^+}{mCS^+ + mCS^-}$$

$$\text{Direct aversion} = \frac{CS^- - CS^+}{CS^+ + CS^-}$$

Statistical significance was set at $p = 0.05$.

Figure 2.5. Experimental timelines of the different variants used to evaluate representation-mediated aversion ("reality testing"). All versions began with water habituation, followed by odor–taste (O/T) pairings, a devaluation phase using LiCl-induced malaise, and subsequent test sessions. **(Top and middle)** Extended protocols using 6 or 3 O/T pairings with 1 or 3 devaluation pairings, respectively. **(Bottom)** Minimal-conditioning version with reduced preconditioning and devaluation phases. Pairing sessions are shown in light colors, devaluation sessions in dark colors. W = water; A = almond (odor); M = maltodextrin (taste); B = banana (odor); S = sucrose (taste); O/T = odor–taste; LiCl = lithium chloride.

Chapter 3

Results

3.1 Study 1A Sign- vs. goal-tracking in the 3-CRIT model of cocaine addiction

This section includes results and figures previously published in Pohořalá et al. (2021), reproduced here with minimal changes. The introductory text has been revised to align with the structure of the present thesis.

3.1.1 Introduction

One of the primary challenges in managing SUD is the heightened vulnerability to relapse. It is estimated that up to 60 % of individuals with SUD resume drug use within the first year following treatment (McLellan et al., 2000). Moreover, relapse can occur even after extended periods of abstinence (Leshner, 1997; Moos and Moos, 2006; Sinha and Li, 2007). A recent meta-analysis (Vafaie and Kober, 2022) suggests that drug-associated cues and indicators of craving play a significant role in the mechanisms underlying SUD, particularly in triggering relapse behavior. For instance, detoxified cocaine patients reported experiencing cravings and euphoria in response to a video simulating crack-cocaine use, whereas no such reactions were observed when they viewed a non-drug-related video (Childress et al., 1999). Similarly, both contextual and discrete stimuli previously associated with drug use—such as specific environments, individuals, and objects—can provoke drug-seeking behavior and cravings in humans (Mayo et al., 2013; O'Brien, 2005) and animals (Crombag and Shaham, 2002). Furthermore, reactivity to drug-related cues and subsequent cue-induced craving may, to some extent, predict relapse in patients with SUD (Back et al., 2010; Niaura et al.,

1988; Witteman et al., 2015). Therefore, developing behavioral assays to better understand an individual's susceptibility to relapse in the presence of drug-related cues is crucial and could promote the development of more effective treatment strategies for SUD.

Sign- and goal-tracking procedures in humans have been established to classify individuals based on their attentional behaviors, typically using eye-tracking to measure responses to CS or US (Cope et al., 2023; Garofalo and di Pellegrino, 2015; Le Pelley et al., 2016; Schad et al., 2020). Research suggests that STs exhibit an attentional bias toward reward-paired stimuli due to the attribution of incentive salience to such stimuli (Anderson et al., 2013; Le Pelley et al., 2016). For example, STs are more likely to respond to task-irrelevant CS when the US is unavailable (Garofalo and di Pellegrino, 2015) and demonstrate greater attentional approach behavior towards CS (Schad et al., 2020), driven by dopaminergic model-free learning via reward prediction error (Schultz et al., 1997), while GTs rely on non-dopaminergic model-based learning via state prediction error (Gläscher et al., 2010). These findings suggest two distinct learning processes within individuals, influencing their responses to new and future experiences (Schad et al., 2020).

Despite the growing body of research, studies on human sign- and goal-tracking behaviors, particularly in the context of SUD, remain relatively limited. Consequently, animal models have been crucial for investigating the effects of conditioned stimuli on instrumental behaviors. In rodents, an animal´s reaction to CS is assessed through PCA procedures. STs typically approach and fixate on the CS itself (e.g., a lever), by licking, gnawing and biting, while GTs approach the location of the US (e.g., food), focusing more on the predictive properties of the stimuli (Bolles, 1972; Enkel et al., 2019; Yager and Robinson, 2010). Importantly, the reward is delivered independently of the animal's behavior, and both groups learn a conditioned orienting response, indicating their understanding of the CS-US association (Robinson et al., 2014; Yager and Robinson, 2013). Studies have shown that STs exhibit stronger drug-seeking behaviors and increased responsiveness to drug-related cues, suggesting a potential connection between the ST phenotype and addiction (Flagel et al., 2009; Saunders and Robinson, 2013). For instance, STs work harder for cocaine under a PR schedule (Saunders and Robinson, 2011), display a preference for cocaine-associated cues (Meyer et al., 2012), and are more prone to cue- and drug-induced reinstatement of drug-seeking behaviors compared to GTs (Saunders and Robinson, 2010, 2011; Yager and Robinson, 2013). However, the majority of these studies involved relatively limited exposure to drugs of abuse (ranging from 5 to $\sim$20 SA sessions) and it seems that prolonged drug exposure may diminish these differences. For example, Kawa et al. (2016) reported significantly higher motivation to self-administer cocaine in STs compared to GTs after limited drug-taking period; however, this difference was no longer present following prolonged

drug experience, indicating that the degree of drug access might be a crucial factor in addiction vulnerability among ST and GT phenotypes (Colaizzi et al., 2020).

The aim of this study was to investigate the potential differential vulnerability of STs and GTs to develop addiction-like behaviors following extended cocaine access using the 3-CRIT model of cocaine addiction in rats. The behavioral measures of this model closely aligns with several addiction criteria defined in DSM, evaluating the following 3 criteria: the persistence of drug-seeking, motivation for drug taking and drug-taking despite adverse consequences (Deroche-Gamonet et al., 2004).

3.1.2 Results

3.1.2.1 PCA

Following 7 d of PCA, distinct patterns of approach behaviors to the lever, the food cup, or some intermediate combination had developed. Animals were then divided into 3 groups according to their PCA scores: rats with PCA scores < -0.5 were categorized as GTs (n = 8), rats with PCA scores between -0.5 and 0.5 were categorized as INTs (n = 20) and rats with PCA scores > 0.5 were categorized as STs (n = 17). Mean ($\pm$ SEM) PCA scores for GTs, INTs and STs on day 7 were -0.76 ± 0.05, -0.02 ± 0.05, and 0.81 ± 0.03, respectively. Figure 3.1A shows the mean ($\pm$ SEM) development of PCA scores over 7 d for for groups GT, INT, and ST. A two-way ANOVA of PCA scores (group $\times$ day) revealed significant main effects of group [$F(2, 42) = 51.1,\ p < 0.0005$] and day [$F(3.7, 155.3) = 8.1,\ p < 0.0005$], and a significant group $\times$ day interaction [$F(3.7, 155.3) = 6.5,\ p < 0.0005$], indicating a difference in the development of GT, INT, and ST behaviors, and a difference between the groups. A one-way ANOVA of data from days 1 and 7 confirmed that PCA scores among the groups did not differ significantly on day 1 [$F(2, 42) = 3.1,\ p > 0.05$], but differed on day 7 [$F(2, 42) = 202.6,\ p < 0.0005$; Bonferroni post hoc: GT vs. INT, $p < 0.0005$; GT vs. ST, $p < 0.0005$; INT vs. ST, $p < 0.0005$]. The mean number of CR was similar in all three groups on both days 1 and day 7, indicating similar levels of learning and performance. Figure 3.1B shows the mean ($\pm$ SEM) number of CR for groups GT, INT, and ST on days 1 and 7, indicating similar levels of learning and performance in all groups. A two-way ANOVA of CR (group $\times$ day) revealed a significant main effect of day [$F(1, 42) = 46.2,\ p < 0.0005$], but no other significant effects [group: $F(2, 42) = 0.9,\ p > 0.05$; group $\times$ day: $F(2, 42) = 0.3,\ p > 0.05$]. These findings indicate similar performance in all groups at the beginning and end of training, as well as an increase in learning of the task, demonstrated by the significant increase in the number of CR from

day 1 to 7. On day 7, the mean number of lever approaches and food cup head entries, respectively, were 6.1 ± 2.4 and 149.1 ± 21.7 for GT rats, 35.8 ± 3.3 and 90.0 ± 13.5 for INT rats, and 95.8 ± 7.3 and 4.6 ± 1.5 for ST rats.

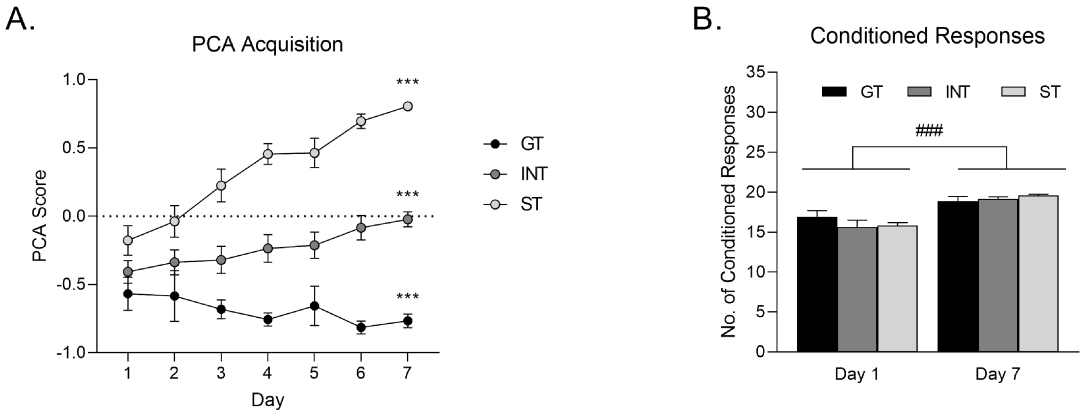

Figure 3.1. Development of PCA score and conditioned responses. **(A)** Rats characterized according their PCA scores on day 7 as GT (n = 8), INT (n = 20) and ST (n = 17) plotted as the development of these phenotypes. Rats showed no difference in PCA scores on day 1, but a significant difference on day 7. Data represent mean (± SEM) PCA score across 7 d of PCA sessions. **(B)** The number of conditioned responses did not differ among GT, INT and ST groups on day 1 or 7 of PCA training; however, the number of conditioned responses increased significantly from day 1 to day 7. Data represent mean (± SEM) number of conditioned responses on days 1 and 7. *** $p < 0.0005$ vs. all other groups; ### $p < 0.0005$. PCA = Pavlovian conditioned approach; GT = goal-tracker; INT = intermediate; ST = sign-tracker.

3.1.2.2 Self-administration

GT, INT, and ST rats did not differ in SA behavior during drug periods, cocaine infusions received, or SA behavior during non-drug periods. Figure 3.2A shows the mean (± SEM) number of cocaine infusions across all FR5 SA sessions for GT, INT, and ST groups. A two-way ANOVA (PCA group × day) found no significant main effect of group [$F(2, 42) = 0.5,\ p > 0.05$] or group × day interaction in cocaine intake [$F(7.8, 163.9) = 0.9,\ p > 0.05$], indicating no difference between GT, INT, and ST groups across sessions. There was, however, a main effect of day [$F(3.9, 163.9) = 3.3,\ p < 0.05$], indicating a significant change in cocaine intake across sessions. A paired samples t-test suggests that cocaine intake significant increased over time, as intake on the final FR5 session was significantly higher than that on the first FR5 session [$t(44) = 4.8,\ p < 0.0005$]. Figure 3.2B shows the mean (± SEM) number of active and inactive NPs during drug periods across all FR5

SA sessions for GT, INT, and ST groups. A three-way ANOVA (PCA group × day × NP) revealed a significant effect of NP [$F(1, 42) = 80.7,\ p < 0.0005$], but no other significant effects [group: $F(2, 42) = 0.02,\ p > 0.05$; day: $F(4.4, 185.1) = 1.2,\ p > 0.05$; group × day: $F(8.8, 185.1) = 0.9,\ p > 0.05$; group × NP: $F(2, 42) = 1.4,\ p > 0.05$; day × NP: $F(4.3, 178.8) = 0.6,\ p > 0.05$; group × day × NP: $F(8.5, 178.8) = 1.0,\ p > 0.05$], indicating a distinction between the active and inactive NPs, but no other differences between the groups across sessions. Figure 3.2C shows the mean (± SEM) number of active and inactive NPs during no-drug periods across all FR5 SA sessions for GT, INT, and ST groups. A three-way ANOVA (PCA group × day × NP) revealed a significant effect of NP [$F(1, 42) = 80.7,\ p < 0.0005$], but no other significant effects [group: $F(2, 42) = 0.02,\ p > 0.05$; day: $F(4.4, 185.1) = 1.2,\ p > 0.05$; group × day: $F(8.8, 185.1) = 0.9,\ p > 0.05$; group × NP: $F(2, 42) = 1.4,\ p > 0.05$; day × NP: $F(4.3, 178.8) = 0.6,\ p > 0.05$; group × day × NP: $F(8.5, 178.8) = 1.0,\ p > 0.05$], indicating a distinction between the active and inactive NPs, but no other differences between the groups across sessions.

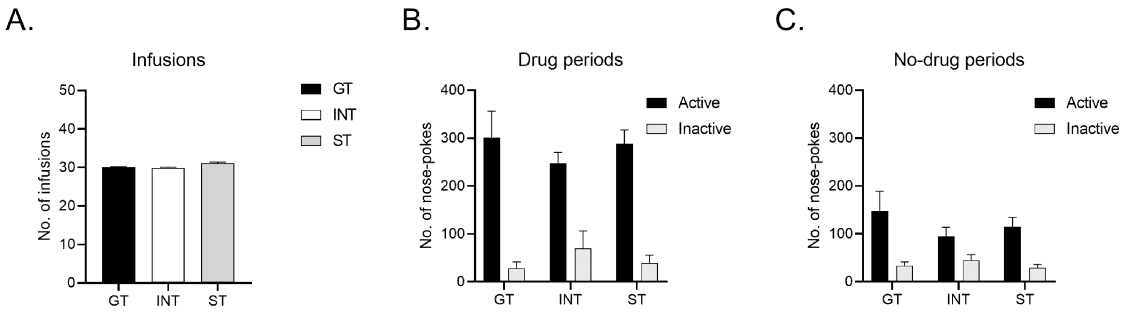

Figure 3.2. Cocaine self-administration in GTs, INTs and STs across all FR5 sessions. There were no differences in the number of **(A)** reinforcers achieved, **(B)** NPs during drug periods, or **(C)** NPs during no-drug periods between GTs, INTs and STs across sessions. Data represent the mean (± SEM) number of active and inactive NPs reinforcers achieved for groups GT, INT, and ST. GT = goal-tracker; INT = intermediate; ST = sign-tracker; NP = nose-poke.

3.1.2.3 3-CRIT

Following cocaine SA training, animals were scored and divided into 4 groups corresponding with the number of positive criteria they met—0crit [n = 8 (18 %)], 1crit [n = 21 (47 %)], 2crit [(n = 11 (24 %)] and 3crit [n = 5 (11 %)]. Figure 3.3A-D shows the distribution according to the 0, 1, 2, or 3 positive criteria (crit). Independent samples t-tests revealed that 0 and 3crit animals differed significantly on all 4 measures: (A) persistence to drug-seeking

as indicated by non-drug period responding [$t(11) = 4.4,\ p < 0.005$], (B) breakpoint in PR [$t(11) = 4.2,\ p < 0.005$], (C) resistance to punishment [$t(11) = 11.9,\ p < 0.0005$], and (D) addiction score [$t(11) = 9.4,\ p < 0.0005$]. The prevalence of each PCA phenotype in the crit groups: 0crit (3 GT, 3 INT, 2 ST), 1crit (3 GT, 10 INT, 8 ST), 2crit (2 GT, 5 INT, 4 ST) and 3crit (0 GT, 2 INT, 3 ST).

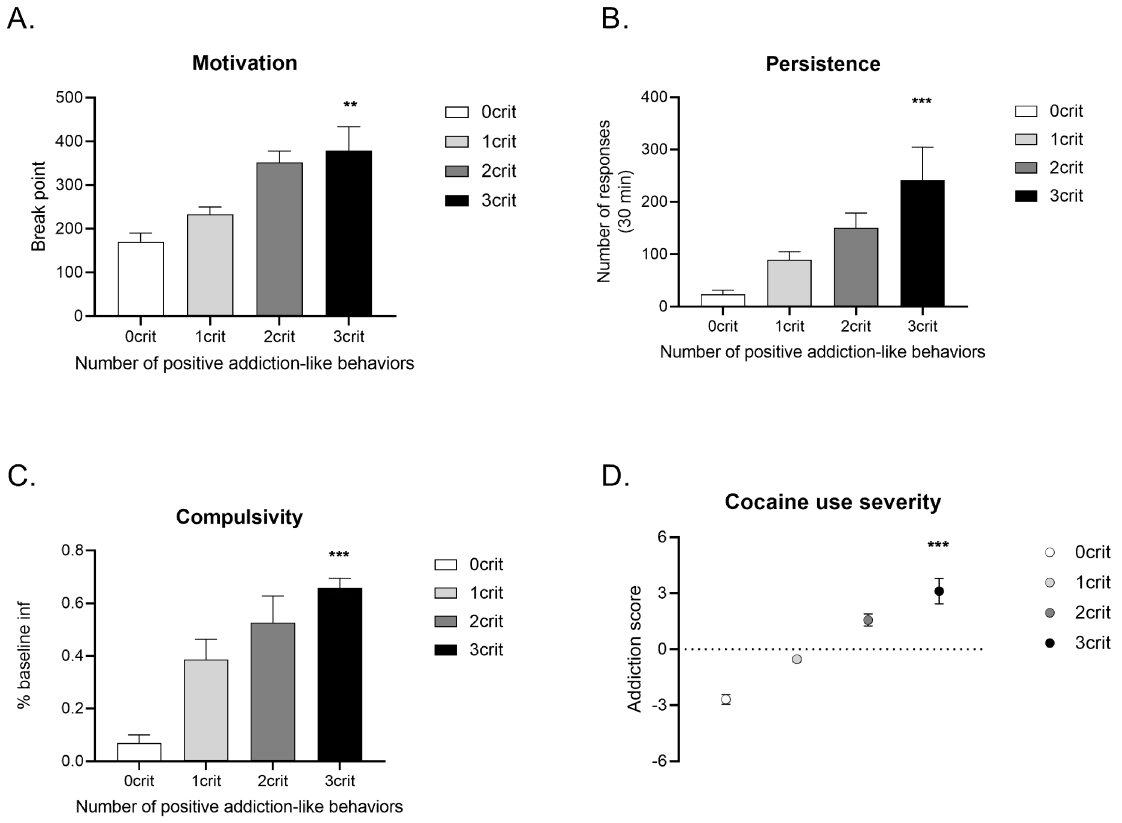

Figure 3.3. Addiction-like behavior in rats exhibiting 0, 1, 2, or 3 positive criteria (crit). As compared to 0crit rats (n=8), 3crit rats (n=5) displayed a higher score in every criterion for addiction-like behavior. **(A)** Persistence of cocaine seeking, measured by the number of active NPs when cocaine is signaled as unavailable. **(B)** Motivation for cocaine taking, measured as the break point during PR. **(C)** Cocaine taking and seeking despite adverse consequences, measured by resistance to punishment. **(D)** Cocaine-use severity, indicated by the addiction score. 0 and 3crit animals differed significantly in all measurements. Data represent the mean ($\pm$ SEM) of each criteria/addiction score. ** $p < 0.005$, *** $p < 0.0005$, relative to 0crit. NP = nose-poke; PR = progressive ratio.

3.1.2.4 PCA 3-CRIT scores analyses

There were no significant correlations between PCA score and addiction criteria as measured using the 3-CRIT model. Figure 3.4A-D shows Spearman's correlation of PCA

scores versus (A) persistence to drug-seeking as indicated by non-drug period responding [$r = 0.06,\ p > 0.05$], (B) breakpoint in PR [$r = 0.12,\ p > 0.05$], (C) resistance to punishment [$r = 0.24,\ p > 0.05$], and (D) addiction score [$r = 0.22,\ p > 0.05$]. Thus, PCA score in the current experiment was not predictive of subsequent addictive-like behavior.

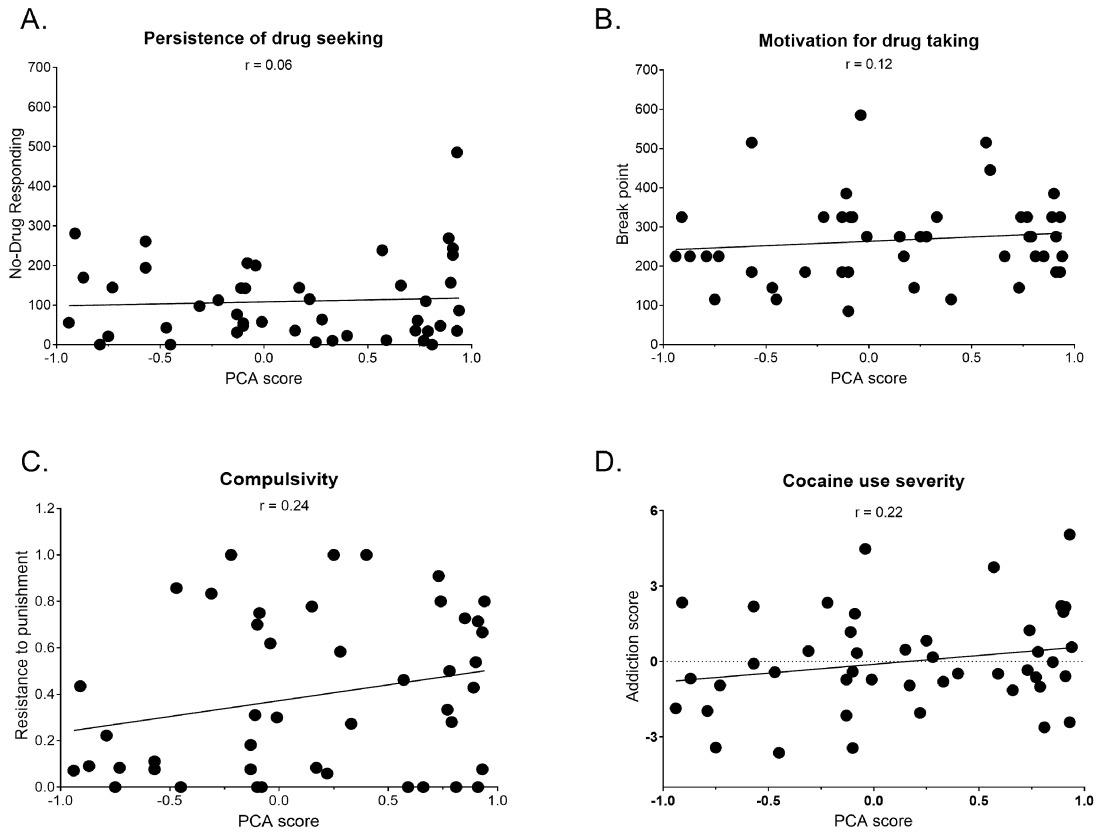

Figure 3.4. Correlation between PCA score and addiction criteria. There were no significant positive correlations between PCA score and **(A)** persistence of cocaine seeking **(B)** motivation for cocaine taking **(C)** resistance to punishment, or **(D)** addiction score. Data represent Pearson's correlation coefficient (r) between pre-cocaine SA PCA scores and addiction criteria. PCA = Pavlovian conditioned approach.

3-CRIT scores between GTs and STs were analyzed to determine whether the two extremes differed in addiction-like characteristics. There were no differences between GTs and STs on persistence of drug-seeking or PR breakpoint, but a significant difference in resistance to punishment. Figure 3.5A-D shows the mean ($\pm$ SEM) non-drug period responding, PR BP, resistance to punishment, and addiction scores, respectively, in GTs and STs. Independent-samples t-tests revealed that GTs and STs did not differ on non-drug period responding [$t(23) = 0.2,\ p > 0.05$], PR breakpoint [$t(23) = 0.7,\ p > 0.05$], or addiction score [$t(23) = 1.8,\ p > 0.05$], but STs showed significantly greater resistance to punishment than GTs [$t(23) = 2.4,\ p < 0.05$].

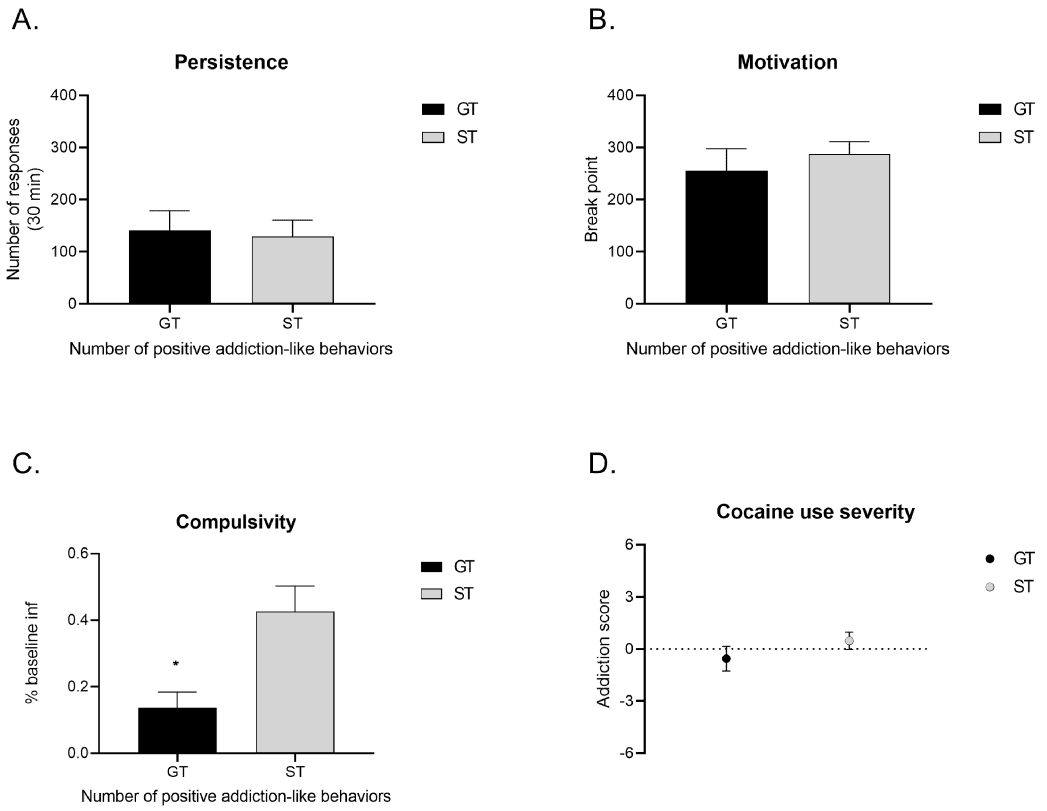

Figure 3.5. Addiction-like criteria in rats characterized as sign- and goal-trackers. There were no differences in **(A)** persistence of cocaine seeking, or **(B)** motivation for cocaine taking between GTs and STs. Data represent the mean ($\pm$ SEM) number of NPs during non-drug periods and break point, respectively. **(C)** There was a significant difference between GTs and STs during the resistance to punishment session, with STs showing a higher resistance to punishment. Data represent the mean ($\pm$ SEM) percentage of infusions relative to baseline. **(D)** There was no difference in addiction score between GTs and STs. Data represent mean ($\pm$ SEM). $*p < 0.05$. GT = goal-tracker; ST = sign-tracker; NP = nose-poke.

3.2 Study 1B Sex differences in the 3-CRIT model of cocaine addiction

3.2.1 Introduction

SUD is a harmful condition that affects individuals across both sexes. Although only a small percentage of drug users in the general population progress to fully developed addiction, research suggests that women, despite making up a smaller proportion of overall cocaine users, may be at greater risk at various stages of addiction development process (Becker and Koob, 2016; Center for Substance Abuse Treatment, 2009; Greenfield et al., 2010). A particularly striking example is a phenomenon called the "telescoping effect", which suggests that, following initial drug use, women meet the criteria for SUD and pursue treatment considerably sooner than men (Becker et al., 2017; Brady and Randall, 1999; Greenfield et al., 2010; Haas and Peters, 2000; Towers et al., 2022b). In the context of CUD, women are more susceptible to developing dependence during adolescence (Chen and Kandel, 2002), are more likely to use dangerous routes of administration (Kosten et al., 1993), and tend to escalate cocaine intake more rapidly than men (McCance-Katz et al., 1999). After progressing to SUD, women often experience stronger cravings for cocaine (Elman et al., 2001; Moran-Santa Maria et al., 2014; Robbins et al., 1999; Waldrop et al., 2010), although some studies have reported no significant sex differences (for review, see Nicolas et al., 2022). Moreover, during early abstinence, women frequently experience more severe and distressing symptoms, encounter a greater burden of drug-related medical and psychological complications (Becker and Chartoff, 2019; Becker et al., 2017; Sanvicente-Vieira et al., 2019), and exhibit higher relapse rates, with shorter periods of abstinence between relapses compared to men (Becker and Hu, 2008; Gallop et al., 2007; Hudson and Stamp, 2011). Taken together, these data suggest that women may be more severely affected by cocaine use than men.

Similarly, preclinical studies with rodents have also revealed significant sex-specific differences in drug-related behaviors. Female rodents, in comparison to their male counterparts, tend to escalate their drug use to a greater extent (Roth and Carroll, 2004), and they exhibit more rapid acquisition of drug-seeking behaviors (Carroll et al., 2002; Hu et al., 2004; Jackson et al., 2006; Lynch and Carroll, 1999). This accelerated acquisition is accompanied by a more pronounced binge-like pattern of drug intake, further highlighting the heightened vulnerability of female rodents to substance use (Hu et al., 2004; Lynch and Taylor, 2004). In addition to these behavioral differences, female rats exhibit a stronger motivation for drug-taking, as indicated by higher BPs in PR schedules (Carroll et al., 2002; Roberts et al.,

1989b), and show a greater propensity for relapse-like behavior, as observed in reinstatement tests, during which female rodents are more likely to revert to drug-seeking after periods of abstinence (Lynch et al., 2010; Swalve et al., 2016) Moreover, sex-specific variability extends to physiological and behavioral responses to dose-dependent drug effects. For example, following the administration of psychomotor stimulants, female rats are more prone to exhibit increased behavioral sensitization and heightened locomotor responses compared to males (Cailhol and Mormède, 1999; Robinson and Becker, 1986; Sell et al., 2002).

Findings from both clinical and preclinical studies indicate that sex plays a significant role in the development and treatment of SUD. However, historically, the majority of preclinical research in this field has predominantly focused on male subjects, with relatively few studies investigating their female counterparts and the 3-CRIT model of cocaine addiction is no exception. This gender discrepancy has only begun to be addressed in recent years, as the scientific community increasingly recognizes the importance of including female animals in experimental paradigms. To the best of my knowledge, all published studies utilizing this model to date have exclusively employed male rats. To address this sex bias, the present study was designed to systematically investigate potential behavioral differences between male and female rats within the 3-CRIT model of cocaine addiction. By doing so, the research aims to contribute to a more nuanced and comprehensive understanding of substance use disorders. Such insights are crucial for the development of more effective, sex-specific therapeutic strategies.

3.2.2 Results

3.2.2.1 Sex differences in acquisition of cocaine self-administration behavior

Operant Responding

Drug periods Male rats demonstrated significantly higher active NPs compared to female rats during the drug periods of all FR5 training sessions (Session 7–Session 45) of the 3-CRIT protocol. Figure 3.6, left panel, shows the mean ($\pm$ SEM) number of active and inactive NPs performed by male and female rats during drug periods only (3 $\times$ 40-min periods/session). A four-way ANOVA (session $\times$ NP $\times$ group $\times$ sex) revealed a significant main effect of NP [$F(1, 85) = 34.1,\ p < 0.001$], indicating an overall distinction between the active and inactive NPs. A significant main effect of sex [$F(1, 85) = 8.5,\ p < 0.005$] indicated differences in operant responding between male and female rats. An independent samples t-test further revealed that male rats had a significantly higher number of active

NPs compared to females [$t(91) = 2.6,\ p < 0.05$]. Additionally, a significant main effect of group [$F(3, 85) = 4.9,\ p < 0.005$] was found. Post hoc analyses (Bonferroni corrected) showed that the 3crit group had significantly higher nose-poking behavior than the 0crit group ($\Delta M = 426.4,\ p < 0.001$), the 1crit group ($\Delta M = 331.0,\ p = 0.001$), and the 2crit group ($\Delta M = 279.5,\ p < 0.01$). No significant differences were observed between the 0crit and 1crit groups ($\Delta M = -95.4,\ p > 0.05$), the 0crit and 2crit groups ($\Delta M = -146.9,\ p > 0.05$), or the 1crit and 2crit groups ($\Delta M = -51.5,\ p > 0.05$). No other significant effects were found (session [$F(3.1, 262.0) = 2.0,\ p > 0.05$]; session × group [$F(9.2, 262.0) = 2.0,\ p > 0.05$]; session × sex [$F(3.1, 262.0) = 0.8,\ p > 0.05$]; session × group × sex [$F(9.2, 262.0) = 0.7,\ p > 0.05$]; NP × group [$F(3, 85) = 1.5,\ p > 0.05$]; NP × sex [$F(1, 85) = 0.0,\ p > 0.05$]; NP × group × sex [$F(3, 85) = 0.0,\ p > 0.05$]; session × NP [$F(3.2, 271.1) = 1.5,\ p > 0.05$]; session × NP × group [$F(9.6, 271.1) = 1.1,\ p > 0.05$]; session × NP × sex [$F(3.2, 271.1) = 1.3,\ p > 0.05$]; session × NP × group × sex [$F(9.6, 271.1) = 1.4,\ p > 0.05$]; and group × sex [$F(3, 85) = 1.6,\ p > 0.05$]).

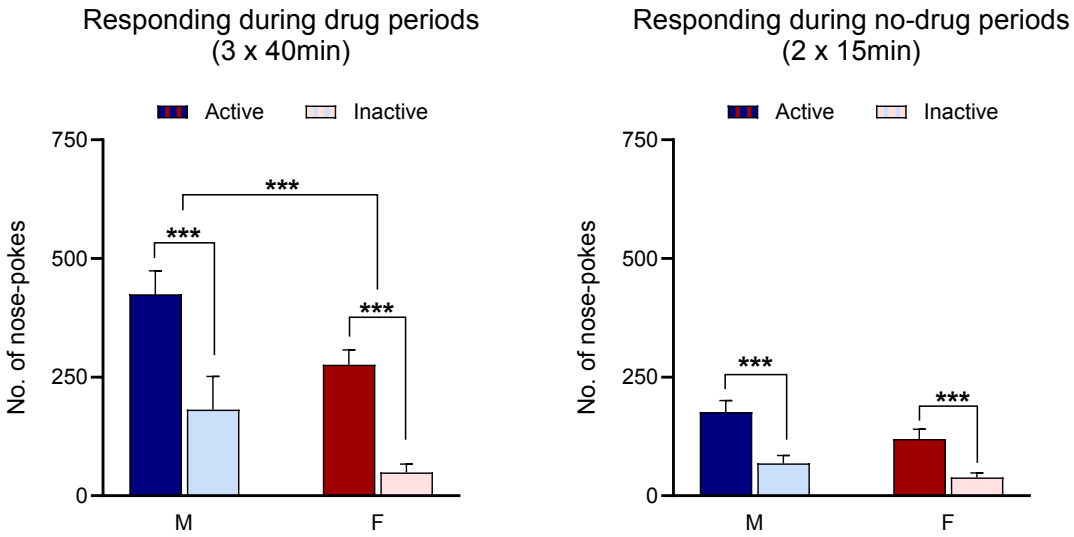

Figure 3.6. Active (dark) and inactive (light) NPs responding during drug periods *(left)* and no-drug periods *(right)* across all FR5 sessions for male (blue) and female (red) rats. During drug periods, male rats performed significantly more active NPs compared to females, with males showing a slightly stronger preference for active over inactive responding. Data represented as mean (± SEM). *** $p < 0.001$, main effect of NP, main effect of sex. F = female; M = male; NP = nose-poke.

No-drug periods No significant differences in overall operant responding were observed between male and female rats during the no-drug periods of the FR5 training sessions (Session 7 – Session 45) of the 3-CRIT protocol. Figure 3.6, right panel, shows the mean (± SEM) number of active and inactive NPs performed by male and female rats during no-drug

periods only (2 × 15-min periods/session). A four-way ANOVA (session × NP × group × sex) revealed a significant main effect of session [$F(7.7, 651.0) = 7.3,\ p < 0.001$] and NP [$F(1, 85) = 49.7,\ p < 0.001$], indicating changes in the pattern of operant responding over time and a significant distinction between active and inactive NPs, respectively. A significant main effect of group [$F(3, 85) = 9.1,\ p < 0.001$] revealed differences in operant responding across the crit groups. Post hoc analyses (Bonferroni-adjusted) revealed that the 3crit group exhibited significantly higher nose-poking behavior compared to the 0crit, 1crit, and 2crit groups ($p < 0.001,\ p < 0.001,$ and $p < 0.05$, respectively). The 2crit group also exhibited significantly higher nose-poking behavior compared to the 0crit group ($p < 0.05$), but no significant differences were found between the 0crit and 1crit groups ($p > 0.05$) or between the 1crit and 2crit groups ($p > 0.05$). A significant session × group interaction [$F(23.0, 651.0) = 3.6,\ p < 0.001$], NP × group interaction [$F(3, 85) = 10.2,\ p < 0.001$], and session × NP interaction [$F(3.2, 271.1) = 3.3,\ p < 0.005$] were found, indicating that the difference in operant responding varied across groups and sessions. A significant session × NP × sex interaction [$F(21.6, 611.0) = 2.3,\ p < 0.001$] suggested that the variation in nose-poking behavior between active and inactive responses differed between males and females over sessions. Furthermore, a significant session × NP × group × sex interaction [$F(21.6, 611.0) = 1.6,\ p < 0.05$] indicated that the development of active and inactive nose-poking behavior across sessions depended on both group (e.g., 0crit vs. 3crit) and sex (male vs. female). However, the main effect of sex was not significant [$F(1, 85) = 2.3,\ p > 0.05$], indicating no overall differences in nose-poking behavior between males and females during no-drug periods. Similarly, no other significant effects were found (session × sex [$F(7.7, 651.0) = 1.8,\ p > 0.05$]; session × group × sex [$F(23.0, 651.0) = 0.9,\ p > 0.05$]; NP × sex [$F(1, 85) = 0.0,\ p > 0.05$]; NP × group × sex [$F(3, 85) = 0.7,\ p > 0.05$]; and group × sex [$F(3, 85) = 0.6,\ p > 0.05$]).

Cocaine intake

Male rats demonstrated significantly higher cocaine intake across all FR5 training sessions in the 3-CRIT protocol. Figure 3.7 displays the mean (± SEM) number of cocaine infusions received during the 2.5-hour FR5 training sessions (Session 7–Session 45) for male and female rats. A three-way ANOVA (sex × session × group) revealed a significant main effect of sex [$F(1, 85) = 5.7,\ p < 0.05$], indicating that males consumed more cocaine overall than females. A significant main effect of group [$F(3, 85) = 9.4,\ p < 0.0005$] was also observed. Post hoc analyses (Bonferroni-adjusted) indicated that the 3crit and 2crit groups exhibited significantly higher cocaine intake compared to the 0crit and 1crit groups

($p < 0.001$ and $p < 0.05$, respectively), while no significant differences were observed between the 0crit and 1crit groups or between the 2crit and 3crit groups. A significant main effect of session [$F(9.7, 823.4) = 7.7,\ p < 0.0005$] indicated that cocaine intake increased significantly over time. Further analysis using a paired-samples t-test confirmed this, with cocaine intake in the final FR5 session (Session 45) being significantly higher than in the first FR5 session (Session 7) [$t(92) = 4.52,\ p < 0.001$]. Furthermore, a significant session $\times$ sex interaction [$F(9.7, 823.4) = 2.6,\ p = 0.005$] was identified, suggesting that trends in cocaine intake over time differed between males and females. Post hoc pairwise comparisons (Bonferroni-corrected) revealed that males consumed significantly more cocaine than females in early and mid-training sessions. Specifically, significant differences were observed in Session 12 ($\Delta M = 3.4,\ p < 0.005$) and Session 13 ($\Delta M = 2.2,\ p < 0.005$). From Session 14 to Session 30, males consistently exhibited higher intake compared to females, with significant differences ranging from $\Delta M = 2.4$ in Session 29 ($p < 0.05$) to $\Delta M = 3.7$ in Session 23 ($p < 0.001$). In later sessions (Sessions 31–45), the differences between males and females diminished, and no significant differences were observed. No other significant effects were found (session $\times$ group [$F(29.1, 823.4) = 1.1,\ p > 0.05$]; session $\times$ sex $\times$ group [$F(29.1, 823.4) = 0.9,\ p > 0.05$]; sex $\times$ group [$F(3, 85) = 0.9,\ p > 0.05$]).

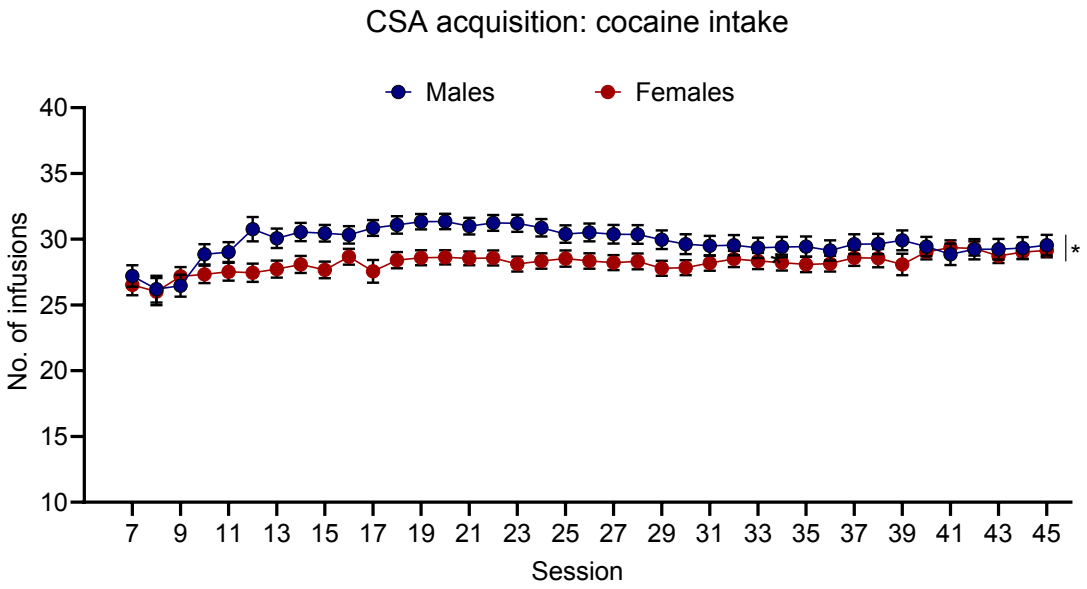

Figure 3.7. Acquisition of cocaine intake across all FR5 sessions for male (blue) and female (red) rats. Across sessions, female rats had significantly lower cocaine intake . Data represented as mean ($\pm$ SEM). * $p < 0.05$, main effect of sex.

3.2.2.2 Sex differences in 3-CRIT

Following cocaine SA training, both male and female rats were scored and divided into 4 groups corresponding to the number of positive criteria they met—0crit [males: n = 10 (22 %); females: n = 8 (17 %)], 1crit [males: n = 17 (37 %); females: n = 17 (37 %)], 2crit [males: n = 12 (26 %); females: n = 19 (40 %)] and 3crit [males: n = 7 (15 %); females: n = 3 (6 %)] as shown in Figure 3.8.

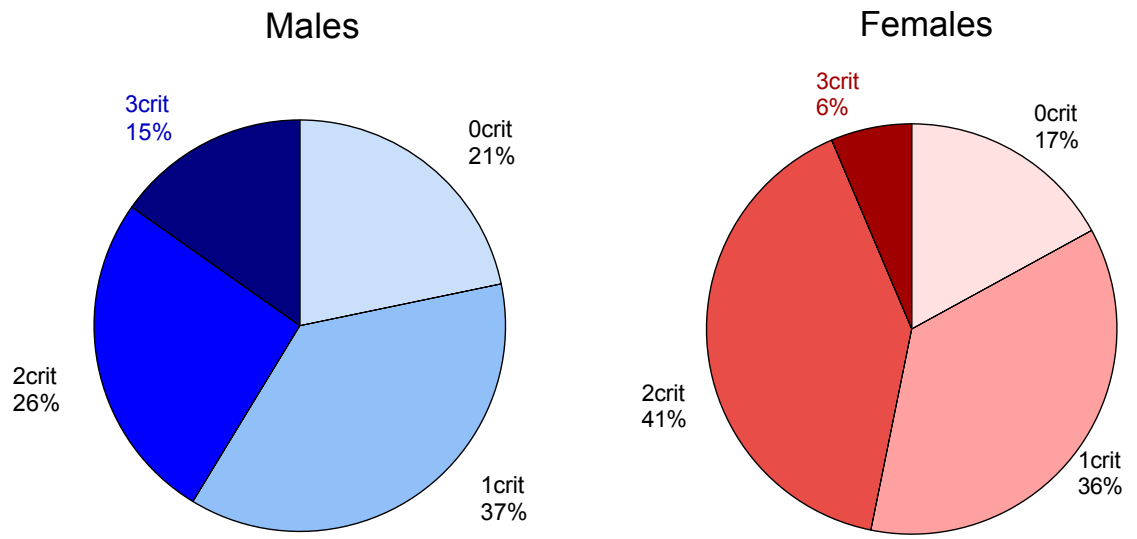

Figure 3.8. Population distribution of male (blue) and female (red) rats exhibiting 0, 1, 2, or 3 positive criteria (crit).

Figure 3.9A-D shows the distribution of of male and female rats meeting 0 (addiction-resilient) vs. 3 (addiction-vulnerable) criteria. A two-way ANOVA (sex × group) revealed a significant main effect of group in all 3 criteria tested in the 3-CRIT protocol and the overall addiction score, meaning that, across sexes, the addiction-resilient (0crit) and addiction-vulnerable (3crit) rats differed significantly on all 4 measures: (A) Persistence [$F(1,24) = 36.7,\ p < 0.001$], (B) Motivation [$F(1,24) = 50.1,\ p < 0.001$], (C) Compulsivity [$F(1,24) = 72.1,\ p < 0.001$], and (D) Addiction score [$F(1,24) = 95.7,\ p < 0.001$]. The figure displays sex-specific means for illustration purposes. Additionally, a significant main effect of sex [$F(1,24) = 8.8,\ p < 0.05$] and sex × group interaction [$F(1,24) = 7.0,\ p < 0.05$] was found in the overall Addiction score. No significant main effects of sex were observed for Persistence, Motivation, and Compulsivity [$F(1,24) = 1.7,\ p > 0.05$; $F(1,24) = 0.0,\ p > 0.05$; $F(1,24) = 0.3,\ p > 0.05$, respectively], and no significant sex × group interactions were found for these measures [$F(1,24) = 2.4,\ p > 0.05$; $F(1,24) = 1.0,\ p > 0.05$; $F(1,24) = 0.1,\ p > 0.05$, respectively]. Assumptions of normality (Shapiro–Wilk) and homogeneity of variances (Lev-

ene's) were evaluated. Some variables deviated from these assumptions; however, given the widespread use of ANOVA in preclinical behavioral research and the need for consistency across measures, this method was retained and results interpreted with caution.

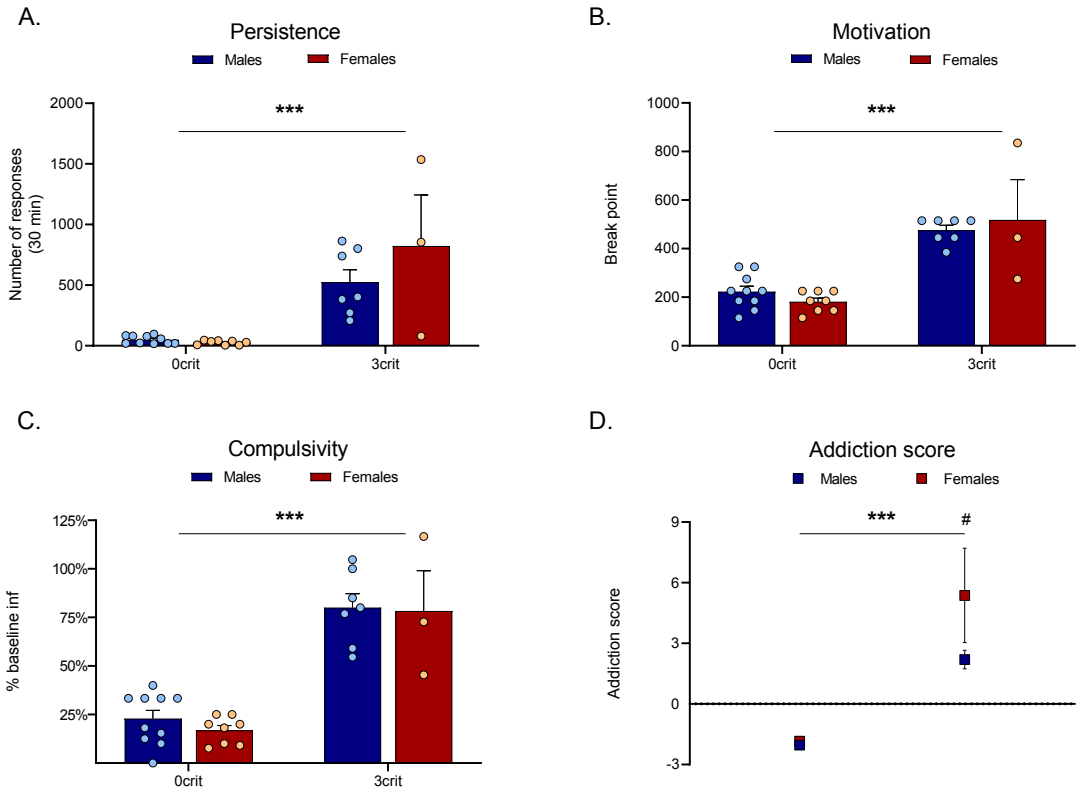

Figure 3.9. Addiction-like behavior in both male (blue) and female (red) rats exhibiting 0 and 3 positive criteria (crit). As compared to 0crit rats (male: n = 10; female: n = 8), 3crit rats (male: n = 7; female: n = 3) displayed a higher score in every criterion for addiction-like behavior and overall addiction score. **(A)** Persistence of cocaine seeking, measured by the number of active NPs when cocaine is signaled as unavailable. **(B)** Motivation for cocaine taking, measured as the break point during PR. **(C)** Cocaine taking and seeking despite adverse consequences, measured by resistance to punishment. **(D)** Cocaine-use severity, indicated by the addiction score. 0crit and 3crit animals differed significantly in all measurements. Data represent the mean (± SEM) of each criteria/addiction score. *** $p < 0.0001$, main effect of group (0crit vs. 3crit); # $p < .05$, main effect of sex and sex x group interaction. NP = nose-poke; PR = progressive ratio.

To further examine sex differences independent of group, independent samples t-tests were conducted across all subjects. Males exhibited significantly higher motivation than females $[t(91) = 2.6,\ p < 0.05]$, whereas no significant sex differences were observed for persistence $[t(91) = 1.2,\ p > 0.05]$ or compulsivity $[t(91) = 1.7,\ p > 0.05]$. Because the BP data for females deviated from normality, the motivation result should be interpreted with

caution.

3.3 Study 2A Metabotropic glutamate receptor 2: Stage-specific regulation during cocaine self-administration

3.3.1 Introduction

The $mGluR_2$ has gained attention in addiction research due to its significant role in neuroplasticity and its abundant expression in brain regions implicated in drug-seeking behaviors, such as PFC and VS, including the NAc (Niedzielska-Andres et al., 2021; Ohishi et al., 1998, 1993). $mGluR_2$ functions as an autoreceptor, modulating glutamatergic transmission by inhibiting presynaptic glutamate release, thereby maintaining excitatory balance in addiction-relevant circuits (Kalivas, 2009; Moussawi et al., 2011). Since addiction is characterized by dysregulated glutamatergic activity following chronic drug exposure, $mGluR_2$ has been increasingly investigated as a potential therapeutic target for substance use disorders.

Previous studies using rodent models have revealed a downregulation of $mGluR_2$ in brain areas closely associated with addiction, particularly in the prefrontal cortex and nucleus accumbens, following exposure to and withdrawal from various substances of abuse, including cocaine (Kasanetz et al., 2013; Logan et al., 2020), morphine (Qian et al., 2019), and alcohol (Meinhardt et al., 2013, 2021). This reduction in $mGluR_2$ expression has been implicated in the persistence of relapse-like behaviors. Notably, this downregulation appears to be reversible, as demonstrated by Meinhardt and colleagues (2021), who showed that psilocybin treatment was able to restore $mGluR_2$ expression and concurrently reduce relapse-like behavior in alcohol-exposed rodents. Similarly, Logan et al. (2020) successfully reversed cocaine-induced $mGluR_2$ downregulation using ceftriaxone, a beta-lactam antibiotic, which was accompanied by a significant reduction in cocaine-seeking behavior during reinstatement tests.

Pharmacological modulation of $mGluR_2$ has been explored as a potential strategy for reducing drug-seeking behavior, with particular interest in the $mGluR_{2/3}$ agonist LY379268. This compound has been shown to attenuate cocaine-seeking in various preclinical models (Adewale et al., 2006; Baptista et al., 2004; Cannella et al., 2013; Justinova et al., 2016; Martin-Fardon and Weiss, 2012). However, its clinical applicability remains limited due

to poor brain penetration, potential off-target effects via $mGluR_3$, and concerns about tolerance development with prolonged use (Galici et al., 2005; Liechti et al., 2007; Sheffler et al., 2011). Moreover, while previous studies have reported $mGluR_2$ downregulation following cocaine exposure, it remains unclear whether this reduction is a consequence of chronic drug use or a pre-existing vulnerability trait that predisposes individuals to addiction (Yang et al., 2017). These uncertainties underscore the need for a deeper understanding of $mGluR_2$ alterations across different stages of addiction, as explored in this study.

Together, these findings highlight $mGluR_2$ as a promising target for addiction research, particularly in the context of relapse prevention. The ability to modulate its expression and influence relapse-like behavior suggests that therapies aimed at restoring or enhancing $mGluR_2$ function could offer novel strategies for treating substance use disorders. To further investigate this potential, the present study was designed to examine $mGluR_2$ expression in Sprague-Dawley rats across different stages of CSA and withdrawal. Specifically, expression levels were assessed following short-term CSA (CSA), extinction (CSA+EXT), and abstinence (CSA+ABST), as well as long-term CSA. Furthermore, the study also compared $mGluR_2$ levels between addiction-prone (3crit) and addiction-resilient (0crit) phenotypes identified using the 3-CRIT model of cocaine addiction.

The main aim was to replicate and extend previous findings of cocaine-induced $mGluR_2$ downregulation, while also investigating the effects of LY379268 on cue-induced reinstatement of cocaine-seeking behavior. Importantly, by including the 3-CRIT model, this study provides a unique opportunity to directly compare addiction-prone and addiction-resilient phenotypes, a distinction that is not captured by many traditional self-administration models. Given the growing interest in pharmacological strategies targeting glutamatergic dysregulation, characterizing $mGluR_2$ alterations across different stages of drug exposure and withdrawal is particularly relevant for the development of mGluR2-based or phenotype-tailored treatments.

3.3.2 Results

3.3.2.1 Short-term cocaine self-administration – different conditions

Behavioral data

Operant responding: Response patterns during the final 10 SA sessions were stable and showed no differences between cocaine-exposed and control groups. Figure 3.10A shows responding, measured as the mean number (± SEM) of active and inactive NPs, across

all cocaine-exposed groups: CSA (n = 7), EXT (n = 10), and ABST (n = 8). A three-way ANOVA (session × NP × group) revealed a significant main effect of NP [$F(1, 22) = 19.6,\ p < 0.0005$] and a session × NP interaction [$F(2.2, 47) = 3.1,\ p < 0.05$], indicating a clear distinction between active and inactive NPs, with their patterns of responding differing over time. There were no other significant effects (session [$F(2.4, 53.3) = 1.3,\ p > 0.05$]; group [$F(2, 22) = 1.8,\ p > 0.05$]; session × group [$F(4.8, 53.3) = 0.6,\ p > 0.05$]; NP × group [$F(2, 23) = 0.5,\ p > 0.05$]; and session × NP × group [$F(4.3, 47) = 0.8,\ p > 0.05$]).

Figure 3.10B shows responding of the EXT group during 10 days of extinction. A two-way ANOVA revealed a significant main effect of session [$F(3.0, 27.3) = 13.0,\ p < 0.0005$], indicating a change in operant responding over time. Subsequent paired *t*-tests confirmed a significant reduction in both active and inactive NPs between the first and last extinction session. Specifically, active NPs decreased significantly [$t(9) = 3.58,\ p < 0.005$], as did inactive NPs [$t(9) = 3.60,\ p < 0.005$], indicating an effective suppression of cocaine-seeking behavior through extinction training. There were no other significant effects (NP [$F(1, 9) = 0.8,\ p > 0.05$]; session × NP [$F(3.2, 28.6) = 0.5,\ p > 0.05$]).

Figure 3.10C presents responding for control groups, measured as the mean number (± SEM) of active and inactive NPs during the last 10 days of training (Sessions 6–15) for SA CTRL (n = 8), EXT CTRL (n = 10), and ABST CTRL (n = 8). A three-way ANOVA (session × NP × group) revealed a significant main effect of NP [$F(1, 23) = 6.1,\ p < 0.05$], indicating a distinction between active and inactive NPs, likely due to stimulus reinforcement (Everitt and Robbins, 2005). However, no other significant effects were observed (group [$F(2, 23) = 0.1,\ p > 0.05$]; session [$F(4.6, 105.5) = 1.3,\ p > 0.05$]; session × group [$F(9.2, 105.5) = 1.7,\ p > 0.05$]; NP × group [$F(2, 23) = 0.3,\ p > 0.05$]; session × NP [$F(5.8, 133.8) = 1.5,\ p > 0.05$]; and session × NP × group [$F(11.6, 133.8) = 0.8,\ p > 0.05$]).

Figure 3.10D shows the operant responding of the EXT CTRL group during 10 days of extinction. A two-way ANOVA did not reveal any significant effects [session: $F(3.0, 26.8) = 1.4,\ p > 0.05$; NP: $F(1, 9) = 4.9,\ p > 0.05$; session × NP: $F(3.5, 31.3) = 2.3,\ p > 0.05$], indicating that responding in the EXT CTRL did not follow the typical pattern of seeking behavior seen with cocaine (high initial responding followed by a gradual decrease in responding), consistent with a lack of reinforcement during training.

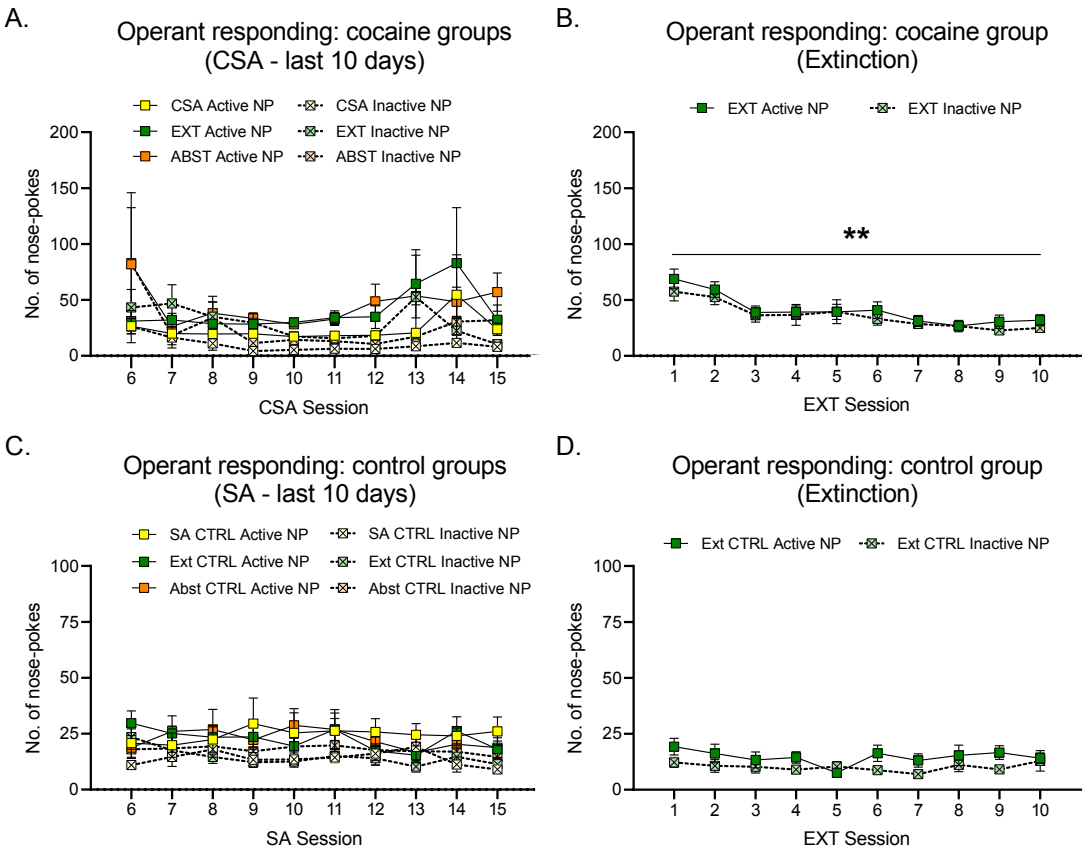

Figure 3.10. (A) Operant responding (active and inactive NPs) during last 10 sessions of self-administration across all cocaine-exposed groups. CSA EXT and ABST groups did not differ in overall operant respoding. **(B)** Operant responding (active and inactive NPs) during extinction training in the EXT group. A significant decrease in both active and inactive NPs was observed over time, indicating successful extinction of cocaine-seeking behavior. **(C)** Operant responding during the last 10 self-administration sessions across control groups (SA CTRL, Ext CTRL, Abst CTRL). No significant differences in operant responding were observed among groups. **(D)** Operant responding during extinction training in the Ext CTRL group. Data represent mean ($\pm$ SEM). ** $p < 0.005$ (first vs. final extinction session). CSA = cocaine self-administration; EXT = extinction; ABST = abstinence; CTRL = control; NP = nose-poke.

Cocaine intake: Following 15 days of CSA, rats in the CSA, EXT, and ABST groups displayed stable cocaine intake. Figure 3.11A illustrates the mean number ($\pm$ SEM) of cocaine infusions during the last 10 days of self-administration training (Sessions 6–15) across all cocaine-exposed groups: CSA (n = 7), EXT (n = 10), and ABST (n = 8). A two-way ANOVA (session $\times$ group) revealed a significant main effect of group [$F(2, 22) = 3.9,\ p < 0.05$]. Bonferroni-corrected post hoc comparisons indicated that the ABST group had significantly higher cocaine intake than the CSA group ($p < 0.05$), while differences

between the CSA and EXT groups ($p > 0.05$) and between the EXT and ABST groups ($p > 0.05$) were not statistically significant. Despite the significant difference in total cocaine intake between the ABST and CSA groups, the lack of a significant main effect of session [$F(4.1, 91) = 2.3,\ p > 0.05$] and the session × group interaction [$F(8.3, 91) = 0.7,\ p > 0.05$] indicates that intake remained stable over time and followed a similar pattern across groups.

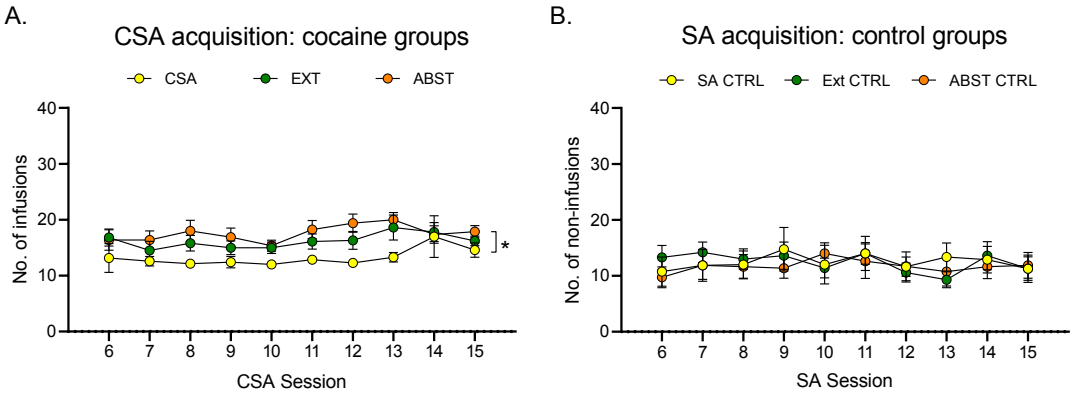

Figure 3.11. (A.) Mean number (± SEM) of cocaine infusions during the last 10 sessions of self-administration across cocaine-exposed groups (CSA, EXT, and ABST). The CSA group received significantly fewer cocaine infusions compared to the ABST group, while no significant differences were observed between CSA and EXT or between EXT and ABST groups. (B.) Mean number (± SEM) of scheduled but non-administered reinforcers ("non-infusions") during the last 10 self-administration sessions across control groups (SA CTRL, EXT CTRL, and ABST CTRL). No significant differences were found among control groups. *$p < 0.05$, main effect of group (CSA vs. ABST). CSA = cocaine self-administration; SA = self-administration; EXT = extinction; ABST = abstinence; CTRL = protocol-matched control

For each cocaine-exposed group, a corresponding non-infused control group (SA CTRL, EXT CTRL, and ABST CTRL) was included. These rats were placed in the same operant chambers using the same programs but did not have catheter implants, preventing cocaine infusions. Figure 3.11B presents the mean number (± SEM) of "non-infusions" (i.e., scheduled but non-administered reinforcers) during the last 10 days of training (Sessions 6–15) for all control groups: SA CTRL (n = 8), EXT CTRL (n = 10), and ABST CTRL (n = 8). A two-way ANOVA (session × group) did not reveal any significant main or interaction effects (session [$F(4.7, 109.0) = 1.5,\ p > 0.05$]; group [$F(2, 23) = 0.0\ p > 0.05$]; and session × group [$F(9.5, 109.0) = 1.1,\ p > 0.05$]), indicating that the number of scheduled but non-administered reinforcers remained stable over the last 10 training sessions, with no significant differences among control groups.

Molecular data

Cocaine self-administration significantly reduced the *Grm2* expression in PrLC. A linear mixed-effects model (LMM) with Brain Region (CgC, PrLC, ILC, OFC, INS, VS and DS) and Group (CSA vs. SA CTRL) as fixed effects, and Subject ID as a random effect, was applied to assess the effect of short-term CSA on *Grm2* expression, the $mGluR_2$ coding gene. This model was chosen to account for repeated measures (various brain regions) within subjects, and log-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions sufficiently, with log transformation improving model fit (AIC = -107.8, BIC = -102.8). Figure 3.12A depicts *Grm2* expression levels (mean ± SEM) across 7 brain regions in short-term CSA group (CSA, n = 7) and cocaine-naïve operant box control group (SA CTRL, n = 8). The LMM revealed a significant main effect of Brain Region [$F(6, 78) = 3.8,\ p < 0.005$] and a significant Brain Region × Group interaction [$F(6, 78) = 2.5,\ p < 0.05$], indicating that the effect of CSA on *Grm2* expression differed across brain regions. However, there was no significant main effect of Group [$F(1, 13) = 1.5,\ p > 0.05$], suggesting that overall cocaine exposure did not significantly alter global *Grm2* expression. Bonferroni-corrected post hoc pairwise comparisons identified a significant reduction in *Grm2* expression in the PrLC in CSA animals compared to SA CTRL ($\Delta M = -0.176 \pm 0.073,\ p < 0.05$). A trend towards reduced expression in the DS was observed ($\Delta M = -0.142 \pm 0.073,\ p = 0.059$). No significant differences were detected in the CgC, ILC, OFC, INS, or VS (all $p > 0.05$).

Pairwise group differences are reported as $\Delta M \pm SE$, where SE denotes the standard error of the mean difference estimated from the mixed model. Because the LMM relies on a pooled variance estimate, identical SE values occur across some brain regions, and this applies to all LMM-based comparisons reported throughout.

Extinction training after short-term CSA led to a significant and general increase in *Grm2* expression across all examined brain regions. A LMM was used to assess the effects of extinction training on *Grm2* expression in animals that underwent 15 days of CSA followed by 10 days of extinction (EXT) to cocaine-naïve operant box controls (Ext CTRL), with Brain Region (CgC, PrLC, ILC, OFC, INS, VS, DS) and Group (EXT vs. Ext CTRL) as fixed effects and Subject ID as a random effect. Log-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions, with log transformation improving model fit (AIC = -90.3, BIC = -84.9). Figure 3.12B illustrates *Grm2* expression levels (mean ± SEM) across 7 brain regions in extinction group (EXT, n = $10^{\dagger}$) and cocaine-naïve operant box control group (Ext CTRL, n = $10^{\dagger}$). The LMM revealed a significant main effect of Group [$F(1, 105) = 27.7,\ p < 0.001$], indicating that extinction training led to a global increase in

Grm2 expression compared to Ext CTRL. However, there was no significant main effect of Brain Region [$F(6, 96) = 0.5,\ p > 0.05$] and no significant Brain Region $\times$ Group interaction [$F(6, 96) = 1.0,\ p > 0.05$], suggesting that the upregulation of *Grm2* was consistent across brain regions. Bonferroni-corrected post hoc comparisons confirmed that *Grm2* expression was significantly higher in the EXT group compared to Ext CTRL across all brain regions (CgC: $\Delta M = 0.236 \pm 0.069,\ p < 0.005$; PrLC: $\Delta M = 0.267 \pm 0.069,\ p < 0.001$; ILC: $\Delta M = 0.356 \pm 0.069,\ p < 0.0001$; OFC: $\Delta M = 0.306 \pm 0.094,\ p < 0.005$; INS: $\Delta M = 0.229 \pm 0.069,\ p < 0.005$; VS: $\Delta M = 0.232 \pm 0.069,\ p < 0.005$; DS: $\Delta M = 0.319 \pm 0.069,\ p < 0.0001$). For OFC†, the sample size was smaller (*n* = 4 per group), which should be considered when interpreting results. Effect size calculations (Cohen's *d*) showed large to very large effect sizes, ranging from 1.58 (INS) to 2.44 (ILC), further confirming that these differences were not only statistically significant but also biologically meaningful. Unlike short-term CSA, which had region-specific effects, extinction training resulted in a broad increase in *Grm2* expression.

***Grm2* expression did not significantly differ between ABST and Abst CTRL groups.** A LMM was used to compare *Grm2* expression in animals that underwent 15 days of CSA followed by 10 days of abstinence (ABST) to cocaine-naïve operant box controls (Abst CTRL). Brain Region (CgC, PrLC, ILC, OFC, INS, VS, DS) and Group (ABST vs. Abst CTRL) were included as fixed effects, with Subject ID as a random effect to account for repeated measures within subjects. Log-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions (AIC = -90.414, BIC = -85.244). Figure 3.12C portrays *Grm2* expression levels (mean $\pm$ SEM) across 7 brain regions in abstinence group (ABST, n = 8) and cocaine-naïve operant box control group (Abst CTRL, n = 8). The LMM revealed no significant main effect of Group [$F(1, 14) = 0.4,\ p > 0.05$] or Brain Region $\times$ Group interaction [$F(6, 84) = 1.3,\ p > 0.05$], indicating that overall *Grm2* expression did not differ between ABST and Abst CTRL groups. A trend toward a main effect of Brain Region was observed [$F(6, 84) = 2.1,\ p = 0.067$]. Post hoc comparisons revealed no significant group differences in any individual brain region (all $p > 0.05$). These results suggest that 10 days of abstinence following CSA does not significantly alter *Grm2* expression relative to cocaine-naïve operant box controls.

Extinction training increased *Grm2* expression in prefrontal regions, while abstinence led to higher levels in DS and lower in OFC compared to the CSA group. A LMM was used to examine the effects of extinction training (EXT) and abstinence (ABST) on *Grm2* expression relative to the CSA only (CSA) group, which underwent 15 days of CSA and was sacrificed 24 hours after the final session. Group (CSA, EXT, ABST) and Brain Region

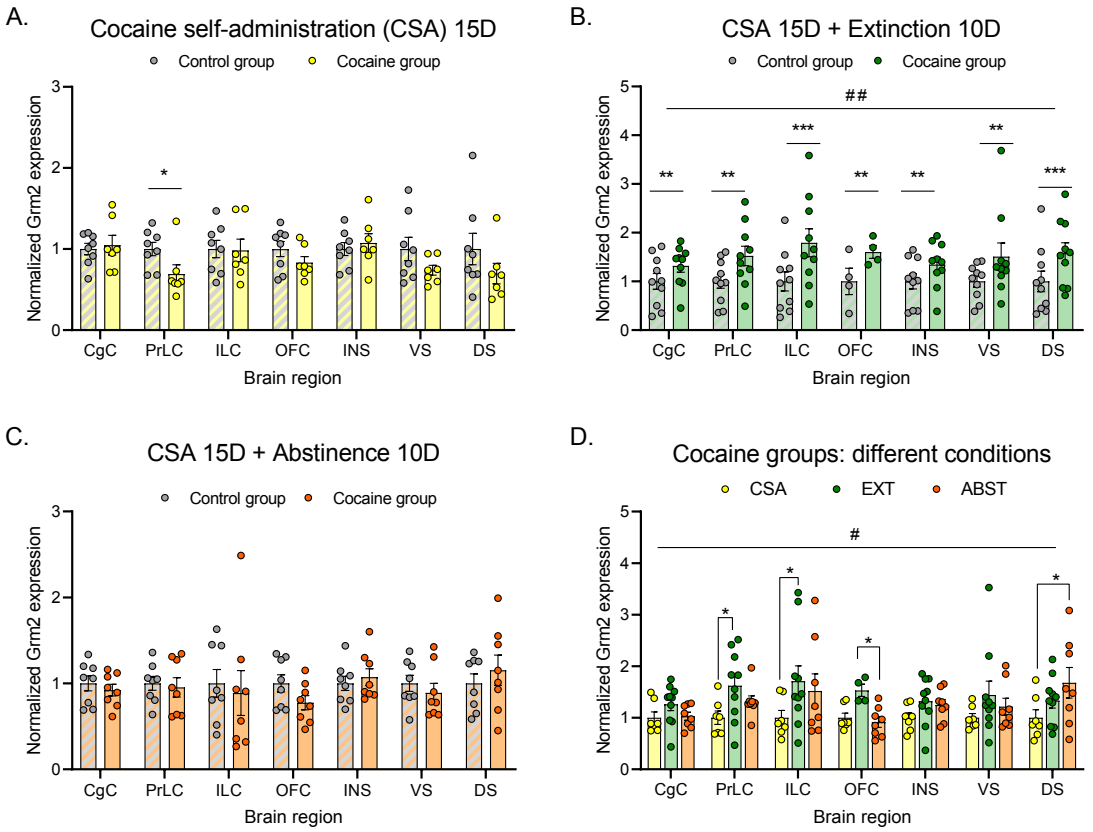

Figure 3.12. **(A)** *Grm2* expression across brain regions in rats after CSA only (CSA, n = 7) vs. cocaine-naïve operant controls (SA CTRL, n = 8). A significant Brain Region × Group interaction was observed, with reduced expression in the PrLC in CSA rats. **(B)** *Grm2* expression in extinction-trained rats (EXT, n = 10†) vs. cocaine-naïve operant controls (Ext CTRL, n = 10†). Extinction led to a global upregulation of *Grm2* across all regions. **(C)** *Grm2* expression in abstinent rats (ABST, n = 8) vs. cocaine-naïve operant controls (Abst CTRL, n = 8). No significant group differences were observed. **(D)** *Grm2* expression across cocaine-exposed groups (CSA, EXT, ABST), normalized to CSA. EXT rats showed elevated expression in PrLC and ILC. ABST rats exhibited increased DS expression (vs. CSA), and decreased OFC expression (vs. EXT). Data shown as mean± SEM (original, untransformed values). † sample size for OFC: n = 4 per group (EXT and Ext CTRL). * $p < 0.05$ ** $p < 0.005$, *** $p < 0.0001$ control vs. cocaine. # $p < 0.05$, ## $p < 0.001$ main effect of group. CgC =ngulate cortex; PrLC = prelimbic cortex; ILC = infralimbic cortex; OFC = orbitofrontal cortex; INS = insula; DS = dorsal striatum; VS = ventral striatum.

(CgC, PrLC, ILC, OFC, INS, VS, DS) were included as fixed effects, with Subject ID as a random intercept to account for individual variability. Given the violation of normality in the log-transformed data, a square-root transformation was applied to improve model fit. Residual diagnostics confirmed that this transformation improved normality assumptions (AIC = -43.2, BIC = -37.8). Figure 3.12D illustrates *Grm2* expression levels (mean ± SEM) across brain regions in all cocaine groups: CSA (n = 7), EXT (n = 10), and ABST (n = 8). The LMM revealed a significant main effect of Brain Region [$F(6, 96) = 2.2,\ p < 0.05$] and Group [$F(2, 16) = 4.8,\ p < 0.05$], indicating that *Grm2* expression differed across brain regions and was modulated by the behavioral history of the animals. Although the Brain Region × Group interaction was not statistically significant [$F(12, 96) = 1.6,\ p = 0.110$], a weak trend suggests that certain brain regions may be differentially affected by extinction training and abstinence, warranting further investigation. Bonferroni-corrected post hoc comparisons revealed significant differences in *Grm2* expression between groups in specific brain regions. The EXT group exhibited higher *Grm2* expression compared to CSA in the ILC ($\Delta M = 0.368 \pm 0.116,\ p < 0.01$) and PrLC ($\Delta M = 0.347 \pm 0.116,\ p < 0.05$), while the ABST group showed increased *Grm2* expression compared to CSA in the DS ($\Delta M = 0.275 \pm 0.096,\ p < 0.05$). Additionally, *Grm2* expression was significantly lower in the ABST group compared to EXT in the OFC ($\Delta M = -0.289 \pm 0.114,\ p < 0.05$). No significant differences were observed in CgC, INS, or VS between groups ($p > 0.05$). For OFC, the sample size was smaller in the EXT group (*n* = 4), which should be considered when interpreting results.

Note that all statistical analyses were performed on log-transformed or square root-transformed data to meet normality assumptions; however, for clarity, all data in Figure 3.12 are presented in their original (non-log/square root-transformed) normalized form.

Effect of LY379268 on Cue-induced reinstatement of cocaine seeking

To ensure the experimental groups were behaviorally comparable prior to LY379268 treatment, the active and inactive NPs over 15 CSA sessions were analyzed using a three-way ANOVA (Session x NP x Group). There was no significant main effect of group [$F(1, 4) = 0.8,\ p > 0.05$] and no Session × Group interaction [$F(14, 56) = 1.0,\ p > 0.05$], indicating similar response patterns across sessions. A trend-level main effect of NP type (active > inactive) was observed [$F(1, 4) = 5.2,\ p = 0.085$], consistent with expectations for the SA task; however, the small sample size (n = 3 per group) likely limited the ability to detect significance. Similarly, cocaine intake was assessed—a two-way ANOVA (Session × Group) showed no significant main effect or interaction (Group [$F(1, 4) = 0.0,\ p > 0.05$]; Session [$F(14, 56) = 1.1,\ p > 0.05$]; Session × Group [$F(14, 56) = 0.9,\ p > 0.05$]), indicating

that cocaine intake did not differ across sessions or between groups. Together, these results support the interpretation that both groups were behaviorally matched at baseline prior to any drug administration (data not shown).

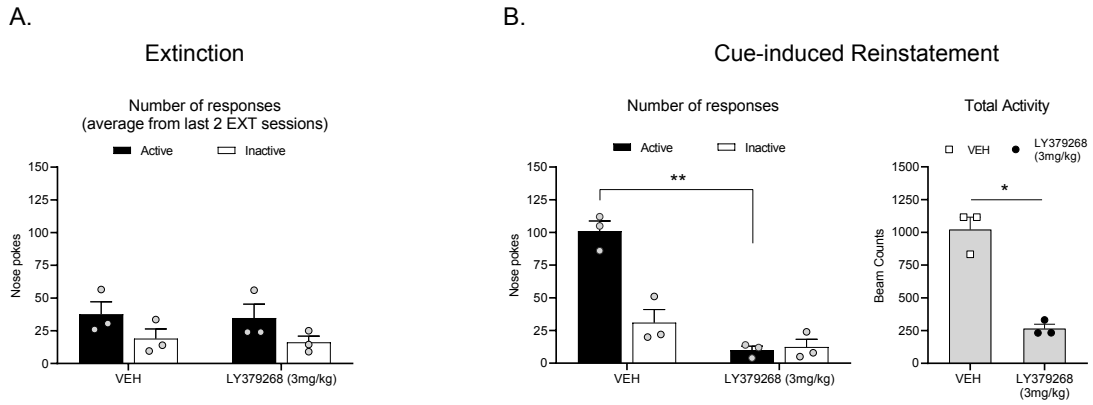

Figure 3.13. (A) Operant responding (active and inactive NPs) during the last two extinction sessions in vehicle- and LY379268-treated (3 mg/kg) rats. No significant group differences were observed. **(B)** *Left.* Active and inactive NPs during the cue-induced reinstatement. LY379268 significantly reduced active responding. *Right.* Total activity (measured as beam counts) during reinstatement. LY379268 significantly decreased activity. Data represent mean ($\pm$ SEM). * $p < 0.05$ ** $p < 0.005$. NP = nose poke; VEH = vehicle.

To ensure the experimental groups were behaviorally comparable prior to LY379268 treatment, the active and inactive NPs over 10 extinction (EXT) sessions were analyzed using a three-way ANOVA (Session $\times$ NP $\times$ Group). A significant main effect of NP [$F(1,4) = 18.7,\ p < 0.05$] and session [$F(1.7,6.6) = 8.3,\ p < 0.05$] was found, consistent with extinction learning over time. Importantly, there was no significant main effect of group [$F(1,4) = 0.3,\ p > 0.05$], and no significant interactions involving group (NP $\times$ Group, Session $\times$ Group, and Session $\times$ NP $\times$ Group; all $p > 0.3$). These results confirm that the VEH and LY379268 groups were behaviorally equivalent at baseline, showing similar extinction rates and NP preference patterns prior to treatment.

To assess the effect of the mGluR$_{2/3}$ agonist LY379268 (3 mg/kg) on cue-induced reinstatement of cocaine-seeking behavior, a two-way ANOVA was conducted (NP $\times$ Group). There was a significant main effect of NP [$F(1,4) = 54.3,\ p < 0.005$], confirming that rats exhibited higher responding on the previously active NP. A significant main effect of treatment group [$F(1,4) = 36.1,\ p < 0.005$] indicated reduced overall responding in LY379268-treated rats. A significant NP $\times$ Group interaction was observed [$F(1,4) = 58.6,\ p < 0.005$], indicating a greater suppressive effect of LY379268 on

active NP responding. However, this effect should be interpreted with caution given the pronounced motor suppression induced by LY379268.

LY379268 also produced a robust decrease in overall locomotor activity during the reinstatement session, as measured by total beam breaks (Fig. 3.13B, right panel). A Welch's t-test (unequal variances and small sample sizes) revealed a significant reduction in the LY379268 group compared to VEH ($\Delta M = 1021.67 \pm 94.4$ vs. $\Delta M = 265.33 \pm 32.8$) [$t(\approx 2) = 7.6,\ p < 0.05$]. These findings suggest that the behavioral effects of LY379268 may be largely attributable to non-specific sedation rather than selective attenuation of drug-seeking behavior.

3.3.2.2 Short-term vs. long-term cocaine self-administration

Behavioral data

Cocaine intake: Cocaine intake in both the short-term CSA group that underwent 15 CSA sessions in total and the long-term CSA group that underwent more than 50 CSA sessions in total was assessed by analyzing the number of infusions obtained during the first 40 minutes of the final three self-administration sessions (Session 13–15 and Session 50–52, respectively). Importantly, session structures differed substantially between the two protocols: short-term CSA sessions lasted 1 hour and used FR1 schedule, while long-term CSA sessions followed the structure of the 3-CRIT protocol, lasting 2.5 hours and consisting of three 40-minute drug-available periods (FR5) separated by two 15-minute no-drug periods. Due to these differences in session design and reinforcement schedule—especially the higher response requirement (FR5) and session segmentation in the long-term group—only the first 40 minutes of each session were used for comparison, corresponding to the initial drug-available period in the 3-CRIT protocol. This was done to standardize the time window for intake measurement and reduce confounding effects introduced by task structure and effort-related variables. Additionally, cocaine intake (infusions) was selected as the primary behavioral metric, as NP counts are not directly comparable between the FR1 and FR5 conditions.

As illustrated in Figure 3.14A, rats in the short-term (n = 7) and long-term (n = 21) CSA groups exhibited similarly stable levels of cocaine intake during this period. A Student's t-test revealed no statistically significant difference between the groups [$t(26) = 1.26,\ p > 0.05$]. The mean number ($\pm$ SEM) of infusions was 10.6 ± 1.1 in the short-term group and 12.9 ± 1.0 in the long-term group. These findings indicate that despite differences in overall training duration and schedule structure, both groups demonstrated comparable levels of

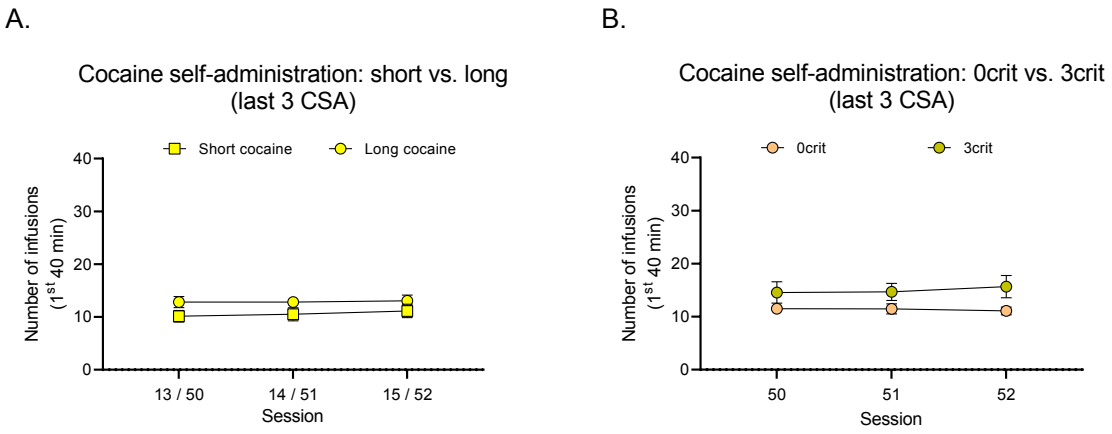

Figure 3.14. Comparison of cocaine intake between short- and long-term self-administration cohorts. **(A)** Cocaine infusions during the first 40 minutes of the final three sessions in short-term (n = 7; Sessions 13–15; square) and long-term (n = 21; Sessions 50–52; circle) CSA groups. Both groups showed comparable intake during this time window. **(B)** Cocaine infusions during the first 40 minutes of Sessions 50–52 in 0crit (n = 12; orange) and 3crit (n = 9; khaki) rats. No significant differences were observed between addiction-resilient and addiction-prone phenotypes. Data represent mean (± SEM). CSA = cocaine self-administration.

cocaine intake in the final phase of training, justifying subsequent molecular comparisons. Similarly, the cocaine intake of the 0crit and 3crit animals during the first 40 minutes of the final three CSA sessions (session 50–52) was assessed. As shown in Figure 3.14B, both groups exhibited stable levels of cocaine intake across sessions. A Welch's t-test revealed no statistically significant difference between 0crit and 3crit groups [$t(\approx 12) = -1.80,\ p > 0.05$]. The mean number (± SEM) of infusions was 11.3 ± 0.9 in the 0crit group and 15.0 ± 1.8 in the 3crit group. These results suggest that while 0crit and 3crit animals differ in addiction phenotype classification, their cocaine intake during the first 40 min of final 3 CSA sessions was stable and at a similar level.

Molecular data

Short-term CSA reduced mGluR$_2$ expression in the PrLC and had a region-specific effect in the DS. A LMM with Brain Region (CgC, PrLC, ILC, INS, VS and DS) and Group (naïve vs. cocaine) as fixed effects, and Subject ID as a random effect, was applied to assess the effect of short-term CSA on *Grm2* expression, the mGluR$_2$ coding gene. This model was chosen to account for repeated measures (various brain regions) within subjects, and log-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions sufficiently, with log trans-

formation improving model fit (AIC = -22.80, BIC = -18.42). Figure 3.15A illustrates *Grm2* expression levels (mean ± SEM), normalized to the control group, across 6 brain regions in short-term CSA group (cocaine, n = 7) and cocaine-naïve home-caged control group (naïve, n = 6). The LMM revealed a significant Brain Region × Group interaction [$F(5, 55) = 3.0,\ p < 0.05$], indicating that the effect of cocaine exposure on *Grm2* expression varied across brain regions. There was also a trend toward a main effect of Brain Region [$F(5, 55) = 2.33,\ p = 0.055$]. In contrast, the main effect of Group was not significant [$F(1, 11) = 0.7,\ p > 0.05$], suggesting that short-term cocaine exposure did not produce a global change in *Grm2* expression. Bonferroni-corrected post hoc pairwise comparisons did not reveal any statistically significant differences in individual brain regions between cocaine and naïve groups (all $p > 0.05$). A trend towards reduced expression in the PrLC was observed ($\Delta M = -0.236 \pm 0.131,\ p = 0.086$). No significant differences were found in the CgC ($\Delta M = -0.124 \pm 0.131,\ p > 0.05$), DS ($\Delta M = 0.119 \pm 0.131,\ p > 0.05$), ILC ($\Delta M = 0.000 \pm 0.131,\ p > 0.05$), INS ($\Delta M = -0.128 \pm 0.131,\ p > 0.05$), or VS ($\Delta M = -0.181 \pm 0.131,\ p > 0.05$). To further explore the reduction in *Grm2* expression in the PrLC, an independent samples t-test was performed comparing cocaine and naïve control groups. This analysis revealed a significant reduction in the cocaine group compared to naïve controls [$t(11) = 2.3,\ p < 0.05$], supporting the possibility of a region-specific downregulation consistent with the trend observed in the mixed model. However, this result should be interpreted with caution.

Long-term CSA did not significantly affect overall $mGluR_2$ expression, although a weak region-specific trend in the ILC was observed. A LMM with Brain Region (CgC, PrLC, ILC, INS, VS and DS) and Group (naïve vs. cocaine) as fixed effects, and Subject ID as a random effect, was applied to assess the effect of long-term CSA on *Grm2* expression, the $mGluR_2$ coding gene. This model was chosen to account for repeated measures (various brain regions) within subjects, and square root-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions sufficiently, with sqrt-transformation improving model fit (AIC = -26.68, BIC = -21.77). Figure 3.15B depicts *Grm2* expression levels (mean ± SEM), normalized to the control group, across 6 brain regions in the long-term CSA group (cocaine, n = 9‡) and cocaine-naïve home-caged control group (naïve, n = 4‡). The LMM revealed a significant main effect of Brain Region [$F(5, 52.0) = 2.8,\ p < 0.05$], indicating that *Grm2* expression differs across regions. However, there was no significant main effect of Group [$F(1, 35.8) = 0.2,\ p > 0.05$], suggesting that long-term cocaine exposure does not alter overall *Grm2* expression across the brain. A trend toward a Brain Region × Group interaction was observed [$F(5, 52.0) = 2.3,\ p = 0.062$], indicating a possible region-specific effect of cocaine exposure on *Grm2* expression, although this did not reach statistical signif-

icance. Bonferroni-corrected post hoc pairwise comparisons did not reveal any significant differences in individual brain regions between cocaine and naïve groups (all $p > 0.05$). Specifically, differences were nonsignificant in the CgC ($\Delta M = 0.105 \pm 0.115,\ p = 0.364$), DS ($\Delta M = 0.121 \pm 0.109,\ p = 0.273$), ILC ($\Delta M = -0.132 \pm 0.097,\ p = 0.180$), INS ($\Delta M = 0.096 \pm 0.115,\ p = 0.406$), PrLC ($\Delta M = 0.088 \pm 0.115,\ p = 0.448$), and VS ($\Delta M = -0.031 \pm 0.115,\ p = 0.787$).

Addiction severity (0crit vs. 3crit) did not significantly affect mGluR$_2$ expression overall, although a significant reduction in the ILC was observed in 0crit animals. A LMM with Brain Region (CgC, PrLC, ILC, INS, VS, and DS) and Group (0crit vs. 3crit) as fixed effects, and Subject ID as a random effect, was used to assess the impact of addiction severity on *Grm2* expression (encoding mGluR$_2$). This model was chosen to account for repeated measures (various brain regions) within subjects, and square root-transformed *Grm2* expression levels were used to improve normality. Residual diagnostics confirmed that the model met normality assumptions sufficiently, with sqrt-transformation improving model fit (AIC = -6.46, BIC = -2.41). Figure 3.15C displays normalized *Grm2* expression (mean ± SEM) across six brain regions in addiction-resilient (0crit, n = 6‡) and addiction-vulnerable (3crit, n = 3‡) animals. The LMM revealed no significant main effect of Brain Region [$F(5, 38.4) = 1.15,\ p > 0.05$], no main effect of Group [$F(1, 27.7) = 1.55,\ p > 0.05$], and no Brain Region × Group interaction [$F(5, 38.4) = 0.88,\ p > 0.05$], indicating no broad or region-specific differences in *Grm2* expression due to addiction severity. Bonferroni-corrected post hoc pairwise comparisons identified a significant reduction in *Grm2* expression in the ILC in 0crit animals compared to 3crit ($\Delta M = -0.213 \pm 0.091,\ p < 0.05$). A non-significant trend toward increased expression in the DS in 3crit animals was also observed ($\Delta M = -0.214 \pm 0.119,\ p = 0.079$). No significant group differences were detected in the CgC ($\Delta M = 0.026 \pm 0.132,\ p > 0.05$), INS ($\Delta M = -0.021 \pm 0.132,\ p > 0.05$), PrLC ($\Delta M = -0.079 \pm 0.132,\ p > 0.05$), or VS ($\Delta M = -0.101 \pm 0.132,\ p > 0.05$). To further explore these effects, independent-samples t-tests were performed. The analysis revealed a significant reduction in *Grm2* expression in 0crit animals compared to 3crit in both ILC [$t(19) = -2.16,\ p < 0.05$] and DS [$t(9) = -2.53,\ p < 0.05$], supporting the findings suggested by the mixed model. However, given the small sample sizes and multiple comparisons, these findings should be interpreted with caution.

Note: Sample sizes were increased in two brain regions (‡)—the ILC and DS. For the ILC, sample sizes were: naïve (n = 8), long cocaine (n = 21), 0crit (n = 12), and 3crit (n = 9); for the DS: naïve (n = 6), long cocaine (n = 11), 0crit (n = 6), and 3crit (n = 5). These adjustments should be taken into account when interpreting the results. All statistical analyses were performed on transformed data (log- or square root-transformed) to ensure model assumptions

were met; however, for clarity, all data in Figure 3.15 are presented in their original (non-log/square root-transformed) normalized form.

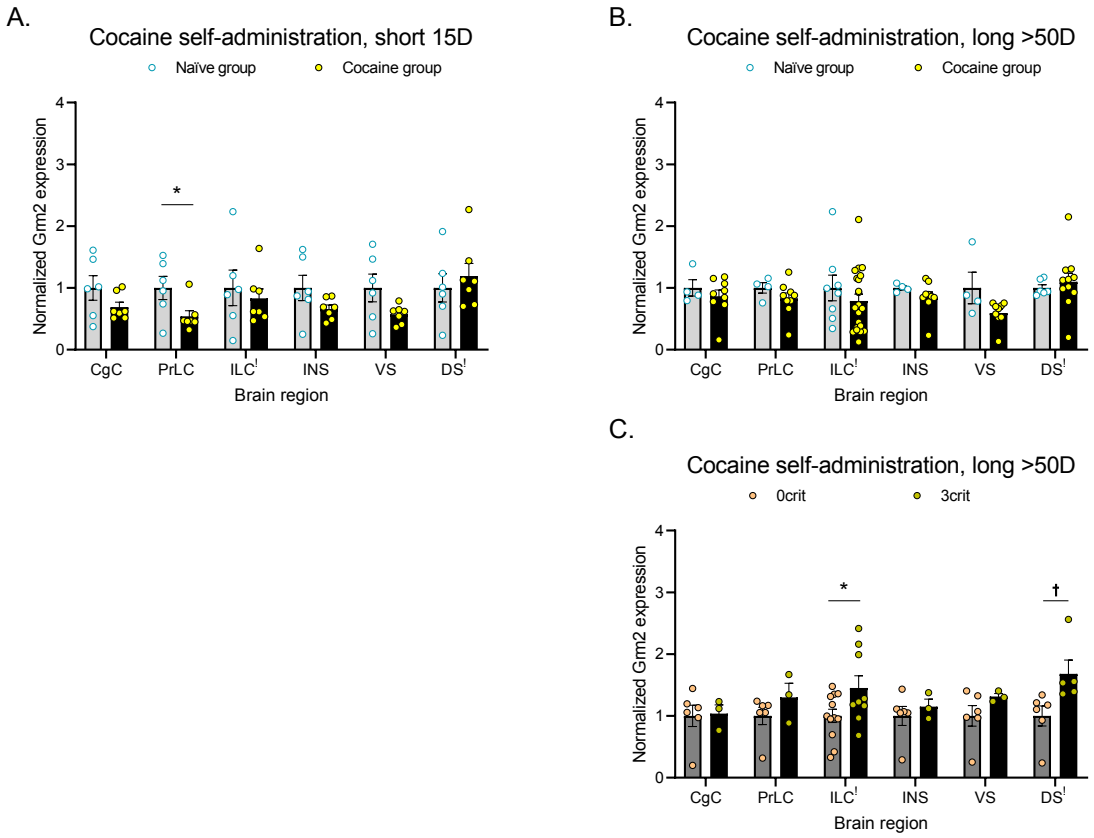

Figure 3.15. *Grm2* expression across different durations of cocaine exposure and addiction-like phenotypes. **(A)** Normalized *Grm2* levels in selected brain regions in rats following short-term cocaine self-administration (CSA; yellow data points; n = 7) relative to age-matched, cocaine-naïve, home-caged controls (blue data points; n = 6). *Grm2* expression was significantly reduced in the PrLC of cocaine-exposed rats. **(B)** Normalized *Grm2* levels in rats trained under the 3-CRIT model of addiction-like behavior (>50 CSA sessions; yellow data points; n = 9) compared to age-matched home-caged controls (blue data points; n = 4). No significant group differences were found **(C)** Normalized Comparison of *Grm2* expression between addiction-resilient (0crit; orange data points; n = 6‡) and addiction-vulnerable (3crit; khaki data points; n = 3‡) rats. 3crit animals exhibited significantly increased expression in the ILC and DS. Data represent mean ± SEM. * $p < 0.05$ (LMM), naïve vs. cocaine, 0crit vs. 3crit. † $p < 0.05$ (*t*-test), 0crit vs. 3crit. ‡ indicates regions with extended sample size (n > 5). CSA = cocaine self-administration; CgC = cingulate cortex; PrLC = prelimbic cortex; ILC = infralimbic cortex; INS = insula; DS = dorsal striatum; VS = ventral striatum.

3.4 Study 2B *Slc6a3*_N157K mutation: Effects on cocaine self-administration

3.4.1 Introduction

The dopamine transporter, encoded by the *Slc6a3* gene, is a key regulator of dopamine neurotransmission and plays a pivotal role in controlling synaptic dopamine levels by facilitating dopamine reuptake into presynaptic neurons. This process is crucial for maintaining dopamine homeostasis, which influences a variety of behaviors, including reward processing, motivation, and reinforcement (Dreher et al., 2009; Salamone and Correa, 2012; Wise and Jordan, 2021). Dysregulation of dopamine signaling has been implicated in various neuropsychiatric disorders (Grace, 2016; Reith et al., 2022), including drug addiction (Bressan and Crippa, 2005; Dreher et al., 2009; Merims and Giladi, 2008; Wise and Jordan, 2021; Wise and Robble, 2020). Cocaine, a potent psychostimulant, exerts its reinforcing effects by binding to and inhibiting the DAT, leading to increased extracellular DA levels in reward-related brain regions such as the nucleus accumbens (Giros et al., 1996; Kuhar et al., 1991; Ritz et al., 1987). This surge in dopaminergic signaling enhances the reinforcing properties of cocaine, thereby promoting repeated drug use and ultimately contributing to the development of addiction (Anderson and Pierce, 2005; Nestler, 2005a).

Several transgenic preclinical models with modified expression of DAT have been previously established and used to study various processes, such as DAT functioning, pharmacodynamics of psychotropic drugs, the interplay of neurotransmitter systems, and pathological mechanisms underlying various disorders, including schizophrenia, ADHD, and addiction (Efimova et al., 2016; Savchenko et al., 2023). One DAT alteration is knock-out (KO) of the DAT encoding gene (*Slc6a3*), by complete deletion or inactivation, which results in the absence of functional DAT and a subsequent hyperdopaminergic state as DA cannot be cleared from the synaptic cleft (Giros et al., 1996; Sora et al., 1998). Indeed, basal extracellular levels of DA in DAT-KO mice are significantly higher and remain there longer than in their WT littermates (Gainetdinov et al., 1999a; Giros et al., 1996; Jones et al., 1998). As a result, DAT-KO mice were found to exhibit atypical motor behaviors, such as pronounced locomotor hyperactivity or the increased occurrence of stereotypies, and impaired cognitive functions (Carpenter et al., 2012; Gainetdinov et al., 1999a; Giros et al., 1996; Sora et al., 1998). Furthermore, DAT-KO mice showed antihyperkinetic responses to psychostimulants, all of which makes them a valuable model for ADHD (Gainetdinov and Caron, 2000; Lee and Yoon, 2023). However, DAT-KO mice suffer from dwarfism and have a higher prevalence of premature deaths, presumably due to abnormal development and function of the

pituitary gland (Bossé et al., 1997; Giros et al., 1996), which ultimately limits phenotyping.

Compared to mice, rat models offer several advantages, for example larger brain and body size for surgeries, lower aggression and a closer overall resemblance to humans, including enhanced sociability. Rats also demonstrate a broader behavioral range combined with more robust and consistent performance in cognitive tasks (Abbott, 2004). These characteristics make rat models particularly valuable for investigating cognitive-behavioral processes critical for modeling neuropsychiatric conditions, such as addiction. Recent advances in gene-editing technologies have enabled the independent development of two transgenic rat lines deficient in the DAT (Leo et al., 2018b; Vengeliene et al., 2017); for detailed comparison see (Leo et al., 2018a). Vengeliene and colleagues developed a mutant rat line carrying a loss-of-function point mutation in the DAT gene (*Slc6a3*_N157K) (Vengeliene et al., 2017). Although the mutant *Slc6a3*_N157K protein is transcribed and translated, it fails to undergo proper trafficking to the cell surface, resulting in severely impaired DAT function ($>$ 95 % reduction) in these animals. Previous data from our laboratory demonstrated increased alcohol consumption in *Slc6a3*_N157K mutant rats compared to WT animals (Hirth et al., 2016). However, to the best of my knowledge, the effects of this mutation have not yet been explored in the context of cocaine use and addiction. Therefore, the present study investigated the impact of *Slc6a3*_N157K point mutation on the acquisition of CSA and to assess motivation for cocaine-taking behavior in mutant *Slc6a3*_N157K rats as compared to their WT counterparts.

3.4.2 Results

*Slc6a3*_N157K rats did not self-administer cocaine. Figure 3.16A shows the mean ($\pm$ SEM) responding on the active and inactive NPs in *Slc6a3*_N157K (n=8) and wild-type rats (n=5) during 13 daily CSA sessions. Figure 3.16B shows the mean ($\pm$ SEM) number of infusions obtained per session across the 13 daily CSA sessions for *Slc6a3*_N157K (n = 8) and WT rats (n = 5). Data shown are for the drug periods only (2 hr).

For FR1, a three-way ANOVA (NP $\times$ session $\times$ group) revealed significant main effects of group [$F(1, 11) = 10.2,\ p < 0.05$] and NP [$F(1, 11) = 6.3,\ p < 0.05$], and a significant NP $\times$ group interaction [$F(1, 11) = 8.0,\ p < 0.05$], but no other significant effects [session: $F(1.5, 17) = 1.2,\ p > 0.05$; session $\times$ group: $F(1.5, 17) = 0.6,\ p > 0.05$; NP $\times$ session: $F(1.6, 17.4) = 0.7,\ p > 0.05$; NP $\times$ session $\times$ group: $F(1.6, 17.4) = 0.9,\ p > 0.05$]. These findings indicate a significant reduction in responding for cocaine in *Slc6a3*_N157K rats relative to WT rats during the 7 training sessions on the FR1 schedule. Follow-up

independent-samples t-tests indicated that *Slc6a3*_N157K rats showed a significant reduction in responding in the active NP relative to the WT rats [$t(11) = -3.0,\ p < 0.05$], but no difference in responding in the inactive NP was found [$t(11) = -1.0,\ p > 0.05$]. Similarly, a two-way ANOVA (session $\times$ group) of cocaine intake revealed a significant main effect of group [$F(1, 11) = 56.4,\ p < 0.0005$] and a session $\times$ group interaction [$F(1.5, 17) = 5.4,\ p < 0.05$], indicating a significant reduction in cocaine intake in *Slc6a3*_N157K rats relative to WT rats across the 7 FR1 sessions.

For FR3, a three-way ANOVA (NP $\times$ session $\times$ group) revealed significant main effects of group [$F(1, 11) = 73.6,\ p < 0.0005$] and NP [$F(1, 11) = 91.4,\ p < 0.0005$], and a significant NP $\times$ group interaction [$F(1, 11) = 106.3,\ p < 0.0005$], but no other significant effects [session: $F(1.6, 17.7) = 1.7,\ p > 0.05$; session $\times$ group: $F(1.6, 17.7) = 3.6,\ p > 0.05$; NP $\times$ session: $F(1.6, 18) = 0.7,\ p > 0.05$; NP $\times$ session $\times$ group: $F(1.6, 18) = 1.2,\ p > 0.05$]. These findings indicate a significant reduction in responding for cocaine in *Slc6a3*_N157K rats relative to WT rats during the 3 training sessions on the FR3 schedule. Follow-up independent-samples t-tests indicated that *Slc6a3*_N157K rats showed a significant reduction in responding in the active NP relative to the WT rats [$t(11) = -11.9,\ p < 0.0005$], but no difference in responses on the inactive NP was found [$t(11) = 0.54,\ p > 0.05$]. A two-way ANOVA (day $\times$ group) of cocaine intake revealed a significant main effect of group [$F(1, 11) = 467.7,\ p < 0.0005$], indicating a significantly lower cocaine intake in *Slc6a3*_N157K rats relative to WT rats.

For FR5, a three-way ANOVA (NP $\times$ session $\times$ group) revealed significant main effects of group [$F(1, 11) = 43.9,\ p < 0.0005$], NP [$F(1, 11) = 35.2,\ p < 0.0005$], and session [$F(1.3, 13.8) = 7.8,\ p < 0.05$], and significant NP $\times$ group [$F(1, 11) = 36.5,\ p < 0.0005$], session $\times$ group [$F(1.3, 13.8) = 10.3,\ p < 0.005$], NP $\times$ session [$F(1.3, 14.7) = 5.7,\ p < 0.05$], and NP $\times$ session $\times$ group [$F(1.3, 14.7) = 7.4,\ p < 0.05$] interactions. These findings indicate a significant reduction in responding for cocaine in *Slc6a3*_N157K rats relative to WT rats during the 3 training sessions on the FR5 schedule. Follow-up independent-samples t-tests indicated that *Slc6a3*_N157K rats showed a significant reduction in responding to the active NP relative to the WT rats [$t(11) = -6.4,\ p < 0.0005$], but no difference in responses on the inactive NP was found [$t(11) = -1.5,\ p > 0.05$]. A two-way ANOVA (day $\times$ group) of cocaine intake revealed a significant main effect of group [$F(1, 11) = 218.6,\ p < 0.0005$], indicating a significantly lower cocaine intake in *Slc6a3*_N157K rats relative to WT rats.

Figure 3.16C shows the mean ($\pm$ SEM) BP of *Slc6a3*_N157K (n = 8) and WT rats (n = 5) during the 2-hour PR test. An independent-samples t-test confirmed a significant difference in motivation between genotypes [*Slc6a3*_N157K: 299.0 ± 79.0; WT: 1.3 ± 1.25; $t(11) =$

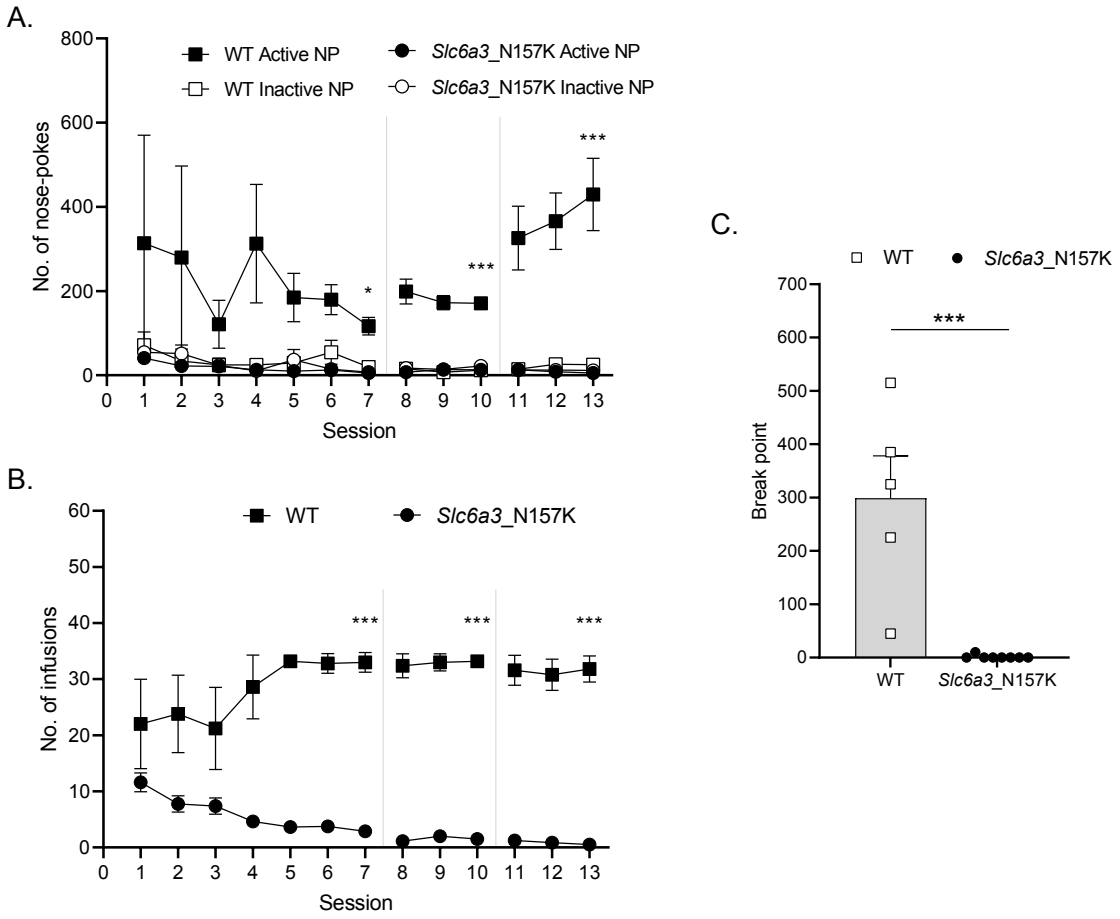

Figure 3.16. Acquisition of cocaine self-administration behavior and final PR test. **(A)** Acquisition of active and inactive NPs responding across all CSA sessions for *Slc6a3*_N157K and WT rats. *Slc6a3*_N157K rats rats showed significantly lower active NP responding across FR1, FR3, and FR5 schedules. **(B)** Acquisition of cocaine intake of *Slc6a3*_N157K and WT rats. Cocaine intake in *Slc6a3*_N157K rats was markedly reduced and virtually absent, indicating a failure to maintain self-administration behavior. **(C)** PR test. *Slc6a3*_N157K rats failed to respond under the PR schedule, reflecting a total lack of motivation to obtain cocaine, underscoring a complete absence of cocaine-seeking behavior following failed acquisition of cocaine intake. Data represent mean ($\pm$ SEM) for the drug periods only (2 hr). * $p < 0.05$, *** $p < 0.0005$, main effect of group. CSA = cocaine self-administration; NP = nose-poke; FR = fixed ratio; PR = progressive ratio; WT = wild-type.

$4.9,\ p < 0.0005$]. This striking lack of responding in *Slc6a3*_N157K rats is consistent with their complete failure to acquire and maintain CSA, resulting in an absence of motivation to seek the drug under PR conditions.

3.5 Study 3A Psilocybin and cocaine-seeking in rodents

This section includes previously published figures and associated results (Pohořalá et al., 2024), reproduced here with minimal changes. The surrounding text has been reformulated to ensure consistency with the thesis structure.

3.5.1 Introduction

Psychedelic compounds, particularly serotonergic tryptamines such as psilocybin, have recently reemerged as promising candidates in the treatment of various neuropsychiatric conditions (Hadar et al., 2023; Murnane, 2018). The primary psychoactive effects of psilocybin, or more precisely its active metabolite psilocin, are mediated predominantly through agonist activity at serotonin 5-HT_{2A} receptors (Madsen et al., 2019; Nichols, 2004; Vollenweider et al., 1998). However, psilocin exhibits affinity for a broader range of serotonin receptor subtypes, including 5-HT_{1A}, 5-HT_{2B}, and 5-HT_{2C}, implicating more complex downstream pharmacodynamics (Halberstadt and Geyer, 2011; McKenna et al., 1990).

In clinical settings, psilocybin, typically administered under psychotherapeutic guidance, has shown potential in treating various psychiatric conditions, such as major depressive disorder (Carhart-Harris et al., 2021, 2018; Ellis et al., 2025; Goodwin et al., 2022), anxiety and depression in life-threatening cancer patients (Agrawal et al., 2025; Griffiths et al., 2016; Ross et al., 2016), obsessive-compulsive disorder (Collins, 2024; Kelmendi et al., 2022; Moreno et al., 2006), or substance use disorder (Bogenschutz et al. 2022; Garcia-Romeu et al. 2019, 2015; Johnson et al. 2017; for reviews, see Hogea et al. 2025; Meshkat et al. 2025). Despite this swiftly expanding clinical literature, preclinical work remains limited, especially in the context of psychostimulant addiction.

To date, most rodent studies investigating psychedelic effects on addiction-like behavior have focused on alcohol, and have reported inconsistent findings. For instance, a study using the Alcohol Deprivation Effect (ADE) model found no or only temporary effects of psilocybin administered in various doses and schedules on the reduction of relapse-like drinking (Meinhardt et al., 2020). Additionally, psilocybin did not decrease alcohol consumption or alcohol-seeking in Marchigian Sardinian alcohol-preferring (msP) rats (Benvenuti et al., 2023). However, when administered immediately following a brief reconsolidation-like context re-exposure trial, psilocybin (5 mg/kg) reduced alcohol-seeking in msP rats (Benvenuti et al., 2023). Furthermore, a single injection of psilocybin (1 and 2.5 mg/kg) given 4 hours before a relapse session was found to reduce relapse-like behavior in alcohol-dependent rats after a period of abstinence (Meinhardt et al., 2021). A recent study in C57BL/6J

mice found that psilocybin reduced alcohol intake and preference in males, but not females, in a dose-dependent manner, indicating sex-specific effects. However, the reduction was transient and disappeared after alcohol reintroduction following a brief abstinence period (Alper et al., 2023).

Notably, sex-dependent effects of psychedelics have also been observed in learning paradigms involving memory and extinction. For example, the psilocin analogue 4-Hydroxy-N,N-diisopropyltryptamine (4-OH-DiPT) reduced conditioned freezing responses during fear extinction only in female mice (Kelly et al., 2024), suggesting sex-specific modulation of extinction learning. Psilocybin itself has been shown to accelerate extinction of auditory and trace fear conditioning in mice (Catlow et al., 2013; Du et al., 2023), and reduce alcohol-seeking when administered following a reconsolidation-like context re-exposure (Benvenuti et al., 2023). Together, these findings indicate that psychedelics may influence maladaptive learning and memory processes thought to contribute to relapse and compulsive drug-seeking behavior.

Despite this, preclinical research into the effects of psilocybin in the context of psychostimulant use, and particularly cocaine, remain limited. This is notable given that cue-induced relapse poses a major challenge in the management of CUD, as environmental and drug-related cues can trigger reinstatement of drug-seeking behavior even after prolonged abstinence (Bossert et al., 2013; Sanchis-Segura and Spanagel, 2006; Shaham et al., 2003). In addition to its effects on extinction learning (Catlow et al., 2013; Du et al., 2023), psilocybin has been reported to enhance cognitive flexibility (Doss et al., 2021; Sayalı and Barrett, 2023; Torrado Pacheco et al., 2023). Together, these characteristics suggest that psilocybin may modulate processes relevant to relapse. To investigate this, the present study examined whether administration of psilocybin following extinction training would reduce cue-induced reinstatement of cocaine-seeking behavior in rodents. Using intravenous CSA paradigms in both mice and rats, and including both male and female subjects, the study aimed to identify potential species- and sex-dependent effects.

3.5.2 Results

3.5.2.1 Cocaine self-administration in mice

Female mice Female VEH and PSI 1.0 mice did not differ in cocaine SA prior to treatment. Figure 3.17A shows the mean (± SEM) responding on the active and inactive levers in VEH (n = 8) and PSI 1.0 (n = 8) mice during 8 daily 1-h sessions of cocaine SA. A three-way ANOVA (lever × day × treatment group) revealed a significant main effect of lever

[$F(1, 14) = 93.3,\ p < 0.0005$], indicating a distinction between active and inactive responding, and a lever × day interaction [$F(2.9, 40.7) = 4.1,\ p < 0.05$], but no other significant effects [all $F < 1$ except lever × treatment group: $F(1, 14) = 3.6,\ p > 0.05$], indicating no difference in lever responding between groups prior to extinction and treatment. Figure 3.17B shows the mean (± SEM) number of cocaine infusions received during 8 d of cocaine SA in the VEH and PSI 1.0 groups. A two-way ANOVA (day × treatment group) revealed no significant effects [day × treatment group: $F < 1$; day: $F(2.6, 36.5) = 1.4,\ p > 0.05$; treatment group: $F(1, 14) = 1.6,\ p > 0.05$], indicating no difference in cocaine intake prior to extinction and treatment.

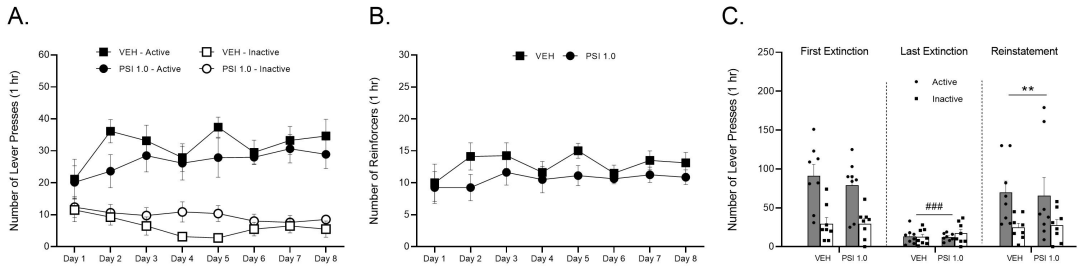

Figure 3.17. Cocaine SA, extinction, and reinstatement in female mice. **(A)** VEH (n = 8) and PSI 1.0 (n = 8) mice did not differ in lever responding during cocaine SA. Data represent mean number of presses (± SEM) on the active and inactive levers during 8 daily 1 h sessions of cocaine SA (0.5 mg/kg/14 µl infusion). **(B)** VEH and PSI 1.0 mice did not differ in the number of cocaine reinforcers achieved during cocaine SA. Data represent mean number of cocaine reinforcers (± SEM) achieved during 8 daily 1 h sessions of CSA. **(C)** VEH and PSI 1.0 mice did not differ in extinction responding (*left 2 panels*). Data represent mean number of presses (± SEM) on the active and inactive levers during the 1st and final 1 h extinction sessions. VEH and PSI 1.0 mice did not differ in reinstatement responding following the completion of the extinction criterion (*right panel*). Data represent mean number of presses (± SEM) on the active and inactive levers during reinstatement sessions. ### $p < 0.0005$, lever x session interaction, final extinction day vs. Extinction Day 1; ** $p < 0.005$, lever x session interaction, reinstatement vs. final extinction day. VEH = vehicle; PSI = psilocybin; CSA = cocaine self-administration.

Female VEH and PSI 1.0 mice did not differ in cue-induced reinstatement following extinction. An independent-samples *t*-test revealed no difference in the number of days required to reach the extinction criterion in female PSI 1.0 mice relative to VEH mice [VEH = 13.0±2.4, PSI 1.0 = 8.3±1.3; $t(14) = 1.8,\ p > 0.05$]. Because the completion of extinction was based on each mouse achieving a criterion level of active lever responding, as described above, Figure 3.17C, left 2 panels, shows the mean (± SEM) responding on the active and inactive

levers in VEH and PSI 1.0 mice during the first 2 and final 2 days of 1-h daily extinction sessions. A three-way ANOVA (lever × session × treatment group) of the 1st and last extinction sessions revealed significant main effects of lever [$F(1,14) = 31.4,\ p < 0.0005$] and session [$F(1,14) = 55.1,\ p < 0.0005$], and a significant lever × session interaction [$F(1,14) = 30.0,\ p < 0.0005$], indicating a significant change in responding from the 1st to the last extinction session that was irrespective of group. There were no other significant effects [all $F < 1$, except lever × treatment group: $F(1,14) = 1.7,\ p > 0.05$; session × treatment group: $F(1,14) = 1.1,\ p > 0.05$]. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive levers across groups confirmed that active [$t(15) = 7.9,\ p < 0.0005$], but not inactive [$t(15) = 2.5,\ p > 0.025$], responding decreased significantly from Extinction Day 1 to the final extinction day across groups.

Figure 3.17C, right panel, also shows the mean (± SEM) responding on the active and inactive levers in VEH and PSI 1.0 mice during cue-induced reinstatement testing 24 h following the completion of extinction. A three-way ANOVA (lever × session × treatment group) of final extinction values and reinstatement testing revealed significant main effects of lever [$F(1,14) = 8.1,\ p < 0.05$] and session [$F(1,14) = 21.0,\ p < 0.0005$], and a lever × session interaction [$F(1,14) = 12.2,\ p < 0.005$], but no other significant effects [all $F < 1$], indicating a significant change in responding from the last extinction session to reinstatement that was irrespective of group. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive levers confirmed that active [$t(15) = 4.4,\ p < 0.005$] responding increased significantly during the reinstatement session relative to final extinction values, indicative of a cocaine cue reinstatement across groups; inactive responding also increased across sessions [$t(15) = 3.0,\ p < 0.025$], but increases in responding were largely selective for the active lever.

Male mice Male VEH and PSI 1.0 mice did not differ in cocaine SA prior to treatment. Figure 3.18A shows the mean (± SEM) responding on the active and inactive levers in VEH (n = 9) and PSI 1.0 (n = 10) mice during 8 daily 1-h sessions of cocaine SA. A three-way ANOVA (lever × day × treatment group) revealed a significant main effect of lever [$F(1,17) = 85.0,\ p < 0.0005$], indicating a distinction between active and inactive responding, and a significant main effect of day [$F(3.0,51.3) = 3.4,\ p < 0.05$], but no other significant effects [all $F < 1$, except day × treatment group: $F(3.0,51.3) = 1.9,\ p > 0.05$], indicating no difference in lever responding between groups prior to extinction and treatment. Figure 3.18B shows the mean (± SEM) number of cocaine infusions received during 8 d of cocaine SA in the VEH and PSI 1.0 groups. A two-way ANOVA (day × treatment group) revealed no significant effects [all $F < 1$, except day: $F(1.9,32.0) = 2.0,\ p >$

0.05], indicating no difference in cocaine intake prior to extinction and treatment.

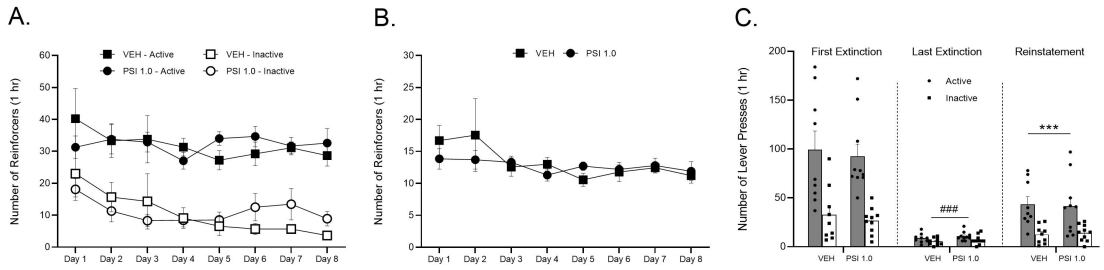

Figure 3.18. Cocaine SA, extinction, and reinstatement in male mice. **(A)** VEH (n = 9) and PSI 1.0 (n = 10) mice did not differ in lever responding during CSA. Data represent mean number of presses (± SEM) on the active and inactive levers during 8 daily 1 h sessions of cocaine SA (0.5 mg/kg/14 µl infusion). **(B)** VEH and PSI 1.0 mice did not differ in the number of cocaine reinforcers achieved during cocaine SA. Data represent mean number of cocaine reinforcers (± SEM) achieved during 8 daily 1 h sessions of CSA. **(C)** VEH and PSI 1.0 mice did not differ in extinction responding (*left 2 panels*). Data represent mean number of presses (± SEM) on the active and inactive levers during the 1st and final 1 h extinction sessions. VEH and PSI 1.0 mice did not differ in reinstatement responding 24 h following the completion of the extinction criterion (*right panel*). Data represent mean number of presses (± SEM) on the active and inactive levers during reinstatement sessions. ### $p < 0.0005$, lever x session interaction, final extinction day vs. Extinction Day 1; *** $p < 0.0005$, lever x session interaction, reinstatement vs. final extinction day. VEH = vehicle; PSI = psilocybin; CSA = cocaine self-administration.

Male VEH and PSI 1.0 mice did not differ in cue-induced reinstatement following extinction. An independent-samples t-test revealed no difference in the number of days required to reach the extinction criterion in male PSI 1.0 mice relative to VEH mice [VEH = 4.6 ± 0.42, PSI 1.0 = 6.0 ± 1.2; $t(17) = 1.0,\ p > 0.05$]. Because the completion of extinction was based on each mouse achieving a criterion level of active lever responding, as described above, Figure 3.18C, left 2 panels, shows the mean (± SEM) responding on the active and inactive levers in VEH and PSI 1.0 mice during the first and final days of 1 h daily extinction sessions. A three-way ANOVA (lever × session × treatment group) of the 1st and last extinction sessions revealed significant main effects of lever [$F(1, 17) = 32.5,\ p < 0.0005$] and session [$F(1, 17) = 85.7,\ p < 0.0005$], and a significant lever × session interaction [$F(1, 17) = 27.9,\ p < 0.0005$], indicating a significant change in responding from the 1st to the last extinction session that was irrespective of group. There were no other significant effects [all $F < 1$]. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive levers across groups revealed that both active [$t(18) = 8.1,\ p < 0.0005$] and inactive [$t(18) = 5.0,\ p < 0.0005$] responding decreased significantly from

Extinction Day 1 to the final extinction day.

Figure 3.18C, right panel, also shows the mean (± SEM) responding on the active and inactive levers in VEH and PSI 1.0 mice during cue-induced reinstatement testing 24 h following the completion of extinction. A three-way ANOVA (lever × session × treatment group) of final extinction values and reinstatement testing revealed significant main effects of lever [$F(1, 17) = 27.5,\ p < 0.0005$] and session [$F(1, 17) = 28.9,\ p < 0.0005$], and a lever × session interaction [$F(1, 17) = 18.6,\ p < 0.0005$], but no other significant effects [all $F < 1$], indicating a significant change in responding from the last extinction session to reinstatement that was irrespective of group. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive levers revealed that active [$t(18) = 5.2,\ p < 0.0005$] responding increased significantly during the reinstatement session relative to final extinction values, indicative of a cocaine cue reinstatement across groups; inactive responding also increased across sessions [$t(18) = 4.2,\ p < 0.0005$], but increases in responding were largely selective for the active lever.

3.5.2.2 Cocaine self-administration in rats

Female rats Female VEH, PSI 1.0, and PSI 2.5 rats did not differ in cocaine SA. Figure 3.19A shows the mean (± SEM) responding on the active (upper panel) and inactive (lower panel) NPs in VEH (n = 8), PSI 1.0 (n = 8), and PSI 2.5 (n = 8) rats during 15 daily 1-h sessions of cocaine SA. A three-way ANOVA (nose poke × day × treatment group) revealed significant main effects of NP [$F(1, 21) = 46.3,\ p < 0.0005$], indicating a distinction between active and inactive responding, and day [$F(3.0, 63.8) = 5.4,\ p < 0.005$], but no other significant effects [all $F < 1$], indicating no difference in NP responding between groups prior to extinction and treatment. Figure 3.19B shows the mean (± SEM) number of cocaine infusions received during 15 d of cocaine SA in the VEH, PSI 1.0, and PSI 2.5 groups. A two-way ANOVA (day × treatment group) revealed a significant main effect of day [$F(4.9, 103.5) = 2.8,\ p < 0.05$], but no other significant effects [all $F < 1$], indicating no difference in cocaine intake prior to extinction and treatment.

Female VEH, PSI 1.0, and PSI 2.5 rats did not differ in cue-induced reinstatement following extinction. Figure 3.19C shows the mean (± SEM) responding on the active (upper panel) and inactive (lower panel) NPs in VEH, PSI 1.0, and PSI 2.5 rats during 7 daily extinction sessions. A three-way ANOVA (NP × session × treatment group) of Extinction Days 1 and 7 revealed significant main effects of NP [$F(1, 21) = 54.3,\ p < 0.0005$] and session [$F(1, 21) = 103.8,\ p < 0.0005$], and a significant NP × session interaction [$F(1, 21) = 33.7,\ p < 0.0005$], indicating a significant change in responding from the 1st to the last

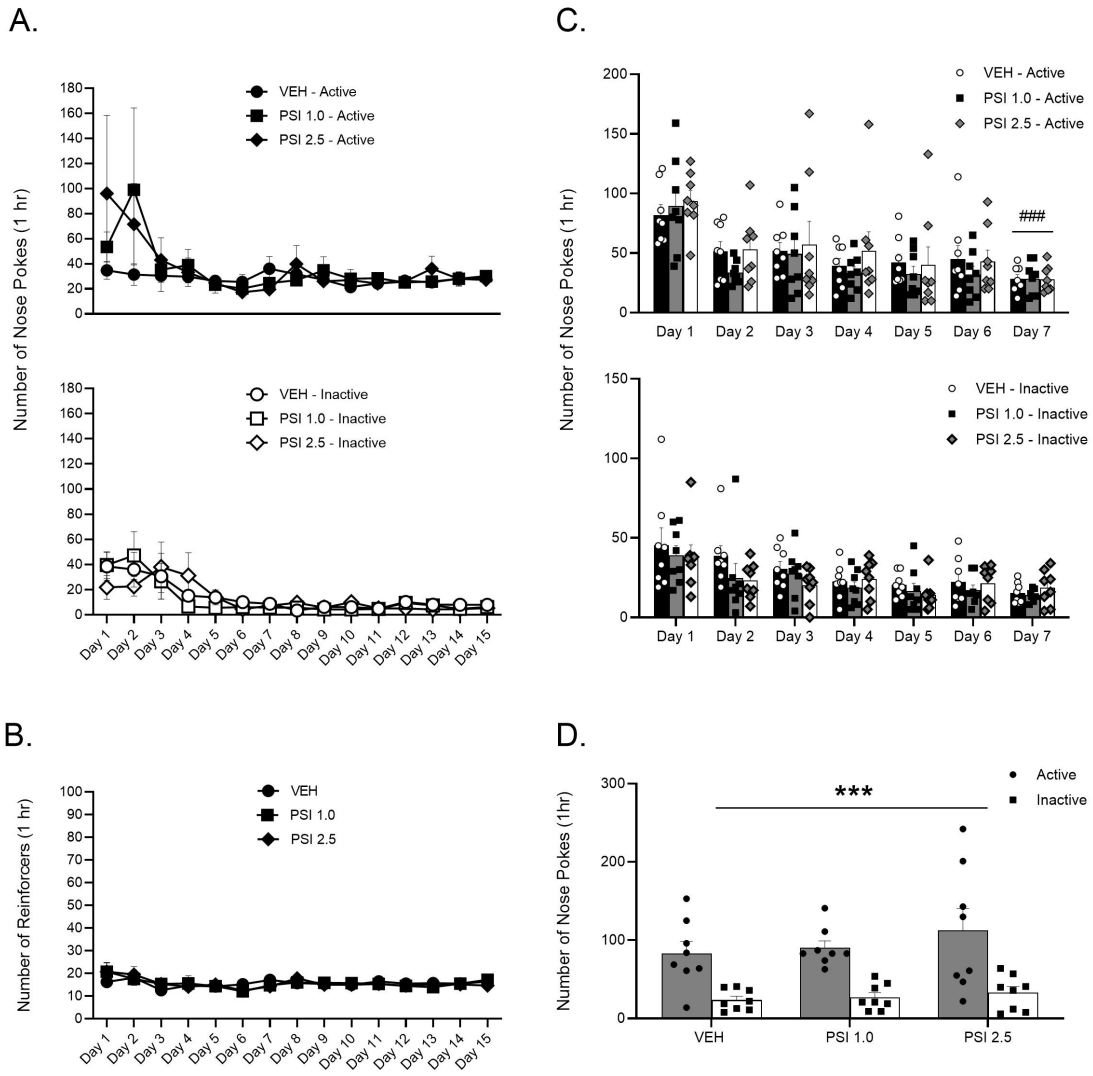

Figure 3.19. Cocaine SA, extinction, and reinstatement in female rats. **(A)** VEH (n = 8), PSI 1.0 (n = 8), and PSI 2.5 (n = 8) rats did not differ in NP responding during CSA. Data represent mean number of responses (± SEM) on the active (*upper panel*) and inactive (*lower panel*) NPs during 15 daily 1-h sessions of cocaine SA (0.8 mg/kg/36 µl infusion). **(B)** VEH, PSI 1.0, and PSI 2.5 rats did not differ in the number of cocaine reinforcers achieved during cocaine SA. Data represent mean number of cocaine reinforcers (± SEM) achieved during 15 daily 1-h sessions of CSA. **(C)** VEH, PSI 1.0, and PSI 2.5 rats did not differ in extinction responding. Data represent mean number of responses (± SEM) on the active (*upper panel*) and inactive (*lower panel*) NPs during 7 daily extinction sessions. (D) VEH, PSI 1.0, and PSI 2.5 rats did not differ in reinstatement responding 24 h following the completion of extinction sessions. Data represent mean number of responses (± SEM) on the active and inactive NPs during the reinstatement session. ### $p < 0.0005$, NP x session interaction, Extinction Day 7 vs. Extinction Day 1; *** $p < 0.0005$, NP x session interaction, Reinstatement vs. Extinction Day 7. VEH = vehicle; PSI = psilocybin; NP = nose-poke; CSA = cocaine self-administration.

extinction session that was irrespective of group. There were no other significant effects [all $F < 1$, except NP $\times$ session $\times$ treatment group: $F(2, 21) = 1.2,\ p > 0.05$]. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive NPs across groups revealed that both active [$t(23) = 11.9,\ p < 0.0005$] and inactive [$t(23) = 5.0,\ p < 0.0005$] responding decreased significantly from Extinction Day 1 to 7.

Figure 3.19D shows the mean ($\pm$ SEM) responding on the active and inactive NPs in VEH, PSI 1.0, and PSI 2.5 rats during cue-induced reinstatement. A three-way ANOVA (NP $\times$ session $\times$ treatment group) of responding during the final extinction day and reinstatement testing revealed significant main effects of NP [$F(1, 21) = 56.8,\ p < 0.0005$] and session [$F(1, 21) = 45.2,\ p < 0.0005$], and a significant session $\times$ NP interaction [$F(1, 21) = 29.4,\ p < 0.0005$], but no other significant effects [all $F < 1$], indicating no effect of psilocybin across extinction and reinstatement measurements. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive NPs revealed that active [$t(23) = 6.4,\ p < 0.0005$] responding increased significantly during the reinstatement session relative to final extinction values, indicative of a cocaine cue reinstatement across groups; inactive responding also increased across sessions [$t(23) = 4.3,\ p < 0.0005$], but increases in responding were largely selective for the active NP.

Male rats Male VEH, PSI 1.0, and PSI 2.5 rats did not differ in cocaine SA. Figure 3.20A shows the mean ($\pm$ SEM) responding on the active (upper panel) and inactive (lower panel) NPs in VEH (n = 9), PSI 1.0 (n = 8), and PSI 2.5 (n = 6) rats during 15 daily 1 h sessions of cocaine SA. A three-way ANOVA (NP $\times$ day $\times$ treatment group) revealed significant main effects of NP [$F(1, 20) = 36.9,\ p < 0.0005$], indicating a distinction between active and inactive responding, and day [$F(3.2, 63.5) = 8.5,\ p < 0.0005$], but no other significant effects [all $F < 1$, except day $\times$ treatment group: $F(6.3, 63.5) = 1.8,\ p > 0.05$; NP $\times$ day: $F(3.3, 66.9) = 1.5,\ p > 0.05$], indicating no difference in NP responding between groups prior to extinction and treatment. Figure 3.20B shows the mean ($\pm$ SEM) number of cocaine infusions received during 15 d of cocaine SA in the VEH, PSI 1.0, and PSI 2.5 groups. A two-way ANOVA (day $\times$ treatment group) revealed no significant effects [all $F < 1$, except day: $F(4.1, 81.2) = 2.4,\ p > 0.05$], indicating no difference in cocaine intake prior to extinction and treatment.

Male VEH, PSI 1.0, and PSI 2.5 rats did not differ in cue-induced reinstatement following extinction. Figure 3.20C shows the mean ($\pm$ SEM) responding on the active (upper panel) and inactive (lower panel) NPs in VEH, PSI 1.0, and PSI 2.5 rats during 7 daily extinction sessions. A three-way ANOVA (NP $\times$ session $\times$ treatment group) of Extinction Days 1 and 7 revealed significant main effects of NP [$F(1, 20) = 18.8,\ p < 0.0005$] and session

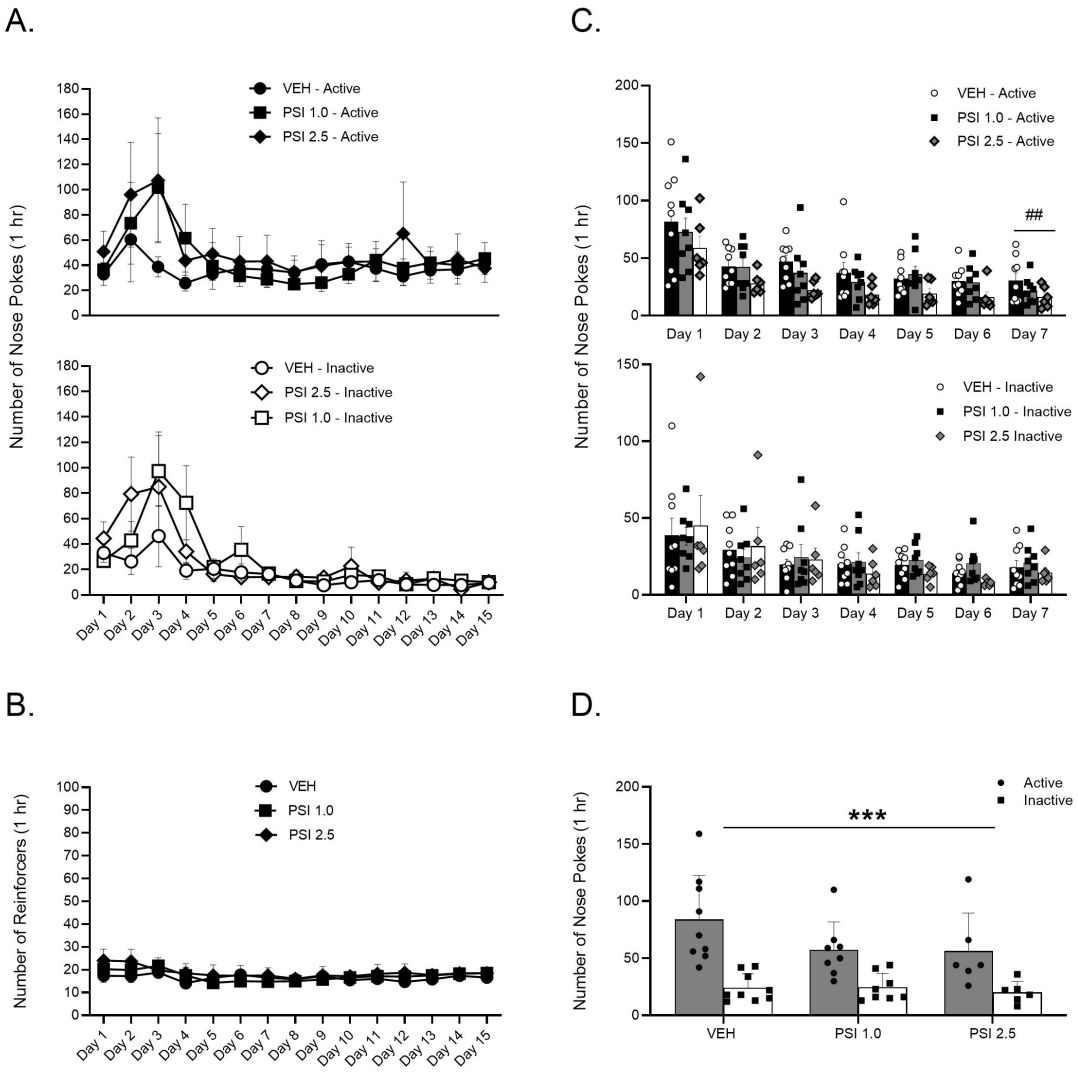

Figure 3.20. Cocaine SA, extinction, and reinstatement in male rats. **(A)** VEH (n = 9), PSI 1.0 (n = 8), and PSI 2.5 (n = 6) rats did not differ in NP responding during CSA. Data represent mean number of responses (± SEM) on the active (*upper panel*) and inactive (*lower panel*) NPs during 15 daily 1-h sessions of CSA (0.8 mg/kg/36 µl infusion). **(B)** VEH, PSI 1.0, and PSI 2.5 rats did not differ in the number of cocaine reinforcers achieved during CSA. Data represent mean number of cocaine reinforcers (± SEM) achieved during 15 daily 1-h sessions of CSA. **(C)** VEH, PSI 1.0, and PSI 2.5 rats did not differ in extinction responding. Data represent mean number of responses (± SEM) on the active (*upper panel*) and inactive (*lower panel*) NPs during 7 daily extinction sessions. **(D)** VEH, PSI 1.0, and PSI 2.5 rats did not differ in reinstatement responding 24 h following the completion of extinction sessions. Data represent mean number of responses (± SEM) on the active and inactive NPs during the reinstatement session. ### $p < 0.0005$, NP x session interaction, Extinction Day 7 vs. Extinction Day 1; *** $p < 0.0005$, NP x session interaction, Reinstatement vs. Extinction Day 7. VEH = vehicle; PSI = psilocybin; NP = nose-poke; CSA = cocaine self-administration.

[$F(1,20) = 30.1,\ p < 0.0005$], and a significant NP $\times$ session interaction [$F(1,20) = 15.6,\ p < 0.005$], indicating a significant change in responding from the 1st to the last extinction session that was irrespective of group. There were no other significant effects [all $F < 1$, except NP $\times$ treatment group: $F(2,20) = 2.0,\ p > 0.05$]. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive NPs across groups revealed that both active [$t(22) = 8.3,\ p < 0.0005$] and inactive [$t(22) = 2.9,\ p < 0.025$] responding decreased significantly from Extinction Day 1 to 7.

Figure 3.20D shows the mean ($\pm$ SEM) responding on the active and inactive NPs in VEH, PSI 1.0, and PSI 2.5 rats during cue-induced reinstatement. A three-way ANOVA (NP $\times$ session $\times$ treatment group) of responding during the final extinction day and reinstatement testing revealed significant main effects of NP [$F(1,20) = 31.0,\ p < 0.0005$] and session [$F(1,20) = 53.0,\ p < 0.0005$], and a significant NP $\times$ session interaction [$F(1,20) = 55.9,\ p < 0.0005$], but no other significant effects [all $F < 1$, except treatment group: $F(2,20) = 1.6,\ p > 0.05$; NP $\times$ treatment group: $F(2,20) = 2.3,\ p > 0.05$; NP $\times$ session $\times$ treatment group: $F(2,20) = 1.0,\ p > 0.05$], indicating no effect of psilocybin across extinction and reinstatement measurements. Follow-up paired-samples t-tests (Bonferroni-corrected $\alpha = 0.05/2$) of the active and inactive NPs confirmed that active [$t(22) = 8.8,\ p < 0.0005$], but not inactive [$t(22) = 1.8,\ p > 0.025$], responding increased significantly during the reinstatement session relative to final extinction values, indicative of cocaine cue reinstatement across groups. Because active responding at the start of extinction sessions was not equal among the three groups of male rats, and thus to mitigate against any potential bias resulting from differences in baseline extinction responding, we also include here the percent increase in active responding during reinstatement relative to final extinction values: VEH = 3.3 ± 0.47, PSI 1.0 = 2.8 ± 0.34, and PSI 2.5 = 3.8 ± 0.51. A one-way ANOVA revealed no difference in percent active responding during reinstatement relative to responding on the final extinction day [$F(2,20) = 1.1,\ p > 0.05$]. These findings further confirm a lack of effect of psilocybin on reinstatement.

3.6 Study 3B Reality testing in rats: Adaptation of representation-mediated aversion protocol

3.6.1 Introduction

The term "reality testing" (RT) originates from psychiatry and refers to the mental ability to distinguish between external perception and fantasy (Noshpitz, 1982)—in simpler terms, what is real versus what is imagined. This crucial cognitive function is impaired in various psychiatric disorders, most notably schizophrenia and psychosis, where such impairments manifest as "positive symptoms," including hallucinations, delusions, and other disturbances in perception. These symptoms prevent affected individuals from accurately interpreting their surroundings, severely impacting their quality of life (Andreasen and Olsen, 1982; Seabury et al., 2021).

Modeling RT preclinically in animals has been particularly challenging, as they cannot directly communicate their internal experiences. However, research in learning and associative processes has demonstrated that animals are capable of forming internal representations of their surroundings. These representations enable them to predict and respond to environmental changes. For example, in PCA paradigms, ST show a preference for cues associated with rewards, often engaging with the cues more than the rewards themselves (Fitzpatrick and Morrow, 2016; Morrison et al., 2015; Pohořalá et al., 2021). This behavior suggests that animals form robust internal representations of cue-reward associations, highlighting a potential cognitive parallel to human RT.

Schizophrenia and psychedelic research in rodents use various models and tests to examine its distinct features, symptoms and effects. For example, Morris Water Maze or T-Maze tests can be used to assess cognitive deficits, pre-pulse inhibition (PPI) can evaluate impairments in sensory gating, and social interaction tests can assess negative symptoms of schizophrenia, such as social withdrawal (Geyer et al., 2001; Jones et al., 2011; Winship et al., 2019). Similarly, psychedelic research often relies on tools like head-twitch response (HTR) and wet-dog shakes, which correlate with activation of serotonin 5-HT_{2A} receptors (Bedard and Pycock, 1977; Halberstadt and Geyer, 2013; Hanks and González-Maeso, 2013) as well as PPI, which is disrupted by serotonergic hallucinogens and modulated in a receptor-dependent and sex-specific manner (Halberstadt and Geyer, 2013; Ouagazzal et al., 2001; Vohra et al., 2022). Although these methods are certainly valuable, they do not address the core issue of RT—the ability to distinguish between mental representations and real stimuli. As this function is central to hallucinations and delusions in both schizophre-

nia and psychedelic states, establishing a reliable RT protocol in rodents would provide a unique foundation for investigating drug-induced cognitive alterations.

In fact, building upon Pavlovian conditioning and associative learning research, McDannald and Schoenbaum (2009) introduced an idea to use representation-mediated taste aversion (RMTA) for the assessment of RT in animals. RMTA was developed by Holland (1981; 1990; 2005) and utilizes a modified conditioned taste aversion paradigm (Chambers, 2018; Garcia and Koelling, 1966), in which an animal makes an association between taste and an aversive stimulus, e.g., malaise caused by LiCl, resulting in aversion to that particular taste. In RMTA, however, a neutral cue (e.g., tone or odor) is first paired with the taste and later with an aversive stimulus (LiCl-induced malaise) in the absence of taste. The neutral cue induces a mental representation of the taste and this representation becomes associated with the aversive stimulus, manifesting as mediated aversion. It is important to note here that the number of initial pairings is crucial, as mediated aversion in healthy animals emerges only after a relatively short pairing phase and disappears with further training (Holland, 1998, 1990, 2005; McDannald and Schoenbaum, 2009). This pattern suggests a transition from epresentation-driven behavior in early training to reward expectancy with prolonged training, when healthy animals can clearly distinguish between the two, whereas animals with impaired RT cannot. Indeed, McDannald et al. (2011) later applied this concept by testing the RMTA in one of the animal models of schizophrenia and demonstrated an impaired RT in rats with neonatal ventral hippocampal lesion, a comprehensive schizophrenia model (Tseng et al., 2009). Similarly, other preclinical studies in rodents have reported impairments in RT using different models of schizophrenia and modified RMTA protocols (Busquets-Garcia et al., 2018, 2017a,b; Fry et al., 2020; Kim and Koh, 2016; Koh et al., 2018; Wheeler et al., 2013).

The present study aimed to establish a reliable protocol for RT in rats, building on the work of Busquets-Garcia et al. (2017a; 2017b), whose protocol was originally developed for mice. Given the intended applications in examining the cognitive effects of psychedelics, particularly psilocybin, in addiction-relevant contexts, the protocol was adapted for rats to extend its translational utility and to enable systematic investigation of drug-induced changes in cognition.

3.6.2 Results

Direct aversion was consistently established across all experiments, whereas mediated aversion did not emerge under any experimental condition. Prior to the start of testing, basal

consumption of each odor- and taste-flavored solution was assessed to control for any preference. Independent-samples t-tests revealed no differences in basal intake of odor-flavored [$t(6) = 1.0,\ p > 0.05$] or taste-flavored [$t(6) = 0.1,\ p > 0.05$] solutions (data not shown), confirming no pre-existing preference.

Figure 3.21A, left panel, shows mean ($\pm$ SEM) consumption of taste-flavored solutions (mediated aversion test) and odor-flavored solutions (direct aversion test) after 3 or 6 odor–taste pairings and three devaluation sessions. Significant differences were observed during direct aversion tests both after 3 pairings [$t(14) = 21.5,\ p < 0.0001$] and 6 pairings [$t(14) = 13.0,\ p < 0.0001$], but not during mediated aversion tests [3 pairings: $t(14) = 0.6,\ p > 0.05$; 6 pairings: $t(14) = 0.1,\ p > 0.05$]. Panel A, right, shows the corresponding aversion indices. Figure 3.21B, left panel, shows mean ($\pm$ SEM) consumption following 3 or 6 odor–taste pairings and a single devaluation session. Direct aversion was again significant for 3 pairings [$t(14) = 13.1,\ p < 0.0001$] and 6 pairings [$t(14) = 7.4,\ p < 0.0001$], while mediated aversion remained absent [3 pairings: $t(14) = 1.7,\ p > 0.05$; 6 pairings: $t(14) = 1.2,\ p > 0.05$]. Panel B, right, shows the corresponding indices. Figure 3.21C, left panel, shows mean ($\pm$ SEM) consumption after a single odor–taste pairing and one devaluation session. Direct aversion was significant [$t(14) = 17.0,\ p < 0.0001$], but mediated aversion was absent [$t(14) = 1.5,\ p > 0.05$]. Panel C, right, shows the indices. For all panels, aversion indices were calculated as $(\text{mCS}^- - \text{mCS}^+)/(\text{mCS}^+ + \text{mCS}^-)$ for mediated aversion and $(\text{CS}^- - \text{CS}^+)/(\text{CS}^+ + \text{CS}^-)$ for direct aversion.

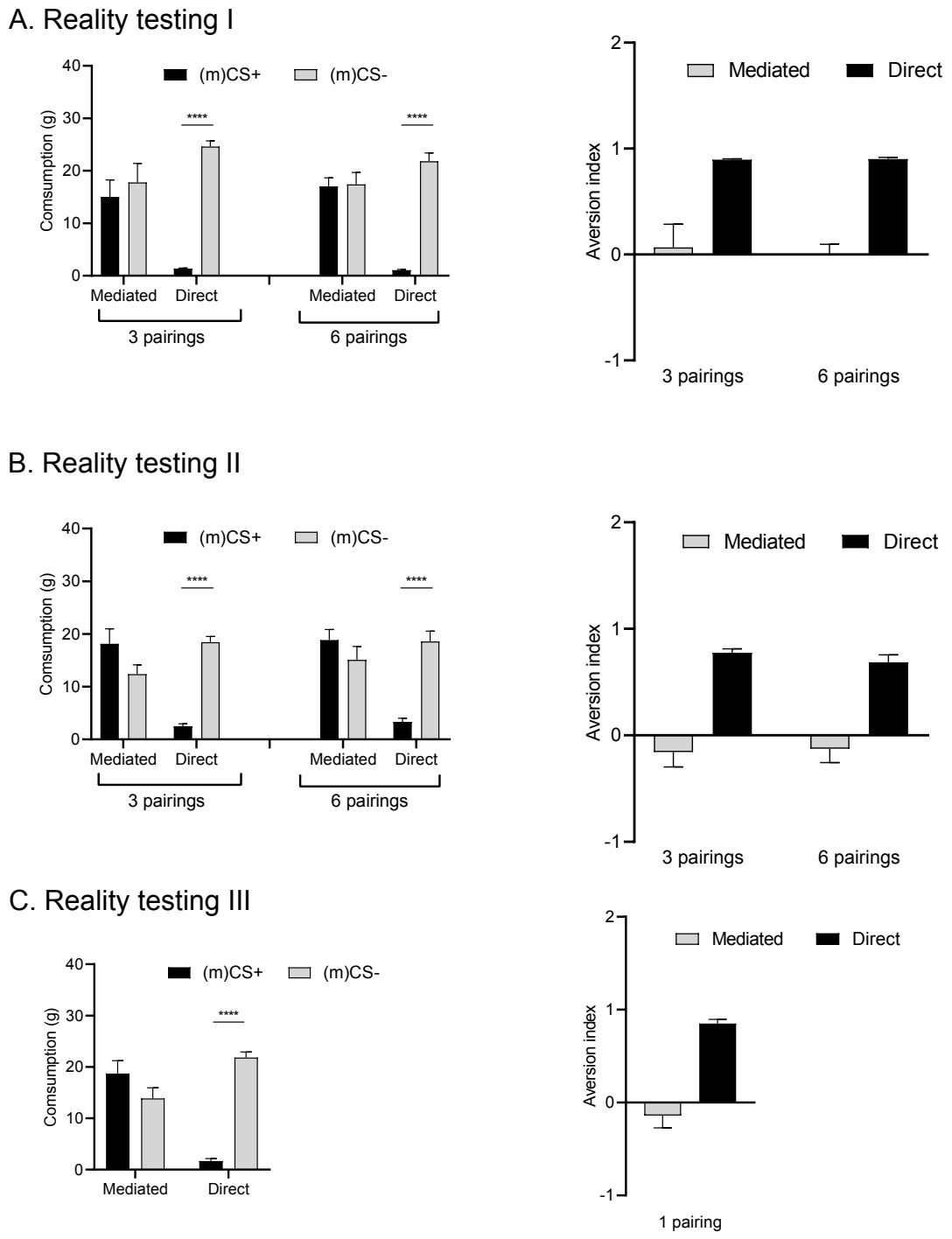

Figure 3.21. Mean consumption during mediated and direct aversion tests (*left panels*) and the corresponding aversion indices (*right panels*). **(A)** Reality testing I (3 or 6 pairings, three devaluation sessions). **(B)** Reality testing II (3 or 6 pairings, one devaluation session). **(C)** Reality testing III (one pairing, one devaluation session). Data represent mean (± SEM). mCS^+ = mediated conditioned stimulus; mCS^- = devaluated mediated conditioned stimulus; CS^+ = conditioned stimulus; CS^- = devaluated conditioned stimulus.

Chapter 4

Discussion

4.1 Study 1A Sign- vs. goal-tracking in the 3-CRIT model of cocaine addiction

This discussion is based substantially on the author's previous publication (Pohořalá et al., 2021), with minor paraphrasing for clarity and consistency.

This study was conducted to investigate whether extended daily CSA resulted in differential expression of addiction-like behaviors among goal-trackers, intermediates and sign-trackers, and whether these differences correlated with PCA scores characterizing these subgroups. The findings revealed no correlations between PCA scores and any of the criteria measured by 3-CRIT model: persistence of drug-seeking, motivation for CSA and drug-taking despite adverse consequences, or overall addiction score. These results align with prior research using different study designs and metrics to evaluate addiction-like behavior in rats (Kawa et al., 2016). Considering that addiction is a chronic condition, often persisting for a lifetime, the lack of correlation between PCA scores and addiction-like behaviors is particularly noteworthy as it suggests that PCA phenotyping along the continuum of GT, INT, and ST scores may not be a reliable predictor of vulnerability to addiction-like characteristics as measured by the 3-CRIT model of cocaine addiction.

In the present cohort, INTs were the most common phenotype, followed by STs, with GTs least represented. This distribution is consistent with Fitzpatrick and Morrow (2016) but contrasts with earlier results from our lab, which found a higher proportion of GTs than STs (Enkel et al., 2019), although such a reversal in phenotype prevalence has been documented previously (T. Enkel, unpublished). This fluctuating distribution pattern may be

influenced, at least in part, by the variability of PCA behaviors exhibited by Sprague-Dawley rats as reported by Fitzpatrick and colleagues (2013) in a paper emphasizing significant variability in PCA outcomes even among animals from the same vendor but different colonies.

Only a minority of animals met all three addiction-like criteria (3crit), which corresponds to previously reported data from our lab (Cannella et al., 2013) and others (Belin et al., 2009; Kasanetz et al., 2013). Although the percentage of rats classified as 0crit was lower than previously reported results from other 3-CRIT studies, comparable distribution was reported by Kasanetz et al. (2013). Moreover, some studies have combined the four phenotypes into fewer groups based on their addiction scores. For instance, Jadhav et al. (2017) in their study on alcohol use disorder grouped 0 crit and 1 crit rats together and found 66 % of their cohort to be addiction resilient, a finding that resembles the results of the current cocaine study and aligns with previous research (Belin et al., 2009; Cannella et al., 2017, 2013; Deroche-Gamonet et al., 2004; Kasanetz et al., 2013). Additionally, across all 3-CRIT groups, baseline cocaine intake prior to testing was similar, consistent with reports from previous 3-CRIT studies (Belin et al., 2009; Cannella et al., 2013; Deroche-Gamonet et al., 2004).

In recent years, STs have been suggested by various studies, primarily involving short drug exposure, to be more prone to developing addiction-like behaviors than GTs. This objective is grounded in the incentive-sensitization theory of addiction (Berridge and Robinson, 2016; Robinson and Berridge, 1993, 2000), which posits that addiction progresses into compulsive behavior due to the sensitization of the mesolimbic system. This sensitization enhances the "wanting" of drugs and drug-associated cues by amplifying their incentive motivational properties (Berridge and Robinson, 2016; Robinson and Berridge, 1993, 2000). In line with this objective, recent studies have demonstrated higher initial motivation to self-administer cocaine (Saunders and Robinson, 2011) and impulsivity in STs when compared to GTs (Flagel et al., 2010; Lovic et al., 2011). Additionally, STs were found to prioritize drugs over non-drug rewards, such as choosing cocaine over food (Tunstall and Kearns, 2015), being more responsive to discrete cocaine cues (Tunstall and Kearns, 2015), and having weaker top-down attentional control (Enkel et al., 2019; Paolone et al., 2013; Sarter and Phillips, 2018). On the contrary, GTs appear more reactive to contextual cues or "occasion setters" (e.g., discriminative stimuli), that are capable of restoring drug-seeking behavior in both rats (Crombag and Shaham, 2002; Fuchs et al., 2005) and humans (Mayo et al., 2013; O'Brien, 2005), as they are believed to exert higher-order hierarchical regulation of behavior (Sarter and Phillips, 2018). Furthermore, elevated conditioned hyperactivity and reinstatement of cocaine-seeking in response to the original drug context after extinction in a novel context have been observed in GTs relative to STs (Saunders et al., 2014). Taken

together, substance dependence likely emerges through various triggers and pathways, and the aforementioned findings point to a distinctive involvement of numerous mechanisms and neural systems underlying the processing of information with high motivational value. Although these factors are significant for understanding addiction, they were not addressed in the present study.

When comparing STs and GTs across the three criteria measured by the 3-CRIT model and the overall addiction score, no significant differences were found in persistence of drug-seeking, motivation for drug-taking, or overall addiction score. However, a significant difference emerged in the resistance to punishment criterion, with STs showing greater resistance to punishment than GTs. This finding indicates that STs, may, in fact, be more vulnerable to certain aspects of addiction; however, this is contrary to previous data reporting no difference in cocaine intake following a foot-shock punishment among STs and GTs (Saunders et al., 2013), although those results were obtained in a short-access rather than extended-access context. In the same study, STs rather than GTs were more likely to nose poke in the presence of a noncontingent cue light previously paired with cocaine following an incubation period (Saunders et al., 2013). Furthermore, a number of studies have also explored links between PCA phenotype and fear conditioning. For example, Morrow et al. (2011) demonstrated stronger freezing responses to discrete shock-predictive cues in STs, whereas GTs displayed higher freezing responses to the overall context. In a later study from the same group, STs showed lower freezing levels than GTs three days after conditioning; however, when tested 30 days later, both groups displayed comparable freezing, indicating a fear incubation effect present in STs but not in GTs (Morrow et al., 2015). The differences in resistance to punishment observed in the current study, along with the aforementioned findings, are likely linked to the stronger valence that STs assign to discrete stimuli. This enhanced focus, which has been discussed both here and previously (e.g., reviewed by Flagel and Robinson, 2017; Sarter and Phillips, 2018), results in elevated conditioned freezing behavior in response to fear-related cues and a greater motivational drive for drug-seeking when exposed to drug-associated cues (Milton and Everitt, 2010). Nevertheless, the specific conditions that give rise to these phenotype-dependent behaviors remain unclear, highlighting the need for further research to elucidate the mechanisms underlying the greater resistance to punishment in STs compared to GTs.

Sign- and goal-tracking behaviors are well characterized in rodents through standardized PCA paradigms (Enkel et al., 2019; Flagel et al., 2009; Saunders et al., 2013), yet their investigation in humans remains limited. These studies have used diverse and often indirect approaches, such as eye-tracking (Garofalo and di Pellegrino, 2015; Schad et al., 2020), touchscreen or lever-based tasks (Colaizzi et al., 2023; Joyner et al., 2018), and attentional

or behavioral indices of cue reactivity (Cope et al., 2023; Dinu et al., 2024; Watson et al., 2019, 2024). The substantial methodological variability among these approaches may limit their ability to capture the incentive motivational processes measured by rodent autoshaping (Colaizzi et al., 2020; Joyner et al., 2018). While some findings link ST-like tendencies in humans to addiction-relevant traits such as impulsivity (Colaizzi et al., 2023), cue-driven decision-making (Cherkasova et al., 2024), and compulsivity (Albertella et al., 2019), these associations remain correlational and lack longitudinal validation. In the present study, no consistent association was found between PCA phenotype and most 3-CRIT criteria, aside from resistance to punishment. Whether this absence of predictive value is also true in humans remains unclear, as no validated translational assays currently exist to assess ST/GT phenotypes in a manner directly comparable to rodent PCA procedures. Therefore, developing human paradigms that more closely parallel rodent PCA procedures could help clarify whether the observed null associations reflect a genuine cross-species pattern or a limitation of current animal-to-human comparisons.

In the context of the present study, the 3-CRIT model remains a valuable tool for assessing addiction-like behavior, as it integrates DSM-IV–derived diagnostic features of human addiction into a single experimental framework (American Psychiatric Association, 2000; Belin and Deroche-Gamonet, 2012; Deroche-Gamonet et al., 2004). This design addresses core behavioral features of addiction and provides strong translational relevance. Nonetheless, important aspects of SUD such as relapse, tolerance, withdrawal, or social context are not incorporated. Relapse-related assays (e.g., cue-induced reinstatement, incubation of craving) have revealed ST–GT differences in other contexts (Everett et al., 2020; Pitchers et al., 2017; Saunders et al., 2013). As relapse was not assessed in the present study, future incorporation of such measures could increase sensitivity to phenotype-specific vulnerabilities and yield a more comprehensive assessment of addiction-like behavior while preserving the model's existing strengths.

4.1.1 Summary

In general, no significant correlations were observed between PCA scores and the addiction-like criteria assessed by the 3-CRIT model following extended CSA, suggesting that PCA-derived phenotypes of GTs, INTs, and STs did not reliably predict the expression of these behaviors. The only phenotype-dependent difference was detected in resistance to punishment, indicating that this measure may represent a sensitive indicator of phenotype-related variation within the 3-CRIT framework and could be incorporated into future studies. In conclusion, these data contribute to the growing body of research on goal- and sign-tracking

behaviors and enhance understanding of the role of these phenotypes in preclinical addiction models.

4.2 Study 1B Sex differences in the 3-CRIT model of cocaine addiction

This study examined a female Sprague–Dawley rat cohort in the 3-CRIT model of cocaine addiction and directly compared their addiction-like behaviors to those of a similarly-sized cohort of males. Based on currently available published literature, this appears to be the first application of the 3-CRIT model in females. In the present female cohort, a subset of animals met the 3crit criterion, indicating the development of an addiction-like phenotype. Mirroring the 3-CRIT model profile, 3crit females and males scored higher across all three criteria and the overall Addiction score compared to their respective 0crit groups, consistent with previous reports (Belin et al., 2008; Cannella et al., 2017, 2013; Deroche-Gamonet et al., 2004; Kasanetz et al., 2013; Pohořalá et al., 2021). The proportion of 3crit animals was lower in females, while those that met the 3crit threshold showed greater addiction severity compared to males.

During CSA, female rats exhibited significantly lower intake than males. While these results seem contradictory to several preclinical studies reporting higher cocaine intake in females (Hu et al., 2004; Kawa and Robinson, 2019; Lynch and Carroll, 1999; Towers et al., 2022a), other work found no sex differences in cocaine intake (Bender and Torregrossa, 2023; Khoo and Samaha, 2023; Lynch and Taylor, 2004; Sun et al., 2021). These discrepancies may be due to variations in experimental protocols and cocaine doses used (Becker and Koob, 2016; Carroll and Lynch, 2016). However, the findings of lower cocaine intake in female rats presented in the current study are similar to previous work demonstrating that female rodents exhibit higher sensitivity to the behavioral effects of cocaine (Quinones-Jenab and Jenab, 2012; Sircar and Kim, 1999). For instance, female rats display enhanced cocaine-induced behavioral responses, including greater locomotor sensitization, following both acute and chronic cocaine administration across various experimental paradigms (Algallal et al., 2020; Chin et al., 2001; Festa et al., 2003; Khoo and Samaha, 2023; van Haaren and Meyer, 1991; Walker et al., 2001). Moreover, females require lower doses of cocaine to elicit behavioral responses comparable to those observed in males (Festa et al., 2004). In line with this heightened sensitivity, studies using the conditioned place preference paradigm have shown that female rodents develop cocaine-induced CPP at lower doses and after shorter conditioning periods compared to males (Russo et al., 2003; Zakharova et al.,

2009). Although Bobzean and colleagues (2010) did not report significant sex differences in CPP acquisition, their findings showed that at the highest cocaine doses tested (15, 20, and 25 mg/kg), females exhibited stronger reinstatement responses than males. In fact, reinstatement levels in females exceeded those observed during initial CPP testing. This suggests that females may form more robust associative learning between environmental cues and drug reward, potentially contributing to their increased susceptibility to relapse (Becker et al., 2017; Smith et al., 2023).

It is important to note here that male rats displayed significantly higher active nose-poke responding during drug periods across all FR5 sessions compared to their female counterparts. However, no significant sex-specific differences were observed in responding during no-drug periods, indicating that the difference in operant behavior was likely related to cocaine's reinforcing effects rather than baseline activity or exploratory tendencies. This suggests that both sexes exhibited comparable baseline response rates in the absence of drug reinforcement.

In both sexes, the 3crit group represented the smallest proportion of the cohort, consistent with previous applications of the 3-CRIT model (Belin et al., 2008; Cannella et al., 2017, 2013; Kasanetz et al., 2013; Pohořalá et al., 2021). In the present study, the percentage of 3crit animals was lower in females (6 %) than in males (15 %). Despite this lower prevalence, 3crit females a displayed higher addiction score than their male counterparts, suggesting a more severe addiction-like phenotype. This pattern aligns with prior rat studies (de Guglielmo et al., 2024) and parallels human epidemiological data, where women generally show lower prevalence of substance use disorders than men (Grant et al., 2016; Substance Abuse and Mental Health Services Administration, 2020, 2024) but, when affected, often present with a more severe course. Women progress more rapidly from initial cocaine use to dependence ("telescoping") (Becker et al., 2017; Fattore et al., 2014; Gallop et al., 2007; McCance-Katz et al., 1999), face greater difficulty achieving abstinence (Back et al., 2005), experience stronger cocaine craving (Elman et al., 2001; Moran-Santa Maria et al., 2014; Robbins et al., 1999; Waldrop et al., 2010), and relapse more frequently and after shorter abstinence periods than men (Gallop et al., 2007; Kosten et al., 1993; Smith et al., 2023).

As expected for the 3-CRIT model, 3crit animals of both sexes scored higher than 0crit animals across all criteria. Within this pattern, no significant sex differences were observed between 0crit and 3crit rats for any individual measure. However, when the cohorts were compared by sex irrespective of 3-CRIT classification, males showed higher BP in the PR test (Motivation) and a tendency toward greater compulsivity. This outcome is somewhat unexpected, as previous studies have frequently reported higher break points in females (Al-

gallal et al., 2020; Roberts et al., 1989a; Towers et al., 2021). Other work, however, has described no sex differences in motivation for cocaine-taking (Algallal et al., 2020; Doyle et al., 2014; Ramôa et al., 2013), and such discrepancies have been attributed to differences in cocaine dose or experimental protocol (Carroll and Lynch, 2016). The PR schedule used in the 3-CRIT model is also more behaviorally demanding than those typically applied in motivation testing (Belin and Deroche-Gamonet, 2012; Richardson and Roberts, 1996), which may have influenced the present findings.

Although the 3-CRIT model is designed to generate distinct addiction-like phenotypes independent of overall cocaine intake (Deroche-Gamonet et al., 2004; Deroche-Gamonet and Piazza, 2014), the present study found that 3crit and 2crit animals self-administered significantly more cocaine than 0crit and 1crit animals. This deviation from the original model's assumption should be noted, as greater cocaine intake may have contributed to the development of addiction-like behaviors. Nevertheless, all animals—regardless of phenotype—received extended CSA and accumulated high lifetime intake. Therefore, the emergence of the 3crit phenotype still reflects an individual difference in addiction-vulnerability, rather than being solely attributable to reduced exposure in the lower-crit groups. Comparable results have been reported in alcohol models. Domi et al. (2024, 2019) found that while higher intake was sometimes associated with greater addiction severity, consumption alone did not explain the emergence of the 3crit phenotype. Instead, the compulsive profile reflected a distinct vulnerability to addiction. Taken together, these findings emphasize that while all animals accumulated substantial lifetime cocaine intake, only a subset developed the compulsive 3crit profile. Thus, elevated exposure may be a precondition for addiction-like behavior, but it is the underlying vulnerability dimension that ultimately defines the validity of the 3-CRIT model.

The lower proportion of 3crit females, combined with the greater addiction severity observed in those that met the 3crit threshold, may, at least in part, be influenced by fluctuations in ovarian hormones, particularly estrogen and progesterone, which exert neuroactive effects (Arunogiri et al., 2021). Preclinical studies suggest that these hormones may have opposing roles in modulating cocaine-seeking behavior (Arunogiri et al., 2021; Feltenstein et al., 2009). Elevated estradiol levels, such as those occurring during the estrus phase, have been associated with increased cocaine intake, heightened drug-seeking, and enhanced reinstatement in female rodents (Feltenstein and See, 2007; Kippin et al., 2005; Lynch, 2008; Nicolas et al., 2019); in addition, exogenous estradiol has been shown to facilitate acquisition and reinstatement in ovariectomized females (Hu et al., 2004; Jackson et al., 2006; Larson and Carroll, 2007). In contrast, progesterone can counteract these effects, as evidenced by its ability to reduce acquisition and reinstatement when administered

exogenously (Anker et al., 2007; Jackson et al., 2006), and by correlations between higher endogenous progesterone levels and decreased cocaine-seeking (Feltenstein and See, 2007).

Notably, similar patterns were observed in humans, with increased cocaine craving during high-estrogen phases of the menstrual cycle and reduced relapse risk with progesterone administration (Evans, 2007; Evans and Foltin, 2006; Fox et al., 2013). Taken together, evidence from both preclinical and clinical research suggests that ovarian hormones play a key role in the heightened vulnerability to cocaine use and addiction observed in females. Therefore, future studies should track estrous cycle stages to assess whether addiction-like behaviors vary with hormonal changes, and examine the effects of hormonal manipulations, such as ovariectomy or hormone replacement, to clarify the hormonal and neurobiological mechanisms contributing to variation in addiction vulnerability as assessed by the 3-CRIT model.

An important consideration is that only three females in the present study met all 3 criteria. Although the observed differences in addiction severity between 3crit females and males reached statistical significance, the small number of affected females warrants cautious interpretation and may limit the generalizability of these findings. The same general limitations of the 3-CRIT model, as discussed in more detail in previous study (1A), also apply here. In summary, while the model captures several core DSM-IV–derived features of addiction, it does not incorporate other important aspects of SUD, including relapse.

4.2.1 Summary

The current study was the first to investigate female rats in the 3-CRIT model of cocaine addiction, with direct comparison to a male cohort to describe both similarities and differences in addiction-like behavior between sexes. The prevalence of the addiction-like phenotype was lower in females; however, those meeting all addiction criteria (3crit) exhibited greater addiction severity than their male counterparts, indicating sex-specific differences in the severity of addiction-like behavior within the affected subgroup. Furthermore, female rats demonstrated lower cocaine intake compared to males, a finding that aligns with previous reports of increased cocaine sensitivity in females.

To complement these behavioral findings, brains from both male and female rats in the 0crit and 3crit groups were collected and are currently being analyzed in an ongoing snRNA-seq study. This molecular analysis is expected to provide valuable insight into sex-specific transcriptional differences associated with addiction vulnerability and severity.

Findings from the present study contribute to the growing body of research on sex differences differences in addiction-related behavior. Future studies should investigate the potential role of hormonal fluctuations, particularly the estrous cycle, in influencing addiction-like behaviors in female rats. Furthermore, increasing the sample size and incorporating additional behavioral measures, such as relapse-related assays, may provide further insight into the mechanisms underlying sex-related differences in addiction severity.

4.3 Study 2A Metabotropic glutamate receptor 2: Stage-specific regulation during cocaine self-administration

The present study examined how CSA and subsequent extinction training or abstinence modulate the expression of *Grm2*, the gene coding for $mGluR_2$, across key corticolimbic and striatal brain regions in male rats. The effects of long-term CSA and addiction-like phenotypes on *Grm2* expression as defined by the 3-CRIT model were also assessed. Additionally, the functional role of $mGluR_2$ in cue-induced reinstatement was analyzed using the non-selective $mGluR_{2/3}$ agonist LY379268. Short-term CSA resulted in a selective reduction of *Grm2* expression in the PrLC, while extinction training following CSA led to a robust and widespread upregulation across all investigated regions. In contrast, abstinence did not significantly alter *Grm2* expression relative to cocaine-naïve controls. Long-term CSA produced no significant expression changes; however, differences emerged between addiction-resilient (0crit) and addiction-prone phenotypes (3crit), with the latter exhibiting increased *Grm2* expression in the infralimbic cortex and dorsal striatum. Pharmacological activation of $mGluR_{2/3}$ receptors with LY379268 reduced cue-induced reinstatement of cocaine-seeking behavior. However, this suppression was accompanied by a marked reduction in overall locomotor activity, raising concerns about behavioral specificity. These findings suggest that the observed effect may reflect non-specific motor suppression rather than targeted attenuation of relapse-related behavior.

The observed downregulation of *Grm2* in the PrLC following short-term CSA is consistent with prior studies demonstrating that early withdrawal ($\sim$ 24h) from cocaine disrupts glutamatergic homeostasis, particularly in medial prefrontal regions involved in executive function and relapse vulnerability (Goldstein and Volkow, 2011; Kalivas, 2009; Moussawi and Kalivas, 2010). A hypoglutamatergic state in NAc has been repeatedly observed following repeated cocaine exposure, as demonstrated by microdialysis studies (McFarland et al., 2003; Miguéns et al., 2008; Moran et al., 2005). This state is believed to result from

increased glutamate reuptake via excitatory amino acid transporters (EAATs) and decreased cystine–glutamate exchange via xCT, although substantial downregulation of xCT appears to emerge more prominently after prolonged withdrawal (Baker et al., 2003; Madayag et al., 2007; Olive, 2016). This imbalance may reduce tonic activation of presynaptic $mGluR_2$ receptors on corticostriatal projections, possibly triggering homeostatic transcriptional downregulation of *Grm2* in upstream cortical regions (Kalivas and Volkow, 2005). Given the established role of the PrLC in cue-driven drug seeking and reinstatement, a reduction in $mGluR_2$-mediated inhibitory feedback could lead to augmented excitatory output, further contributing to relapse vulnerability (Baptista et al., 2004; McFarland et al., 2003).

Several studies have investigated $mGluR_{2/3}$ following short-term SA, reporting inconsistent results ranging from no change to modest increases in $mGluR_{2/3}$ expression, protein levels and activity in the mPFC following cocaine administration (Allain et al. 2017; Hao et al. 2010; Pomierny-Chamiolo et al. 2017; for a comprehensive review, see Niedzielska-Andres et al. 2021). For example, Xie and colleagues (2009) noted a functional desensitization of $mGluR_{2/3}$ receptors in the PFC following repeated non-contingent cocaine administration, whereas Hao et al. (2010) reported enhanced functional activity of these receptors in several brain regions, including the PFC. However, this enhancement was limited to a long-access (6 h) group that exhibited escalated cocaine intake over time. Interestingly, the authors simultaneously showed no change in $mGluR_{2/3}$ protein levels, indicating that the observed alteration cannot be explained solely by a rise in receptor levels (Hao et al., 2010). Such discrepancies may arise from differences in post-transcriptional regulation, temporal dissociation between gene expression and protein synthesis, compensatory neuroadaptations, or methodological variability—including the type of cocaine exposure, withdrawal period, or detection technique. Importantly, not all assays can distinguish between $mGluR_2$ and $mGluR_3$; in contrast, differentiation was feasible in the present study. However, alterations in *Grm2* transcription may not directly reflect changes in receptor availability or function and should be interpreted with caution.

Ten days of extinction training induced a robust increase in *Grm2* expression across all brain regions examined, with the EXT group showing significantly higher *Grm2* mRNA levels than their cocaine-naïve, operant-matched controls (Ext CTRL). Given that Ext CTRL animals experienced the same operant procedures without cocaine exposure, this widespread up-regulation in the EXT group is likely associated with learning the new contingency—that cocaine-seeking no longer results in reinforcement—rather than general effects of training. Importantly, the measurements represent a single time point after the last extinction session and therefore do not capture potential dynamics of *Grm2* expression across the extinction process. By contrast, an equivalent period of passive home-cage abstinence

did not alter *Grm2* expression in any region, suggesting that the observed up-regulation reflects extinction-related processes rather than drug history alone. This pattern aligns with prior findings indicating that behavioral contingency during withdrawal, rather than withdrawal/abstinence alone, guides key glutamate-related neuroadaptations (Schwendt and Knackstedt, 2021). For example, extinction training has been shown to reduce surface expression of $mGluR_5$ (Knackstedt et al., 2010), enhance GluN1 expression (Ghasemzadeh et al., 2009; Pomierny-Chamiolo et al., 2015; Smaga et al., 2020), and restore tyrosine hydroxylase levels in the NAc (Schmidt et al., 2001). Similarly, following escalated methamphetamine SA, extinction reinstated $mGluR_{2/3}$ expression in the NAc and DS, whereas passive abstinence did not, despite persistent reductions in the PFC (Schwendt et al., 2012). Notably, Pomierny-Chamiolo et al. (2017) found that extinction training after CSA reduced the membrane fraction of $mGluR_{2/3}$ in the PFC and NAc, despite no change in total protein expression. This contrasts with the observed extinction-induced rise in *Grm2* mRNA, suggesting that while extinction may enhance transcription of *Grm2*, it could simultaneously promote receptor internalization or limit receptor trafficking to the membrane. Together, these findings point to a potential disconnect between gene expression and functional receptor availability, emphasizing the need to interpret mRNA data cautiously, especially when not paired with protein or functional assessments.

Although *Grm2* expression was not measured in the same animals tested for cue-induced reinstatement, findings from extinction-trained cohorts revealed a robust, region-wide increase in *Grm2* mRNA. As molecular and behavioral data were obtained from separate experimental groups, direct within-subject comparisons cannot be made. However, the observation that reinstatement of cocaine-seeking behavior persisted despite this transcriptional upregulation suggests that elevated *Grm2* expression alone is not sufficient to mitigate drug-seeking behavior. One possibility is that *Grm2* upregulation reflects a compensatory adaptation that does not translate into functional receptor engagement, potentially due to post-transcriptional or translational regulation, receptor trafficking deficits, or altered protein stability. This interpretation is supported by prior work demonstrating discrepancies between mRNA and protein levels in glutamatergic systems (Ferraguti and Shigemoto, 2006; Hao et al., 2010; Pomierny-Chamiolo et al., 2017; Vogel and Marcotte, 2012). Consistent with this interpretation, pharmacological activation of $mGluR_{2/3}$ receptors with LY379268 reduced reinstatement. However, given the pronounced locomotor suppression and the lack of behavioral specificity, it remains unclear whether this effect reflects functional engagement of extinction-associated *Grm2* adaptations or instead a more generalized motor dampening. These findings highlight the importance of integrating molecular and functional assessments when interpreting glutamate-related adaptations in addiction.

The absence of *Grm2* recovery in the ABST group indicates that while drug removal may reverse early PrLC down-regulation, it fails to provide the synaptic activation necessary for full restoration observed with extinction. This contrast underscores that active behavioral engagement during withdrawal—not simply the passage of time—drives $mGluR_2$ recovery. From a therapeutic standpoint, these findings support the idea that pharmacological potentiation of $mGluR_2$ (e.g., with positive allosteric modulators; for review, see Trabanco et al., 2019) may be most effective when paired with cue-exposure or cognitive-behavioral interventions, rather than with passive abstinence alone.

Direct comparisons among the three cocaine-exposed groups further confirmed that the behavioral context during early withdrawal differentially regulates *Grm2* expression across brain regions. Animals sacrificed 24 hours after the last CSA session exhibited suppressed *Grm2* expression in the PrLC, likely reflecting early transcriptional downregulation that may precede reduced $mGluR_2$-mediated signaling at later stages. Extinction training reversed this prelimbic deficit and significantly increased *Grm2* expression in both ILC and PrLC, supporting the role of extinction in engaging prefrontal plasticity (Gass and Chandler, 2013; Kalivas, 2009; Schwendt et al., 2012). In contrast, passive abstinence failed to restore prefrontal *Grm2* expression but increased *Grm2* in the DS, potentially reflecting compensatory adaptations linked to habitual drug-seeking. Consistent with this, Beveridge et al. (2011) reported elevated $mGluR_2$ protein levels in the DS of non-human primates following extended CSA (100 sessions). Moreover, direct intra-DS administration of the $mGluR_{2/3}$ agonist LY379268 reinstated cocaine-seeking in previously extinguished rats, implicating DS $mGluR_2$-sensitive circuits in relapse-related behaviors, although in a distinct pharmacological context. Together, these findings suggest that elevated *Grm2* in the DS may not be inherently protective but could reflect changes in signaling pathways that depend on the behavioral context. Notably, *Grm2* expression in the OFC remained significantly lower in ABST compared to EXT, potentially contributing to reduced behavioral flexibility after drug exposure without extinction-based re-learning (Balleine and O'Doherty, 2010; Lucantonio et al., 2014). Overall, these results highlight how cue-context engagement during withdrawal shapes $mGluR_2$-related plasticity across cortico-striatal networks, with implications for relapse vulnerability and therapeutic strategies (Fuchs et al., 2006; Schwendt and Knackstedt, 2021).

Building on the extinction-induced *Grm2* upregulation, the functional relevance of $mGluR_{2/3}$ signaling was tested in a separate cohort using LY379268. While the compound reduced cue-induced reinstatement of cocaine seeking, this effect was accompanied by robust locomotor suppression and a trend toward decreased inactive responding, suggesting non-specific sedation rather than selective attenuation of drug seeking. This non-specific sup-

pression complicates the interpretation of LY379268's efficacy, as it may not reflect targeted modulation of drug-seeking behavior. High doses of LY379268 ($\geq$3 mg/kg) have been consistently associated with marked reductions in locomotor activity and operant responding across various behavioral tasks, likely driven by sedation rather than specific cognitive or motivational effects (Amitai and Markou, 2010; Imre, 2007; Kufahl et al., 2011). These findings underscore the need to disentangle therapeutic efficacy from general motor suppression when evaluating $mGluR_{2/3}$-targeting compounds for addiction treatment. Although molecular and behavioral outcomes were obtained in different experimental groups, the extinction-associated increase in *Grm2* expression, together with the behavioral suppression observed after LY379268 administration, supports the possibility that $mGluR_{2/3}$-sensitive circuits remain functionally relevant during relapse. Indeed, LY379268 and related compounds have been reported to attenuate drug-seeking not only for cocaine (Baptista et al., 2004; Cannella et al., 2013; Martin-Fardon and Weiss, 2012; Peters and Kalivas, 2006), but also for heroin (Bossert et al., 2005), nicotine (Justinova et al., 2016; Liechti et al., 2007), alcohol (Kufahl et al., 2011; Vengeliene and Spanagel, 2022; Zhao et al., 2006), and methamphetamine (Kufahl et al., 2013; Schwendt et al., 2012). Nonetheless, future studies using more selective pharmacological tools, such as $mGluR_2$-positive allosteric modulators, and a broader dose range will be essential to distinguish motoric suppression from specific anti-relapse efficacy.

Comparative analysis of *Grm2* expression across different stages of cocaine experience (short vs. long) revealed a region-specific dynamic pattern of transcriptional regulation. In animals sacrificed a day after short-term CSA, *Grm2* expression was significantly downregulated in the PrLC, consistent with early disruption of top-down control systems following acute withdrawal (Goldstein and Volkow, 2011; Kalivas, 2009; Moussawi and Kalivas, 2010). In contrast, long-term CSA ($\geq$50 sessions) did not result in significant global alterations; however, a trend toward reduced *Grm2* expression in the ILC was observed. The ILC, in contrast to the PrLC, plays a key role in inhibiting drug-seeking and promoting extinction learning (Gass and Chandler, 2013; Peters et al., 2008). This apparent shift from dorsal (PrLC) to ventral (ILC) prefrontal dysregulation may reflect a temporal progression of cocaine-induced plasticity, during which early phases of cocaine exposure primarily impair executive initiation circuits (PrLC), while chronic use increasingly affects the capacity of the ILC to suppress drug-seeking. This progression mirrors the shift from goal-directed to habitual drug use described in behavioral models (Corbit et al., 2014; Everitt and Robbins, 2005, 2016). Studies have shown that decreased activity of the ILC emerges after repeated cocaine exposure, and that restoring ILC function can reduce relapse behavior (Guercio et al., 2020; Peters et al., 2008). Similarly, in alcohol dependence, Meinhardt et al. (2013, 2021) reported persistent downregulation of $mGluR_2$ in the ILC, which was linked to im-

paired cognitive control and increased relapse; restoring $mGluR_2$ expression in this region normalized both behavior and receptor levels, highlighting the critical role of the ILC across substances.

Notably, analysis of *Grm2* expression revealed a significant increase in mRNA levels within the ILC and DS of addiction-prone (3crit) animals compared to addiction-resilient (0crit) counterparts. Although $mGluR_2$ receptors are typically associated with protective, anti-relapse mechanisms, this increase may reflect a compensatory transcriptional response to underlying receptor dysfunction rather than a restoration of normal function. Supporting this interpretation, Kasanetz et al. (2013) reported reduced $mGluR_{2/3}$ protein levels and a complete loss of $mGluR_{2/3}$-mediated long-term depression (LTD) in the dorsomedial prefrontal cortex of 3crit rats following 2–3 weeks of abstinence, whereas 0crit animals maintained intact synaptic plasticity. In an earlier study, the same group also demonstrated that NMDAR-dependent LTD in the nucleus accumbens, a postsynaptic mechanism involved in behavioral flexibility, was temporarily abolished in all animals during cocaine use, but selectively restored only in 0crit rats following abstinence (Kasanetz et al., 2010). Together, these findings highlight the failure to restore synaptic homeostasis as an important neurobiological feature of vulnerability to addiction.

Therefore, the region-specific (ILC, DS) *Grm2* upregulation observed only in 3crit rats might reflect a localized attempt to compensate for adaptations in circuits implicated in inhibitory control and habit formation. While the ILC is critical for inhibitory control over drug-seeking, the DS is known to mediate stimulus–response learning and compulsive behavior, particularly under conditions of extended drug exposure (Everitt and Robbins, 2016; Zapata et al., 2010). Increased *Grm2* expression in these regions may signal an effort to restore glutamatergic regulation; however, whether this transcriptional adaptation corresponds to functional recovery remains uncertain. Cannella et al. (2013), using the same 3-CRIT model, reported no differences in $mGluR_2$ mRNA between 0crit and 3crit animals, yet found that only 3crit rats required high doses of the $mGluR_{2/3}$ agonist LY379268 to suppress reinstatement, suggesting reduced receptor sensitivity despite comparable transcript levels. However, several methodological differences relative to the present study may account for this discrepancy. In their molecular experiment, completion of the 3-CRIT protocol was followed by a prolonged abstinence period (three weeks), after which animals underwent a within session extinction/cue-induced reinstatement testing protocol, and were sacrificed a week later for molecular analysis. In contrast, tissue collection in the present study occurred 48 hours after the final CSA session, without additional behavioral procedures. Furthermore, Cannella and colleagues employed *in situ* hybridization, whereas qPCR was used in the current analysis—techniques that differ in spatial resolution and analytical

sensitivity. Together, these findings highlight that $mGluR_2$-related plasticity in addiction is shaped not only by the timing of assessments but also by the behavioral and methodological context in which they occur.

While the current study offers valuable insight into $mGluR_2$-related plasticity across different stages of cocaine exposure and behavioral intervention, several limitations should be acknowledged. First, *Grm2* expression was assessed solely at the mRNA level, without accompanying analyses of protein levels, receptor localization, or functional activity—factors that are essential to fully interpret receptor dynamics and their relevance to behavioral outcomes. Second, the use of LY379268, which targets both $mGluR_2$ and $mGluR_3$, limits the ability to draw receptor-specific conclusions. Third, the study was conducted exclusively in male rats, limiting the generalizability of the findings, particularly in light of growing evidence for sex-dependent differences in glutamatergic function and addiction-related behaviors (Fabian et al., 2023; Kniffin and Briand, 2024; Knouse et al., 2023). Including female subjects in future research will be important for improving translational relevance. Finally, relatively small sample sizes, particularly in the 3-CRIT groups, may have reduced the statistical power to detect subtle effects. Therefore, future studies should employ larger cohorts and integrate transcriptomic, proteomic and electrophysiological analyzes to clarify the time course and mechanisms of $mGluR_2$-related adaptations in addiction.

4.3.1 Summary

This study demonstrates that CSA and subsequent behavioral experiences dynamically regulate *Grm2* expression across cortico-striatal circuits. Short-term CSA reduced *Grm2* levels in the PrLC, while extinction training induced a robust, region-wide upregulation—an effect not observed following passive abstinence. Notably, addiction-prone (3crit) rats exhibited elevated *Grm2* in the ILC and DS, likely reflecting compensatory rather than restorative transcriptional responses to persistent receptor dysfunction. Pharmacological activation of $mGluR_{2/3}$ with LY379268 attenuated cue-induced cocaine seeking, supporting this receptor class as a potential therapeutic target, but the high dose used resulted in severe locomotor suppression. Together, these findings underscore the importance of behavioral context in shaping $mGluR_2$-related plasticity and highlight the relevance of timing, circuit engagement, and individual vulnerability in addiction.

4.4 Study 2B *Slc6a3*_N157K mutation: Effects on cocaine self-administration

This study aimed to determine the effects of the *Slc6a3*_N157K point mutation on CSA and motivation to cocaine taking in rats lacking DAT function compared to WT controls. The results demonstrated that while *Slc6a3*_N157K mutant rats initially self-administered cocaine, they failed to maintain this behavior across training sessions and exhibited little to no cocaine intake compared to their WT littermates, indicating that cocaine lacked rewarding properties. Furthermore, *Slc6a3*_N157K rats exhibited significantly lower BP scores in the PR test. However, the interpretive value of this outcome is limited, since these animals never established cocaine reinforcement. In contrast, WT rats continued to self-administer cocaine consistently across FR schedules, in line with their elevated active NP counts at FR5. They also achieved higher BP values in the PR test, indicating intact motivation for cocaine.

The DAT, encoded by the *Slc6a3* gene, is critical for the reuptake of dopamine from the synaptic cleft, thereby regulating dopamine neurotransmission and homeostasis (Gainetdinov et al., 1998; Vaughan and Foster, 2013). The *Slc6a3*_N157K mutation results in severely impaired DAT function, with an over 95 % reduction in activity (Vengeliene et al., 2017). Considering that cocaine exerts its reinforcing effects primarily, though not exclusively, by blocking DAT, leading to elevated extracellular dopamine levels and enhanced dopaminergic signaling (Nestler, 2005a; Volkow et al., 1999), a hyperdopaminergic state caused by impaired DAT could diminish cocaine's reinforcing properties.

Consistent with this interpretation, the present findings align with work in DAT-KO mice, where unsuccessful acquisition and maintenance of CSA were reported (Thomsen et al., 2009). Interestingly, these DAT-KO mice were initially active in the operant chambers, interacting with both active and inactive NP holes, a phenomenon also observed in the current dataset, suggesting a comparable capacity for operant responding in DAT-KO and WT animals (Thomsen et al., 2009). However, earlier work by Rocha et al. (1998) reported sustained CSA in a different DAT-KO line. This discrepancy may reflect differences between DAT-KO lines, particularly in extracellular dopamine regulation. While both studies reported significantly increased basal extracellular dopamine in DAT-KO mice relative to WT controls, the line used by Rocha et al. showed cocaine-induced increases in accumbal dopamine comparable to WT mice (Carboni et al., 2001; Rocha et al., 1998), whereas the line used by Thomsen et al. did not (Shen et al., 2004; Thomsen et al., 2009). These findings support the view that elevated basal dopamine alone is insufficient to support cocaine reinforcement; rather, the ability of cocaine to further elevate accumbal dopamine is critical

for the maintenance of CSA. This principle may also account for the absence of CSA in *Slc6a3*_N157K mutants.

In contrast, *Slc6a3*_N157K mutant rats have been shown to exhibit increased ethanol intake compared to WT controls (Hirth et al., 2016). This apparent discrepancy can be explained by the distinct pharmacological mechanisms of cocaine and ethanol. While cocaine binds directly to DAT, ethanol enhances dopamine release indirectly by modulating other targets and neurotransmitter systems, including $GABA_A$, NMDA, 5-HT_3, nicotinic acetylcholine receptors and non-ligand ion channels (Spanagel, 2009). This mechanism allows dopamine release to be further augmented despite impaired DAT function, thereby enhancing ethanol's rewarding properties.

Beyond differences in drug reinforcement, behavioral traits characteristic of DAT-deficient animals may also help explain aspects of the present data. DAT-KO animals, both mice and rats, have consistently been shown to display abnormal motor behaviors, including distinctly enhanced locomotor activity (Giros et al., 1996; Hall et al., 2009; Leo et al., 2018b; Mallien et al., 2022; Vengeliene et al., 2017), and elevated reactivity to novel stimuli and/or impaired habituation to novelty (Gainetdinov et al., 1999a; Giros et al., 1996; Leo et al., 2018b; Mallien et al., 2022; Vengeliene et al., 2017). This may account for the initially elevated NP responses in *Slc6a3*_N157K mutant rats during early CSA sessions, as each active NP was followed by cue light illumination and drug-pump noise, emphasizing the novelty aspect. Notably, the *Slc6a3*_N157K mutant and WT rats did not differ in inactive NP responses under any FR schedule, indicating that reduced active nose-poking in mutant rats reflects cocaine-specific effects rather than general behavioral or motor deficits. This pattern was mirrored in the PR test, where the *Slc6a3*_N157K rats showed a complete lack of motivation to self-administer cocaine. Given their failure to acquire cocaine reinforcement in the first place, this outcome is consistent with expectations and provides limited additional insight beyond confirming the absence of drug-directed behavior.

The *Slc6a3*_N157K mutant rats used in this study have previously been shown to have elevated basal extracellular dopamine levels compared to wild-types (Vengeliene et al., 2017), consistent with findings in other DAT-impaired rodents (Carboni et al., 2001; Giros et al., 1996; Leo et al., 2018b; Lloyd et al., 2022; Shen et al., 2004). Additionally, amphetamine did not influence the extracellular dopamine in the caudate putamen of *Slc6a3*_N157K mutant rats while causing a significant increase in WT controls. The authors also demonstrated blunted sensitivity to reward (Vengeliene et al., 2017); *Slc6a3*_N157K rats had almost identical preference for sucrose versus water whereas WT rats strongly preferred sucrose solution, an observation corresponding with reduced preference for sucrose/saccharine in a different line of DAT-KO rats (Cinque et al., 2018; Mallien et al., 2022). It is important

to note here that sucrose has been shown to elevate accumbal dopamine levels, suggesting neurochemical similarities between sucrose and drugs of abuse (Bassareo et al., 2017; Hajnal et al., 2004; Rada et al., 2005). Moreover, *Slc6a3*_N157K rats did not approach a reward-predicting stimulus in an autoshaping learning task (Vengeliene et al., 2017). Taken together, these findings may indicate slight anhedonia present in DAT-KO rats, possibly caused by altered or impaired reward processing.

To the best of my knowledge, no microdialysis studies have examined the effects of cocaine on extracellular dopamine levels in *Slc6a3*_N157K mutant rats. However, voltammetric investigations in DAT-KO rats, a model of complete transporter loss, provide relevant comparative evidence. Using fast-scan cyclic voltammetry (FSCV), Leo et al. (2018b) reported that cocaine did not increase stimulus-evoked dopamine release in DAT-KO rats, in contrast to WT controls. More recently, Lloyd et al. (2022), employing both FSCV and fast-scan controlled-adsorption voltammetry (FSCAV), confirmed this lack of effect and additionally reported that cocaine reduced the amplitude of electrically evoked dopamine release in DAT-KO rats. They suggested that this paradoxical reduction reflects anesthetic-like actions of cocaine via sodium channel blockade, which become apparent only when its primary DAT-blocking action is absent, although this interpretation remains unconfirmed. Notably, FSCV and FSCAV offered higher temporal precision than microdialysis, enabling detection of rapid changes in dopamine release and clearance. While extrapolation from DAT-KO rats to *Slc6a3*_N157K mutants should be made cautiously, these findings nonetheless highlight how chronic loss of DAT function can profoundly alter dopaminergic drug responses.

In line with these altered drug responses, the lack of CSA observed in *Slc6a3*_N157K mutant rats may stem from compensatory neuroadaptations resulting from chronic hyperdopaminergia. In DAT-deficient animals, persistently elevated extracellular dopamine levels lead to continuous stimulation of D_1- and D_2-like receptors, including presynaptic D_2 autoreceptors, resulting in their downregulation (Giros et al., 1996; Jones et al., 1999; Leo et al., 2018b; Shen et al., 2004; Vengeliene et al., 2017). Concurrently, the expression of enzymes involved in dopamine synthesis and metabolism is altered. Tyrosine hydroxylase (TH), the rate-limiting enzyme for dopamine biosynthesis, is reduced (Jaber et al., 1999; Leo et al., 2018b; Salvatore et al., 2016; Sora et al., 1998), while catechol-O-methyltransferase (COMT) and monoamine oxidase (MAO) levels are increased, potentially enhancing dopamine degradation (Jones et al., 1998; Leo et al., 2018b; Salvatore et al., 2016).

In addition to dopaminergic alterations, other neurotransmitter systems are also affected. For example, the serotonergic system exhibits increased SERT activity and altered receptor sensitivity, contributing to reduced reward sensitivity (Gainetdinov et al., 1999b; Leo et al., 2018b). The noradrenergic system demonstrates adaptive changes as well; notably,

NET have been shown to compensate for dopamine uptake in regions with low DAT expression (Leo et al., 2018b; Morón et al., 2002; Vengeliene et al., 2017). Furthermore, DAT-deficient states are associated with glutamatergic dysregulation, including changes in NMDA receptor function and reduced glutamate transporter expression, leading to disrupted excitatory signaling (Jones et al., 1999; Kalivas and Volkow, 2005). Alterations in GABAergic systems have also been observed, such as impaired GABAergic transmission and reduced expression of GABA-related markers, suggesting diminished inhibitory control (Savchenko et al., 2023). Together, these compensatory changes highlight that DAT dysfunction triggers a cascade of multi-system neuroadaptations, which may contribute to the absence of cocaine reinforcement and broader alterations in reward processing reported in *Slc6a3*_N157K mutants.

4.4.1 Summary

In conclusion, this study demonstrated that the *Slc6a3*_N157K point mutation, which severely impairs DAT function, resulted in a failure to sustained CSA in mutant rats. These findings support the critical role of DAT in mediating cocaine's reinforcing effects through regulation of extracellular dopamine levels. The inability of *Slc6a3*_N157K mutant rats to maintain CSA suggests that chronic hyperdopaminergia may lead to neuroadaptive changes that diminish the rewarding properties of cocaine. Understanding these mechanisms offers valuable insights into the neurobiology of addiction and the importance of DAT function in shaping responses to psychostimulants.

4.5 Study 3A Psilocybin and cocaine-seeking in rodents

This discussion expands upon the previously published version (Pohořalá et al., 2024), with revised structure and additional context.

The present study investigated whether psilocybin, administered immediately following extinction sessions, modulates subsequent cue-induced reinstatement of cocaine-seeking behavior in male and female mice and rats. Across both species and sexes, post-extinction psilocybin treatment did not produce any measurable effect on reinstatement responding triggered by drug-paired cues. In mice trained to self-administer cocaine, psilocybin (1 mg/kg, i.p.) was delivered immediately after the first two extinction sessions, but no difference was observed between treated and control groups during reinstatement testing. In

rats, psilocybin (1 or 2.5 mg/kg, s.c.) was administered after each of seven extinction sessions, again with no effect on subsequent reinstatement behavior. These findings indicate that, under the tested conditions, psilocybin does not modulate relapse-like behavior triggered by cocaine-associated cues. This outcome contrasts with the initial hypothesis that psilocybin would facilitate extinction-related plasticity and attenuate reinstatement.

This work represents one of the first preclinical efforts to evaluate psilocybin's impact on cocaine-related behaviors. While no significant behavioral effects were detected, the findings are informative in the broader context of psychedelic research. Classical psychedelics like psilocybin elicit their psychological and hallucinogenic effects primarily through agonist activity at serotonin 5-HT_{2A} receptors, which are implicated in a range of cognitive and affective processes, including learning, memory and behavioral flexibility (González-Maeso et al., 2007; Halberstadt, 2015; Madsen et al., 2019; Vollenweider et al., 1998). Several studies have examined the role of 5-HT_{2A} receptor signaling in the modulation of fear and extinction learning. For example, Zhang et al. (2013) demonstrated that 5-HT_{2A} receptor activation facilitates the consolidation and extinction of both trace and delay fear conditioning. Similarly, psychedelics such as psilocybin, 4-OH-DiPT, and DMT have been shown to reduce conditioned fear responses (Hagsäter et al., 2021) and enhance extinction learning in rodent fear paradigms (Cameron et al., 2019; Catlow et al., 2013; Du et al., 2023; Kelly et al., 2024). These findings suggest that 5-HT_{2A} receptor activation may support neural plasticity mechanisms relevant to extinction learning and memory updating. Based on this, the current study tested whether psilocybin, administered immediately after extinction sessions, would modulate cue-induced reinstatement of cocaine-seeking behavior by engaging similar cognitive and memory-related processes.

Recent evidence suggests that psilocybin's impact on memory extends beyond extinction learning. For instance, a study by Benvenuti and colleagues (2023) reported that psilocybin impaired the reconsolidation of alcohol-associated memories in mice, resulting in diminished alcohol-seeking behavior. This finding raises the possibility that psilocybin may interfere with memory updating mechanisms when administered around the time of memory retrieval. However, the broader literature on psilocybin's effects on learning and memory remains limited and often inconsistent. Studies in both humans and animals have reported contradictory outcomes, with some showing enhanced cognitive flexibility or memory performance, while others indicate impairments in memory consolidation or recall (Daws et al., 2022; Doss et al., 2021; Golden and Chadderton, 2022; Healy et al., 2021; Rambousek et al., 2014). This variability complicates efforts to predict how psilocybin might influence drug-associated learning in the context of cocaine-seeking behavior. The present findings suggest that psilocybin, when given post-extinction, fails to engage these mechanisms sufficiently

to influence relapse behavior.

Several features of the extinction and administration protocol used may have limited the potential for psilocybin to affect reinstatement and should be considered when interpreting the results. One important aspect is the design of the extinction training, which was conducted without re-exposure to drug-paired cues or SDs, focusing instead on operant responding. While this approach minimizes potential confounds related to cue salience, it may have limited the extent to which reconsolidation or cue-specific extinction mechanisms were engaged. Indeed, previous studies suggest that extinction protocols involving drug-associated cues are more effective in destabilizing maladaptive memory traces and facilitating reconsolidation-dependent updating (Benvenuti et al., 2023). Another consideration is the variation in response modalities between species, reflecting established protocols routinely used in our laboratory. Mice were trained to respond via lever pressing, while rats were trained using a nose-poke response. Although both are well-established operant procedures, differences in task structure and behavioral output could influence extinction dynamics and drug sensitivity. In addition, mice received psilocybin only after the first two extinction sessions, whereas rats were treated across seven consecutive extinction days. These differences in treatment duration and cumulative drug exposure may have influenced the extent to which psilocybin could interact with extinction-related plasticity. Nonetheless, the lack of behavioral effects was consistent across both models, suggesting it is unlikely to be species- or protocol-specific. Future studies incorporating extinction in the presence of previously conditioned cues, and systematically varying the timing and frequency of psilocybin administration, may help clarify the boundary conditions under which psychedelics modulate cocaine-seeking behavior.

Further insights into the potential mechanisms underlying psilocybin's behavioral effects can be drawn from recent studies investigating its active metabolite, psilocin. Wang et al. (2023) examined the effects of psilocin on methamphetamine CPP in mice and reported a disruption in the acquisition of drug-paired associations following psilocin administration. However, no effects were observed on extinction or reinstatement. In the same study, psilocin attenuated methamphetamine-induced phosphorylation of extracellular signal-regulated kinase (pERK), which is associated with drug learning in brain regions involved in regulating drug-related behaviors, such as PFC, NAc and VTA (Wang et al., 2023). Similarly, the importance of pERK in reward learning linked to cocaine-mediated behaviors, such as cocaine CPP and cocaine SA, has been previously demonstrated (Bernardi et al., 2019; Papale et al., 2016). These findings suggest that psilocin may selectively interfere with early memory formation, possibly through modulation of pERK-related plasticity. In the current study, psilocybin was administered after extinction sessions, a post-acquisition

phase, and in the absence of cue re-exposure. Under these conditions, memory systems required for psilocybin's action may not have been sufficiently engaged. Future work should examine whether psilocybin affects relapse-like behavior in models that incorporate stronger cue reactivation or earlier memory stages.

While the impact of psychedelics on cocaine-related behaviors remains underexplored, several studies have examined the effects of psilocybin on alcohol-related behaviors. For example, a single dose of psilocybin significantly reduced ethanol SA in rats (Jeanblanc et al., 2024) and mice (Alper et al., 2023). However, in rats, the persistence of this effect was not assessed, and in mice, the reduction in alcohol intake was short-lived, lasting only three days. Alper et al. (2023) also reported a sex-specific response, with reduced alcohol intake and preference observed exclusively in male mice. Similarly, Meinhardt and colleagues (2020) investigated relapse-related behavior using different psilocybin dosing regimens. Only subchronic administration produced a short-term reduction in alcohol seeking, while neither high-dose nor microdosing protocols yielded significant effects. This short-term attenuation of relapse-like behavior was replicated in a follow-up study (Meinhardt et al., 2021), which also explored underlying molecular mechanisms.

Meinhardt et al. (2020, 2013) had previously demonstrated that overexpression of $mGluR_2$ receptors in the ILC, achieved via a viral vector, reversed alcohol-induced downregulation of $mGluR_2$ and reduced relapse-like behavior in a cue-induced reinstatement test. Psilocybin treatment produced similar effects, restoring $mGluR_2$ expression and impairing relapse-like behavior (Meinhardt et al., 2021). These findings were attributed to the formation of heteromeric complexes between 5-HT_{2A} and $mGluR_2$ receptors, a mechanism proposed to mediate the therapeutic effects of psychedelics (Baki et al., 2016; Fribourg et al., 2011; Gewirtz and Marek, 2000). Given that chronic cocaine exposure also disrupts $mGluR_2$-mediated signaling (Kasanetz et al., 2013; Logan et al., 2020; Pomierny-Chamiolo et al., 2017), it was hypothesized that psilocybin might suppress cocaine-seeking through a similar pathway. However, in the present study, psilocybin failed to reduce cue-induced reinstatement of cocaine-seeking, even when administered repeatedly following extinction.

In a separate experiment conducted as part of this thesis (Study 2A), extinction training alone produced a robust upregulation of *Grm2* mRNA across several prefrontal and striatal regions, including the ILC and DS. While behavioral and molecular outcomes were obtained from distinct cohorts, the absence of a psilocybin-related behavioral effect suggests that increased *Grm2* expression alone may be insufficient to suppress relapse-like behavior. In contrast, pharmacological activation of $mGluR_{2/3}$ receptors using LY379268 significantly reduced cue-induced reinstatement, reinforcing the relevance of this receptor population in cocaine-seeking. One possibility is that, unlike in alcohol models, psilocybin fails to

adequately engage $mGluR_2$-sensitive circuits in cocaine-experienced animals, possibly due to differences in receptor regulation, circuit specificity, or lack of heteromer formation under these conditions. These findings suggest that while $mGluR_2$ represents a viable therapeutic target, psilocybin may not modulate this system effectively in the context of cocaine relapse.

Taken together, the current findings underscore the importance of identifying the specific behavioral and molecular conditions under which psilocybin may exert measurable effects on drug-related behaviors. Rather than acting as a general anti-relapse agent, its efficacy likely depends on timing, neuroadaptation state, and the behavioral context of administration. The observed dissociation between extinction-induced *Grm2* expression and the lack of psilocybin effect further highlights the need to integrate functional, molecular, and behavioral data within a unified experimental framework. Future studies should explore circuit-specific plasticity, receptor-level dynamics, and cognitive states during intervention to clarify the therapeutic potential of psilocybin in treating cocaine addiction.

4.5.1 Summary

In conclusion, the present experimental results show that the administration of psilocybin after extinction trials did not influence subsequent cue-induced reinstatement in mice and rats previously trained to self-administer cocaine in male or female animals. Although this study did not detect any significant effects, it represents an initial effort to identify preclinical cocaine learning protocols and paradigms in which psilocybin might reduce behaviors related to cocaine taking or seeking, and publishing data from animal model studies, even in the absence of significant effects, is essential for advancing scientific knowledge.

4.6 Study 3B Reality testing in rats: Adaptation of representation-mediated aversion protocol

The aim of the current study was to establish a functional and reliable protocol for reality testing in rats, building on the foundational work in mice by Busquets-Garcia et al. (2017a,b). Although the protocol was originally developed for mice, it was adapted here for use in rats to enable studies examining psychedelic interventions. Successful replication in rats would therefore offer a powerful tool for investigating impairments in reality testing, particularly in the context of psychedelics and psychotic-like states. However, significant challenges emerged during the development of this protocol, which ultimately prevented the successful establishment of mediated aversion, a crucial component of the reality testing paradigm.

While direct aversion was consistently observed, mediated aversion was absent across all experimental conditions, even with variations in the number of pairings and devaluation strategies. This outcome emphasizes the challenges of adapting protocols designed for mice to a rat model and points to potential procedural, cognitive, and neural differences that may underlie this discrepancy.

One of the crucial factors influencing the formation of mediated aversion is the number of initial pairings during the preconditioning phase, as demonstrated as demonstrated in Holland's work (Holland, 1998, 1990, 2005). These findings suggest that mediated aversion is formed after a relatively short pairing phase and diminishes with prolonged training, as animals are believed to transition from forming mental representations to relying on reward expectancy. Similarly, Busquets-Garcia et al. (2017a,b) designed a research protocol with varying durations of the preconditioning phase (1, 3, 6 or 9 pairings) and showed that strong mediated aversion emerged after 3 pairings and disappeared with prolonged pairings. In the current study, however, mediated aversion failed to emerge, even with variations in the preconditioning pairings (1, 3, or 6) and the devaluation pairings (1 or 3). This further emphasizes the potential importance of tailoring protocols to account for species- and strain-specific associative learning processes.

Direct aversion was reliably observed across all experimental conditions, as evidenced by significantly reduced consumption of the odor solution that had been directly paired with LiCl-induced malaise during the devaluation phase. This consistent effect confirms that the 65 mg/kg dose of lithium chloride was effective and that the rats were capable of forming robust aversive associations with directly experienced stimuli. Therefore, the absence of mediated aversion is unlikely to stem from a failure in aversive conditioning itself. Rather, it suggests a more specific deficit in the ability of the odor cue to evoke a mental representation of the previously paired taste during testing—an essential mechanism underlying representation-mediated learning. Furthermore, baseline preference tests conducted prior to conditioning revealed no significant differences in consumption between individual taste and odor stimuli, suggesting that the failure to observe mediated aversion cannot be attributed to pre-existing biases.

Another consideration is the length of training sessions. Rats were allowed one hour of access to solutions during training, which may have been excessive and could potentially lead to decreased task engagement or reduced salience of the stimuli. Shorter sessions, such as the 10-minute access period used in other rat studies (López et al., 2023; Wheeler et al., 2013), may reduce task fatigue and enhance learning. Additionally, the rats may have benefited from additional access to plain water in the evening, several hours after the training session ended (López et al., 2023; Wheeler et al., 2013). This measure could improve

motivation during training sessions by maintaining a more consistent deprivation schedule. Furthermore, the strength or presentation of the odor may also have been insufficient to form strong associative links. Odors in the protocol might require higher concentrations or a different form of presentation to achieve the necessary salience for associative learning. For example, Wheeler et al. (2013) presented odors on filter paper placed near the drinking spout, ensuring the odor remained distinct from the taste cue and consistently perceivable during drinking. This method likely enhanced the salience of the odor as a standalone cue, facilitating its association with the aversive outcome. By contrast, presenting odors directly in the solution, as in the current study, might have weakened the distinctiveness of the odor as an associative cue, making it less likely to form the necessary mental representation to elicit mediated aversion, even though this method was successful in mice.

Additionally, the findings suggest that taste-devaluation protocols might offer a more reliable and effective approach for reality-testing paradigms in rats. Taste is a biologically salient and highly relevant stimulus for rats, as demonstrated by extensive research showing that taste cues are more readily associated with aversive outcomes like illness compared to other sensory cues such as odors, lights, or sounds (Garcia and Koelling, 1966; Holland, 2006). This "cue-to-consequence" principle highlights that taste is naturally prioritized in associative learning due to its evolutionary significance in detecting harmful substances. Moreover, while odors can also form associations with illness, they are generally weaker and require more conditioning trials unless they are potentiated by pairing with a biologically relevant stimulus like taste (Capaldi et al., 2004; Holland, 2006; Saddoris et al., 2009).

Future adaptations of the protocol could incorporate a method of presenting odors near the drinking spout to maintain their distinctiveness as associative cues. Additionally, shortening training sessions and providing evening access to plain water may enhance motivation and reduce task fatigue (López et al., 2023; Wheeler et al., 2013). Finally, shifting to a taste-devaluation procedure could leverage the natural salience of taste for more robust and reliable mediated aversion learning in rats. These adjustments would align the protocol with the sensory and cognitive characteristics of rats and may ultimately increase its suitability for reality-testing paradigms. Importantly, because the present study did not succeed in establishing a functional RT protocol in rats, it was not possible to apply this approach to examine psilocybin-induced alterations in cognition, which had been the intended application. While other behavioral assays, such as HTR and PPI, are commonly used to assess the effects of serotonergic psychedelics, these paradigms primarily index receptor engagement and sensorimotor processing rather than higher-order cognitive functions (Alexander et al., 2024; Halberstadt and Geyer, 2013; Ouagazzal et al., 2001; Vohra et al., 2022). The current work therefore underscores both the challenges of adapting mouse-based procedures to rats

and the continued need for validated paradigms capable of capturing drug-induced changes in perception and cognition.

4.6.1 Summary

In conclusion, this study attempted to adapt a RT protocol from mice to rats, but was not successful in establishing a reliable mediated aversion, likely due to species-specific differences in sensory processing and associative learning. Adjustments such as shorter training sessions, evening water access, odor presentation near the drinking spout, and taste-devaluation procedures are suggested to better align the protocol with the sensory and cognitive characteristics of rats. These refinements may improve the reliability of RT protocols in rats and support their future application to investigating drug-induced alterations in cognition, including those produced by psychedelics and in models of psychotic-like states.

Chapter 5

Summary and Outlook

This thesis investigated vulnerability to cocaine addiction using a combination of behavioral, molecular, and pharmacological approaches in rodent models. Several key experiments applied the 3-CRIT model as a translational tool for identifying addiction-like behavior, while other paradigms explored relapse-relevant mechanisms beyond this framework, including molecular adaptations and responses to novel interventions.

The predictive value of individual traits such as sign- versus goal-tracking was limited. Although sign-trackers showed greater punishment resistance, these trends did not reliably predict full 3-CRIT classification. Observed sex differences in addiction-like behavior echoed clinical patterns, where severity does not always correlate with prevalence. These findings underscore the complex, multifactorial nature of addiction vulnerability and the need for models that capture this variability.

Cocaine exposure led to changes in glutamatergic signaling, with *Grm2* expression modulated by both behavioral context and addiction-like phenotype. Pharmacological activation of $mGluR_{2/3}$ receptors attenuated cue-induced cocaine seeking, although the specificity of this effect remains unclear. In parallel, impaired DAT function abolished cocaine self-administration, supporting the critical role of DAT-mediated dopamine reuptake in cocaine's reinforcing effects. Together, these findings highlight the complementary roles of glutamatergic and dopaminergic systems in shaping addiction-related behavior.

The final part examined novel pharmacological and methodological approaches. Psilocybin did not reduce cue-induced reinstatement under the tested conditions. Nevertheless, the study provides insight into the feasibility and limitations of applying psychedelic-based interventions in preclinical addiction models. In addition, the development of a rat-adapted paradigm intended to assess reality testing underscored both the challenges of cross-species

translation and the importance of refining behavioral tools to probe drug-induced changes in cognition. These efforts highlight the difficulty of modeling higher-order cognitive dysfunction and the ongoing need for improved assays in preclinical addiction research.

Overall, these findings illustrate that cocaine addiction vulnerability is not driven by isolated behavioral traits or single molecular mechanisms, but by their interactions. Addiction emerges from the interplay of individual traits, sex differences, and drug-induced neuroplasticity, with both protective and risk factors shaping outcomes. While some hypotheses were only partially supported, the results identify promising molecular targets, point to methodological limitations, and support a shift toward individualized, circuit-level models of addiction.

Future research should build on the limitations identified in this work. Improved behavioral phenotyping is needed, ideally through longitudinal tracking and multidimensional trait profiling, to better capture stable predictors of addiction vulnerability beyond single traits such as sign- versus goal-tracking. Circuit-level tools such as chemogenetics and optogenetics will be essential for testing causal mechanisms underlying the glutamatergic and dopaminergic adaptations observed here. Pharmacological interventions should continue to be evaluated for both efficacy and potential behavioral liabilities. While activation of $mGluR_{2/3}$ receptors with LY379268 reliably reduced cue-induced cocaine seeking, psilocybin produced no measurable effect under the present conditions. These findings suggest that treatment outcomes may depend strongly on pharmacological specificity, timing, and experimental context. Studies of emerging treatments like psychedelics should therefore be designed to more directly engage their proposed mechanisms of action, for example by incorporating extinction paradigms with drug-associated cues or by systematically varying timing and re-exposure conditions to improve translational relevance. A particularly promising avenue is the selective breeding of addiction-resilient and addiction-vulnerable populations, based on stable behavioral traits identified in the 3-CRIT model—or at minimum, on resistance to punishment. This approach could help elucidate heritable risk factors, clarify the neurobiological mechanisms driving individual susceptibility, and ultimately support the development of targeted therapeutic interventions. Finally, the refinement of cognitive assays remains critical for linking addiction-related behaviors to neurocognitive dysfunction and for enabling targeted treatment strategies. Together, these directions offer a framework for more refined preclinical studies that better reflect the complexity of human addiction.

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Bibliography

Abbott, A. (2004). The Renaissance rat. *Nature*, 428(6982):464–466.

Adewale, A. S., Platt, D. M., and Spealman, R. D. (2006). Pharmacological stimulation of group ii metabotropic glutamate receptors reduces cocaine self-administration and cocaine-induced reinstatement of drug seeking in squirrel monkeys. *The Journal of pharmacology and experimental therapeutics*, 318(2):922–931.

Agrawal, M., Roddy, K., Jenkins, B., Leeks, C., and Emanuel, E. (2025). Long-term benefits of single-dose psilocybin in depressed patients with cancer. *Cancer*, 131(12):e35889.

Aharonovich, E., Hasin, D. S., Nunes, E. V., Stohl, M., Cannizzaro, D., Sarvet, A., Bolla, K., Carroll, K. M., and Genece, K. G. (2018). Modified cognitive behavioral therapy (M-CBT) for cocaine dependence: Development of treatment for cognitively impaired users and results from a Stage 1 trial. *Psychology of Addictive Behaviors*, 32(7):800–811.

Albertella, L., Le Pelley, M. E., Chamberlain, S. R., Westbrook, F., Fontenelle, L. F., Segrave, R., Lee, R., Pearson, D., and Yücel, M. (2019). Reward-related attentional capture is associated with severity of addictive and obsessive-compulsive behaviors. *Psychology of addictive behaviors : journal of the Society of Psychologists in Addictive Behaviors*, 33(5):495–502.

Alexander, L., Anderson, D., Baxter, L., Claydon, M., Rucker, J., and Robinson, E. S. J. (2024). Preclinical models for evaluating psychedelics in the treatment of major depressive disorder. *British journal of pharmacology*.

Alexander Morton, W. (1999). Cocaine and psychiatric symptoms. *Primary Care Companion to the Journal of Clinical Psychiatry*, 1(4):109–113.

Algallal, H., Allain, F., Ndiaye, N. A., and Samaha, A.-N. (2020). Sex differences in cocaine self-administration behaviour under long access versus intermittent access conditions. *Addiction biology*, 25(5):e12809.

Allain, F., Roberts, D. C. S., Lévesque, D., and Samaha, A.-N. (2017). Intermittent intake of rapid cocaine injections promotes robust psychomotor sensitization, increased incentive motivation for the drug and mGlu2/3 receptor dysregulation. *Neuropharmacology*, 117:227–237.

Alper, K., Cange, J., Sah, R., Schreiber-Gregory, D., Sershen, H., and Vinod, K. Y. (2023). Psilocybin sex-dependently reduces alcohol consumption in C57BL/6J mice. *Frontiers in Pharmacology*, 13:1074633.

Altman, A. J., Albert, D. M., and Fournier, G. A. (1985). Cocaine's use in ophthalmology: Our 100-year heritage. *Survey of Ophthalmology*, 29(4):300–306.

American Psychiatric Association (2000). *Diagnostic and statistical manual of mental disorders : DSM-IV-TR*. American Psychiatric Association, Washington, DC, 4th ed., t edition.

American Psychiatric Association (2013). *Diagnostic and Statistical Manual of Mental Disorders (DSM-5®)*. American Psychiatric Publishing, Arlington, VA, 5th edition.

Amerio, A., Baccino, C., Breda, G., Cortesi, D., Spiezio, V., Magnani, L., De Berardis, D., Conio, B., Costanza, A., De Paola, G., Rocca, G., Arduino, G., Aguglia, A., Amore, M., and Serafini, G. (2023). Effects of transcranial magnetic stimulation on cocaine addiction: A systematic review of randomized controlled trials. *Psychiatry Research*, 329:115491.

Amitai, N. and Markou, A. (2010). Effects of metabotropic glutamate receptor 2/3 agonism and antagonism on schizophrenia-like cognitive deficits induced by phencyclidine in rats. *European journal of pharmacology*, 639(1-3):67–80.

Anderson, B. A., Faulkner, M. L., Rilee, J. J., Yantis, S., and Marvel, C. L. (2013). Attentional bias for nondrug reward is magnified in addiction. *Experimental and clinical psychopharmacology*, 21(6):499–506.

Anderson, E. and Hearing, M. (2019). Neural Circuit Plasticity in Addiction. In *Neural Mechanisms of Addiction*, pages 35–60. Elsevier.

Anderson, S. M. and Pierce, R. C. (2005). Cocaine-induced alterations in dopamine receptor signaling: Implications for reinforcement and reinstatement. *Pharmacology & Therapeutics*, 106(3):389–403.

Andreasen, N. C. and Olsen, S. (1982). Negative v Positive Schizophrenia: Definition and Validation. *Archives of General Psychiatry*, 39(7):789–794.

Andrews, P. (1997). Cocaethylene toxicity. *Journal of Addictive Diseases*, 16(3):75–84.

Anker, J. J., Larson, E. B., Gliddon, L. A., and Carroll, M. E. (2007). Effects of progesterone on the reinstatement of cocaine-seeking behavior in female rats. *Experimental and clinical psychopharmacology*, 15(5):472–480.

Anthony, J. C., Warner, L. A., and Kessler, R. C. (1994). Comparative epidemiology of dependence on tobacco, alcohol, controlled substances, and inhalants: Basic findings from the National Comorbidity Survey. *Experimental and Clinical Psychopharmacology*, 2(3):244–268.

Arunogiri, S., Crossin, R., Rizzo, D., Walker, L., Ridley, K., and Gurvich, C. (2021). A systematic review of the effect of ovarian sex hormones on stimulant use in females. *Addiction biology*, 26(6):e13079.

Back, S. E., Brady, K. T., Jackson, J. L., Salstrom, S., and Zinzow, H. (2005). Gender differences in stress reactivity among cocaine-dependent individuals. *Psychopharmacology*, 180(1):169–176.

Back, S. E., Hartwell, K., DeSantis, S. M., Saladin, M., McRae-Clark, A. L., Price, K. L., Moran-Santa Maria, M. M., Baker, N. L., Spratt, E., Kreek, M. J., and Brady, K. T. (2010). Reactivity to laboratory stress provocation predicts relapse to cocaine. *Drug and alcohol dependence*, 106(1):21–27.

Badiani, A. and Spagnolo, P. (2013). Role of Environmental Factors in Cocaine Addiction. *Current Pharmaceutical Design*, 19(40):6996–7008.

Baker, D. A., McFarland, K., Lake, R. W., Shen, H., Tang, X. C., Toda, S., and Kalivas, P. W. (2003). Neuroadaptations in cystine-glutamate exchange underlie cocaine relapse. *Nature Neuroscience*, 6(7):743–749.

Baki, L., Fribourg, M., Younkin, J., Eltit, J. M., Moreno, J. L., Park, G., Vysotskaya, Z., Narahari, A., Sealfon, S. C., Gonzalez-Maeso, J., and Logothetis, D. E. (2016). Cross-signaling in metabotropic glutamate 2 and serotonin 2A receptor heteromers in mammalian cells. *Pflugers Archiv : European journal of physiology*, 468(5):775–793.

Balleine, B. W. and O'Doherty, J. P. (2010). Human and rodent homologies in action control: corticostriatal determinants of goal-directed and habitual action. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(1):48–69.

Baptista, M. A. S., Martin-Fardon, R., and Weiss, F. (2004). Preferential Effects of the Metabotropic Glutamate 2/3 Receptor Agonist LY379268 on Conditioned Reinstatement

versus Primary Reinforcement: Comparison between Cocaine and a Potent Conventional Reinforcer. *The Journal of Neuroscience*, 24(20):4723–4727.

Barnett, G., Hawks, R., and Resnick, R. (1981). Cocaine pharmacokinetics in humans. *Journal of Ethnopharmacology*, 3(2):353–366.

Bassareo, V., Cucca, F., Frau, R., and Di Chiara, G. (2017). Changes in Dopamine Transmission in the Nucleus Accumbens Shell and Core during Ethanol and Sucrose Self-Administration. *Frontiers in Behavioral Neuroscience*, 11.

Becker, J. B. and Chartoff, E. (2019). Sex differences in neural mechanisms mediating reward and addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 44(1):166–183.

Becker, J. B. and Hu, M. (2008). Sex differences in drug abuse. *Frontiers in Neuroendocrinology*, 29(1):36–47.

Becker, J. B. and Koob, G. F. (2016). Sex Differences in Animal Models: Focus on Addiction. *Pharmacological Reviews*, 68(2):242–263.

Becker, J. B., McClellan, M. L., and Reed, B. G. (2017). Sex differences, gender and addiction. *Journal of Neuroscience Research*, 95(1-2):136–147.

Bedard, P. and Pycock, C. J. (1977). 'Wet-Dog' shake behaviour in the rat: A possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology*, 16(10):663–670.

Belin, D., Balado, E., Piazza, P. V., and Deroche-Gamonet, V. (2009). Pattern of Intake and Drug Craving Predict the Development of Cocaine Addiction-like Behavior in Rats. *Biological Psychiatry*, 65(10):863–868.

Belin, D. and Deroche-Gamonet, V. (2012). Responses to novelty and vulnerability to cocaine addiction: Contribution of a multi-symptomatic animal model. *Cold Spring Harbor Perspectives in Medicine*, 2(11).

Belin, D., Mar, A. C., Dalley, J. W., Robbins, T. W., and Everitt, B. J. (2008). High Impulsivity Predicts the Switch to Compulsive Cocaine-Taking. *Science*, 320(5881):1352–1355.

Bender, B. N. and Torregrossa, M. M. (2023). Intermittent cocaine self-administration has sex-specific effects on addiction-like behaviors in rats. *Neuropharmacology*, 230:109490.

Benowitz, N. L. (1993). Clinical Pharmacology and Toxicology of Cocaine. *Pharmacology & Toxicology*, 72(1):3–12.

Benvenuti, F., Colombo, D., Soverchia, L., Cannella, N., Domi, E., and Ciccocioppo, R. (2023). Psilocybin prevents reinstatement of alcohol seeking by disrupting the reconsolidation of alcohol-related memories. *Psychopharmacology*, 240(7):1521–1530.

Bernardi, R. E. and Lattal, K. M. (2012). Prazosin differentially affects extinction of cocaine conditioned place preference on the basis of dose and initial preference. *Neuroreport*, 23(18):1048–1051.

Bernardi, R. E., Olevska, A., Morella, I., Fasano, S., Santos, E., Brambilla, R., and Spanagel, R. (2019). The Inhibition of RasGRF2, But Not RasGRF1, Alters Cocaine Reward in Mice. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 39(32):6325–6338.

Bernardi, R. E. and Spanagel, R. (2014). Enhanced extinction of contextual fear conditioning in ClockΔ19 mutant mice. *Behavioral neuroscience*, 128(4):468–473.

Berridge, K. C. and Robinson, T. E. (1998). What is the role of dopamine in reward: hedonic impact, reward learning, or incentive salience? *Brain Research Reviews*, 28(3):309–369.

Berridge, K. C. and Robinson, T. E. (2016). Liking, wanting, and the incentive-sensitization theory of addiction. *American Psychologist*, 71(8):670–679.

Beveridge, T. J. R., Smith, H. R., Nader, M. A., and Porrino, L. J. (2011). Group II metabotropic glutamate receptors in the striatum of non-human primates: dysregulation following chronic cocaine self-administration. *Neuroscience letters*, 496(1):15–19.

Bierut, L. J., Strickland, J. R., Thompson, J. R., Afful, S. E., and Cottler, L. B. (2008). Drug use and dependence in cocaine dependent subjects, community-based individuals, and their siblings. *Drug and Alcohol Dependence*, 95(1-2):14–22.

Biondich, A. S. and Joslin, J. D. (2016). Coca: The History and Medical Significance of an Ancient Andean Tradition. *Emergency Medicine International*, 2016:1–5.

Blanca, M. J., Alarcón, R., Arnau, J., Bono, R., and Bendayan, R. (2017). Non-normal data: Is ANOVA still a valid option? *Psicothema*, 29(4):552–557.

Blanca, M. J., Arnau, J., García-Castro, F. J., Alarcón, R., and Bono, R. (2023). Non-normal Data in Repeated Measures ANOVA: Impact on Type I Error and Power. *Psicothema*, 35(1):21–29.

Bobzean, S. A. M., Dennis, T. S., Addison, B. D., and Perrotti, L. I. (2010). Influence of sex on reinstatement of cocaine-conditioned place preference. *Brain research bulletin*, 83(6):331–336.

Bogenschutz, M. P. and Johnson, M. W. (2016). Classic hallucinogens in the treatment of addictions. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 64:250–258.

Bogenschutz, M. P., Ross, S., Bhatt, S., Baron, T., Forcehimes, A. A., Laska, E., Mennenga, S. E., O'Donnell, K., Owens, L. T., Podrebarac, S., Rotrosen, J., Tonigan, J. S., and Worth, L. (2022). Percentage of Heavy Drinking Days Following Psilocybin-Assisted Psychotherapy vs Placebo in the Treatment of Adult Patients With Alcohol Use Disorder. *JAMA Psychiatry*, 79(10):953.

Bolles, R. C. (1972). Reinforcement, expectancy, and learning. *Psychological Review*, 79(5):394–409.

Bossé, R., Fumagalli, F., Jaber, M., Giros, B., Gainetdinov, R. R., Wetsel, W. C., Missale, C., and Caron, M. G. (1997). Anterior Pituitary Hypoplasia and Dwarfism in Mice Lacking the Dopamine Transporter. *Neuron*, 19(1):127–138.

Bossert, J. M., Ghitza, U. E., Lu, L., Epstein, D. H., and Shaham, Y. (2005). Neurobiology of relapse to heroin and cocaine seeking: An update and clinical implications. *European Journal of Pharmacology*, 526(1-3):36–50.

Bossert, J. M., Marchant, N. J., Calu, D. J., and Shaham, Y. (2013). The reinstatement model of drug relapse: Recent neurobiological findings, emerging research topics, and translational research. *Psychopharmacology*, 229(3):453–476.

Brady, K. T., Lydiard, R. B., Malcolm, R., and Ballenger, J. C. (1991). Cocaine-induced psychosis. *The Journal of clinical psychiatry*, 52(12):509–12.

Brady, K. T. and Randall, C. L. (1999). Gender differences in substance use disorders. *The Psychiatric clinics of North America*, 22(2):241–252.

Brandt, L., Chao, T., Comer, S. D., and Levin, F. R. (2021). Pharmacotherapeutic strategies for treating cocaine use disorder—what do we have to offer? *Addiction*, 116(4):694–710.

Bressan, R. A. and Crippa, J. A. (2005). The role of dopamine in reward and pleasure behaviour–review of data from preclinical research. *Acta psychiatrica Scandinavica. Supplementum*, (427):14–21.

Buchholz, J. and Saxon, A. J. (2019). Medications to treat cocaine use disorders: Current options. *Current Opinion in Psychiatry*, 32(4):275–281.

Bunzow, J. R., Tol, H. H. M. V., Grandy, D. K., Albert, P., Salon, J., Christie, M., Machida, C. A., Neve, K. A., and Civelli, O. (1988). Cloning and expression of a rat D2 dopamine receptor cDNA. *Nature*, 336(6201):783–787.

Busquets-Garcia, A., Oliveira da Cruz, J. F., Terral, G., Pagano Zottola, A. C., Soria-Gómez, E., Contini, A., Martin, H., Redon, B., Varilh, M., Ioannidou, C., Drago, F., Massa, F., Fioramonti, X., Trifilieff, P., Ferreira, G., and Marsicano, G. (2018). Hippocampal CB1 Receptors Control Incidental Associations. *Neuron*, 99(6):1247–1259.

Busquets-Garcia, A., Soria-Gómez, E., Ferreira, G., and Marsicano, G. (2017a). Representation-mediated Aversion as a Model to Study Psychotic-like States in Mice. *Bio-Protocol*, 7(12).

Busquets-Garcia, A., Soria-Gómez, E., Redon, B., Mackenbach, Y., Vallée, M., Chaouloff, F., Varilh, M., Ferreira, G., Piazza, P.-V., and Marsicano, G. (2017b). Pregnenolone blocks cannabinoid-induced acute psychotic-like states in mice. *Molecular Psychiatry*, 22(11):1594–1603.

Cabana-Domínguez, J., Shivalikanjli, A., Fernàndez-Castillo, N., and Cormand, B. (2019). Genome-wide association meta-analysis of cocaine dependence: Shared genetics with comorbid conditions. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 94:109667.

Cailhol, S. and Mormède, P. (1999). Strain and sex differences in the locomotor response and behavioral sensitization to cocaine in hyperactive rats. *Brain Research*, 842(1):200–205.

Cameron, L. P., Benson, C. J., Defelice, B. C., Fiehn, O., and Olson, D. E. (2019). Chronic, Intermittent Microdoses of the Psychedelic N, N-Dimethyltryptamine (DMT) Produce Positive Effects on Mood and Anxiety in Rodents. *ACS Chemical Neuroscience*, 10(7):3261–3270.

Cannella, N., Cosa-Linan, A., Roscher, M., Takahashi, T. T., Vogler, N., Wängler, B., and Spanagel, R. (2017). [18F]-Fluorodeoxyglucose-positron emission tomography in rats with prolonged cocaine self-administration suggests potential brain biomarkers for addictive behavior. *Frontiers in Psychiatry*, 8(NOV):1–14.

Cannella, N., Halbout, B., Uhrig, S., Evrard, L., Corsi, M., Corti, C., Deroche-Gamonet, V., Hansson, A. C., and Spanagel, R. (2013). The mGluR2/3 agonist LY379268 induced

anti-reinstatement effects in rats exhibiting addiction-like behavior. *Neuropsychopharmacology*, 38(10):2048–2056.

Capaldi, E. D., Hunter, M. J., and Privitera, G. J. (2004). Odor of taste stimuli in conditioned ”taste” aversion learning. *Behavioral neuroscience*, 118(6):1400–1408.

Carboni, E., Spielewoy, C., Vacca, C., Nosten-Bertrand, M., Giros, B., and Di Chiara, G. (2001). Cocaine and Amphetamine Increase Extracellular Dopamine in the Nucleus Accumbens of Mice Lacking the Dopamine Transporter Gene. *Journal of Neuroscience*, 21(9):RC141–RC141.

Cardoso, F. E. C. and Jankovic, J. (1993). Cocaine□related movement disorders. *Movement Disorders*, 8(2):175–178.

Carhart-Harris, R., Giribaldi, B., Watts, R., Baker-Jones, M., Murphy-Beiner, A., Murphy, R., Martell, J., Blemings, A., Erritzoe, D., and Nutt, D. J. (2021). Trial of Psilocybin versus Escitalopram for Depression. *New England Journal of Medicine*, 384(15):1402–1411.

Carhart-Harris, R. L., Bolstridge, M., Day, C. M. J., Rucker, J., Watts, R., Erritzoe, D. E., Kaelen, M., Giribaldi, B., Bloomfield, M., Pilling, S., Rickard, J. A., Forbes, B., Feilding, A., Taylor, D., Curran, H. V., and Nutt, D. J. (2018). Psilocybin with psychological support for treatment-resistant depression: six-month follow-up. *Psychopharmacology*, 235(2):399–408.

Carlsson, A. (1959). The occurrence, distribution and physiological role of catecholamines in the nervous system. *Pharmacological reviews*, 11(2 , Part 2):490–493.

Carlsson, A., Lindqvist, M., Magnusson, T., and Waldeck, B. (1958). On the presence of 3-hydroxytyramine in brain. *Science*, 127(3296):471.

Carpenter, A. C., Saborido, T. P., and Stanwood, G. D. (2012). Development of hyperactivity and anxiety responses in dopamine transporter-deficient mice. *Developmental neuroscience*, 34(2-3):250–257.

Carroll, K. M., Ball, S. A., Martino, S., Nich, C., Babuscio, T. A., Nuro, K. F., Gordon, M. A., Portnoy, G. A., and Rounsaville, B. J. (2008). Computer-assisted delivery of cognitive-behavioral therapy for addiction: A randomized trial of CBT4CBT. *American Journal of Psychiatry*, 165(7):881–888.

Carroll, K. M., Kiluk, B. D., Nich, C., Gordon, M. A., Portnoy, G. A., Marino, D. R., and Ball, S. A. (2014). Computer-assisted delivery of cognitive-behavioral therapy:

Efficacy and durability of cbt4cbt among cocaine-dependent individuals maintained on methadone. *American Journal of Psychiatry*, 171(4):436–444.

Carroll, M., Morgan, A., Lynch, W., Campbell, U., and Dess, N. (2002). Intravenous cocaine and heroin self-administration in rats selectively bred for differential saccharin intake: phenotype and sex differences. *Psychopharmacology*, 161(3):304–313.

Carroll, M. E. and Lynch, W. J. (2016). How to study sex differences in addiction using animal models. *Addiction biology*, 21(5):1007–1029.

Catlow, B. J., Song, S., Paredes, D. A., Kirstein, C. L., and Sanchez-Ramos, J. (2013). Effects of psilocybin on hippocampal neurogenesis and extinction of trace fear conditioning. *Experimental Brain Research*, 228(4):481–491.

Center for Substance Abuse Treatment (2009). *Substance Abuse Treatment: Addressing the Specific Needs of Women. Report No.: (SMA) 09-4426. SAMHSA/CSAT Treatment Improvement Protocols.*

Chambers, K. C. (2018). Conditioned taste aversions. *World journal of otorhinolaryngology - head and neck surgery*, 4(1):92–100.

Chan, B., Kondo, K., Freeman, M., Ayers, C., Montgomery, J., and Kansagara, D. (2019). Pharmacotherapy for Cocaine Use Disorder—a Systematic Review and Meta-analysis. *Journal of General Internal Medicine*, 34(12):2858–2873.

Chen, K. and Kandel, D. (2002). Relationship between extent of cocaine use and dependence among adolescents and adults in the United States. *Drug and Alcohol Dependence*, 68(1):65–85.

Cherkasova, M. V., Clark, L., Barton, J. J. S., Stoessl, A. J., and Winstanley, C. A. (2024). Risk-promoting effects of reward-paired cues in human sign- and goal-trackers. *Behavioural brain research*, 461:114865.

Childress, A. R., Mozley, P. D., McElgin, W., Fitzgerald, J., Reivich, M., and O'Brien, C. P. (1999). Limbic Activation During Cue-Induced Cocaine Craving. *American Journal of Psychiatry*, 156(1):11–18.

Chin, J., Sternin, O., Wu, H. B., Fletcher, H., Perrotti, L. I., Jenab, S., and Quiñones-Jenab, V. (2001). Sex differences in cocaine-induced behavioral sensitization. *Cellular and molecular biology (Noisy-le-Grand, France)*, 47(6):1089–1095.

Cinque, S., Zoratto, F., Poleggi, A., Leo, D., Cerniglia, L., Cimino, S., Tambelli, R., Alleva, E., Gainetdinov, R. R., Laviola, G., and Adriani, W. (2018). Behavioral Phenotyping

of Dopamine Transporter Knockout Rats: Compulsive Traits, Motor Stereotypies, and Anhedonia. *Frontiers in Psychiatry*, 9.

Coe, M. A., Jufer Phipps, R. A., Cone, E. J., and Walsh, S. L. (2018). Bioavailability and Pharmacokinetics of Oral Cocaine in Humans. *Journal of Analytical Toxicology*, 42(5):285–292.

Colaizzi, J. M., Flagel, S. B., Gearhardt, A. N., Borowitz, M. A., Kuplicki, R., Zotev, V., Clark, G., Coronado, J., Abbott, T., and Paulus, M. P. (2023). The propensity to sign-track is associated with externalizing behavior and distinct patterns of reward-related brain activation in youth. *Scientific Reports*, 13(1):4402.

Colaizzi, J. M., Flagel, S. B., Joyner, M. A., Gearhardt, A. N., Stewart, J. L., and Paulus, M. P. (2020). Mapping sign-tracking and goal-tracking onto human behaviors. *Neuroscience and Biobehavioral Reviews*, 111:84–94.

Collins, H. M. (2024). Psychedelics for the Treatment of Obsessive-Compulsive Disorder: Efficacy and Proposed Mechanisms. *The international journal of neuropsychopharmacology*, 27(12).

Cone, E. J. (1995). Pharmacokinetics and pharmacodynamics of cocaine. *Journal of Analytical Toxicology*, 19(6):459–478.

Cope, L. M., Gheidi, A., Martz, M. E., Duval, E. R., Khalil, H., Allerton, T., and Morrow, J. D. (2023). A mechanical task for measuring sign- and goal-tracking in humans: A proof-of-concept study. *Behavioural Brain Research*, 436:114112.

Corbit, L. H., Chieng, B. C., and Balleine, B. W. (2014). Effects of Repeated Cocaine Exposure on Habit Learning and Reversal by N-Acetylcysteine. *Neuropsychopharmacology*, 39(8):1893–1901.

Cornish, J. W. and O'Brien, C. P. (1996). Crack cocaine abuse: an epidemic with many public health consequences. *Annual review of public health*, 17:259–273.

Costa, K. M. and Schoenbaum, G. (2022). Dopamine. *Current Biology*, 32(15):R817–R824.

Crombag, H. S. and Shaham, Y. (2002). Renewal of drug seeking by contextual cues after prolonged extinction in rats. *Behavioral Neuroscience*, 116(1):169–173.

Cunha-Oliveira, T., Rego, A. C., Carvalho, F., and Oliveira, C. R. (2013). Medical Toxicology of Drugs of Abuse. In *Principles of Addiction: Comprehensive Addictive Behaviors and Disorders, Volume 1*, pages 159–175. Elsevier.

Currie, S. R., Patten, S. B., Williams, J. V., Wang, J., Beck, C. A., El-Guebaly, N., and Maxwell, C. (2005). Comorbidity of Major Depression with Substance Use Disorders. *The Canadian Journal of Psychiatry*, 50(10):660–666.

Daras, M., Tuchman, A. J., Koppel, B. S., Samkoff, L. M., Weitzner, I., and Marc, J. (1994). Neurovascular complications of cocaine. *Acta Neurologica Scandinavica*, 90(2):124–129.

Davis, A. K., Barrett, F. S., May, D. G., Cosimano, M. P., Sepeda, N. D., Johnson, M. W., Finan, P. H., and Griffiths, R. R. (2021). Effects of Psilocybin-Assisted Therapy on Major Depressive Disorder. *JAMA Psychiatry*, 78(5):481–489.

Daws, R. E., Timmermann, C., Giribaldi, B., Sexton, J. D., Wall, M. B., Erritzoe, D., Roseman, L., Nutt, D., and Carhart-Harris, R. (2022). Increased global integration in the brain after psilocybin therapy for depression. *Nature medicine*, 28(4):844–851.

de Guglielmo, G., Carrette, L. L. G., Kallupi, M., Brennan, M., Boomhower, B., Maturin, L., Conlisk, D., Sedighim, S., Tieu, L., Fannon, M. J., Martinez, A. R., Velarde, N., Othman, D., Sichel, B., Ramborger, J., Lau, J., Kononoff, J., Kimbrough, A., Simpson, S., Smith, L. C., Shankar, K., Bonnet-Zahedi, S., Sneddon, E. A., Avelar, A., Plasil, S. L., Mosquera, J., Crook, C., Chun, L., Vang, A., Milan, K. K., Schweitzer, P., Lin, B., Peng, B., Chitre, A., Polleskaya, O., Solberg Woods, L. C., Palmer, A. A., and George, O. (2024). Large-scale characterization of cocaine addiction-like behaviors reveals that escalation of intake, aversion-resistant responding, and breaking-points are highly correlated measures of the same construct.

de Wit, H. (1996). Priming effects with drugs and other reinforcers. *Experimental and Clinical Psychopharmacology*, 4(1):5–10.

Dearry, A., Gingrich, J. A., Falardeau, P., Fremeau, R. T., Bates, M. D., and Caron, M. G. (1990). Molecular cloning and expression of the gene for a human D1 dopamine receptor. *Nature*, 347(6288):72–76.

Deneau, G., Yanagita, T., and Seevers, M. H. (1969). Self-administration of psychoactive substances by the monkey. *Psychopharmacologia*, 16(1):30–48.

Deroche-Gamonet, V., Belin, D., and Piazza, P. V. (2004). Evidence for addiction-like behavior in the rat. *Science*, 305(5686):1014–1017.

Deroche-Gamonet, V. and Piazza, P. V. (2014). Psychobiology of cocaine addiction: Contribution of a multi-symptomatic animal model of loss of control. *Neuropharmacology*, 76:437–449.

Di Chiara, G. (1999). Drug addiction as dopamine-dependent associative learning disorder. *European journal of pharmacology*, 375(1-3):13–30.

Di Chiara, G. and 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(14):5274–5278.

Dillehay, T. D., Rossen, J., Ugent, D., Karathanasis, A., Vásquez, V., and Netherly, P. J. (2010). Early Holocene coca chewing in northern Peru. *Antiquity*, 84(326):939–953.

Dinu, L.-M., Georgescu, A.-L., Singh, S. N., Byrom, N. C., Overton, P. G., Singer, B. F., and Dommett, E. J. (2024). Sign-tracking and goal-tracking in humans: Utilising eye-tracking in clinical and non-clinical populations. *Behavioural brain research*, 461:114846.

Domi, A., Cadeddu, D., Lucente, E., Gobbo, F., Edvardsson, C., Petrella, M., Jerlhag, E., Ericson, M., Söderpalm, B., and Adermark, L. (2024). Pre- and postsynaptic signatures in the prelimbic cortex associated with "alcohol use disorder" in the rat. *Neuropsychopharmacology*, 49(12):1851–1860.

Domi, A., Stopponi, S., Domi, E., Ciccocioppo, R., and Cannella, N. (2019). Subdimensions of Alcohol Use Disorder in Alcohol Preferring and Non-preferring Rats, a Comparative Study. *Frontiers in behavioral neuroscience*, 13:3.

Doss, M. K., Považan, M., Rosenberg, M. D., Sepeda, N. D., Davis, A. K., Finan, P. H., Smith, G. S., Pekar, J. J., Barker, P. B., Griffiths, R. R., and Barrett, F. S. (2021). Psilocybin therapy increases cognitive and neural flexibility in patients with major depressive disorder. *Translational psychiatry*, 11(1):574.

Doyle, S. E., Ramôa, C., Garber, G., Newman, J., Toor, Z., and Lynch, W. J. (2014). A shift in the role of glutamatergic signaling in the nucleus accumbens core with the development of an addicted phenotype. *Biological psychiatry*, 76(10):810–815.

Drake, L. R. and Scott, P. J. (2018). DARK Classics in Chemical Neuroscience: Cocaine. *ACS Chemical Neuroscience*, 9(10):2358–2372.

Dreher, J.-C., Kohn, P., Kolachana, B., Weinberger, D. R., and Berman, K. F. (2009). Variation in dopamine genes influences responsivity of the human reward system. *Proceedings of the National Academy of Sciences*, 106(2):617–622.

D'Souza, M. S. (2015). Glutamatergic transmission in drug reward: implications for drug addiction. *Frontiers in Neuroscience*, 9.

Du, Y., Li, Y., Zhao, X., Yao, Y., Wang, B., Zhang, L., and Wang, G. (2023). Psilocybin facilitates fear extinction in mice by promoting hippocampal neuroplasticity. *Chinese Medical Journal*, 136(24):2983–2992.

Ducci, F. and Goldman, D. (2012). The Genetic Basis of Addictive Disorders. *Psychiatric Clinics of North America*, 35(2):495–519.

Edinoff, A. N., Sall, S., Roberts, T. D., Tomlinson, H. H., Soileau, L. G., Jackson, E. D., Murnane, K. S., Wenger, D. M., Cornett, E. M., Toms, J., Kumbhare, D., Kaye, A. M., and Kaye, A. D. (2023). Transcranial Stimulation for the Treatment of Stimulant Use Disorder. *Neurology International*, 15(1):325–338.

Efimova, E. V., Gainetdinov, R. R., Budygin, E. A., and Sotnikova, T. D. (2016). Dopamine transporter mutant animals: a translational perspective. *Journal of neurogenetics*, 30(1):5–15.

Ellis, S., Bostian, C., Donnelly, A., Feng, W., Eisen, K., Lean, M., Conlan, E., Ostacher, M., Aaronson, S., and Suppes, T. (2025). Long-term outcomes of single-dose psilocybin for U.S. military Veterans with severe treatment-resistant depression - 12-month data from an open-label pilot study. *Journal of affective disorders*, page 119655.

Ellison, G. (1994). Stimulant-induced psychosis, the dopamine theory of schizophrenia, and the habenula. *Brain Research Reviews*, 19(2):223–239.

Elman, I., Karlsgodt, K. H., and Gastfriend, D. R. (2001). Gender differences in cocaine craving among non-treatment-seeking individuals with cocaine dependence. *American Journal of Drug and Alcohol Abuse*, 27(2):193–202.

Enkel, T., Bartsch, D., and Bähner, F. (2019). Sign- and goal-tracking rats show differences in various executive functions: Authors. *Behavioural Brain Research*, 371:111979.

Epstein, D. H., Preston, K. L., Stewart, J., and Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. *Psychopharmacology*, 189(1):1–16.

European drug report (2021). *European drug report 2021 – Trends and developments*. Publications Office of the European Union.

Evans, S. M. (2007). The role of estradiol and progesterone in modulating the subjective effects of stimulants in humans. *Experimental and clinical psychopharmacology*, 15(5):418–426.

Evans, S. M. and Foltin, R. W. (2006). Exogenous Progesterone Attenuates the Subjective Effects of Smoked Cocaine in Women, but not in Men. *Neuropsychopharmacology*, 31(3):659–674.

Everett, N. A., Carey, H. A., Cornish, J. L., and Baracz, S. J. (2020). Sign tracking predicts cue-induced but not drug-primed reinstatement to methamphetamine seeking in rats: Effects of oxytocin treatment. *Journal of psychopharmacology (Oxford, England)*, 34(11):1271–1279.

Everitt, B. J. and Robbins, T. W. (2005). Neural systems of reinforcement for drug addiction: From actions to habits to compulsion. *Nature Neuroscience*, 8(11):1481–1489.

Everitt, B. J. and Robbins, T. W. (2016). Drug Addiction: Updating Actions to Habits to Compulsions Ten Years On. *Annual Review of Psychology*, 67(1):23–50.

Fabian, C. B., Seney, M. L., and Joffe, M. E. (2023). Chapter Seven - Sex differences and hormonal regulation of metabotropic glutamate receptor synaptic plasticity. In Knackstedt, L. A. and Schwendt, M., editors, *Metabotropic Glutamate Receptors in Psychiatric and Neurological Disorders*, volume 168 of *International Review of Neurobiology*, pages 311–347. Academic Press.

Fattore, L., Melis, M., Fadda, P., and Fratta, W. (2014). Sex differences in addictive disorders. *Frontiers in Neuroendocrinology*, 35(3):272–284.

Feltenstein, M. W., Byrd, E. A., Henderson, A. R., and See, R. E. (2009). Attenuation of cocaine-seeking by progesterone treatment in female rats. *Psychoneuroendocrinology*, 34(3):343–352.

Feltenstein, M. W. and See, R. E. (2007). Plasma progesterone levels and cocaine-seeking in freely cycling female rats across the estrous cycle. *Drug and alcohol dependence*, 89(2-3):183–189.

Fernàndez-Castillo, N., Cabana-Domínguez, J., Corominas, R., and Cormand, B. (2022). Molecular genetics of cocaine use disorders in humans. *Molecular Psychiatry*, 27(1):624–639.

Ferraguti, F. and Shigemoto, R. (2006). Metabotropic glutamate receptors. *Cell and tissue research*, 326(2):483–504.

Festa, E. D., Jenab, S., Chin, J., Gazi, F. M., Wu, H. B. K., Russo, S. J., and Quinones-Jenab, V. (2003). Frequency of cocaine administration affects behavioral and endocrine responses in male and female Fischer rats. *Cellular and molecular biology (Noisy-le-Grand, France)*, 49(8):1275–1280.

Festa, E. D., Russo, S. J., Gazi, F. M., Niyomchai, T., Kemen, L. M., Lin, S.-N., Foltz, R., Jenab, S., and Quinones-Jenab, V. (2004). Sex differences in cocaine-induced behavioral responses, pharmacokinetics, and monoamine levels. *Neuropharmacology*, 46(5):672–687.

Fitzpatrick, C. J., Gopalakrishnan, S., Cogan, E. S., Yager, L. M., Meyer, P. J., Lovic, V., Saunders, B. T., Parker, C. C., Gonzales, N. M., Aryee, E., Flagel, S. B., Palmer, A. A., Robinson, T. E., and Morrow, J. D. (2013). Variation in the form of Pavlovian conditioned approach behavior among outbred male Sprague-Dawley rats from different vendors and colonies: sign-tracking vs. goal-tracking. *PloS one*, 8(10):e75042.

Fitzpatrick, C. J. and Morrow, J. D. (2016). Pavlovian Conditioned Approach Training in Rats. *Journal of visualized experiments : JoVE*, (108):e53580.

Flagel, S. B., Akil, H., and Robinson, T. E. (2009). Individual differences in the attribution of incentive salience to reward-related cues: Implications for addiction. *Neuropharmacology*, 56(SUPPL. 1):139–148.

Flagel, S. B. and Robinson, T. E. (2017). Neurobiological Basis of Individual Variation in Stimulus-Reward Learning. *Current opinion in behavioral sciences*, 13:178–185.

Flagel, S. B., Robinson, T. E., Clark, J. J., Clinton, S. M., Watson, S. J., Seeman, P., Phillips, P. E. M., and Akil, H. (2010). An animal model of genetic vulnerability to behavioral disinhibition and responsiveness to reward-related cues: implications for addiction. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 35(2):388–400.

Fox, H. C., Sofuoglu, M., Morgan, P. T., Tuit, K. L., and Sinha, R. (2013). The effects of exogenous progesterone on drug craving and stress arousal in cocaine dependence: impact of gender and cue type. *Psychoneuroendocrinology*, 38(9):1532–1544.

Freud, S. (1885). Ueber Coca [On Coca]. *Zentralbl Ges Ther*, 2:289–314.

Fribourg, M., Moreno, J. L., Holloway, T., Provasi, D., Baki, L., Mahajan, R., Park, G., Adney, S. K., Hatcher, C., Eltit, J. M., Ruta, J. D., Albizu, L., Li, Z., Umali, A., Shim, J., Fabiato, A., MacKerell, A. D. J., Brezina, V., Sealfon, S. C., Filizola, M., González-Maeso, J., and Logothetis, D. E. (2011). Decoding the signaling of a GPCR heteromeric complex reveals a unifying mechanism of action of antipsychotic drugs. *Cell*, 147(5):1011–1023.

Fry, B. R., Russell, N., Gifford, R., Robles, C. F., Manning, C. E., Sawa, A., Niwa, M., and Johnson, A. W. (2020). Assessing Reality Testing in Mice Through Dopamine-Dependent

Associatively Evoked Processing of Absent Gustatory Stimuli. *Schizophrenia bulletin*, 46(1):54–67.

Fuchs, R. A., Branham, R. K., and See, R. E. (2006). Different neural substrates mediate cocaine seeking after abstinence versus extinction training: a critical role for the dorsolateral caudate-putamen. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(13):3584–3588.

Fuchs, R. A., Evans, K. A., Ledford, C. C., Parker, M. P., Case, J. M., Mehta, R. H., and See, R. E. (2005). The role of the dorsomedial prefrontal cortex, basolateral amygdala, and dorsal hippocampus in contextual reinstatement of cocaine seeking in rats. *Neuropsychopharmacology*, 30(2):296–309.

Gainetdinov, R. R. and Caron, M. G. (2000). An animal model of attention deficit hyperactivity disorder. *Molecular medicine today*, 6(1):43–44.

Gainetdinov, R. R., Jones, S. R., and Caron, M. G. (1999a). Functional hyperdopaminergia in dopamine transporter knock-out mice. *Biological Psychiatry*, 46(3):303–311.

Gainetdinov, R. R., Jones, S. R., Fumagalli, F., Wightman, R. M., and Caron, M. G. (1998). Re-evaluation of the role of the dopamine transporter in dopamine system homeostasis. *Brain research. Brain research reviews*, 26(2-3):148–153.

Gainetdinov, R. R., Wetsel, W. C., Jones, S. R., Levin, E. D., Jaber, M., and Caron, M. G. (1999b). Role of serotonin in the paradoxical calming effect of psychostimulants on hyperactivity. *Science (New York, N.Y.)*, 283(5400):397–401.

Galici, R., Echemendia, N. G., Rodriguez, A. L., and Conn, P. J. (2005). A selective allosteric potentiator of metabotropic glutamate (mGlu) 2 receptors has effects similar to an orthosteric mGlu2/3 receptor agonist in mouse models predictive of antipsychotic activity. *The Journal of pharmacology and experimental therapeutics*, 315(3):1181–1187.

Gallop, R. J., Crits-Christoph, P., Ten Have, T. R., Barber, J. P., Frank, A., Griffin, M. L., and Thase, M. E. (2007). Differential transitions between cocaine use and abstinence for men and women. *Journal of Consulting and Clinical Psychology*, 75(1):95–103.

Garcia, J. and Koelling, R. A. (1966). Relation of cue to consequence in avoidance learning. *Psychonomic Science*, 4(1):123–124.

Garcia-Romeu, A., Davis, A. K., Erowid, F., Erowid, E., Griffiths, R. R., and Johnson, M. W. (2019). Cessation and reduction in alcohol consumption and misuse after psychedelic use. *Journal of Psychopharmacology*, 33(9):1088–1101.

Garcia-Romeu, A., Griffiths, R. R., and Johnson, M. W. (2015). Psilocybin-Occasioned Mystical Experiences in the Treatment of Tobacco Addiction. *Current Drug Abuse Reviews*, 7(3):157–164.

Garofalo, S. and di Pellegrino, G. (2015). Individual differences in the influence of task-irrelevant Pavlovian cues on human behavior. *Frontiers in behavioral neuroscience*, 9:163.

Gass, J. T. and Chandler, L. J. (2013). The Plasticity of Extinction: Contribution of the Prefrontal Cortex in Treating Addiction through Inhibitory Learning. *Frontiers in psychiatry*, 4:46.

Gay, G. R., Inaba, D. S., Sheppard, C. W., Newmeyer, J. A., and Rappolt, R. T. (1975). Cocaine: History, epidemiology, human pharmacology, and treatment. A perspective on a new debut for an old girl. *Clinical Toxicology*, 8(2):149–178.

Gewirtz, J. C. and Marek, G. J. (2000). Behavioral evidence for interactions between a hallucinogenic drug and group II metabotropic glutamate receptors. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 23(5):569–576.

Geyer, M. A., Krebs-Thomson, K., Braff, D. L., and Swerdlow, N. R. (2001). Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology*, 156(2-3):117–154.

Ghasemzadeh, M. B., Mueller, C., and Vasudevan, P. (2009). Behavioral sensitization to cocaine is associated with increased glutamate receptor trafficking to the postsynaptic density after extended withdrawal period. *Neuroscience*, 159(1):414–426.

Giros, B., Jaber, M., Jones, S. R., Wightman, R. M., and Caron, M. G. (1996). Hyperlocomotion and indifference to cocaine and amphetamine in mice lacking the dopamine transporter. *Nature*, 379(6566):606–612.

Gläscher, J., Daw, N., Dayan, P., and O'Doherty, J. P. (2010). States versus rewards: dissociable neural prediction error signals underlying model-based and model-free reinforcement learning. *Neuron*, 66(4):585–595.

Golden, C. T. and Chadderton, P. (2022). Psilocybin reduces low frequency oscillatory power and neuronal phase-locking in the anterior cingulate cortex of awake rodents. *Scientific reports*, 12(1):12702.

Goldstein, R. A., DesLauriers, C., and Burda, A. M. (2009). Cocaine: History, Social Implications, and Toxicity—A Review. *Disease-a-Month*, 55(1):6–38.

Goldstein, R. Z. and Volkow, N. D. (2011). Dysfunction of the prefrontal cortex in addiction: neuroimaging findings and clinical implications. *Nature Reviews Neuroscience*, 12(11):652–669.

González-Maeso, J., Weisstaub, N. V., Zhou, M., Chan, P., Ivic, L., Ang, R., Lira, A., Bradley-Moore, M., Ge, Y., Zhou, Q., Sealfon, S. C., and Gingrich, J. A. (2007). Hallucinogens recruit specific cortical 5-HT(2A) receptor-mediated signaling pathways to affect behavior. *Neuron*, 53(3):439–452.

Goodwin, G. M., Aaronson, S. T., Alvarez, O., Arden, P. C., Baker, A., Bennett, J. C., Bird, C., Blom, R. E., Brennan, C., Brusch, D., Burke, L., Campbell-Coker, K., Carhart-Harris, R., Cattell, J., Daniel, A., DeBattista, C., Dunlop, B. W., Eisen, K., Feifel, D., Forbes, M., Haumann, H. M., Hellerstein, D. J., Hoppe, A. I., Husain, M. I., Jelen, L. A., Kamphuis, J., Kawasaki, J., Kelly, J. R., Key, R. E., Kishon, R., Knatz Peck, S., Knight, G., Koolen, M. H., Lean, M., Licht, R. W., Maples-Keller, J. L., Mars, J., Marwood, L., McElhiney, M. C., Miller, T. L., Mirow, A., Mistry, S., Mletzko-Crowe, T., Modlin, L. N., Nielsen, R. E., Nielson, E. M., Offerhaus, S. R., O'Keane, V., Páleníček, T., Printz, D., Rademaker, M. C., van Reemst, A., Reinholdt, F., Repantis, D., Rucker, J., Rudow, S., Ruffell, S., Rush, A. J., Schoevers, R. A., Seynaeve, M., Shao, S., Soares, J. C., Somers, M., Stansfield, S. C., Sterling, D., Strockis, A., Tsai, J., Visser, L., Wahba, M., Williams, S., Young, A. H., Ywema, P., Zisook, S., and Malievskaia, E. (2022). Single-Dose Psilocybin for a Treatment-Resistant Episode of Major Depression. *New England Journal of Medicine*, 387(18):1637–1648.

Gorelick, P. B. and Zych, D. (1985). Corning, Cocaine, and Local Anesthesia. *Archives of Ophthalmology*, 103(3):325–325.

Gossop, M., Griffiths, P., Powis, B., and Strang, J. (1994). Cocaine: Patterns of Use, Route of Administration, and Severity of Dependence. *British Journal of Psychiatry*, 164(5):660–664.

Grace, A. (1991). Phasic versus tonic dopamine release and the modulation of dopamine system responsivity: A hypothesis for the etiology of schizophrenia. *Neuroscience*, 41(1):1–24.

Grace, A. A. (2016). Dysregulation of the dopamine system in the pathophysiology of schizophrenia and depression. *Nature reviews. Neuroscience*, 17(8):524–532.

Grandy, D. K., Zhang, Y. A., Bouvier, C., Zhou, Q. Y., Johnson, R. A., Allen, L., Buck, K., Bunzow, J. R., Salon, J., and Civelli, O. (1991). Multiple human D5 dopamine receptor genes: a functional receptor and two pseudogenes. *Proceedings of the National Academy of Sciences*, 88(20):9175–9179.

Grant, B. F., Saha, T. D., Ruan, W. J., Goldstein, R. B., Chou, S. P., Jung, J., Zhang, H., Smith, S. M., Pickering, R. P., Huang, B., and Hasin, D. S. (2016). Epidemiology of DSM-5 Drug Use Disorder: Results From the National Epidemiologic Survey on Alcohol and Related Conditions–III. *JAMA Psychiatry*, 73(1):39–47.

Greenfield, S. F., Back, S. E., Lawson, K., and Brady, K. T. (2010). Substance abuse in women. *The Psychiatric clinics of North America*, 33(2):339–355.

Greenslade, R. G. and Mitchell, S. N. (2004). Selective action of (−)-2-oxa-4-aminobicyclo[3.1.0]hexane-4,6-dicarboxylate (LY379268), a group II metabotropic glutamate receptor agonist, on basal and phencyclidine-induced dopamine release in the nucleus accumbens shell. *Neuropharmacology*, 47(1):1–8.

Griffiths, R. R., Johnson, M. W., Carducci, M. A., Umbricht, A., Richards, W. A., Richards, B. D., Cosimano, M. P., and Klinedinst, M. A. (2016). Psilocybin produces substantial and sustained decreases in depression and anxiety in patients with life-threatening cancer: A randomized double-blind trial. *Journal of Psychopharmacology*, 30(12):1181–1197.

Guercio, L. A., Wimmer, M. E., Schmidt, H. D., Swinford-Jackson, S. E., Pierce, R. C., and Vassoler, F. M. (2020). Deep brain stimulation of the infralimbic cortex attenuates cocaine priming-induced reinstatement of drug seeking. *Brain research*, 1746:147011.

Haas, A. L. and Peters, R. H. (2000). Development of substance abuse problems among drug-involved offenders. Evidence for the telescoping effect. *Journal of substance abuse*, 12(3):241–253.

Hadar, A., David, J., Shalit, N., Roseman, L., Gross, R., Sessa, B., and Lev-Ran, S. (2023). The Psychedelic Renaissance in Clinical Research: A Bibliometric Analysis of Three Decades of Human Studies with Psychedelics. *Journal of Psychoactive Drugs*, 55(1):1–10.

Hagsäter, S. M., Pettersson, R., Pettersson, C., Atanasovski, D., Näslund, J., and Eriksson, E. (2021). A Complex Impact of Systemically Administered 5-HT2A Receptor Ligands on Conditioned Fear. *The international journal of neuropsychopharmacology*, 24(9):749–757.

Hajnal, A., Smith, G. P., and Norgren, R. (2004). Oral sucrose stimulation increases accumbens dopamine in the rat. *American journal of physiology. Regulatory, integrative and comparative physiology*, 286(1):31–7.

Halberstadt, A. L. (2015). Recent advances in the neuropsychopharmacology of serotonergic hallucinogens. *Behavioural brain research*, 277:99–120.

Halberstadt, A. L. and Geyer, M. A. (2011). Multiple receptors contribute to the behavioral effects of indoleamine hallucinogens. *Neuropharmacology*, 61(3):364–381.

Halberstadt, A. L. and Geyer, M. A. (2013). Characterization of the head-twitch response induced by hallucinogens in mice: detection of the behavior based on the dynamics of head movement. *Psychopharmacology*, 227(4):727–739.

Hall, F. S., Li, X.-F., Randall-Thompson, J., Sora, I., Murphy, D. L., Lesch, K.-P., Caron, M., and Uhl, G. R. (2009). Cocaine-conditioned locomotion in dopamine transporter, norepinephrine transporter and 5-HT transporter knockout mice. *Neuroscience*, 162(4):870–880.

Hamid, A. (1992). The developmental cycle of a drug epidemic: the cocaine smoking epidemic of 1981-1991. *Journal of psychoactive drugs*, 24(4):337–348.

Hanks, J. B. and González-Maeso, J. (2013). Animal models of serotonergic psychedelics. *ACS Chemical Neuroscience*, 4(1):33–42.

Hansen, K. B., Wollmuth, L. P., Bowie, D., Furukawa, H., Menniti, F. S., Sobolevsky, A. I., Swanson, G. T., Swanger, S. A., Greger, I. H., Nakagawa, T., McBain, C. J., Jayaraman, V., Low, C.-M., Dell'Acqua, M. L., Diamond, J. S., Camp, C. R., Perszyk, R. E., Yuan, H., and Traynelis, S. F. (2021). Structure, Function, and Pharmacology of Glutamate Receptor Ion Channels. *Pharmacological Reviews*, 73(4):1469–1658.

Hao, Y., Martin-Fardon, R., and Weiss, F. (2010). Behavioral and Functional Evidence of Metabotropic Glutamate Receptor 2/3 and Metabotropic Glutamate Receptor 5 Dysregulation in Cocaine-Escalated Rats: Factor in the Transition to Dependence. *Biological Psychiatry*, 68(3):240–248.

Harris, D. S., Everhart, E. T., Mendelson, J., and Jones, R. T. (2003). The pharmacology of cocaethylene in humans following cocaine and ethanol administration. *Drug and Alcohol Dependence*, 72(2):169–182.

Hart, C. L., Jatlow, P., Sevarino, K. A., and McCance-Katz, E. F. (2000). Comparison of intravenous cocaethylene and cocaine in humans. *Psychopharmacology*, 149(2):153–162.

Hart, C. L. and Ksir, C. (2012). *Drugs, society, \& human behavior*. McGraw-Hill Professional, New York, NY, 15 edition.

Healy, C. J., Lee, K. A., and D'Andrea, W. (2021). Using Psychedelics With Therapeutic Intent Is Associated With Lower Shame and Complex Trauma Symptoms in Adults With Histories of Child Maltreatment. *Chronic stress (Thousand Oaks, Calif.)*, 5:24705470211029881.

Heimer, L. and Van Hoesen, G. W. (2006). The limbic lobe and its output channels: implications for emotional functions and adaptive behavior. *Neuroscience and biobehavioral reviews*, 30(2):126–147.

Hirth, N., Meinhardt, M. W., Noori, H. R., Salgado, H., Torres-Ramirez, O., Uhrig, S., Broccoli, L., Vengeliene, V., Roßmanith, M., Perreau-Lenz, S., Köhr, G., Sommer, W. H., Spanagel, R., and Hansson, A. C. (2016). Convergent evidence from alcohol-dependent humans and rats for a hyperdopaminergic state in protracted abstinence. *Proceedings of the National Academy of Sciences of the United States of America*, 113(11):3024–3029.

Hodos, W. (1961). Progressive Ratio as a Measure of Reward Strength. *Science*, 134(3483):943–944.

Hogea, L., Tabugan, D. C., Costea, I., Albai, O., Nussbaum, L., Cojocaru, A., Corsaro, L., and Anghel, T. (2025). The Therapeutic Potential of Psychedelics in Treating Substance Use Disorders: A Review of Clinical Trials. *Medicina*, 61(2).

Holland, P. (1998). Amount of training affects associatively-activated event representation. *Neuropharmacology*, 37(4):461–469.

Holland, P. C. (1981). Acquisition of representation-mediated conditioned food aversions. *Learning and Motivation*, 12(1):1–18.

Holland, P. C. (1990). Event representation in Pavlovian conditioning: Image and action. *Cognition*, 37(1):105–131.

Holland, P. C. (2005). Amount of training effects in representation-mediated food aversion learning: no evidence of a role for associability changes. *Learning & behavior*, 33(4):464–478.

Holland, P. C. (2006). Limitations on representation-mediated potentiation of flavour or odour aversions. *Quarterly journal of experimental psychology (2006)*, 59(2):233–250.

Hu, H. (2016). Reward and Aversion. *Annual Review of Neuroscience*, 39(1):297–324.

Hu, M., Crombag, H. S., Robinson, T. E., and Becker, J. B. (2004). Biological Basis of Sex Differences in the Propensity to Self-administer Cocaine. *Neuropsychopharmacology*, 29(1):81–85.

Hudson, A. and Stamp, J. A. (2011). Ovarian hormones and propensity to drug relapse: a review. *Neuroscience and biobehavioral reviews*, 35(3):427–436.

Hunt, W. A., Barnett, L. W., and Branch, L. G. (1971). Relapse rates in addiction programs. *Journal of Clinical Psychology*, 27(4):455–456.

Ikemoto, S. (2007). Dopamine reward circuitry: two projection systems from the ventral midbrain to the nucleus accumbens-olfactory tubercle complex. *Brain research reviews*, 56(1):27–78.

Imre, G. (2007). The preclinical properties of a novel group II metabotropic glutamate receptor agonist LY379268. *CNS drug reviews*, 13(4):444–464.

Jaber, M., Dumartin, B., Sagné, C., Haycock, J. W., Roubert, C., Giros, B., Bloch, B., and Caron, M. G. (1999). Differential regulation of tyrosine hydroxylase in the basal ganglia of mice lacking the dopamine transporter. *European Journal of Neuroscience*, 11(10):3499–3511.

Jackson, L. R., Robinson, T. E., and Becker, J. B. (2006). Sex differences and hormonal influences on acquisition of cocaine self-administration in rats. *Neuropsychopharmacology*, 31(1):129–138.

Jadhav, K. S., Magistretti, P. J., Halfon, O., Augsburger, M., and Boutrel, B. (2017). A preclinical model for identifying rats at risk of alcohol use disorder. *Scientific Reports*, 7(1):9454.

Javaid, J. I., Musa, M. N., Fischman, M., Schuster, C. R., and Davis, J. M. (1983). Kinetics of cocaine in humans after intravenous and intranasal administration. *Biopharmaceutics & drug disposition*, 4(1):9–18.

Jeanblanc, J., Bordy, R., Fouquet, G., Jeanblanc, V., and Naassila, M. (2024). Psilocybin reduces alcohol self-administration via selective left nucleus accumbens activation in rats. *Brain : a journal of neurology*.

Jentsch, J. D. and Taylor, J. R. (1999). Impulsivity resulting from frontostriatal dysfunction in drug abuse: implications for the control of behavior by reward-related stimuli. *Psychopharmacology*, 146(4):373–390.

Johnson, M. W., Garcia-Romeu, A., and Griffiths, R. R. (2017). Long-term follow-up of psilocybin-facilitated smoking cessation. *The American journal of drug and alcohol abuse*, 43(1):55–60.

Jones, C. A., Watson, D. J. G., and Fone, K. C. F. (2011). Animal models of schizophrenia. *British journal of pharmacology*, 164(4):1162–1194.

Jones, S. R., Gainetdinov, R. R., Hu, X.-T., Cooper, D. C., Wightman, R. M., White, F. J., and Caron, M. G. (1999). Loss of autoreceptor functions in mice lacking the dopamine transporter. *Nature Neuroscience*, 2(7):649–655.

Jones, S. R., Gainetdinov, R. R., Wightman, R. M., and Caron, M. G. (1998). Mechanisms of amphetamine action revealed in mice lacking the dopamine transporter. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 18(6):1979–1986.

Joyner, M. A., Gearhardt, A. N., and Flagel, S. B. (2018). A Translational Model to Assess Sign-Tracking and Goal-Tracking Behavior in Children. *Neuropsychopharmacology*, 43(1):228–229.

Justinova, Z., Le Foll, B., Redhi, G. H., Markou, A., and Goldberg, S. R. (2016). Differential effects of the metabotropic glutamate 2/3 receptor agonist LY379268 on nicotine versus cocaine self-administration and relapse in squirrel monkeys. *Psychopharmacology*, 233(10):1791–1800.

Kahlig, K. M. and Galli, A. (2003). Regulation of dopamine transporter function and plasma membrane expression by dopamine, amphetamine, and cocaine. *European Journal of Pharmacology*, 479(1-3):153–158.

Kalivas, P. W. (2004). Recent understanding in the mechanisms of addiction. *Current Psychiatry Reports*, 6(5):347–351.

Kalivas, P. W. (2009). The glutamate homeostasis hypothesis of addiction. *Nature Reviews Neuroscience*, 10(8):561–572.

Kalivas, P. W. and O'Brien, C. (2008). Drug Addiction as a Pathology of Staged Neuroplasticity. *Neuropsychopharmacology*, 33(1):166–180.

Kalivas, P. W. and Volkow, N. D. (2005). The neural basis of addiction: A pathology of motivation and choice. *American Journal of Psychiatry*, 162(8):1403–1413.

Kampman, K. M. (2019). The treatment of cocaine use disorder. *Science Advances*, 5(10).

Karasawa, J.-i., Yoshimizu, T., and Chaki, S. (2006). A metabotropic glutamate 2/3 receptor antagonist, MGS0039, increases extracellular dopamine levels in the nucleus accumbens shell. *Neuroscience Letters*, 393(2-3):127–130.

Karch, S. B., Green, G. S., and Young, S. (1995). Myocardial Hypertrophy and Coronary Artery Disease in Male Cocaine Users. *Journal of Forensic Sciences*, 40(4):13831J.

Kasanetz, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., and Vincenzo Piazza, P. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. *Science*, 328(5986):1709–1712.

Kasanetz, F., Lafourcade, M., Deroche-Gamonet, V., Revest, J. M., Berson, N., Balado, E., Fiancette, J. F., Renault, P., Piazza, P. V., and Manzoni, O. J. (2013). Prefrontal synaptic markers of cocaine addiction-like behavior in rats. *Molecular Psychiatry*, 18(6):729–737.

Kawa, A. B., Bentzley, B. S., and Robinson, T. E. (2016). Less is more: prolonged intermittent access cocaine self-administration produces incentive-sensitization and addiction-like behavior. *Psychopharmacology*, 233(19-20):3587–3602.

Kawa, A. B. and Robinson, T. E. (2019). Sex differences in incentive-sensitization produced by intermittent access cocaine self-administration. *Psychopharmacology*, 236(2):625–639.

Kebabian, J. W. and Calne, D. B. (1979). Multiple receptors for dopamine. *Nature*, 277(5692):93–96.

Kelley, A. E. (2004). Ventral striatal control of appetitive motivation: role in ingestive behavior and reward-related learning. *Neuroscience and biobehavioral reviews*, 27(8):765–776.

Kelly, T. J., Bonniwell, E. M., Mu, L., Liu, X., Hu, Y., Friedman, V., Yu, H., Su, W., McCorvy, J. D., and Liu, Q. S. (2024). Psilocybin analog 4-OH-DiPT enhances fear extinction and GABAergic inhibition of principal neurons in the basolateral amygdala. *Neuropsychopharmacology*, 49(5):854–863.

Kelmendi, B., Kichuk, S. A., DePalmer, G., Maloney, G., Ching, T. H. W., Belser, A., and Pittenger, C. (2022). Single-dose psilocybin for treatment-resistant obsessive-compulsive disorder: A case report. *Heliyon*, 8(12):e12135.

Khoo, S. Y.-S. and Samaha, A.-N. (2023). Metabotropic glutamate group II receptor activation in the ventrolateral dorsal striatum suppresses incentive motivation for cocaine in rats. *Psychopharmacology*, 240(6):1247–1260.

Kiluk, B. D. (2019). Computerized Cognitive Behavioral Therapy for Substance Use Disorders: A Summary of the Evidence and Potential Mechanisms of Behavior Change. *Perspectives on Behavior Science*, 42(3):465–478.

Kim, H.-J. and Koh, H.-Y. (2016). Impaired Reality Testing in Mice Lacking Phospholipase Cβ1: Observed by Persistent Representation-Mediated Taste Aversion. *PloS one*, 11(1):e0146376.

Kippin, T. E., Fuchs, R. A., Mehta, R. H., Case, J. M., Parker, M. P., Bimonte-Nelson, H. A., and See, R. E. (2005). Potentiation of cocaine-primed reinstatement of drug seeking in female rats during estrus. *Psychopharmacology*, 182(2):245–252.

Knackstedt, L. A., Moussawi, K., Lalumiere, R., Schwendt, M., Klugmann, M., and Kalivas, P. W. (2010). Extinction training after cocaine self-administration induces glutamatergic plasticity to inhibit cocaine seeking. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(23):7984–7992.

Kniffin, A. R. and Briand, L. A. (2024). Sex differences in glutamate transmission and plasticity in reward related regions. *Frontiers in behavioral neuroscience*, 18:1455478.

Knouse, M. C., Deutschmann, A. U., Nenov, M. N., Wimmer, M. E., and Briand, L. A. (2023). Sex differences in pre- and post-synaptic glutamate signaling in the nucleus accumbens core. *Biology of Sex Differences*, 14(1):52.

Koh, M. T., Ahrens, P. S., and Gallagher, M. (2018). A greater tendency for representation mediated learning in a ketamine mouse model of schizophrenia. *Behavioral neuroscience*, 132(2):106–113.

Koob, G. (2001). Drug Addiction, Dysregulation of Reward, and Allostasis. *Neuropsychopharmacology*, 24(2):97–129.

Koob, G. F. and Le Moal, M. (2006). Animal Models of Drug Addiction. In *Neurobiology of Addiction*, pages 23–67. Elsevier.

Koob, G. F. and Le Moal, M. (2008). Addiction and the brain antireward system. *Annual Review of Psychology*, 59(1):29–53.

Koob, G. F. and Volkow, N. D. (2010). Neurocircuitry of addiction. *Neuropsychopharmacology*, 35(1):217–238.

Kosten, T. A., Gawin, F. H., Kosten, T. R., and Rounsaville, B. J. (1993). Gender differences in cocaine use and treatment response. *Journal of Substance Abuse Treatment*, 10(1):63–66.

Kufahl, P. R., Martin-Fardon, R., and Weiss, F. (2011). Enhanced sensitivity to attenuation of conditioned reinstatement by the mGluR 2/3 agonist LY379268 and increased functional activity of mGluR 2/3 in rats with a history of ethanol dependence. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 36(13):2762–2773.

Kufahl, P. R., Watterson, L. R., Nemirovsky, N. E., Hood, L. E., Villa, A., Halstengard, C., Zautra, N., and Olive, M. F. (2013). Attenuation of methamphetamine seeking by the mGluR2/3 agonist LY379268 in rats with histories of restricted and escalated self-administration. *Neuropharmacology*, 66:290–301.

Kuhar, M. J., Ritz, M. C., and Boja, J. W. (1991). The dopamine hypothesis of the reinforcing properties of cocaine. *Trends in Neurosciences*, 14(7):299–302.

Kuhn, B. N., Kalivas, P. W., and Bobadilla, A.-C. (2019). Understanding Addiction Using Animal Models. *Frontiers in Behavioral Neuroscience*, 13.

Larson, E. B. and Carroll, M. E. (2007). Estrogen receptor beta, but not alpha, mediates estrogen's effect on cocaine-induced reinstatement of extinguished cocaine-seeking behavior in ovariectomized female rats. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 32(6):1334–1345.

Lau, A. and Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflügers Archiv - European Journal of Physiology*, 460(2):525–542.

Le Pelley, M. E., Mitchell, C. J., Beesley, T., George, D. N., and Wills, A. J. (2016). Attention and associative learning in humans: An integrative review. *Psychological bulletin*, 142(10):1111–1140.

Lee, W.-S. and Yoon, B.-E. (2023). Necessity of an Integrative Animal Model for a Comprehensive Study of Attention-Deficit/Hyperactivity Disorder. *Biomedicines*, 11(5).

Leo, D., Sukhanov, I., and Gainetdinov, R. (2018a). Novel translational rat models of dopamine transporter deficiency. *Neural Regeneration Research*, 13(12):2091.

Leo, D., Sukhanov, I., Zoratto, F., Illiano, P., Caffino, L., Sanna, F., Messa, G., Emanuele, M., Esposito, A., Dorofeikova, M., Budygin, E. A., Mus, L., Efimova, E. V., Niello, M., Espinoza, S., Sotnikova, T. D., Hoener, M. C., Laviola, G., Fumagalli, F., Adriani, W., and Gainetdinov, R. R. (2018b). Pronounced Hyperactivity, Cognitive Dysfunctions, and BDNF Dysregulation in Dopamine Transporter Knock-out Rats. *The Journal of Neuroscience*, 38(8):1959–1972.

Leshner, A. I. (1997). Addiction is a brain disease, and it matters. *Science*, 278(5335):45–47.

Levitz, J., Habrian, C., Bharill, S., Fu, Z., Vafabakhsh, R., and Isacoff, E. Y. (2016). Mechanism of Assembly and Cooperativity of Homomeric and Heteromeric Metabotropic Glutamate Receptors. *Neuron*, 92(1):143–159.

Liechti, M. E., Lhuillier, L., Kaupmann, K., and Markou, A. (2007). Metabotropic glutamate 2/3 receptors in the ventral tegmental area and the nucleus accumbens shell are involved in behaviors relating to nicotine dependence. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(34):9077–9085.

Lile, J. and Nader, M. (2005). The Abuse Liability and Therapeutic Potential of Drugs Evaluated for Cocaine Addiction as Predicted by Animal Models. *Current Neuropharmacology*, 1(1):21–46.

Liu, X. and Weiss, F. (2002). Additive Effect of Stress and Drug Cues on Reinstatement of Ethanol Seeking: Exacerbation by History of Dependence and Role of Concurrent Activation of Corticotropin-Releasing Factor and Opioid Mechanisms. *The Journal of Neuroscience*, 22(18):7856–7861.

Liu, Y., Cheong, J., Vaddiparti, K., and Cottler, L. B. (2020). The association between quantity, frequency and duration of cocaine use during the heaviest use period and DSM-5 cocaine use disorder. *Drug and Alcohol Dependence*, 213:108114.

Lloyd, J. T., Yee, A. G., Kalligappa, P. K., Jabed, A., Cheung, P. Y., Todd, K. L., Karunasinghe, R. N., Vlajkovic, S. M., Freestone, P. S., and Lipski, J. (2022). Dopamine Dysregulation and Altered Responses to Drugs Affecting Dopaminergic Transmission in a New Dopamine Transporter Knockout (DAT-KO) Rat Model. *Neuroscience*, 491:43–64.

Logan, C. N., Bechard, A. R., Hamor, P. U., Wu, L., Schwendt, M., and Knackstedt, L. A. (2020). Ceftriaxone and mGlu2/3 interactions in the nucleus accumbens core affect the reinstatement of cocaine-seeking in male and female rats. *Psychopharmacology*, 237(7):2007–2018.

López, M., Dwyer, D. M., Gasalla, P., Begega, A., and Jove, C. (2023). Odor-taste pairings lead to the acquisition of negative hedonic qualities by the odor in aversion learning. *Physiology and Behavior*, 269:114269.

Lopez-Quintero, C., Cobos, J. P. d. l., Hasin, D. S., Okuda, M., Wang, S., Grant, B. F., and Blanco, C. (2011). Probability and predictors of transition from first use to dependence on nicotine, alcohol, cannabis, and cocaine: Results of the National Epidemiologic Survey

on Alcohol and Related Conditions (NESARC). *Drug and Alcohol Dependence*, 115(1-2):120–130.

López-Valverde, A., De Vicente, J., and Cutando, A. (2011). The surgeons Halsted and Hall, cocaine and the discovery of dental anaesthesia by nerve blocking. *British Dental Journal*, 211(10):485–487.

Lovic, V., Saunders, B. T., Yager, L. M., and Robinson, T. E. (2011). Rats prone to attribute incentive salience to reward cues are also prone to impulsive action. *Behavioural Brain Research*, 223(2):255–261.

Lucantonio, F., Takahashi, Y. K., Hoffman, A. F., Chang, C. Y., Bali-Chaudhary, S., Shaham, Y., Lupica, C. R., and Schoenbaum, G. (2014). Orbitofrontal activation restores insight lost after cocaine use. *Nature neuroscience*, 17(8):1092–1099.

Lüscher, C. and Malenka, R. C. (2011). Drug-Evoked Synaptic Plasticity in Addiction: From Molecular Changes to Circuit Remodeling. *Neuron*, 69(4):650–663.

Lynch, W. J. (2008). Acquisition and maintenance of cocaine self-administration in adolescent rats: effects of sex and gonadal hormones. *Psychopharmacology*, 197(2):237–246.

Lynch, W. J. and Carroll, M. E. (1999). Sex differences in the acquisition of intravenously self-administered cocaine and heroin in rats. *Psychopharmacology*, 144(1):77–82.

Lynch, W. J., Nicholson, K. L., Dance, M. E., Morgan, R. W., and Foley, P. L. (2010). Animal models of substance abuse and addiction: Implications for science, animal welfare, and society. *Comparative Medicine*, 60(3):177–188.

Lynch, W. J. and Taylor, J. R. (2004). Sex differences in the behavioral effects of 24-h/day access to cocaine under a discrete trial procedure. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 29(5):943–951.

Madayag, A., Lobner, D., Kau, K. S., Mantsch, J. R., Abdulhameed, O., Hearing, M., Grier, M. D., and Baker, D. A. (2007). Repeated N-acetylcysteine administration alters plasticity-dependent effects of cocaine. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 27(51):13968–13976.

Madsen, M. K., Fisher, P. M., Burmester, D., Dyssegaard, A., Stenbæk, D. S., Kristiansen, S., Johansen, S. S., Lehel, S., Linnet, K., Svarer, C., Erritzoe, D., Ozenne, B., and Knudsen, G. M. (2019). Psychedelic effects of psilocybin correlate with serotonin 2A receptor occupancy and plasma psilocin levels. *Neuropsychopharmacology*, 44(7):1328–1334.

Mallien, A. S., Becker, L., Pfeiffer, N., Terraneo, F., Hahn, M., Middelman, A., Palme, R., Creutzberg, K. C., Begni, V., Riva, M. A., Leo, D., Potschka, H., Fumagalli, F., Homberg, J. R., and Gass, P. (2022). Dopamine Transporter Knockout Rats Show Impaired Well-being in a Multimodal Severity Assessment Approach. *Frontiers in Behavioral Neuroscience*, 16.

Mantegazza, P. (1859). Sull'introduzione in Europa della coca, nuovo elemento nervoso. *Annali di Chimica applicata alla Medicina*, 29:18–21.

Maraj, S., Figueredo, V. M., and Morris, D. L. (2010). Cocaine and the heart. *Clinical Cardiology*, 33(5):264–269.

Markel, H. (2011). Über Coca: Sigmund Freud, Carl Koller, and Cocaine. *JAMA*, 305(13):1360.

Marsden, C. A. (2006). Dopamine: the rewarding years. *British Journal of Pharmacology*, 147(S1).

Martin-Fardon, R. and Weiss, F. (2012). (-)-2-Oxa-4-aminobicylco[3.1.0]hexane-4,6-dicarboxylic acid (LY379268) and 3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]piperidine (MTEP) similarly attenuate stress-induced reinstatement of cocaine seeking. *Addiction Biology*, 17(3):557–564.

Mayo, L. M., Fraser, D., Childs, E., Momenan, R., Hommer, D. W., De Wit, H., and Heilig, M. (2013). Conditioned preference to a methamphetamine-associated contextual cue in humans. *Neuropsychopharmacology*, 38(6):921–929.

McCance-Katz, E. F., Carroll, K. M., and Rounsaville, B. J. (1999). Gender differences in treatment-seeking cocaine abusers - Implications for treatment and prognosis. *American Journal on Addictions*, 8(4):300–311.

McDannald, M. and Schoenbaum, G. (2009). Toward a Model of Impaired Reality Testing in Rats. *Schizophrenia Bulletin*, 35(4):664–667.

McDannald, M. A., Whitt, J. P., Calhoon, G. G., Piantadosi, P. T., Karlsson, R.-M., O'Donnell, P., and Schoenbaum, G. (2011). Impaired reality testing in an animal model of schizophrenia. *Biological psychiatry*, 70(12):1122–1126.

McEntee, W. J. and Crook, T. H. (1993). Glutamate: its role in learning, memory, and the aging brain. *Psychopharmacology*, 111(4):391–401.

McFarland, K., Davidge, S. B., Lapish, C. C., and Kalivas, P. W. (2004). Limbic and Motor Circuitry Underlying Footshock-Induced Reinstatement of Cocaine-Seeking Behavior. *The Journal of Neuroscience*, 24(7):1551–1560.

McFarland, K., Lapish, C. C., and Kalivas, P. W. (2003). Prefrontal Glutamate Release into the Core of the Nucleus Accumbens Mediates Cocaine-Induced Reinstatement of Drug-Seeking Behavior. *The Journal of Neuroscience*, 23(8):3531–3537.

McKenna, D. J., Repke, D. B., Lo, L., and Peroutka, S. J. (1990). Differential interactions of indolealkylamines with 5-hydroxytryptamine receptor subtypes. *Neuropharmacology*, 29(3):193–198.

McKinney, W. T. (1969). Animal Model of Depression. *Archives of General Psychiatry*, 21(2):240.

McLellan, A. T., Lewis, D. C., O'Brien, C. P., and Kleber, H. D. (2000). Drug Dependence, a Chronic Medical Illness. *JAMA*, 284(13):1689.

Meil, W. and See, R. (1996). Conditioned cued recovery of responding following prolonged withdrawal from self-administered cocaine in rats: an animal model of relapse. *Behavioural pharmacology*, 7(8):754–763.

Meinhardt, M. W., Güngör, C., Skorodumov, I., Mertens, L. J., and Spanagel, R. (2020). Psilocybin and LSD have no long-lasting effects in an animal model of alcohol relapse. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 45(8):1316–1322.

Meinhardt, M. W., Hansson, A. C., Perreau-Lenz, S., Bauder-Wenz, C., Stählin, O., Heilig, M., Harper, C., Drescher, K. U., Spanagel, R., and Sommer, W. H. (2013). Rescue of infralimbic mGluR2 deficit restores control over drug-seeking behavior in alcohol dependence. *Journal of Neuroscience*, 33(7):2794–2806.

Meinhardt, M. W., Pfarr, S., Fouquet, G., Rohleder, C., Meinhardt, M. L., Barroso-Flores, J., Hoffmann, R., Jeanblanc, J., Paul, E., Wagner, K., Hansson, A. C., Köhr, G., Meier, N., Von Bohlen und Halbach, O., Bell, R. L., Endepols, H., Neumaier, B., Schönig, K., Bartsch, D., Naassila, M., Spanagel, R., and Sommer, W. H. (2021). Psilocybin targets a common molecular mechanism for cognitive impairment and increased craving in alcoholism. *Science Advances*, 7(47).

Merikangas, K. R., Stolar, M., Stevens, D. E., Goulet, J., Preisig, M. A., Fenton, B., Zhang, H., O'Malley, S. S., and Rounsaville, B. J. (1998). Familial Transmission of Substance Use Disorders. *Archives of General Psychiatry*, 55(11):973.

Merims, D. and Giladi, N. (2008). Dopamine dysregulation syndrome, addiction and behavioral changes in Parkinson's disease. *Parkinsonism & Related Disorders*, 14(4):273–280.

Meshkat, S., Malik, G., Zeifman, R. J., Swainson, J., Balachandra, K., Reichelt, A. C., Zhang, Y., Burback, L., Winkler, O., Greenshaw, A., Vermetten, E., Mayo, L. M., Tanguay, R., Jetly, R., and Bhat, V. (2025). Efficacy and safety of psilocybin for the treatment of substance use disorders: A systematic review. *Neuroscience & Biobehavioral Reviews*, 173:106163.

Meyer, P. J., Ma, S. T., and Robinson, T. E. (2012). A cocaine cue is more preferred and evokes more frequency-modulated 50-kHz ultrasonic vocalizations in rats prone to attribute incentive salience to a food cue. *Psychopharmacology*, 219(4):999–1009.

Miguéns, M., Del Olmo, N., Higuera-Matas, A., Torres, I., García-Lecumberri, C., and Ambrosio, E. (2008). Glutamate and aspartate levels in the nucleus accumbens during cocaine self-administration and extinction: a time course microdialysis study. *Psychopharmacology*, 196(2):303–313.

Milton, A. L. and Everitt, B. J. (2010). The psychological and neurochemical mechanisms of drug memory reconsolidation: implications for the treatment of addiction. *The European journal of neuroscience*, 31(12):2308–2319.

Mirchandani, H. G., Rorke, L. B., Sekula-Perlman, A., and Hood, I. C. (1994). Cocaine-Induced Agitated Delirium, Forceful Struggle, and Minor Head Injury. *The American Journal of Forensic Medicine and Pathology*, 15(2):95–99.

Mladěnka, P., Applová, L., Patočka, J., Costa, V. M., Remiao, F., Pourová, J., Mladěnka, A., Karlíčková, J., Jahodář, L., Vopršalová, M., Varner, K. J., and Štěrba, M. (2018). Comprehensive review of cardiovascular toxicity of drugs and related agents. *Medicinal Research Reviews*, 38(4):1332–1403.

Montagu, K. A. (1957). Catechol compounds in rat tissues and in brains of different animals. *Nature*, 180(4579):244–245.

Moore, J. M. and Casale, J. F. (1994). In-depth chromatographic analyses of illicit cocaine and its precursor, coca leaves. *Journal of Chromatography A*, 674(1-2):165–205.

Moos, R. H. and Moos, B. S. (2006). Rates and predictors of relapse after natural and treated remission from alcohol use disorders. *Addiction (Abingdon, England)*, 101(2):212–222.

Moran, M. M., McFarland, K., Melendez, R. I., Kalivas, P. W., and Seamans, J. K. (2005). Cystine/Glutamate Exchange Regulates Metabotropic Glutamate Receptor Presynaptic

Inhibition of Excitatory Transmission and Vulnerability to Cocaine Seeking. *The Journal of Neuroscience*, 25(27):6389–6393.

Moran-Santa Maria, M. M., McRae-Clark, A., Baker, N. L., Ramakrishnan, V., and Brady, K. T. (2014). Yohimbine administration and cue-reactivity in cocaine-dependent individuals. *Psychopharmacology*, 231(21):4157–4165.

Moreno, F. A., Wiegand, C. B., Taitano, E. K., and Delgado, P. L. (2006). Safety, Tolerability, and Efficacy of Psilocybin in 9 Patients With Obsessive-Compulsive Disorder. *The Journal of Clinical Psychiatry*, 67(11):1735–1740.

Morón, J. A., Brockington, A., Wise, R. A., Rocha, B. A., and Hope, B. T. (2002). Dopamine uptake through the norepinephrine transporter in brain regions with low levels of the dopamine transporter: evidence from knock-out mouse lines. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 22(2):389–395.

Morrison, S. E., Bamkole, M. A., and Nicola, S. M. (2015). Sign Tracking, but Not Goal Tracking, is Resistant to Outcome Devaluation. *Frontiers in Neuroscience*, 9.

Morrow, J. D., Maren, S., and Robinson, T. E. (2011). Individual variation in the propensity to attribute incentive salience to an appetitive cue predicts the propensity to attribute motivational salience to an aversive cue. *Behavioural brain research*, 220(1):238–243.

Morrow, J. D., Saunders, B. T., Maren, S., and Robinson, T. E. (2015). Sign-tracking to an appetitive cue predicts incubation of conditioned fear in rats. *Behavioural brain research*, 276:59–66.

Moussawi, K. and Kalivas, P. W. (2010). Group II metabotropic glutamate receptors (mGlu2/3) in drug addiction. *European Journal of Pharmacology*, 639(1-3):115–122.

Moussawi, K., Zhou, W., Shen, H., Reichel, C. M., See, R. E., Carr, D. B., and Kalivas, P. W. (2011). Reversing cocaine-induced synaptic potentiation provides enduring protection from relapse. *Proceedings of the National Academy of Sciences*, 108(1):385–390.

Murnane, K. S. (2018). The renaissance in psychedelic research: What do preclinical models have to offer. pages 25–67.

Natekar, A., Motok, I., Walasek, P., Rao, C., Clare-Fasullo, G., and Koren, G. (2012). Cocaethylene as a biomarker to predict heavy alcohol exposure among cocaine users. *Journal of population therapeutics and clinical pharmacology = Journal de la therapeutique des populations et de la pharmacologie clinique*, 19(3):466–72.

Nestler, E. (2005a). The Neurobiology of Cocaine Addiction. *Science & Practice Perspectives*, 3(1):4–10.

Nestler, E. J. (2005b). Is there a common molecular pathway for addiction? *Nature Neuroscience*, 8(11):1445–1449.

Niaura, R. S., Rohsenow, D. J., Binkoff, J. A., Monti, P. M., Pedraza, M., and Abrams, D. B. (1988). Relevance of cue reactivity to understanding alcohol and smoking relapse. *Journal of abnormal psychology*, 97(2):133–152.

Nichols, D. E. (2004). Hallucinogens. *Pharmacology & therapeutics*, 101(2):131–181.

Nicolas, C., Russell, T. I., Pierce, A. F., Maldera, S., Holley, A., You, Z.-B., McCarthy, M. M., Shaham, Y., and Ikemoto, S. (2019). Incubation of Cocaine Craving After Intermittent-Access Self-administration: Sex Differences and Estrous Cycle. *Biological Psychiatry*, 85(11):915–924.

Nicolas, C., Zlebnik, N. E., Farokhnia, M., Leggio, L., Ikemoto, S., and Shaham, Y. (2022). Sex Differences in Opioid and Psychostimulant Craving and Relapse: A Critical Review. *Pharmacological reviews*, 74(1):119–140.

Niedzielska-Andres, E., Pomierny-Chamioło, L., Andres, M., Walczak, M., Knackstedt, L. A., Filip, M., and Przegaliński, E. (2021). Cocaine use disorder: A look at metabotropic glutamate receptors and glutamate transporters. *Pharmacology & Therapeutics*, 221:107797.

Niemann, A. (1860). Ueber eine neue organische Base in den Cocablättern. *Archiv der Pharmazie*, 153(2):129–155.

Niswender, C. M. and Conn, P. J. (2010). Metabotropic glutamate receptors: Physiology, pharmacology, and disease. *Annual Review of Pharmacology and Toxicology*, 50(1):295–322.

Noshpitz, J. D. (1982). Reality testing: a neurophysiological fantasy. *Comprehensive psychiatry*, 23(1):25–32.

Nutt, D., King, L. A., Saulsbury, W., and Blakemore, C. (2007). Development of a rational scale to assess the harm of drugs of potential misuse. *Lancet*, 369(9566):1047–1053.

O'Brien, C. P. (2005). Anticraving medications for relapse prevention: A possible new class of psychoactive medications. *American Journal of Psychiatry*, 162(8):1423–1431.

O'Brien, C. P., Childress, A. R., Ehrman, R., and Robbins, S. J. (1998). Conditioning factors in drug abuse: Can they explain compulsion? *Journal of Psychopharmacology*, 12(1):15–22.

O'Brien, C. P., Childress, A. R., McLellan, A. T., and Ehrman, R. (1992). Classical conditioning in drug-dependent humans. *Annals of the New York Academy of Sciences*, 654(1):400–15.

Ohishi, H., Neki, A., and Mizuno, N. (1998). Distribution of a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat and mouse: an immunohistochemical study with a monoclonal antibody. *Neuroscience Research*, 30(1):65–82.

Ohishi, H., Shigemoto, R., Nakanishi, S., and Mizuno, N. (1993). Distribution of the messenger RNA for a metabotropic glutamate receptor, mGluR2, in the central nervous system of the rat. *Neuroscience*, 53(4):1009–1018.

Olds, J. and Milner, P. (1954). Positive Reinforcement Produced By Electrical Stimulation of Septal Area and Other Regions of Rat Brain. *Journal of Comparative and Physiological Psychology*, 47(6):419–427.

Olive, M. (2009). Metabotropic Glutamate Receptor Ligands as Potential Therapeutics for Addiction. *Current Drug Abuse Reviewse*, 2(1):83–98.

Olive, M. F. (2016). Glutamate Receptors and Drug Addiction. In *Neuropathology of Drug Addictions and Substance Misuse*, pages 102–110. Elsevier.

Oliveira, N. G. and Dinis-Oliveira, R. J. (2018). Drugs of abuse from a different toxicological perspective: an updated review of cocaine genotoxicity. *Archives of Toxicology*, 92(10):2987–3006.

OpenAI (2024). ChatGPT (GPT□4o model).

Ouagazzal, A., Grottick, A. J., Moreau, J., and Higgins, G. A. (2001). Effect of LSD on prepulse inhibition and spontaneous behavior in the rat. A pharmacological analysis and comparison between two rat strains. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 25(4):565–575.

Panlilio, L. V. and Goldberg, S. R. (2007). Self-administration of drugs in animals and humans as a model and an investigative tool. *Addiction*, 102(12):1863–1870.

Paolone, G., Angelakos, C. C., Meyer, P. J., Robinson, T. E., and Sarter, M. (2013). Cholinergic control over attention in rats prone to attribute incentive salience to reward cues. *Journal of Neuroscience*, 33(19):8321–8335.

Papale, A., Morella, I. M., Indrigo, M. T., Bernardi, R. E., Marrone, L., Marchisella, F., Brancale, A., Spanagel, R., Brambilla, R., and Fasano, S. (2016). Impairment of cocaine-mediated behaviours in mice by clinically relevant Ras-ERK inhibitors. *eLife*, 5.

Pavlov, I. P. (1927). Conditioned reflexes: An investigation of the physiological activity of the cerebral cortex. *Annals of neurosciences*, 17(3).

Paxinos, G. and Watson, C. (2006). *The rat brain in stereotaxic coordinates*. Academic Press, San Diego, CA, 6 edition.

Pergolizzi, J., Breve, F., Magnusson, P., LeQuang, J. A. K., and Varrassi, G. (2022). Cocaethylene: When Cocaine and Alcohol Are Taken Together. *Cureus*.

Perry, C. J., Zbukvic, I., Kim, J. H., and Lawrence, A. J. (2014). Role of cues and contexts on drug□seeking behaviour. *British Journal of Pharmacology*, 171(20):4636–4672.

Peters, J. and Kalivas, P. W. (2006). The group II metabotropic glutamate receptor agonist, LY379268, inhibits both cocaine- and food-seeking behavior in rats. *Psychopharmacology*, 186(2):143–149.

Peters, J., LaLumiere, R. T., and Kalivas, P. W. (2008). Infralimbic prefrontal cortex is responsible for inhibiting cocaine seeking in extinguished rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 28(23):6046–6053.

Petrželová, M. (2018). Individuálně připravované léčivé přípravky s obsahem lokálních anestetik v otorhinolaryngologii. *Praktické lékárenství*, 14(4):179–184.

Pierce, R. C., Fant, B., Swinford-Jackson, S. E., Heller, E. A., Berrettini, W. H., and Wimmer, M. E. (2018). Environmental, genetic and epigenetic contributions to cocaine addiction. *Neuropsychopharmacology*, 43(7):1471–1480.

Pierce, R. C. and Kumaresan, V. (2006). The mesolimbic dopamine system: The final common pathway for the reinforcing effect of drugs of abuse? *Neuroscience and Biobehavioral Reviews*, 30(2):215–238.

Piñeiro-Dieguez, B., Balanzá-Martínez, V., García-García, P., and Soler-López, B. (2016). Psychiatric Comorbidity at the Time of Diagnosis in Adults With ADHD. *Journal of Attention Disorders*, 20(12):1066–1075.

Pinheiro, P. S. and Mulle, C. (2008). Presynaptic glutamate receptors: physiological functions and mechanisms of action. *Nature Reviews Neuroscience*, 9(6):423–436.

Pitchers, K. K., Phillips, K. B., Jones, J. L., Robinson, T. E., and Sarter, M. (2017). Diverse Roads to Relapse: A Discriminative Cue Signaling Cocaine Availability Is More Effective in Renewing Cocaine Seeking in Goal Trackers Than Sign Trackers and Depends on Basal Forebrain Cholinergic Activity. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 37(30):7198–7208.

Plowman, T. and Rivier, L. (1983). Cocaine and cinnamoylcocaine content of Erythroxylum species. *Annals of Botany*, 51(5):641–659.

Pohořalá, V., Enkel, T., Bartsch, D., Spanagel, R., and Bernardi, R. E. (2021). Sign- and goal-tracking score does not correlate with addiction-like behavior following prolonged cocaine self-administration. *Psychopharmacology*, 238(8):2335–2346.

Pohořalá, V., Kuchař, M., Spanagel, R., and Bernardi, R. E. (2024). Psilocybin administered following extinction sessions does not affect subsequent cocaine cue reinstatement in male and female rats and mice. *Neuroscience*, 559:156–165.

Politi, E., Fauci, E., Santoro, A., and Smeraldi, E. (2008). Daily Sessions of Transcranial Magnetic Stimulation to the Left Prefrontal Cortex Gradually Reduce Cocaine Craving. *The American Journal on Addictions*, 17(4):345–346.

Pomierny-Chamiolo, L., Miszkiel, J., Frankowska, M., Mizera, J., and Filip, M. (2017). Neuroadaptive changes in metabotropic glutamate mGlu2/3R expression during different phases of cocaine addiction in rats. *Pharmacological reports : PR*, 69(5):1073–1081.

Pomierny-Chamiolo, L., Miszkiel, J., Frankowska, M., Pomierny, B., Niedzielska, E., Smaga, I., Fumagalli, F., and Filip, M. (2015). Withdrawal from Cocaine Self-administration and Yoked Cocaine Delivery Dysregulates Glutamatergic mGlu5 and NMDA Receptors in the Rat Brain. *Neurotoxicity Research*, 27(3):246–258.

Potvin, S., Stavro, K., Rizkallah, ., and Pelletier, J. (2014). Cocaine and Cognition. *Journal of Addiction Medicine*, 8(5):368–376.

Qian, Z., Wu, X., Qiao, Y., Shi, M., Liu, Z., Ren, W., Han, J., and Zheng, Q. (2019). Down-regulation of mGluR2/3 receptors during morphine withdrawal in rats impairs mGluR2/3- and NMDA receptor-dependent long-term depression in the nucleus accumbens. *Neuroscience Letters*, 690:76–82.

Quinones-Jenab, V. and Jenab, S. (2012). Influence of Sex Differences and Gonadal Hormones on Cocaine Addiction. *ILAR Journal*, 53(1):14–22.

Rada, P., Avena, N. M., and Hoebel, B. G. (2005). Daily bingeing on sugar repeatedly releases dopamine in the accumbens shell. *Neuroscience*, 134(3):737–744.

Rambousek, L., Palenicek, T., Vales, K., and Stuchlik, A. (2014). The effect of psilocin on memory acquisition, retrieval, and consolidation in the rat. *Frontiers in behavioral neuroscience*, 8:180.

Ramôa, C. P., Doyle, S. E., Naim, D. W., and Lynch, W. J. (2013). Estradiol as a mechanism for sex differences in the development of an addicted phenotype following extended access cocaine self-administration. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 38(9):1698–1705.

Rapinesi, C., Del Casale, A., Di Pietro, S., Ferri, V. R., Piacentino, D., Sani, G., Raccah, R. N., Zangen, A., Ferracuti, S., Vento, A. E., Angeletti, G., Brugnoli, R., Kotzalidis, G. D., and Girardi, P. (2016). Add-on high frequency deep transcranial magnetic stimulation (dTMS) to bilateral prefrontal cortex reduces cocaine craving in patients with cocaine use disorder. *Neuroscience Letters*, 629:43–47.

Reiner, A. and Levitz, J. (2018). Glutamatergic Signaling in the Central Nervous System: Ionotropic and Metabotropic Receptors in Concert. *Neuron*, 98(6):1080–1098.

Reith, M. E. A., Kortagere, S., Wiers, C. E., Sun, H., Kurian, M. A., Galli, A., Volkow, N. D., and Lin, Z. (2022). The dopamine transporter gene SLC6A3: multidisease risks. *Molecular Psychiatry*, 27(2):1031–1046.

Rescorla, R. A. and Wagner, A. R. (1972). A Theory of Pavlovian Conditioning: Variations in the Effectiveness of Reinforcement and Nonreinforcement. *Clasical conditioning II: current research and theory*, Vol. 2:64–99.

Richardson, N. R. and Roberts, D. C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. *Journal of neuroscience methods*, 66(1):1–11.

Riezzo, I., Fiore, C., De Carlo, D., Pascale, N., Neri, M., Turillazzi, E., and Fineschi, V. (2012). Side Effects of Cocaine Abuse: Multiorgan Toxicity and Pathological Consequences. *Current Medicinal Chemistry*, 19(33):5624–5646.

Rikoon, S. H., Cacciola, J. S., Carise, D., Alterman, A. I., and McLellan, A. T. (2006). Predicting DSM-IV dependence diagnoses from Addiction Severity Index composite scores. *Journal of Substance Abuse Treatment*, 31(1):17–24.

Ritz, M. C., Lamb, R. J., Goldberg, S. R., and Kuhar, M. J. (1987). Cocaine Receptors on Dopamine Transporters Are Related to Self-Administration of Cocaine. *Science*, 237(4819):1219–1223.

Robbins, S. J., Ehrman, R. N., Childress, A. R., and O'Brien, C. P. (1999). Comparing levels of cocaine cue reactivity in male and female outpatients. *Drug and Alcohol Dependence*, 53(3):223–230.

Roberts, D. C., Bennett, S. A., and Vickers, G. J. (1989a). The estrous cycle affects cocaine self-administration on a progressive ratio schedule in rats. *Psychopharmacology*, 98(3):408–411.

Roberts, D. C., Morgan, D., and Liu, Y. (2007). How to make a rat addicted to cocaine. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, 31(8):1614–1624.

Roberts, D. C. S., Loh, E. A., and Vickers, G. (1989b). Self-administration of cocaine on a progressive ratio schedule in rats: dose-response relationship and effect of haloperidol pretreatment. *Psychopharmacology*, 97(4):535–538.

Robinson, T. E. and Becker, J. B. (1986). Enduring changes in brain and behavior produced by chronic amphetamine administration: A review and evaluation of animal models of amphetamine psychosis. *Brain Research*, 396(2):157–198.

Robinson, T. E. and Berridge, K. C. (1993). The neural basis of drug craving: An incentive-sensitization theory of addiction. *Brain Research Reviews*, 18(3):247–291.

Robinson, T. E. and Berridge, K. C. (2000). The psychology and neurobiology of addiction: an incentive-sensitization view. *Addiction (Abingdon, England)*, 95 Suppl 2:91–117.

Robinson, T. E. and Berridge, K. C. (2003). Addiction. *Annual Review of Psychology*, 54(1):25–53.

Robinson, T. E. and Berridge, K. C. (2008). The incentive sensitization theory of addiction: some current issues. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 363(1507):3137–3146.

Robinson, T. E. and Kolb, B. (2004). Structural plasticity associated with exposure to drugs of abuse. *Neuropharmacology*, 47 Suppl 1:33–46.

Robinson, T. E., Yager, L. M., Cogan, E. S., and Saunders, B. T. (2014). On the motivational properties of reward cues: Individual differences. *Neuropharmacology*, 76(PART B):450–459.

Rocha, B. A., Fumagalli, F., Gainetdinov, R. R., Jones, S. R., Ator, R., Giros, B., Miller, G. W., and Caron, M. G. (1998). Cocaine self-administration in dopamine-transporter knockout mice. *Nature neuroscience*, 1(2):132–137.

Roque Bravo, R., Faria, A. C., Brito-da Costa, A. M., Carmo, H., Mladěnka, P., Dias da Silva, D., and Remião, F. (2022). Cocaine: An Updated Overview on Chemistry, Detection, Biokinetics, and Pharmacotoxicological Aspects including Abuse Pattern. *Toxins*, 14(4):278.

Ross, S., Bossis, A., Guss, J., Agin-Liebes, G., Malone, T., Cohen, B., Mennenga, S. E., Belser, A., Kalliontzi, K., Babb, J., Su, Z., Corby, P., and Schmidt, B. L. (2016). Rapid and sustained symptom reduction following psilocybin treatment for anxiety and depression in patients with life-threatening cancer: a randomized controlled trial. *Journal of Psychopharmacology*, 30(12):1165–1180.

Roth, M. E. and Carroll, M. E. (2004). Sex differences in the escalation of intravenous cocaine intake following long- or short-access to cocaine self-administration. *Pharmacology, biochemistry, and behavior*, 78(2):199–207.

Russo, S. J., Jenab, S., Fabian, S. J., Festa, E. D., Kemen, L. M., and Quinones-Jenab, V. (2003). Sex differences in the conditioned rewarding effects of cocaine. *Brain research*, 970(1-2):214–220.

Saddoris, M. P., Holland, P. C., and Gallagher, M. (2009). Associatively learned representations of taste outcomes activate taste-encoding neural ensembles in gustatory cortex. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 29(49):15386–15396.

Salamone, J. D. and Correa, M. (2012). The mysterious motivational functions of mesolimbic dopamine. *Neuron*, 76(3):470–485.

Salvatore, M. F., Calipari, E. S., and Jones, S. R. (2016). Regulation of Tyrosine Hydroxylase Expression and Phosphorylation in Dopamine Transporter-Deficient Mice. *ACS chemical neuroscience*, 7(7):941–951.

Sams-Dodd, F. (1999). Phencyclidine in the Social Interaction Test: An Animal Model of Schizophrenia with Face and Predictive Validity. *Reviews in the Neurosciences*, 10(1).

Sanchis-Segura, C. and Spanagel, R. (2006). Behavioural assessment of drug reinforcement and addictive features in rodents: An overview. *Addiction Biology*, 11(1):2–38.

Sanvicente-Vieira, B., Rovaris, D. L., Ornell, F., Sordi, A., Rothmann, L. M., Niederauer, J. P. O., Schuch, J. B., von Diemen, L., Kessler, F. H. P., and Grassi-Oliveira, R. (2019). Sex-based differences in multidimensional clinical assessments of early-abstinence crack cocaine users. *PLOS ONE*, 14(6):e0218334.

Sarter, M. and Phillips, K. B. (2018). The neuroscience of cognitive-motivational styles: Sign- and goal-trackers as animal models. *Behavioral neuroscience*, 132(1):1–12.

Saunders, B. T., O'Donnell, E. G., Aurbach, E. L., and Robinson, T. E. (2014). A cocaine context renews drug seeking preferentially in a subset of individuals. *Neuropsychopharmacology*, 39(12):2816–2823.

Saunders, B. T. and Robinson, T. E. (2010). A Cocaine Cue Acts as an Incentive Stimulus in Some but not Others: Implications for Addiction. *Biological Psychiatry*, 67(8):730–736.

Saunders, B. T. and Robinson, T. E. (2011). Individual variation in the motivational properties of cocaine. *Neuropsychopharmacology : official publication of the American College of Neuropsychopharmacology*, 36(8):1668–1676.

Saunders, B. T. and Robinson, T. E. (2012). The role of dopamine in the accumbens core in the expression of pavlovian-conditioned responses. *European Journal of Neuroscience*, 36(4):2521–2532.

Saunders, B. T. and Robinson, T. E. (2013). Individual variation in resisting temptation: Implications for addiction. *Neuroscience and Biobehavioral Reviews*, 37(9):1955–1975.

Saunders, B. T., Yager, L. M., and Robinson, T. E. (2013). Cue-Evoked Cocaine "Craving": Role of Dopamine in the Accumbens Core. *Journal of Neuroscience*, 33(35):13989–14000.

Savchenko, A., Targa, G., Fesenko, Z., Leo, D., Gainetdinov, R. R., and Sukhanov, I. (2023). Dopamine Transporter Deficient Rodents: Perspectives and Limitations for Neuroscience. *Biomolecules*, 13(5).

Sayalı, C. and Barrett, F. S. (2023). The costs and benefits of psychedelics on cognition and mood. *Neuron*, 111(5):614–630.

Schad, D. J., Rapp, M. A., Garbusow, M., Nebe, S., Sebold, M., Obst, E., Sommer, C., Deserno, L., Rabovsky, M., Friedel, E., Romanczuk-Seiferth, N., Wittchen, H. U., Zimmermann, U. S., Walter, H., Sterzer, P., Smolka, M. N., Schlagenhauf, F., Heinz, A., Dayan, P., and Huys, Q. J. (2020). Dissociating neural learning signals in human sign- and goal-trackers. *Nature Human Behaviour*, 4(2):201–214.

Schmidt, E. F., Sutton, M. A., Schad, C. A., Karanian, D. A., Brodkin, E. S., and Self, D. W. (2001). Extinction training regulates tyrosine hydroxylase during withdrawal from cocaine self-administration. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 21(7):RC137.

Schmidt, H. D. and Pierce, R. C. (2010). Cocaine-induced neuroadaptations in glutamate transmission: Potential therapeutic targets for craving and addiction. *Annals of the New York Academy of Sciences*, 1187(1):35–75.

Schultz, W. (2015). Neuronal Reward and Decision Signals: From Theories to Data. *Physiological Reviews*, 95(3):853–951.

Schultz, W., Dayan, P., and Montague, P. R. (1997). A neural substrate of prediction and reward. *Science (New York, N.Y.)*, 275(5306):1593–1599.

Schuster, C. R. and Thompson, T. (1969). Self Administration of and Behavioral Dependence on Drugs. *Annual Review of Pharmacology*, 9(1):483–502.

Schwartz, E. K. C., Wolkowicz, N. R., De Aquino, J. P., MacLean, R. R., and Sofuoglu, M. (2022). Cocaine Use Disorder (CUD): Current Clinical Perspectives. *Substance Abuse and Rehabilitation*, Volume 13:25–46.

Schwendt, M. and Knackstedt, L. A. (2021). Extinction vs. Abstinence: A Review of the Molecular and Circuit Consequences of Different Post-Cocaine Experiences. *International Journal of Molecular Sciences*, 22(11).

Schwendt, M., Reichel, C. M., and See, R. E. (2012). Extinction-Dependent Alterations in Corticostriatal mGluR2/3 and mGluR7 Receptors following Chronic Methamphetamine Self-Administration in Rats. *PLOS ONE*, 7(3):e34299.

Scülfort, S. A., Bartsch, D., and Enkel, T. (2016). Dopamine antagonism does not impair learning of Pavlovian conditioned approach to manipulable or non-manipulable cues but biases responding towards goal tracking. *Behavioural Brain Research*, 314:1–5.

Seabury, R. D., Bearden, C. E., Ventura, J., Subotnik, K. L., Nuechterlein, K. H., and Cannon, T. D. (2021). Confident memory errors and disrupted reality testing in early psychosis. *Schizophrenia Research*, 238:170–177.

Sell, S. L., Thomas, M. L., and Cunningham, K. A. (2002). Influence of estrous cycle and estradiol on behavioral sensitization to cocaine in female rats. *Drug and Alcohol Dependence*, 67(3):281–290.

Shaham, Y., Shalev, U., Lu, L., de Wit, H., and Stewart, J. (2003). The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology*, 168(1-2):3–20.

Shaham, Y. and Stewart, J. (1995). Stress reinstates heroin-seeking in drug-free animals: An effect mimicking heroin, not withdrawal. *Psychopharmacology*, 119(3):334–341.

Sheffler, D. J., Pinkerton, A. B., Dahl, R., Markou, A., and Cosford, N. D. P. (2011). Recent progress in the synthesis and characterization of group II metabotropic glutamate receptor allosteric modulators. *ACS chemical neuroscience*, 2(8):382–393.

Shen, H.-w., Hagino, Y., Kobayashi, H., Shinohara-Tanaka, K., Ikeda, K., Yamamoto, H., Yamamoto, T., Lesch, K.-P., Murphy, D. L., Hall, F. S., Uhl, G. R., and Sora, I. (2004). Regional Differences in Extracellular Dopamine and Serotonin Assessed by In Vivo Microdialysis in Mice Lacking Dopamine and/or Serotonin Transporters. *Neuropsychopharmacology*, 29(10):1790–1799.

Sinha, R. (2007). The role of stress in addiction relapse. *Current Psychiatry Reports*, 9(5):388–395.

Sinha, R. and Li, C. S. R. (2007). Imaging stress- and cue-induced drug and alcohol craving: association with relapse and clinical implications. *Drug and alcohol review*, 26(1):25–31.

Sircar, R. and Kim, D. (1999). Female gonadal hormones differentially modulate cocaine-induced behavioral sensitization in Fischer, Lewis, and Sprague-Dawley rats. *Journal of Pharmacology and Experimental Therapeutics*, 289(1):54–65.

Skinner, B. F. (1938). *The behavior of organisms: an experimental analysis.* Appleton-Century, Oxford, England.

Smaga, I., Wydra, K., Frankowska, M., Fumagalli, F., Sanak, M., and Filip, M. (2020). Cocaine Self-Administration and Abstinence Modulate NMDA Receptor Subunits and Active Zone Proteins in the Rat Nucleus Accumbens. *Molecules*, 25(15).

Smith, K., Lacadie, C. M., Milivojevic, V., Fogelman, N., and Sinha, R. (2023). Sex differences in neural responses to stress and drug cues predicts future drug use in individuals with substance use disorder. *Drug and alcohol dependence*, 244:109794.

Sokoloff, P., Giros, B., Martres, M.-P., Bouthenet, M.-L., and Schwartz, J.-C. (1990). Molecular cloning and characterization of a novel dopamine receptor (D3) as a target for neuroleptics. *Nature*, 347(6289):146–151.

Solomon, R. L. and Corbit, J. D. (1973). An opponent-process theory of motivation: II. Cigarette addiction. *Journal of Abnormal Psychology*, 81(2):158–171.

Sora, I., Wichems, C., Takahashi, N., Li, X. F., Zeng, Z., Revay, R., Lesch, K. P., Murphy, D. L., and Uhl, G. R. (1998). Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice. *Proceedings of the National Academy of Sciences of the United States of America*, 95(13):7699–7704.

Spanagel, R. (2009). Alcoholism: A Systems Approach From Molecular Physiology to Addictive Behavior. *Physiological Reviews*, 89(2):649–705.

Spanagel, R. (2017). Animal models of addiction. *Dialogues in clinical neuroscience*, 19(3):247–258.

Speranza, L., di Porzio, U., Viggiano, D., de Donato, A., and Volpicelli, F. (2021). Dopamine: The Neuromodulator of Long-Term Synaptic Plasticity, Reward and Movement Control. *Cells*, 10(4):735.

Spronk, D. B., van Wel, J. H., Ramaekers, J. G., and Verkes, R. J. (2013). Characterizing the cognitive effects of cocaine: A comprehensive review. *Neuroscience and Biobehavioral Reviews*, 37(8):1838–1859.

Steinhauer, J. R. and Caulfield, J. B. (2001). Spontaneous coronary artery dissection associated with cocaine use: A case report and brief review. *Cardiovascular Pathology*, 10(3):141–145.

Stewart, J. and de Wit, H. (1987). Reinstatement of Drug-Taking Behavior as a Method of Assessing Incentive Motivational Properties of Drugs. In *Methods of Assessing the Reinforcing Properties of Abused Drugs*, pages 211–227. Springer New York, New York, NY.

Substance Abuse and Mental Health Services Administration (2020). Key substance use and mental health indicators in the United States: Results from the 2019 National Survey on Drug Use and Health.

Substance Abuse and Mental Health Services Administration (2024). Key substance use and mental health indicators in the United States: Results from the 2023 National Survey on Drug Use and Health.

Sughondhabirom, A., Jain, D., Gueorguieva, R., Coric, V., Berman, R., Lynch, W. J., Self, D., Jatlow, P., and Malison, R. T. (2005). A paradigm to investigate the self-regulation of cocaine administration in humans. *Psychopharmacology*, 180(3):436–446.

Sun, W., Yuill, M. B., and Fan, M. (2021). Behavioral economics of cocaine self-administration in male and female rats. *Behavioural pharmacology*, 32(1):21–31.

Sunahara, R. K., Guan, H.-C., O'Dowd, B. F., Seeman, P., Laurier, L. G., Ng, G., George, S. R., Torchia, J., Van Tol, H. H. M., and Niznik, H. B. (1991). Cloning of the gene for a human dopamine D5 receptor with higher affinity for dopamine than D1. *Nature*, 350(6319):614–619.

Swalve, N., Smethells, J. R., Zlebnik, N. E., and Carroll, M. E. (2016). Sex differences in reinstatement of cocaine-seeking with combination treatments of progesterone and atomoxetine. *Pharmacology Biochemistry and Behavior*, 145:17–23.

Tamargo, J. A., Sherman, K. E., Sékaly, R.-P., Bordi, R., Schlatzer, D., Lai, S., Khalsa, J. H., Mandler, R. N., Ehman, R. L., and Baum, M. K. (2022). Cocaethylene, simultaneous alcohol and cocaine use, and liver fibrosis in people living with and without HIV. *Drug and Alcohol Dependence*, 232:109273.

Terry-McElrath, Y. M., O'Malley, P. M., and Johnston, L. D. (2009). Reasons for Drug Use among American Youth by Consumption Level, Gender, and Race/Ethnicity: 1976–2005. *Journal of Drug Issues*, 39(3):677–713.

Thomsen, M., Hall, F. S., Uhl, G. R., and Caine, S. B. (2009). Dramatically Decreased Cocaine Self-Administration in Dopamine But Not Serotonin Transporter Knock-Out Mice. *Journal of Neuroscience*, 29(4):1087–1092.

Thorndike, E. L. (1898). Animal intelligence: An experimental study of the associative processes in animals. *The Psychological Review: Monograph Supplements*, 2(4):i–109.

Tiffany, S. T. (1990). A cognitive model of drug urges and drug-use behavior: Role of automatic and nonautomatic processes. *Psychological Review*, 97(2):147–168.

Torrado Pacheco, A., Olson, R. J., Garza, G., and Moghaddam, B. (2023). Acute psilocybin enhances cognitive flexibility in rats. *Neuropsychopharmacology*, 48(7):1011–1020.

Torres, G. E., Gainetdinov, R. R., and Caron, M. G. (2003). Plasma membrane monoamine transporters: structure, regulation and function. *Nature Reviews Neuroscience*, 4(1):13–25.

Towers, E. B., Bakhti-Suroosh, A., and Lynch, W. J. (2021). Females develop features of an addiction-like phenotype sooner during withdrawal than males. *Psychopharmacology*, 238(8):2213–2224.

Towers, E. B., Kilgore, M., Bakhti-Suroosh, A., Pidaparthi, L., Williams, I. L., Abel, J. M., and Lynch, W. J. (2022a). Sex differences in the neuroadaptations associated with incubated cocaine-craving: A focus on the dorsomedial prefrontal cortex. *Frontiers in behavioral neuroscience*, 16:1027310.

Towers, E. B., Williams, I. L., Qillawala, E. I., Rissman, E. F., and Lynch, W. J. (2022b). Sex/Gender Differences in The Time-Course for the Development of Substance Use Disorder: A Focus on the Telescoping Effect. *Pharmacological Reviews*, 74(4):217–249.

Trabanco, A. A., Bartolomé, J. M., and Cid, J. M. (2019). mGluR2 positive allosteric modulators: an updated patent review (2013-2018). *Expert opinion on therapeutic patents*, 29(7):497–507.

Trantham-Davidson, H., LaLumiere, R. T., Reissner, K. J., Kalivas, P. W., and Knackstedt, L. A. (2012). Ceftriaxone Normalizes Nucleus Accumbens Synaptic Transmission, Glutamate Transport, and Export following Cocaine Self-Administration and Extinction Training. *The Journal of Neuroscience*, 32(36):12406–12410.

Traynelis, S. F., Wollmuth, L. P., McBain, C. J., Menniti, F. S., Vance, K. M., Ogden, K. K., Hansen, K. B., Yuan, H., Myers, S. J., and Dingledine, R. (2010). Glutamate Receptor Ion Channels: Structure, Regulation, and Function. *Pharmacological Reviews*, 62(3):405–496.

Treadwell, S. D. and Robinson, T. G. (2007). Cocaine use and stroke. *Postgraduate Medical Journal*, 83(980):389–394.

Tritsch, N. X. and Sabatini, B. L. (2012). Dopaminergic Modulation of Synaptic Transmission in Cortex and Striatum. *Neuron*, 76(1):33–50.

Tseng, K. Y., Chambers, R. A., and Lipska, B. K. (2009). The neonatal ventral hippocampal lesion as a heuristic neurodevelopmental model of schizophrenia. *Behavioural brain research*, 204(2):295–305.

Tunstall, B. J. and Kearns, D. N. (2015). Sign-tracking predicts increased choice of cocaine over food in rats. *Behavioural Brain Research*, 281(81270370):222–228.

United Nations: Office on Drugs and Crime (2022). *World drug report 2022*. World Drug Report. United Nations, New York, NY.

Uys, J. and LaLumiere, R. (2008). Glutamate: The New Frontier in Pharmacotherapy for Cocaine Addiction. *CNS & Neurological Disorders - Drug Targets*, 7(5):482–491.

Vafaie, N. and Kober, H. (2022). Association of Drug Cues and Craving With Drug Use and Relapse: A Systematic Review and Meta-analysis. *JAMA psychiatry*, 79(7):641–650.

van Haaren, F. and Meyer, M. E. (1991). Sex differences in locomotor activity after acute and chronic cocaine administration. *Pharmacology, biochemistry, and behavior*, 39(4):923–927.

Van Tol, H. H. M., Bunzow, J. R., Guan, H.-C., Sunahara, R. K., Seeman, P., Niznik, H. B., and Civelli, O. (1991). Cloning of the gene for a human dopamine D4 receptor with high affinity for the antipsychotic clozapine. *Nature*, 350(6319):610–614.

Vaughan, R. A. and Foster, J. D. (2013). Mechanisms of dopamine transporter regulation in normal and disease states. *Trends in pharmacological sciences*, 34(9):489–496.

Vengeliene, V., Bespalov, A., Roßmanith, M., Horschitz, S., Berger, S., Relo, A. L., Noori, H. R., Schneider, P., Enkel, T., Bartsch, D., Schneider, M., Behl, B., Hansson, A. C., Schloss, P., and Spanagel, R. (2017). Towards trans-diagnostic mechanisms in psychiatry: neurobehavioral profile of rats with a loss-of-function point mutation in the dopamine transporter gene. *Disease models & mechanisms*, 10(4):451–461.

Vengeliene, V. and Spanagel, R. (2022). mGlu2 mechanism-based interventions to treat alcohol relapse. *Frontiers in pharmacology*, 13:985954.

Vogel, C. and Marcotte, E. M. (2012). Insights into the regulation of protein abundance from proteomic and transcriptomic analyses. *Nature reviews. Genetics*, 13(4):227–232.

Vohra, H. Z., Saunders, J. M., Jaster, A. M., de la Fuente Revenga, M., Jimenez, J., Fernández-Teruel, A., Wolstenholme, J. T., Beardsley, P. M., and González-Maeso, J. (2022). Sex-specific effects of psychedelics on prepulse inhibition of startle in 129S6/SvEv mice. *Psychopharmacology*, 239(6):1649–1664.

Volkow, N. D. and Fowler, J. S. (2000). Addiction, a disease of compulsion and drive: Involvement of the orbitofrontal cortex. *Cerebral Cortex*, 10(3):318–325.

Volkow, N. D., Fowler, J. S., and Wang, G. J. (1999). Imaging studies on the role of dopamine in cocaine reinforcement and addiction in humans. *Journal of psychopharmacology (Oxford, England)*, 13(4):337–345.

Volkow, N. D., Fowler, J. S., and Wang, G.-J. (2003). The addicted human brain: insights from imaging studies. *The Journal of clinical investigation*, 111(10):1444–1451.

Vollenweider, F. X., Vollenweider-Scherpenhuyzen, M. F. I., Bäbler, A., Vogel, H., and Hell, D. (1998). Psilocybin induces schizophrenia-like psychosis in humans via a serotonin-2 agonist action. *NeuroReport*, 9(17):3897–3902.

Wagner, F. A. and Anthony, J. C. (2002). From first drug use to drug dependence: Developmental periods of risk for dependence upon marijuana, cocaine, and alcohol. *Neuropsychopharmacology*, 26(4):479–488.

Waldrop, A. E., Price, K. L., Desantis, S. M., Simpson, A. N., Back, S. E., McRae, A. L., Spratt, E. G., Kreek, M. J., and Brady, K. T. (2010). Community-dwelling cocaine-dependent men and women respond differently to social stressors versus cocaine cues. *Psychoneuroendocrinology*, 35(6):798–806.

Walker, Q. D., Cabassa, J., Kaplan, K. A., Li, S. T., Haroon, J., Spohr, H. A., and Kuhn, C. M. (2001). Sex differences in cocaine-stimulated motor behavior: Disparate effects of gonadectomy. *Neuropsychopharmacology*, 25(1):118–130.

Wang, J., Liang, M., Shang, Q., Qian, H., An, R., Liu, H., Shao, G., Li, T., and Liu, X. (2023). Psilocin suppresses methamphetamine-induced hyperlocomotion and acquisition of conditioned place preference via D2R-mediated ERK signaling. *CNS neuroscience & therapeutics*, 29(3):831–841.

Watson, P., Pearson, D., Most, S. B., Theeuwes, J., Wiers, R. W., and Le Pelley, M. E. (2019). Attentional capture by Pavlovian reward-signalling distractors in visual search persists when rewards are removed. *PloS one*, 14(12):e0226284.

Watson, P., Prior, K., Ridley, N., Monds, L., Manning, V., Wiers, R. W., and Le Pelley, M. E. (2024). Sign-tracking to non-drug reward is related to severity of alcohol-use problems in a sample of individuals seeking treatment. *Addictive Behaviors*, 154:108010.

Weiss, F., Maldonado-Vlaar, C. S., Parsons, L. H., Kerr, T. M., Smith, D. L., and Ben-Shahar, O. (2000). Control of cocaine-seeking behavior by drug-associated stimuli in rats: Effects on recovery of extinguished operant-responding and extracellular dopamine levels in amygdala and nucleus accumbens. *Proceedings of the National Academy of Sciences of the United States of America*, 97(8):4321–4326.

Westermeyer, J. (2006). Comorbid Schizophrenia and Substance Abuse: A Review of Epidemiology and Course. *The American Journal on Addictions*, 15(5):345–355.

Wheeler, D. S., Chang, S. E., and Holland, P. C. (2013). Odor-mediated taste learning requires dorsal hippocampus, but not basolateral amygdala activity. *Neurobiology of learning and memory*, 101:1–7.

Willner, P., Muscat, R., and Papp, M. (1992). Chronic mild stress-induced anhedonia: A realistic animal model of depression. *Neuroscience & Biobehavioral Reviews*, 16(4):525–534.

Willstätter, R. and Bode, A. (1901). Ueberführung von Tropinon in r□Cocaïn. *Berichte der deutschen chemischen Gesellschaft*, 34(2):1457–1461.

Wilson, L. D., Jeromin, J., Garvey, L., and Dorbandt, A. (2001). Cocaine, ethanol, and cocaethylene cardiotoxity in an animal model of cocaine and ethanol abuse. *Academic Emergency Medicine*, 8(3):211–222.

Winship, I. R., Dursun, S. M., Baker, G. B., Balista, P. A., Kandratavicius, L., Maia-de Oliveira, J. P., Hallak, J., and Howland, J. G. (2019). An Overview of Animal Models Related to Schizophrenia. *Canadian journal of psychiatry. Revue canadienne de psychiatrie*, 64(1):5–17.

Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews Neuroscience*, 5(6):483–494.

Wise, R. A. (2008). Dopamine and reward: The anhedonia hypothesis 30 years on. *Neurotoxicity Research*, 14(2-3):169–183.

Wise, R. A. and Jordan, C. J. (2021). Dopamine, behavior, and addiction. *Journal of Biomedical Science*, 28(1):83.

Wise, R. A. and Robble, M. A. (2020). Dopamine and Addiction. *Annual Review of Psychology*, 71(1):79–106.

Witteman, J., Post, H., Tarvainen, M., De Bruijn, A., Perna, E. D. S. F., Ramaekers, J. G., and Wiers, R. W. (2015). Cue reactivity and its relation to craving and relapse in alcohol dependence: A combined laboratory and field study. *Psychopharmacology*, 232(20):3685–3696.

World Health Organization (2019). International Classification of Diseases, 11th Revision (ICD-11). \url{https://icd.who.int/}.

Xi, Z.-X., Kiyatkin, M., Li, X., Peng, X.-Q., Wiggins, A., Spiller, K., Li, J., and Gardner, E. L. (2010). N-acetylaspartylglutamate (NAAG) inhibits intravenous cocaine self-administration and cocaine-enhanced brain-stimulation reward in rats. *Neuropharmacology*, 58(1):304–313.

Xie, X. and Steketee, J. D. (2009). Effects of repeated exposure to cocaine on group II metabotropic glutamate receptor function in the rat medial prefrontal cortex: behavioral and neurochemical studies. *Psychopharmacology*, 203(3):501–510.

Yager, L. M. and Robinson, T. E. (2010). Cue-induced reinstatement of food seeking in rats that differ in their propensity to attribute incentive salience to food cues. *Behavioural Brain Research*, 214(1):30–34.

Yager, L. M. and Robinson, T. E. (2013). A classically conditioned cocaine cue acquires greater control over motivated behavior in rats prone to attribute incentive salience to a food cue. *Psychopharmacology*, 226(2):217–228.

Yang, H. J., Zhang, H. Y., Bi, G. H., He, Y., Gao, J. T., and Xi, Z. X. (2017). Deletion of Type 2 Metabotropic Glutamate Receptor Decreases Sensitivity to Cocaine Reward in Rats. *Cell Reports*, 20(2):319–332.

Ystrom, E., Reichborn-Kjennerud, T., Neale, M. C., and Kendler, K. S. (2014). Genetic and Environmental Risk Factors for Illicit Substance Use and Use Disorders: Joint Analysis of Self and Co-twin Ratings. *Behavior Genetics*, 44(1):1–13.

Zahm, D. S. (2000). An integrative neuroanatomical perspective on some subcortical substrates of adaptive responding with emphasis on the nucleus accumbens. *Neuroscience and biobehavioral reviews*, 24(1):85–105.

Zakharova, E., Wade, D., and Izenwasser, S. (2009). Sensitivity to cocaine conditioned reward depends on sex and age. *Pharmacology Biochemistry and Behavior*, 92(1):131–134.

Zapata, A., Minney, V. L., and Shippenberg, T. S. (2010). Shift from goal-directed to habitual cocaine seeking after prolonged experience in rats. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 30(46):15457–15463.

Zhang, G., Ásgeirsdóttir, H. N., Cohen, S. J., Munchow, A. H., Barrera, M. P., and Stackman, R. W. J. (2013). Stimulation of serotonin 2A receptors facilitates consolidation and extinction of fear memory in C57BL/6J mice. *Neuropharmacology*, 64(1):403–413.

Zhao, Y., Dayas, C. V., Aujla, H., Baptista, M. A. S., Martin-Fardon, R., and Weiss, F. (2006). Activation of group II metabotropic glutamate receptors attenuates both stress and cue-induced ethanol-seeking and modulates c-fos expression in the hippocampus and amygdala. *The Journal of neuroscience : the official journal of the Society for Neuroscience*, 26(39):9967–9974.

Zhou, Q.-Y., Grandy, D. K., Thambi, L., Kushner, J. A., Tol, H. H. M. V., Cone, R., Pribnow, D., Salon, J., Bunzow, J. R., and Civelli, O. (1990). Cloning and expression of human and rat Dt dopamine receptors. *Nature*, 347(6288):76–80.

Zhou, Y. and Danbolt, N. C. (2014). Glutamate as a neurotransmitter in the healthy brain. *Journal of Neural Transmission*, 121(8):799–817.

Zillich, L., Artioli, A., Pohořalá, V., Zillich, E., Stertz, L., Belschner, H., Jabali, A., Frank, J., Streit, F., Avetyan, D., Völker, M. P., Müller, S., Hansson, A. C., Meyer, T. D., Rietschel, M., Ladewig, J., Spanagel, R., Oliveira, A. M. M., Walss-Bass, C., Bernardi, R. E., Koch, P., and Witt, S. H. (2025). Cell type-specific multi-omics analysis of cocaine use disorder in the human caudate nucleus. *Nature Communications*, 16(1):3381.

Zimmerman, J. L. (2012). Cocaine Intoxication. *Critical Care Clinics*, 28(4):517–526.