Activity of Dorsal Raphe Serotonin Neurons in Mice with Differential TAAR1 Function

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A DISSERTATION

Presented to the Department of Behavioral Neuroscience

Oregon Health & Science University

School of Medicine in Partial Fulfillment of

the Requirements for the Degree of

Doctor of Philosophy

CERTIFICATE OF APPROVAL

School of Medicine

Oregon Health & Science University

This dissertation was presented on

January 31st, 2025

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List of Abbreviations

5-HT — serotonin

5-HT1A — serotonin receptor 1A

5-HT1B — serotonin receptor 1B

AMPA — a-amino-3-hydroxy-5-methyl-4-isoazolepropionic acid

AMPH — amphetamine

ANOVA — analysis of variance

B6 — C57BL/6J

C — Celsius

cAMP — 3', 5' cyclic adenosine monophosphate

CNS — central nervous system

CPA — conditioned place aversion

CPP — conditioned place preference

CRISPR-*Cas9* — clustered regularly interspaced short palindromic repeat (CRISPR)-associated protein 9

CTA — conditioned taste aversion

D2 — DBA/2J

D2R — dopamine D2-receptor

DA — dopamine

DAT — dopamine transporter

DMSO — dimethyl sulfoxide

DR — dorsal raphe

DREADD — designer receptors exclusively activated by designer drugs

EAAT3 — excitatory amino acid transporter 3

EPSC — excitatory postsynaptic current

F2 — second filial generation offspring

g — gram

GABA — glutamic acid decarboxylase

GIRKs — G protein-coupled inwardly-rectifying potassium channels

GPCR — G protein-coupled receptor

h — hour

HEK — human embryonic kidney cells

kg — kilogram

KI — knock-in

Ki — inhibition constant

KO — knock-out

L — liter

LHb — lateral habenula

M — molar

MA — methamphetamine

MADR — methamphetamine drinking

MAHDR — methamphetamine high drinking

MALDR — methamphetamine low drinking

MDMA — 3,4-methylenedioxymethamphetamine

mEPSC — miniature EPSC

mg — milligram

mGluR — metabotropic glutamate receptors

mg/kg — milligram per kilogram

mg/l — milligrams per liter

mL — milliliter

mM — millimolar

mPFC — medial prefrontal cortex

mRNA — messenger ribonucleic acid

µM — micromolar

nM — nanomolar

NE — norepinephrine

NET — norepinephrine transporter

NMDA — N-methyl-D-aspartate

NAc — nucleus accumbens

Oprm1 — mouse µ-opioid receptor gene

OPRM1 — human µ-opioid receptor gene

PAG — periaqueductal gray

PFC — prefrontal cortex

PKA — protein kinase A

PKC — protein kinase C

PPR — paired-pulse ratio

QTL — quantitative trait locus

RhoA — Ras homolog family member A

sEPSC — spontaneous EPSC

SERT — serotonin transporter

SNP — single nucleotide polymorphism

Taar1 — gene encoding trace amine-associated receptor 1

Taar1^{+} — C57BL/6J trace amine-associated receptor 1 allele

Taar1^{m1J} — DBA/2J trace amine-associated receptor 1 allele

TAAR1 — trace amine-associated receptor 1

TpH2 — tryptophan hydroxylase 2**TTX** — tetrodotoxin

VGluT3 — vesicular glutamate transporter 3

VMAT2 — vesicular monoamine transporter 2

VTA — ventral tegmental area

WT — wildtype

Acknowledgements

My time at OHSU did not come without its challenges but there were so many people along the way who helped me persevere. I would first like to express my gratitude to my mentors Dr. Tamara Phillips and Dr. Susan Ingram. When I didn't know where to turn, you welcomed me into your laboratories without hesitation. I admire both of you for your dedication to your science and to all students. Thank you for your unwavering commitment to my training as a scientist.

I am profoundly grateful to my family. I would not be here today without the sacrifices my grandparents made when they left Cuba, giving up everything and everyone they knew so that their children — and future generations — could have more than they did. I think my partner said it best "*You are the dreams of our immigrant parents*" and that is just as true for any grandparents. Abuela, I miss you. I wish you could have seen me cross this finish line, but I know you're somewhere out there, dancing in celebration. I am especially grateful to my father. Thank you for giving me strength when I felt defeated and for celebrating every victory with me, no matter how small. You never failed to remind me that I am a Rios and no matter what, we don't quit. You have supported my love of science in every imaginable way and taught me that nothing is insurmountable. This Ph.D. is dedicated to you dad, it is as much yours as it is mine. And to my little brother Joshua, thank you for all the silly facetimes, the laughter, the singing, and for being your sweet lovable self.

Thank you to my many friends who cheered me on along the way. A special thanks to Caitlynn De Preter and Michelle Palumbo. You both were there for me during the good, the great, the bad, and the brutal. All the late-night conversations, dinners, workouts, nights at Sukis, camping trips, and random adventures made graduate school a brighter experience. I'm beyond grateful for your friendship, it has meant more to me than I can put into words.

And to my partner Joseph Moreno — thank you. Being the partner of a graduate student is no easy task, but you showed up every day, always finding ways both big and small to support

me. You kept me steady when the work felt overwhelming and turned my anxiety about my defense into genuine excitement. Thank you for reminding me that it is ok to step away from work, to take time to do the things I love, and to find joy outside of the constant grind of a Ph.D. And I could never forget your sweet little pup Lola, my good luck charm. Thanks for all the cuddles and countless walks that gave me the chance to enjoy the fresh air and reset my mind.

Abstract

Methamphetamine (MA) is a highly addictive and potent psychostimulant that induces both rewarding and aversive effects that are likely to contribute to variation in voluntary MA intake. Genetic predisposition can play a critical role in influencing individual sensitivity to these effects, shaping patterns of MA consumption. To investigate the contribution of genetic factors on these behaviors, the MA drinking lines (MADRs) were developed from an F2 cross of C57BL/6J and DBA/2J inbred strain mice. The MADRs consist of the MA high drinking (MAHDR) and the MA low drinking (MALDR) lines. In addition to differences in MA intake, MAHDR mice exhibit high sensitivity to the rewarding effects of MA and low sensitivity to the aversive effects of MA. Conversely, MALDR mice exhibit low sensitivity to the rewarding effects of MA and high sensitivity to the aversive effects of MA. A quantitative trait locus analysis identified a region on mouse chromosome 10 that accounts for at least 60% of the genetic variance in MA intake between the lines. Within this region is the trace amine-associated receptor 1 gene (*Taar1*) which encodes the TAAR1 receptor at which MA acts as an agonist. MAHDR mice are homozygous for the DBA/2J mutant *Taar1* allele, $Taar1^{m1J}$, which encodes a non-functional TAAR1 receptor due to a single nucleotide polymorphism. On the other hand, MALDR mice possess at least one copy of the C57BL/6J reference allele, $Taar1^{+}$, which encodes a functional TAAR1 receptor. To assess the causal role of *Taar1* gene variants on MA intake and reward/aversion sensitivities, the laboratory utilized CRISPR-*Cas9*-technology to replace the mutant $Taar1^{m1J}$ allele with the reference $Taar1^{+}$ allele on the MAHDR background and create MAHDR-$Taar1^{+/+}$ knock-in (KI) mice. This approach revealed that *Taar1* gene variants directly influence multiple MA effects. Thus, KI of the $Taar1^{+}$ allele significantly reduced voluntary MA intake to levels comparable to MALDR mice, significantly reduced sensitivity to MA-conditioned reward, and increased sensitivity to MA-conditioned aversion. This is in comparison to the MAHDR-$Taar1^{m1J/m1J}$ control line.

The overall goal of this dissertation was to investigate how TAAR1 functionality influences the activity of dorsal raphe serotonin (5-HT) neurons and their glutamatergic inputs, both in the absence and presence of MA. Additionally, the impact of TAAR1 functionality on binge-level MA intake was examined. The established role of dorsal raphe 5-HT neurons in reward processing, combined with the impact of differential TAAR1 function on MA intake and MA reward sensitivity exhibited by MADR and CRISPR-*Cas9*-generated lines provides a unique opportunity to study the relationship between TAAR1 functionality, 5-HT neuron activity, and MA intake/reward sensitivities.

The first aim of this project focused on characterizing the effect of MA on the activity of dorsal raphe 5-HT neurons in mice with differential TAAR1 functionality and began to investigate the TAAR1-dependent mechanisms underlying these effects. Additionally, I report the impact of CRISPR-*Cas9*-mediated KI of functional TAAR1 on the MAHDR background on binge-level MA intake. Electrophysiological recordings from the MADR lines demonstrated that MA significantly hyperpolarized and decreased the activity of dorsal raphe 5-HT neurons from MALDR mice, but not MAHDR mice. However, the activation of TAAR1 results in increased extracellular monoamine levels which results in activation of 5-HT inhibitory autoreceptors. Therefore, I also examined the effect of MA in the presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists to determine whether activation of these autoreceptors mediates the effects of MA. I found that the MA-induced hyperpolarization and inhibition of MALDR dorsal raphe 5-HT neuron activity is dependent on the activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors. In contrast, MA depolarized and increased firing of MAHDR dorsal raphe 5-HT neurons in the presence of autoreceptor antagonists. To establish a causal link between the activity of dorsal raphe 5-HT neurons in response to MA and TAAR1 functionality, I repeated the experiments on DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines using the autoreceptor antagonists. In the MAHDR-*Taar1*$^{m1J/m1J}$ controls which lack functional TAAR1, MA-induced

depolarization and potentiation of dorsal raphe 5-HT neuron activity persisted. However, KI of functional TAAR1 on the MAHDR background abolished the MA-induced depolarization and potentiation of dorsal raphe 5-HT neurons, indicating that the absence of TAAR1 function is critical for MA-induced excitability of dorsal raphe 5-HT neurons. Furthermore, because MA is a substrate of the 5-HT transporter, I assessed whether the MA-induced depolarization and potentiation of dorsal raphe 5-HT neurons from mice lacking functional TAAR1 is dependent on 5-HT transporters. The 5-HT transporter antagonist fluoxetine blocked MA-induced depolarization and potentiation of dorsal raphe 5-HT neurons from both MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice. Finally, I report that KI of functional TAAR1 on the MAHDR background blocked binge-level MA intake. Overall, these findings highlight the complex relationship between TAAR1 functionality, MA intake/MA reward sensitivity, and the activity of 5-HT neurons in response to MA.

The second aim of this project focused on characterizing how TAAR1 functionality influences glutamatergic synaptic transmission onto dorsal raphe 5-HT neurons, both at baseline and in response to MA. Electrophysiological recordings from dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice demonstrated significantly higher mean spontaneous excitatory post synaptic current (sEPSC) frequencies compared to recordings from MAHDR-*Taar1*$^{m1J/m1J}$ control mice. There was no observed difference in mean sEPSC amplitude between the lines. In response to MA, sEPSC frequency onto dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice significantly increased. In contrast, sEPSC frequency onto dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice decreased in response to MA. I also measured and found no line difference in the paired pulse ratio (PPR) of dorsal raphe 5-HT neurons. However, in response to MA, the PPR of dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice was significantly increased. Conversely, MA significantly decreased the PPR of dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice. Finally, I found that MA-induced depolarization and

potentiation of DR 5-HT neurons in mice lacking functional TAAR1 is dependent on NMDA receptor activation.

Together, these data demonstrate that TAAR1 functionality influences the intrinsic activity of dorsal raphe 5-HT neurons in response to MA, binge-level MA intake, and glutamatergic synaptic transmission onto these neurons at baseline and in presence of MA. These findings establish a critical link between dorsal raphe 5-HT neuron activity, glutamatergic synaptic transmission, and the differences in MA-related behaviors observed in MADR and CRISPR-*Cas9*-generated and control mice.

Chapter 1: General Introduction

History of Methamphetamine

Methamphetamine (MA) is a powerful and highly addictive central nervous system (CNS) stimulant. MA was first synthesized by the Japanese chemist Nagai Nagayoshi while researching ephedrine, an active alkaloid derived from the ephedra plant, which has been used for over five thousand years in traditional Chinese medicine. Nagayoshi determined that the chemical structure of amphetamine (AMPH), first synthesized in 1887 at the University of Berlin by chemist Lazar Edelenau (Chiu & Schenk, 2012; Parsons, 2013), was like ephedrine and produced similar physiological effects. He would go on to synthesize MA from ephedrine in 1893 (Anglin et al., 2000; Chiu & Schenk, 2012; Meredith et al., 2005; Parsons, 2013). In 1919, Akira Ogata created MA hydrochloride by reducing ephedrine using red phosphorus and iodine (Chiu & Schenk, 2012; Khan et al., 2012).

MA has an additional methyl group compared to AMPH which enhances its lipophilicity. This facilitates the ability of MA to more freely cross the blood brain barrier, contributing to its heightened potency, relative to AMPH (Barr et al., 2006; Nordahl et al., 2003). Clinical use of MA did not begin until the 1930's, when MA was used as a bronchodilator and nasal decongestant. The capacity of MA to increase alertness, suppress hunger, and alleviate fatigue rendered it highly sought-after during World War II, when it was administered to soldiers by both Allied and Axis powers (Anglin et al., 2000; Blakemore, 2017; Meredith et al., 2005). The same positive side effects that led the military to distribute MA, along with other benefits, also led to MA being prescribed to the public in the 1940's, although AMPH tablets were available without prescription until 1951. At its peak in 1967, 31 million AMPH prescriptions were written (Anglin et al., 2000). In 1968, after months of investigation, the United States Senate reported that diet pills containing AMPH were linked to at least 60 deaths and numerous adverse effects (Cohen et al., 2012). In the United States,

as pharmaceutical companies were forced to withdraw their AMPH-related drugs, illegal manufacturing labs emerged (Anglin et al., 2000; Cohen et al., 2012; Meredith et al., 2005). The use of AMPHs has only increased in recent decades. In the United States between 2015 and 2019, persons reporting past year MA use increased 43%, the number of adults with MA use disorder increased by 62%, and those reporting frequent MA use (>100d/year) increased by 66%. These trends have resulted in an 180% increase in overdose deaths involving psychostimulants (Han et al., 2021)

Pharmacokinetics of Methamphetamine

MA can be administered in several ways that shape the type of euphoria experienced, bioavailability, time to reach peak plasma concentration, half-life, and time to peak effect. MA is commonly administered by smoking or injection because it allows the drug to quickly enter the bloodstream and brain, producing an immediate but short and intense "rush." If MA is administered via other routes, it produces a delayed, longer lasting, and less intense "high" (Meredith et al., 2005).

Pharmacokinetics in Humans

Intravenous administration of 30 mg of MA yields 100% bioavailability, reaching peak concentration at 6 ± 11 minutes, a half-life ranging from 8-16 hours, and subjective effects peaking within 15 minutes (Cruickshank & Dyer, 2009). MA can also be snorted or ingested orally to produce a longer lasting "high." Oral administration of 30 mg yields 67±3% bioavailability, reaching peak concentrations anywhere from 180-300 minutes, a half-life of 3-17 hours, and subjectively reaching peak effects around 180 minutes after ingestion (Cruickshank & Dyer, 2009).

Studies examining the plasma half-life of MA, the time it takes for the blood concentration of a drug to decrease by 50%, describe variable timing due to inter-individual

variability however most report mean values between 9-12 hours in humans (Cook et al., 1993; Cook et al., 1992; Cruickshank & Dyer, 2009; Harris et al., 2003; Schep et al., 2010; Schepers et al., 2003). Notably, the plasma half-life of MA appeared to be similar across routes of administration (Cook et al., 1993; Cruickshank & Dyer, 2009; Harris et al., 2003; Schep et al., 2010).

MA and its metabolites are predominately excreted in urine. Approximately 70% is excreted within the first 24 hours and 90% within the first four days (Cook et al., 1993; Kim et al., 2004; Schep et al., 2010). However, with repeated or chronic MA dosing, metabolites (byproducts of the body breaking down MA) can be detected in human urine up to 8 days later (Huestis & Cone, 2007; Kim et al., 2004; Oyler et al., 2002). Metabolites of MA include AMPH and norepinephrine; however, none of the metabolites appear to significantly contribute to the primary effects of MA in humans (Caldwell et al., 1972; Perez-Reyes et al., 1991).

Pharmacokinetics in Rodents

In mice, peak MA concentration occurred as early as 5 minutes in blood samples and 20 minutes in brain tissue following intraperitoneal injections and remained detectable up to 4 hours post-injection in both blood samples and brain tissue (Shabani, McKinnon, et al., 2012; Tuv et al., 2021). The plasma half-life of MA, defined as the time required for plasma concentrations to fall by 50%, was approximately 35 minutes in blood samples and 45 min in brain tissue following intravenous injection in mice (Tuv et al., 2021; Wagner et al., 2018). MA produces the same metabolites in rodents as in humans (Caldwell et al., 1972).

Subjective and Physiological Effects of Methamphetamine

The subjective and physiological effects of MA in humans are influenced by a variety of factors including, but not limited to, acute vs prolonged use, frequency of use, and dose. The acute effects of MA span multiple modalities such as physiological and psychological.

Physiologically, there are the "desirable" effects such as loss of appetite for weight loss, increased arousal, and increased energy. There are also "undesirable" physiological effects including tachypnea, hyperthermia and tachycardia (Cruickshank & Dyer, 2009; Hart et al., 2008; Meredith et al., 2005). Psychologically, the "desirable" effects may include euphoria, increased mental focus, and sociability (Cruickshank & Dyer, 2009; Hart et al., 2008). Conversely, there are also "undesirable" psychological side effects such as anxiety, paranoia, and aggression (Anglin et al., 2000; May et al., 2020).

Propensity to continue or discontinue MA use is potentially influenced by the individual's experiences with the drug. An individual's relative sensitivity to the rewarding or aversive effects of MA may predict vulnerability to addiction (Chait, 1993; de Wit et al., 1986; Shabani et al., 2011). Academic research has emphasized studying the rewarding effects associated with MA addiction, often overlooking MA-induced aversion – a potential protective factor that may reduce the likelihood of addiction by reducing a person's desire to engage in repeated use. A stronger aversive response may compel an individual to avoid MA rather than engage in drug seeking behavior. This dissertation focuses on a mouse model with differential sensitivity to the rewarding and aversive effects of MA, illustrating the impact of these contrasting responses on voluntary MA intake. The model and its implications will be explored in greater detail later in this chapter.

Pharmacodynamics and Mechanism of Action of Methamphetamine

MA is a derivative of AMPH, distinct due to the presence of an additional methyl group. This additional methyl group yields a more lipid soluble compound allowing MA to more efficiently pass through the blood brain barrier compared to AMPH (Barr et al., 2006; Nordahl et al., 2003). Importantly, MA and AMPH are structurally similar enough that they possess the same molecular targets, membrane monoamine transporters, vesicular monoamine transporters, and the trace amine-associated receptor 1 (TAAR1).

Membrane Monoamine Transporters

MA shares structural similarities with other monoamines: serotonin (5-HT), dopamine (DA), and norepinephrine (NE). When peripherally taken or administered, after crossing the blood brain barrier, MA competes with these endogenous monoamines for transport through their respective transporters (Cruickshank & Dyer, 2009; Fleckenstein et al., 2007).

However, MA demonstrates varying transporter affinities, meaning the preference for which MA passes through each monoamine transporter varies. In Rothman et al. (2001), transporter affinity was measured using *Ki*. *Ki* represents the inhibition constant and is used below to describe the affinity of MA for binding sites on NE, DA, and 5-HT transporters, thereby blocking the uptake of these neurotransmitters. A lower *Ki* indicates a higher binding affinity for MA at a particular transporter, meaning that MA can occupy 50% of the binding sites on the transporter even when present at a lower concentration. In synaptosomes, (+) MA demonstrated the highest affinity for the NE transporter (NET) ($Ki = 48.0 \pm 5.1$ nM), followed by the dopamine transporter (DAT) ($Ki = 114 \pm 11$ nM), and finally the serotonin transporter (SERT) ($Ki = 2137 \pm 98$ nM). Additionally, (+) MA was most potent at releasing NE ($IC_{50} = 12.3$ nM), followed by DA release ($IC_{50} = 24.5$ nM) and 5-HT release ($IC_{50} = 736$ nM) (Rothman et al., 2001).

Competition for uptake by monoaminergic transporters and MA-induced changes in transporter function disrupts the regulatory balance of these neurotransmitters, allowing for their accumulation in the synapse (Haughey, Brown, et al., 2000; Haughey, Fleckenstein, et al., 2000; Kahlig et al., 2005; Rothman & Baumann, 2003; Sitte et al., 1998). MA also interacts with transporters through a mechanism involving TAAR1, a process that will be discussed in greater detail below.

Vesicular Monoamine Transporters

MA is also a substrate of and binds to the vesicular monoamine transporter 2 (VMAT2) (Fleckenstein et al., 2009; Fleckenstein et al., 2007; Peter et al., 1994; Sulzer et al., 2005). VMAT2 is highly expressed in vesicular membranes within monoaminergic neurons and is responsible for transporting cytosolic monoamines into synaptic vesicles for future neurotransmission (Edwards, 1992; Eiden et al., 2004; Peter et al., 1995; Weihe et al., 1994). Upon being transported into the cell, MA further disrupts the regulatory balance of neurotransmitters. In this case, MA competes with monoamines for vesicle uptake while also triggering monoamine release from vesicles, resulting in increased cytosolic monoamine concentrations (Panenka et al., 2013; Rothman et al., 2001; Sulzer et al., 2005). It is important to note that studies on the effects of AMPHs on VMAT2 function emphasize DA, though there is evidence for effects on 5-HT (Torres & Ruoho, 2014), which is the focus of the work presented in Chapters 2 and 3. At physiologically relevant concentrations, AMPHs bind to VMAT2 and competitively inhibit DA uptake (K. Schwartz et al., 2006). The mechanism by which MA triggers vesicle release remains unclear. One hypothesis relies on the fact that MA is a weak base, the "weak base hypothesis" (Sulzer & Rayport, 1990). Importantly, stereotypical VMAT2 function requires an acidic environment, therefore, when MA accumulates into vesicles it alters the pH, altering the physiological activity of vesicles (Panenka et al., 2013). However, MA concentrations would need to be quite high to significantly disrupt the pH gradient (Floor & Meng, 1996; K. Schwartz et al., 2006).

Trace amine-associated receptor 1

Trace amines are endogenous molecules structurally related to classical biogenic amines like DA and 5-HT, with which they share subcellular localization. However, trace amines are present at lower concentrations, hence the name "trace." Due to their similarity to biogenic amines and their ability to displace these amines from storage vesicles, trace amines are

sometimes referred to as “false transmitters.” However, trace amines also function independently of classical amines, mediating a subset of events via trace amine-associated receptors (TAARs) (Berry, 2004; Borowsky et al., 2001; Lindemann & Hoener, 2005). There are nine TAARs, all of which are G protein-coupled receptors (GPCRs). TAARs are named for their ability to bind endogenous amines at trace, nanomolar concentrations (Berry, 2004; Borowsky et al., 2001; Bunzow et al., 2001; Rutigliano et al., 2017). TAARs are present in various organs including the heart, stomach, and the brain (Borowsky et al., 2001; Chiellini et al., 2007).

The GPCR TAAR1 is of particular interest because MA acts as a potent agonist (Bunzow et al., 2001). Immunofluorescence and *in situ hybridization* histochemistry revealed that TAAR1 is expressed intracellularly, localized to the cytoplasm of neurons in the brain (Borowsky et al., 2001; Bunzow et al., 2001). Importantly, TAAR1 is widely expressed within neurons of the dorsal raphe (DR) (Borowsky et al., 2001; Lindemann et al., 2008; Rutigliano et al., 2017), which is the brain region of interest in Chapters 2 and 3.

The following studies focus on AMPH and MDMA; however, given their structural and functional similarities to MA, these findings are likely applicable to MA as well. Intracellular TAAR1 couples to both $G\alpha_s$ and $G\alpha_{13}$ subunits (Underhill & Amara, 2020; Underhill et al., 2019). AMPH-induced activation of TAAR1 receptors coupled to $G\alpha_{13}$ subunits stimulates the small GTPase Ras homolog family member A (RhoA), leading to the internalization of SERT, DAT, NET, and the neuronal glutamate transporter 3 (EAAT3) in 5-HT, DA, and NE neurons. This transporter internalization resulted in significantly decreased uptake of 5-HT, DA, NE, and glutamate, thereby significantly increasing their extracellular concentrations (Underhill & Amara, 2020; Underhill et al., 2020; Underhill et al., 2019; Underhill et al., 2014; Wheeler et al., 2015). In DA and NE neurons, AMPH-induced activation of TAAR1 coupled to $G\alpha_s$ subunits stimulated cyclic AMP (cAMP) production resulting in the phosphorylation of RhoA by protein kinase A (PKA), which inactivated RhoA and terminated transporter internalization (Underhill et al., 2020;

Underhill et al., 2019). Importantly, AMPH-mediated activation of RhoA and PKA, internalization of EAAT3, and the subsequent reduction in glutamate uptake relies on the co-expression of DAT and EAAT3 (Underhill et al., 2019; Underhill et al., 2014). While the activation of PKA has not yet been confirmed in 5-HT neurons, it is presumed that a similar mechanism may apply.

Interestingly, the $G\alpha_{13}$-mediated signaling occurs in parallel with the $G\alpha_s$-mediated signaling, but there was a delay between the peak activity of each pathway (Underhill et al., 2019). Activity following AMPH-induced activation of the TAAR1 $G\alpha_{13}$ pathway peaked at 10 min and returned to baseline by 30 min. In contrast, activation of the TAAR1 $G\alpha_s$ pathway reached its peak at 30 min, but the time to return to baseline was not measured (Underhill et al., 2019; Wheeler et al., 2015). This temporal separation is crucial as it first allows the $G\alpha_{13}$ pathway to modulate neuronal activity before the $G\alpha_s$ pathway terminates its activity.

MA-induced TAAR1 activation also activates a protein kinase C (PKC) pathway, promoting DA efflux through DAT (Xie & Miller, 2007); however, the relationship between the PKC pathway and the $G\alpha_{13}$ or $G\alpha_s$-coupled pathways remains unclear. PKC can facilitate RhoA complex formation or act independently (Barandier et al., 2003).

A general model for the AMPH-activated TAAR1 signaling pathways in monoaminergic neurons (Figure 1.1).

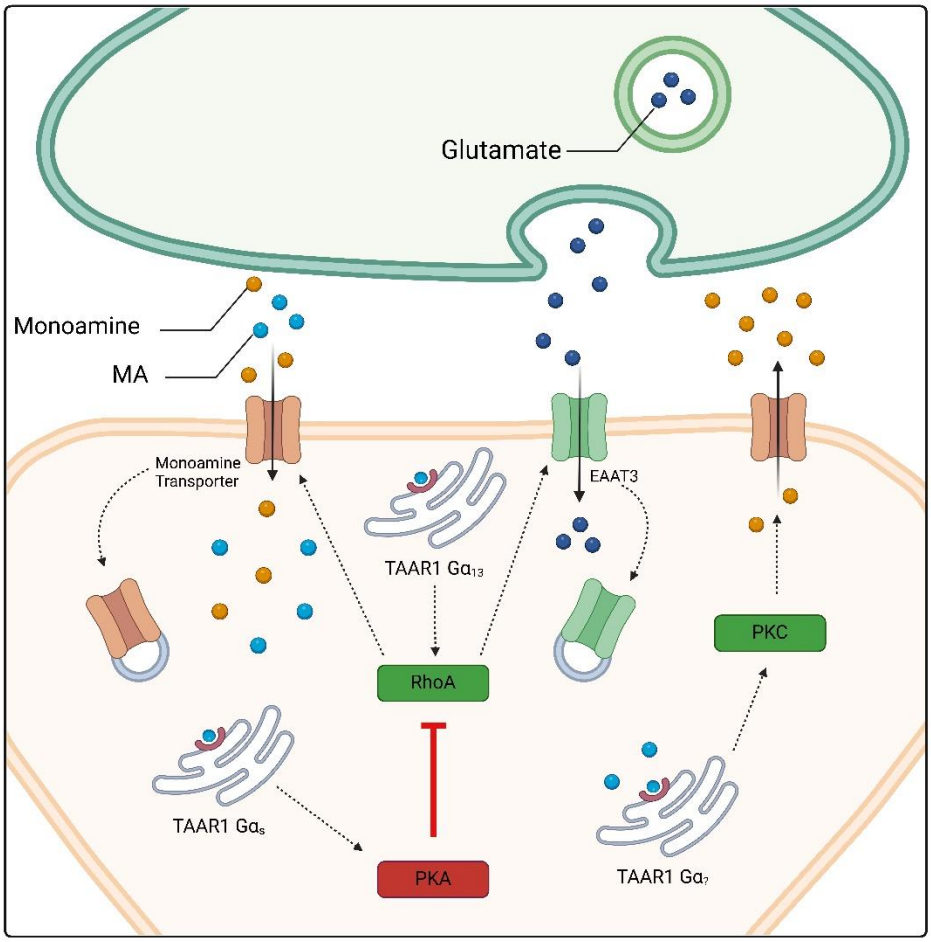


Figure 1.1. Proposed TAAR1 signaling mechanism in monoamine neurons in response to MA and other AMPHs. MA enters monoaminergic neurons (5-HT, DA, NE) through their respective transporters (SERT, DAT, NET). MA activates $G\alpha_{13}$-coupled TAAR1, a PKC pathway, and a delayed $G\alpha_s$-coupled TAAR1 pathway. Activation of $G\alpha_{13}$-coupled TAAR1 receptors stimulates RhoA activation leading to endocytosis of monoaminergic transporters and EAAT3. A TAAR1 mediated PKC pathway is activated either in formation with RhoA or independently and induces monoamine transporter efflux. Activation of $G\alpha_s$-coupled TAAR1 receptors stimulates PKA signaling leading to the phosphorylation of RhoA and the end of monoamine transporter internalization. Notably, the AMPH-indued activation of RhoA and PKA, internalization of EAAT3, and the subsequent decrease in glutamate uptake is dependent on the co-expression of DAT and EAAT3. *Created in BioRender.*

TAAR1: trace amine-associated receptor 1; MA: methamphetamine; AMPHs: amphetamines; 5-HT: serotonin; DA: dopamine; NE: norepinephrine; SERT: serotonin transporter; DAT: dopamine transporter; NET: norepinephrine transporter; PKC: protein kinase C; RhoA: Ras homolog family member A; PKA: protein kinase A

TAAR1 and Its Effect on the Activity of Monoaminergic Neurons

TAAR1 has been extensively investigated for its role in modulating dopaminergic activity, with increasing attention now being given to its effects on serotonergic and noradrenergic activity. TAAR1 activation by endogenous and exogenous agonists reduced the activity of monoaminergic neurons. p-tyramine, the endogenous TAAR1 agonist, decreased the spike frequency of ventral tegmental area (VTA) DA neurons of wildtype (WT) but not TAAR1 knockout (KO) mice (Bradaia et al., 2009; Lindemann et al., 2008). In response to the TAAR1 agonist, RO5166017, VTA DA, DR 5-HT, and locus coeruleus NE neurons expressing TAAR1 exhibited a reduction in firing activity (Revel et al., 2011). In DA, 5-HT, and noradrenergic neurons over-expressing TAAR1, RO5166017 reduced firing activity in all 3 populations. The RO5166017-induced decrease in firing activity was absent in TAAR1 KO cells (Revel et al., 2012).

As outlined previously, TAAR1 activation resulted in increased extracellular monoaminergic concentrations, therefore it is hypothesized that subsequent decreases in neural activity following TAAR1 activation are due to inhibitory autoreceptor activation**.** In support of this hypothesis, the TAAR1-induced increase in intracellular cAMP levels (Borowsky et al., 2001; Bunzow et al., 2001; Lindemann et al., 2005) were attenuated by antagonists for DA, 5-HT, and NE inhibitory autoreceptors (Xie et al., 2008). D2 receptors (D2Rs) function as dopamine inhibitory autoreceptors via activation of GIRKs (Beaulieu & Gainetdinov, 2011; Neve et al., 2004) and in the VTA, TAAR1 activation altered DA firing frequency through activation of GIRK channels (Bradaia et al., 2009). 5-HT_{1A} activation also results in activation of GIRKs (Polter & Li, 2010). In DR 5-HT neurons, activation of TAAR1 resulted in a significant increase in potency of a serotonergic inhibitory autoreceptor 5-HT_{1A} partial agonist (Revel et al., 2011). Additionally, $5HT_{1A}$ agonists alone potently inhibit DR 5-HT neuron activity while 5-HT_{1B} agonists exhibit

weak or irregular effects (Sprouse & Aghajanian, 1987). These findings suggest that TAAR1 regulates the activity of monoaminergic neurons via autoreceptor-mediated pathways.

No experiments have yet examined the effects of AMPHs on the firing rates of monoaminergic neurons in relation to TAAR1. However, we do know that MDMA inhibited DR neuron activity, an effect that was blocked by a SERT antagonist (Sprouse et al., 1989), suggesting a role for SERT in mediating this response. Further evidence in support of inhibitory autoreceptor activation and SERT mediating TAAR1-induced changes in neural activity can be found in Chapter 2.

TAAR1 activation also modulates glutamatergic activity onto monoaminergic neurons. As mentioned previously, TAAR1 activation also resulted in increased extracellular glutamate concentrations. As a result, our laboratory previously observed AMPH-induced potentiation of both AMPA-mediated (Underhill et al., 2014) and NMDA-mediated (Li et al., 2017; Underhill et al., 2019; Underhill et al., 2014) glutamatergic synaptic transmission onto DA neurons. Importantly, these experiments were done in the presence of D2R antagonists. Additionally, the AMPH-induced potentiation of glutamatergic activity was found to be dependent on the internalization of EAAT3 (Underhill et al., 2019; Underhill et al., 2014). These data further support the hypothesis TAAR1's modulation of monoaminergic and glutamatergic activity are intricately linked to autoreceptor feedback mechanisms and transporter dynamics. In Chapter 3, I discuss the preliminary work I have done to elucidate the effect of MA on glutamatergic synaptic transmission onto DR 5-HT neurons.

Selective Breeding as a Tool to Study Addiction

Selectively breeding animals based on a chosen single phenotype is a powerful tool for investigating the genetic basis of that trait. It is important to note that most addiction-related traits are polygenic, meaning they are influenced by multiple genes with small or modest effect sizes

(Crabbe, 2002; Uhl et al., 2008). If variation in a trait is linked to variation in specific genes, then breeding based on a phenotype of interest increases the likelihood that offspring will inherit that phenotype (e.g., a low or high expression of a trait). Then, with successive generations of selective breeding, the frequencies of the alleles influencing the phenotype will increase (Crabbe, 1999; Falconer & Mackay, 1996). Selective breeding from an initial heterogeneous population for a given trait can occur bidirectionally or unidirectionally. Bidirectional selection is when animals with the highest and lowest scores for a given trait are selected for breeding, while unidirectional selection involves selective breeding for only high or low scores. The mouse lines used herein were created using a bidirectional short-term mass selection method. From an initial heterogeneous population, the highest scoring mice were bred together to establish a high line, and the lowest scoring mice were bred together to establish a low line. However, investigators must be cautious of how often a given family contributes to breeders. The contribution of each family to establishing a high or low line must be considered to prevent high rates of inbreeding (Belknap et al., 1997; Crabbe, 1999). Additionally, the number of selection generations should be limited to reduce the risk of genetic drift and random changes in the frequency of gene variants, which may result in the loss of the relevant allele or the fixation of a gene unrelated to the trait of interest. Selected lines should also be replicated using independent progenitor populations to avoid genetic drift, the fixation of a gene or genes unrelated to the selection phenotype in a given replicate (Crabbe, 1999; Falconer & Mackay, 1996; Palmer, 2002). After taking these factors into account, the combination of selective breeding based on values of a phenotype and subsequent quantitative trait locus (QTL) mapping can be used to pinpoint genetic loci that impact the desired trait.

Selective breeding for high versus low ethanol intake has been a common tool used in alcohol research (Belknap et al., 1997; Grahame et al., 1999). Lines have also been selectively bred for binge-level ethanol intake and high blood ethanol concentrations (Barkley-Levenson &

Crabbe, 2014; Crabbe et al., 2009). Selective breeding has also been used to produce lines for a number of ethanol-related phenotypes including high and low sensitivity to ethanol-induced CTA (Phillips et al., 2005) and susceptibility or resistance to the hypothermic effects of ethanol (Crabbe et al., 1987; Feller & Crabbe, 1991). These examples represent only a subset of extensive research utilizing selective breeding in alcohol studies.

Research utilizing selective breeding for MA-related traits is less abundant compared to alcohol studies but has successfully been used to develop lines of mice with high and low sensitivity to MA-induced locomotor sensitization (Scibelli et al., 2011), high and low MA-induced locomotor activation (Kamens et al., 2005), and high and low sensitivity to MA-induced stereotyped chewing (Atkins et al., 2001). The MA drinking lines are an example of mice selectively bred for high and low MA intake and are the model used within this dissertation. They will be described in detail below.

Quantitative Trait Locus Mapping

To investigate the genetic basis of a trait following selective breeding, quantitative trait locus (QTL) mapping is used to identify regions of the genome that account for the genetic variance of a heritable trait. As mentioned previously, addiction-related traits are typically polygenic and can even be under the control of gene interactions (Crabbe, 2002; Palmer, 2002; Uhl et al., 2008). QTL mapping involves measuring a phenotype within a genetically heterogeneous population or multiple populations and correlating variation in the phenotype with genetic markers to identify DNA regions that influence the trait. The potentially influential gene(s) within an identified region can then be analyzed for specific polymorphisms (e.g., differences in DNA sequences between two phenotypically different groups or among phenotypically different individuals) and examined for significance (Mackay, 2001; Palmer, 2002). The precision of QTL mapping improves with the identification of more markers, allowing

for a more focused probe into genes potentially contributing to a heritable trait of interest (Belknap et al., 2001; Palmer, 2002).

QTL analysis has previously been performed for MA-related traits; however, identified QTLs accounted for small amounts of genetic variance impacting the MA-related traits. In fact, potential QTLs were identified on all chromosomes except for mouse chromosomes 11, 13, and 18 (Grisel et al., 1997). This suggests that the various effects of MA involve different genetic factors, highlighting the difficulty of studying genetic influences.

The MA drinking lines used within this dissertation are unique in that QTL mapping identified a single nucleotide polymorphism (SNP) that is a major contributor to the difference in MA intake between the lines. As described next, it was subsequently discovered that several other MA-related behaviors are genetically correlated with MA intake and the same SNP impacts the differences between the lines for these traits. Identification of this SNP within a known gene provided a rare opportunity to explore how genetic variation in a gene with a major impact directly influences MA-related phenotypes in a way that had not been achieved previously and is discussed in more detail in the following section.

The Methamphetamine Drinking Lines

The MA drinking lines (MADRs) were created by selective breeding for high and low voluntary MA intake, measured during a two-bottle choice procedure when mice could freely consume water from one bottle or MA diluted in water (20 and then 40 mg/L for 4 days each) from another bottle (Wheeler et al., 2009). These lines serve as a model of high vs. low genetic risk for MA use. The MADR lines were created from the F2 cross of DBA/2J x C57BL/6J inbred strains and consist of the MA high drinking (MAHDR) and MA low drinking (MALDR) lines. Five replicate sets of the MADR lines have been produced, at a 2-year interval, and have demonstrated reliable outcomes for the selection response and other MA traits that will be

discussed in more detail below (Hitzemann et al., 2019; Phillips & Shabani, 2015; Shabani et al., 2011; Wheeler et al., 2009). Depending on MA concentration, MAHDR mice were found to consume 4-28 times more MA than MALDR mice. As the concentration of MA available increased, so did the dose of MA voluntarily consumed by MAHDR mice, reaching levels consistent with binge-level MA intake. MALDR mice consumed low amounts of MA, regardless of the MA concentration offered. Additionally, if the number of available bottles of MA increased from 1 to 3, with only 1 bottle of water offered, MAHDR mice voluntarily consumed more MA than when they were given only 1 bottle each of water and MA (Shabani et al., 2016).

Taar1

QTL analysis of DNA samples from the MA drinking (MADR) mice, revealed a region on mouse chromosome 10 that explains 60% of their genetic variance in voluntary MA consumption. This highly significant QTL in the chromosome 10 region was validated in a replicate set of lines bred from an independent DBA/2J x C57BL/6J cross (Belknap et al., 2013; Harkness et al., 2015). Numerous genes reside in the mapped interval, but few polymorphisms between the DBA/2J and C57BL/6J progenitors. Subsequent studies focused on the trace amine-associated receptor 1 gene (*Taar1*) because MA is a known agonist for the TAAR1 receptor encoded by this gene (Bunzow et al., 2001) and because a database search identified a non-synonymous SNP differentiating the progenitor strains. Studies from our laboratory found this *Taar1* SNP to be the major contributor to voluntary MA intake differences between the MAHDR and MALDR lines, as well as differences between the lines for other MA-related traits. The lab also determined this SNP (rs33645709) arose spontaneously in the DBA/2J mice sourced from Jackson Laboratories (Reed et al., 2018). At position 229 in the *Taar1* DNA sequence, rs33645709 encodes a proline to threonine mutation at amino-acid position 77 in DBA/2J mice, compared to C57BL/6J mice (Shi et al., 2016) (Harkness et al., 2015). This SNP was not found in DBA/2 mice sourced from 3 other vendors or in 28 other strains genotyped (Harkness et al.,

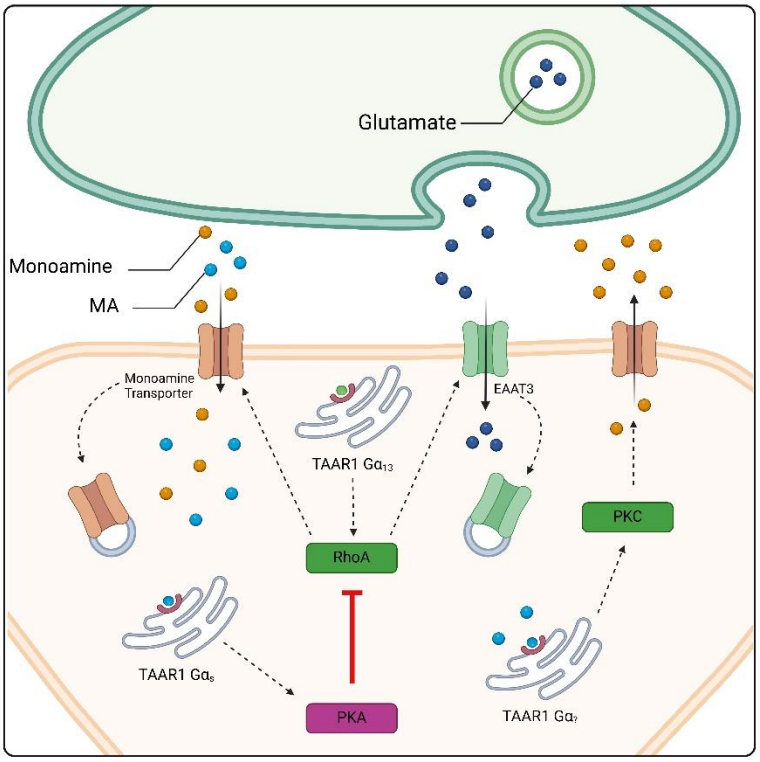
2015; Reed et al., 2018). The mutant DBA/2J allele results in the expression of a TAAR1 receptor that failed to produce an accumulation of cAMP in response to MA or endogenous ligands. This is in comparison to the TAAR1 receptor encoded by the wildtype C57BL/6J allele, which did generate an accumulation of cAMP in response to MA and endogenous ligands (Harkness et al., 2015; Shi et al., 2016). Therefore, the receptor encoded by the mutant allele will be referred to as non-functional throughout this dissertation. After only 1-2 generations of selective breeding, the laboratory found that MAHDR mice were homozygous for the mutated *Taar1* gene (*Taar1*^{m1J}) that encodes a non-functional TAAR1 receptor (Reed et al., 2018). MALDR mice were either homozygous or heterozygous for the wild type allele (*Taar1*$^{+}$), which encodes functional TAAR1 receptors, demonstrating the dominance of the wild type allele for low MA intake (Harkness et al., 2015). Beyond voluntary MA consumption, the laboratory discovered that *Taar1* genotype reliably correlates with differences in sensitivity to the rewarding, aversive and thermal effects of MA (Harkness et al., 2015; Reed et al., 2018; Shabani, Dobbs, et al., 2012; Shabani et al., 2016; Shabani, McKinnon, et al., 2012; Shabani et al., 2011), which are discussed in greater detail below.

To establish a causal link between TAAR1 function and voluntary MA consumption, the Phillips laboratory utilized a gene-editing tool. Clustered regularly interspaced palindromic repeats (CRISPR)-associated protein 9 (CRISPR-*Cas9*) technology has two components, a guide RNA and the endonuclease, *Cas9*. The guide RNA transports *Cas9* to the desired target where it can then initiate a double-stranded DNA break. Once the break is introduced one allele can be substituted for another. CRISPR-*Cas9* was used to replace the mutant *Taar1*^{m1J} allele with the reference *Taar1*$^{+}$ allele to create a homozygous knock-in (KI) on the MAHDR background, denoted MAHDR-*Taar1*$^{+/+}$. As a result, MAHDR-*Taar1*$^{+/+}$ mice express functional TAAR1 receptors. A control mouse line, MAHDR-*Taar1*$^{m1J/m1J}$, was also established using mice in which the mutant *Taar1*^{m1J} allele was not excised and exchanged with the reference allele (Stafford et al.,

2019). Studies confirmed that the replacement of the *Taar1*^{m1J} allele with the *Taar1*$^{+}$ variant significantly reduced voluntary MA intake to amounts observed in the MALDR mice (Stafford et al., 2019). Gene editing was also performed on the two progenitor strains, further establishing the causal link between TAAR1 function and voluntary MA intake. CRISPR-*Cas9* was used to replace the mutant *Taar1*^{m1J} allele with the reference *Taar1*$^{+}$ allele on the DBA/2J background, DBA/2J-*Taar1*$^{+/+}$. A control line was also established using mice in which the alleles were not exchanged, DBA/2J-*Taar1*$^{m1J/m1J}$. Studies again confirmed that replacement of the mutant *Taar1*^{m1J} allele with the wildtype *Taar1*$^{+}$ allele significantly reduced voluntary MA consumption. Finally, CRISPR-*Cas9* technology was used to replace the reference *Taar1*$^{+}$ allele with the mutant *Taar1*^{m1J} allele on the C57BL/6J background, C57BL/6J -*Taar1*$^{m1J/m1J}$, along with a corresponding control line, C57BL/6J -*Taar1*$^{+/+}$. Conversely, replacement of the wildtype *Taar1*$^{+}$ allele with the mutant *Taar1*^{m1J} allele significantly increased voluntary MA consumption (Phillips et al., 2021).

Here we propose a general model for MA-activated TAAR1 signaling pathways in monoaminergic neurons which may partially explain MA-related behavioral differences, based on the differences in *Taar1* genotype and corresponding TAAR1 function between the MADR and CRISPR-*Cas9*-generated lines, (Figure 1.2).

A) MALDR & MAHDR-*Taar1*$^{+/+}$



B) MAHDR & MAHDR-*Taar1*$^{m1J/m1J}$

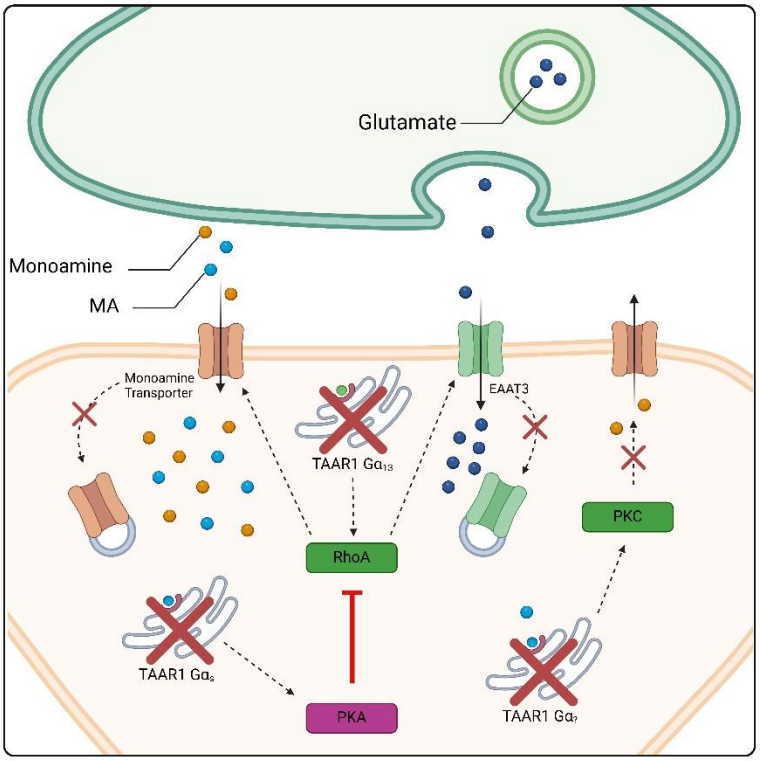


Figure 1.2 Proposed effects of MA-induced activation of TAAR1 receptors in monoaminergic neurons of MADR and the CRISPR-*Cas9*-generated mice. A) In monoaminergic neurons (5-HT, DA, NE) of MALDR and MAHDR-*Taar1*$^{+/+}$ KI mice MA enters the neuron through their respective transporters (SERT, DAT, NET). MA activates $G\alpha_{13}$-coupled TAAR1, a PKC pathway, and a delayed $G\alpha_s$-coupled TAAR1 pathway. Activation of $G\alpha_{13}$-coupled TAAR1 receptors stimulates RhoA activation leading to endocytosis of monoaminergic transporters and EAAT3. A TAAR1 mediated PKC pathway is activated either in formation with RhoA or independently and induces monoamine transporter efflux. Activation of $G\alpha_s$-coupled TAAR1 receptors stimulates PKA signaling leading to the phosphorylation of RhoA and the end of monoamine transporter internalization. B) In monoaminergic neurons (5-HT, DA, NE) of MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice MA enters the neuron through their respective transporters (SERT, DAT, NET). However, because TAAR1 is non-functional in these lines, MA cannot activate the $G\alpha_{13}$- or $G\alpha_s$-coupled TAAR1 signaling pathways which keeps the monoamine transporters and EAAT3 expressed on the cell membrane. This allows for monoamines and glutamate to be continuously trafficked from the extracellular space and prevents their efflux through transporters. *Created in BioRender.*

TAAR1: trace amine-associated receptor 1; MA: methamphetamine; AMPHs: amphetamines; 5-HT: serotonin; DA: dopamine; NE: norepinephrine; SERT: serotonin transporter; DAT: dopamine transporter; NET: norepinephrine transporter; PKC: protein kinase C; RhoA: Ras homolog family member A; PKA: protein kinase A

Methamphetamine-Induced Reward and Aversion Sensitivity in the Methamphetamine Drinking Mice

It was hypothesized that the MADR mice may demonstrate line differences in the balance between sensitivity to the rewarding and aversive effects of MA (Shabani et al., 2011). In humans, individual differences in this balance could potentially explain why not all who experiment with MA escalate their use and develop a substance use disorder (Chait, 1993; de Wit et al., 1986). To validate the MADR drinking lines differential sensitivity to the motivational effects of MA, a battery of behavioral tests was performed in independent sets of mice. A typical tactile cue association conditioned place preference (CPP) procedure in which mice were treated with MA (0.5, 2, and 4 mg/kg) prior to conditioning trials found significant MA-induced CPP in MAHDR but not MALDR mice that was strongest at the lowest dose, when the mice were tested in the absence of MA treatment (Shabani et al., 2011; Wheeler et al., 2009). When assessed in the MA-present state, MALDR mice exhibited profound aversion for all 3 MA doses, whereas MAHDR continued to exhibit reward when treated with the lowest MA dose. A tactile cue association conditioned place aversion (CPA) procedure in which mice were treated with MA (0.5, 2, and 4 mg/kg) after exposure to the tactile cue found significant MA-induced CPA in MALDR at half the dose required to induce CPA in MAHDR mice (Shabani, McKinnon, et al., 2012). MALDR mice also exhibited CPA in response to the TAAR1 agonist RO5256390 (Shabani et al., 2023). A novel taste cue association conditioned taste aversion (CTA) test found significant MA-induced CTA in MALDR mice, whereas MAHDR mice failed to demonstrate MA-induced CTA at the same or higher doses of MA (Shabani, McKinnon, et al., 2012; Wheeler et al., 2009). MALDR mice also exhibit CTA in response to the TAAR1 agonist RO5256390 (Shabani et al., 2023). MA-induced hypothermia, proposed to be an unconditioned aversive physiological effect of MA that discourages MA intake (Mootz et al., 2020; Phillips & Shabani, 2015), was also examined. In response to acute administration of MA, MALDR mice exhibited hypothermia at

early time points after MA administration, a response not observed in MAHDR mice. Both lines exhibited MA-induced hyperthermia after treatment with some doses at some timepoints (Harkness et al., 2015). MALDR mice also exhibited hypothermia in response to the TAAR1 agonist RO5256390 that was not observed in MAHDR mice (Shabani et al., 2023). In addition to voluntarily consuming different amounts of MA, MADR mice have demonstrated reliable outcomes for differential sensitivity to both MA-induced reward and aversion across multiple replicate sets of lines, validating their strength as a model of differential genetic risk for addiction-related traits (Harkness et al., 2015; Hitzemann et al., 2019; Phillips & Shabani, 2015; Shabani et al., 2011; Wheeler et al., 2009).

To establish a causal link between TAAR1 function and sensitivity to the rewarding and aversive properties of MA, the Phillips laboratory again took advantage of the CRISPR-*Cas9*-generated mice. Studies confirmed that replacement of the *Taar1*^{m1J} allele with the *Taar1*$^{+}$ variant on the MAHDR background (MAHDR-*Taar1*$^{+/+}$) significantly reduced sensitivity to MA-conditioned reward, and increased sensitivity to MA-conditioned aversion (Phillips et al., 2021; Shabani et al., 2023). In line with this finding, replacement of the *Taar1*^{m1J} allele with the reference *Taar1*$^{+}$ allele on the DBA/2J background resulted in increased sensitivity to MA-conditioned aversion. Conversely, replacement of the wildtype *Taar1*$^{+}$ allele with the mutant *Taar1*^{m1J} allele on the C57BL/6J background converted MA-induced hypothermia to hyperthermia (Phillips et al., 2021).

Neurotransmitter Systems in the Methamphetamine Drinking Mice

The actions of glutamate play a central role in addiction, including drug sensitization, craving, reinstatement, withdrawal, and relapse (Conrad et al., 2008; Gass & Olive, 2008; Kalivas et al., 2009; Tzschentke & Schmidt, 2003). Consequently, glutamate systems in the MADR mice have garnered significant interest. Based on microdialysis data, the MAHDR mice exhibited a hyper-glutamatergic state within the nucleus accumbens (NAc), both preceding and as a

consequence of MA exposure. Basal extracellular glutamate concentrations were more than two times greater in the NAc of MAHDR mice compared to MALDR mice. There were no line differences in glutamate clearance and reuptake; however, the MAHDR mice exhibited significantly lower expression of EAAT3, which could contribute to the increased extracellular glutamate concentrations. MAHDR mice also expressed greater levels of mGluR5 and the scaffolding protein Homer2a/b compared to MALDR mice within the NAc. In response to an acute dose of MA (2 mg/kg), MAHDR mice exhibited a robust increase in extracellular glutamate levels within the NAc, while no effect was observed in MALDR mice (Szumlinski et al., 2017). In the medial prefrontal cortex (mPFC), MAHDR mice also exhibited greater basal extracellular glutamate concentrations, however unlike in the NAc, EAAT3 was expressed similarly between the lines in the mPFC. MAHDR mice were also found to express lower levels of Homer2a/b and mGluR2 in the mPFC compared to MALDR mice, while there was no line difference in mGluR5 expression. Following an acute dose of MA (2 mg/kg), MAHDR mice exhibited a significant reduction in extracellular glutamate concentrations. In contrast, MA tended to increase glutamate concentrations in MALDR mice, though this effect was not significant. Notably, extracellular glutamate concentrations were significantly higher in MALDR mice compared to MAHDR mice 140-180 minutes after injection of 2 mg/kg MA (Lominac et al., 2016). These findings highlight region-specific differences in glutamate dynamics between the MADR lines.

DA and 5-HT are also well established contributors to addiction, driving behaviors such as drug reinforcement, craving, relapse, impulsivity and withdrawal (Muller & Homberg, 2015; Solinas et al., 2019); therefore, the laboratory has made efforts to examine these systems in the MADR mice. In the NAc, basal DA levels were significantly higher in MALDR mice compared to MAHDR mice when measured using microdialysis. In the NAc shell, MAHDR mice exhibited lower D2R expression, but higher DAT expression compared to MALDR mice. In the NAc core, MADR mice exhibit no difference in D2R expression, but MAHDR mice exhibited higher DAT

expression compared to MALDR mice. With regard to 5-HT, microdialysis revealed that basal levels within the NAc were significantly greater in the MAHDR mice compared to MALDR mice. In the NAc shell, no line difference in 5-HT_{1B} receptor expression was observed, but MAHDR mice expressed higher levels of SERT compared to MALDR mice. In the NAc core, MAHDR mice expressed lower levels of 5-HT_{1B} receptors, but no line difference in SERT expression was observed. In response to 2 mg/kg MA, the amount of DA and 5-HT measured by microdialysis within the NAc was similar between the lines. In the mPFC, basal DA concentrations were significantly greater in MALDR mice compared to MAHDR mice while no difference in basal 5-HT concentrations were observed. No line difference in D2R was observed and MAHDR mice exhibited higher 5-HT_{1B} receptor expression, but this difference was shy of statistical significance. Finally, SERT expression was significantly lower in MAHDR mice compared to MALDR mice. In response to 2 mg/kg MA, the amount of DA measured within the mPFC was significantly higher in MAHDR mice compared to MALDR mice. Conversely, in the mPFC, the amount of 5-HT measured in response to 2 mg/kg MA was significantly higher in the MALDR mice compared to MAHDR mice (Lominac et al., 2014). These findings demonstrate region-specific differences in basal and MA-induced concentrations of DA and 5-HT between the MADR lines, as well as basal receptor and transporter expression.

Much remains unknown about the neurotransmitter systems that contribute to the differences in MA intake and the reward/aversion sensitivities demonstrated by both the MADR and CRISPR-*Cas9*-generated mice. My dissertation aims to further investigate the activity of DR 5-HT neurons in the MADR and MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR-*Taar1*$^{+/+}$ KI mice using slice electrophysiology, with a particular emphasis on the role of TAAR1 in modulating 5-HT signaling. I chose to focus on the DR, the largest serotonergic nucleus in the brain (Hornung, 2010), for several reasons that are described in the sections below.

The Role of the Serotonergic System in Response to Rewarding Stimuli

Serotonin in the Dorsal Raphe

The DR is located within the brainstem at the midline, ventral to the midbrain aqueduct. In mice, the DR is comprised of over 9000 5-HT neurons (Ishimura et al., 1988). Two-thirds of the total neurons in the DR are serotonergic and provide ~70% of the serotonergic neurotransmission to the forebrain. 5-HT neurons are predominantly located in the midline and lateral wings of the DR, confirmed by labelling for the expression of the 5-HT markers tryptophan hydroxylase 2 (TpH2) and 5-HT (Fu et al., 2010; Hioki et al., 2010). Importantly, DR 5-HT neurons are heterogeneous in their morphology, neurochemical markers, projection preferences, functional topography and of relevance, their electrophysiological properties (Abrams et al., 2004; Bang et al., 2012; Calizo et al., 2011; Shikanai et al., 2012). Inputs onto DR 5-HT neurons come from a broad range of forebrain and limbic regions, including regions involved in reward processing (Ogawa et al., 2014; Pollak Dorocic et al., 2014).

Dorsal Raphe Serotonin and Reward

5-HT has long been studied for its role in mental health disorders. More recently, the activity of DR 5-HT neurons has been implicated in reward processing. Fiber photometry recordings of calcium signals from 5-HT neurons found increased activity during sucrose and food intake (Li et al., 2016; Ren et al., 2018). DR 5-HT neuron activity also increased in response to social rewards, such as male mice initiating mating behavior or male to male interactions. Elevated 5-HT neuron activity was also observed upon unexpected sucrose delivery (Li et al., 2016). In a Go/No-go task where mice were rewarded for correct trials with sucrose, DR 5-HT neuron activity significantly increased in response to sucrose (Liu et al., 2014). Studies were also conducted to determine the role of DR 5-HT in reinforcement learning, specifically by nose-poking in an active port to self-stimulate DR Pet-1 neurons. Pet-1 is a transcription factor

involved in the development and function of 5-HT neurons. It is exclusively expressed in colocalization with TPH2+ neurons within raphe nuclei (Hendricks et al., 1999). Mice nose-poked for self-stimulation of DR Pet-1 neurons significantly more when they expressed an excitatory opsin and TpH2 compared to TpH2 KO mice (Liu et al., 2014) and when they expressed an excitatory opsin in TpH2+ neurons compared to a control opsin (Nagai et al., 2020). Additionally, the number of active nose-pokes positively correlated with the strength of the optogenetic stimulation (Liu et al., 2014). Optogenetic activation of DR 5-HT neurons also increased CPP scores, a measure of an animal's preference for an environment associated with a rewarding stimulus, indicating reinforcement of a positive association with that environment. Conversely, optogenetic inhibition of DR 5-HT neurons resulted in decreased CPP scores (Nagai et al., 2020). Single-unit recordings in primates found that the activity of DR putative 5-HT neurons increased with anticipation of and after receipt of reward, with individual neurons responding preferentially to expected reward size (Hayashi et al., 2015; Nakamura, 2013; Nakamura et al., 2008).

Glutamate Neurons in the Dorsal Raphe

A small subset of glutamatergic neurons, identified by expression of the vesicular glutamate transporter 3 (VGluT3), are primarily located along the midline of the DR (Hioki et al., 2010; Liu et al., 2014). However, VGluT3 is also co-expressed in many 5-HT neurons (Fremeau et al., 2002; Gras et al., 2002; Schafer et al., 2002), with an estimated co-expression in approximately 2/3^{rds} of DR 5-HT neurons (Hioki et al., 2010). Whole-cell patch-clamp recordings have shown that DR neurons release glutamate in a VGluT3 dependent manner onto VTA DA neurons (Liu et al., 2014; Qi et al., 2014; Wang et al., 2019). It is not clear whether DR neurons release glutamate within the DR itself.

Importantly, DRN Pet-1 neurons rely on both 5-HT and glutamate to drive reward-motivated behavior. Blocking either 5-HT synthesis or glutamate release led to distinct

impairments in reward behaviors, with partial deficits observed in both a two-bottle choice (sucrose and water) preference measure and a self-stimulation fixed ratio task. However, when both 5-HT synthesis (via TpH2 inhibition) and vesicular transport of glutamate (via VGluT3 KO) were disrupted, the reinforcing effects of DRN Pet-1 neuron stimulation were completely abolished (Liu et al., 2014). Additionally, VTA activation of DR SERT terminals expressing VGluT3 promoted CPP, elicited excitatory glutamatergic currents onto NAc DA neurons and increased their firing frequency, and evoked DA release in the NAc. However, these effects were completely blocked by antagonism of AMPA and 5-HT_3 receptors within the VTA (Wang et al., 2019). Together, these findings suggest that glutamate contributes to the reinforcing potential of DR 5-HT neurons.

Serotonin and Psychostimulants

Psychostimulants interact with SERT to increase extracellular 5-HT levels (Han & Gu, 2006; Rothman et al., 2001; Schmidt et al., 1987; Underhill & Amara, 2020). While studies on the effects of AMPHs in the DR specifically are limited, there are a few studies that provide valuable insight. DR 5-HT concentrations significantly increased in response to intra-DR infusion of AMPH (Ferre et al., 1994). In slice preparations, MDMA application also increased DR 5-HT concentrations, which subsequently inhibited the firing of these neurons. Further supporting the ability of psychostimulants to interact with SERT, the MDMA-induced inhibition of DR 5-HT neuron activity was blocked by application of the SERT antagonist fluoxetine (Sprouse et al., 1989). Behaviorally, SERT antagonists significantly reduced rates of AMPH self-administration (Porrino et al., 1989; Yu et al., 1986), AMPH-induced locomotor activity, and behavioral disinhibition (Olausson et al., 2000). Pre-treatment with SERT antagonists blocked MA-induced CPP, abolished previously established CPP to MA, and reduced MA-induced locomotor sensitization (Takamatsu et al., 2011; Takamatsu et al., 2006; Yamamoto et al., 2011). Beyond AMPHs interactions with SERT and effects on 5-HT concentrations, AMPHS also interact with

different 5-HT receptor subtypes. 5-HT receptor subtypes relevant to AMPH-induced behaviors are discussed below.

$5HT_{1A}$ receptors are highly expressed within the DR, acting as inhibitory autoreceptors on the soma and dendrites of DR 5-HT neurons (Altieri et al., 2013; Gozlan et al., 1983; Khawaja, 1995; Marcinkiewicz et al., 1984; Pompeiano et al., 1992; Riad et al., 2000; Verge et al., 1986). 5-HT-induced activation of 5-HT_{1A} receptors results in activation of GIRKs (Aghajanian & Lakoski, 1984; Penington et al., 1993; Polter & Li, 2010; Williams et al., 1988) and the inhibition of voltage-dependent calcium channels (Penington & Kelly, 1990). Given AMPHs ability to increase extracellular 5-HT concentrations by competing with 5-HT for uptake at SERT and inducing local 5-HT release (Schmidt et al., 1987; Sprouse et al., 1989), it was hypothesized that this increase facilitates activation of $5HT_{1A}$ autoreceptors (Sprouse et al., 1989) that regulate serotonergic tone via an inhibitory feedback mechanism (Altieri et al., 2013). Activation of 5-HT_{1A} receptors regulates extracellular 5-HT levels by controlling the firing rates of neurons. In support of this hypothesis, 5-HT_{1A} agonists potently inhibited spontaneous DR 5-HT neuron activity mimicking the effects of 5-HT and MDMA (Sprouse & Aghajanian, 1986, 1987; Sprouse et al., 1989), an effect that was blocked by several 5-HT_{1A} antagonists (Martin et al., 1999). Such autoreceptor activation may explain AMPH-induced behavioral effects. It is important to note, $5HT_{1A}$ receptors are also expressed post-synaptically in the hippocampus, amygdala, and thalamus among other regions (Albert et al., 1990; Hall et al., 1985; Khawaja, 1995; Marcinkiewicz et al., 1984; Pompeiano et al., 1992; Verge et al., 1986), therefore any observed effects *in vivo* cannot be confirmed as DR-specific. Several 5-HT_{1A} agonists blocked AMPH-induced increases in locomotor activity (Ago et al., 2006; Maj et al., 1987; Millan et al., 1998; Przegalinski & Filip, 1997) and the agonist buspirone reversed MDMA-induced anxiety in an elevated plus maze test (Bhattacharya et al., 1998). Activation of 5-HT_{1A} also inhibited the development and expression of AMPH-induced sensitization (Przegalinski et al., 2000). These

findings suggest that 5-HT_{1A} receptor activation may attenuate the behavioral and neuroadaptive effects of AMPHs.

5-HT_{1B} receptors are predominantly localized on 5-HT axon terminals, but they are also expressed within the DR, where they act as inhibitory autoreceptors (McDevitt & Neumaier, 2011; Tiger et al., 2018). 5-HT_{1B} also regulates serotonergic tone, however it accomplishes this by controlling 5-HT release within the DR. Within the DR, 5-HT_{1B} agonists decreased 5-HT release, an effect that was blocked by a 5-HT_{1B} antagonist (Adell et al., 2001; Davidson & Stamford, 1995). Supporting this distinction, 5-HT_{1B} agonists displayed weak efficacy or inconsistent actions on the firing activity of DR 5-HT neurons (Adell et al., 2001; Sprouse & Aghajanian, 1987). Instead, activation of 5-HT_{1B} receptors within the DR inhibited glutamatergic synaptic activity by decreasing miniature excitatory post synaptic currents (mEPSC) frequency (Lemos et al., 2006). Again, it is important to note 5-HT_{1B} receptors are expressed outside of the DR, including the substantia nigra, striatum, and amygdala (Varnas et al., 2001), therefore any observed effects *in vivo* cannot be confirmed as DR-specific. Stimulating 5-HT_{1B} receptors attenuated AMPH self-administration, an effect that could be significantly reduced by 5-HT_{1B} antagonists (Fletcher & Korth, 1999; Miszkiel et al., 2012). Antagonizing 5-HT_{1B} receptors also attenuated AMPH-primed and AMPH cue-induced reinstatement following extinguished AMPH-seeking behavior (Miszkiel & Przegalinski, 2013). Stimulating 5-HT_{1B} also abolished AMPH-induced increases in responding to a conditioned rewarding stimulus (Fletcher & Korth, 1999). These studies would suggest that activation of 5-HT_{1B} receptors reduces the reinforcing effects of AMPHs.

5-HT_{2A} receptor mRNA are expressed on GABAergic neurons within the periaqueductal gray (PAG) and their terminals onto DR 5-HT neurons (Liu et al., 2000; Wright et al., 1995). 5-HT_{2A} receptors on PAG GABAergic terminals respond to changes in 5-HT within the DR. Local activation of these receptors activate a negative feedback loop that suppresses the firing of DR 5-

HT neurons (Liu et al., 2000). In addition, AMPHs exhibit high affinity and agonist-like properties at 5-HT_{2A} receptors (Battaglia & De Souza, 1989; Teitler et al., 1990). The 5-HT_2 nonspecific antagonist cinanserin decreased rates of AMPH self-administration at high doses (Porrino et al., 1989). The 5-HT_{2A} antagonist MDL 100,907 blocks the stimulant effects of AMPH (Moser et al., 1996). 5-HT_{2A} antagonists also blocked AMPH- and MA-induced CPP (Madden et al., 2020; Nomikos & Spyraki, 1988). However, the antagonist ritanserin showed inconsistent effects on AMPH-induced hyperlocomotion, with one study reporting no effect at higher doses and another demonstrating its ability to block hyperlocomotion at lower doses (Nomikos & Spyraki, 1988; O'Neill et al., 1999).

5-HT_{2B} receptors are expressed in 5-HT neurons of the DR (Belmer, Quentin, et al., 2018; Quentin et al., 2018). Opposite to 5-HT_{1B} receptors, the activation of 5-HT_{2B} receptors stimulates the release of 5-HT (Doly et al., 2008). In *ex-vivo* electrophysiological recordings, the activation of 5-HT_{2B} receptors increased the firing rate of 5-HT Pet1 neurons. Firing rates were also significantly higher in cells overexpressing 5-HT_{2B} receptors compared to control cells when injecting +200 pA of current. In fact, the activation of 5-HT_{2B} receptors counteracts 5-HT_{1A} autoreceptor mediated reductions in firing rate. KO of 5-HT_{2B} receptors in Pet-1 positive 5-HT neurons, resulted in DR 5-HT neurons with a significantly lower firing frequency compared to controls (Belmer, Quentin, et al., 2018). 5-HT_{2B} receptor expression is also observed outside of the DR, including the cerebellum, medial amygdala, hypothalamus, and lateral septum (Hoyer, 2007) –therefore the following effects are not DR specific. Behaviorally, the antagonism of 5-HT_{2B} receptors blocked MDMA-induced increases in locomotor activity (Doly et al., 2008). Antagonizing 5-HT_{2B} receptors also blocked MDMA-induced locomotor sensitization, as well as MDMA-induced reinstatement of CPP following extinction. KO of 5-HT_{2B} receptors also blocked MDMA-induced locomotor sensitization and CPP. (Doly et al., 2009). However, one study specifically assessed the influence of 5-HT_{2B} receptors within raphe nuclei on MDMA-induced

behaviors by using Pet-1 driven KO of 5-HT_{2B} receptors. KO of Pet-1 5-HT_{2B} receptors blocked MDMA-induced increases in locomotor activity and sensitization (Belmer, Quentin, et al., 2018).

Whole-Cell Patch-Clamp Electrophysiology

Whole-cell patch clamp electrophysiology is a technique used to measure the electrical activity of neurons. This technique allows for detailed examination of ion currents and membrane potentials by providing access to the cell interior. There are two configurations for recording neuron activity, 1) current clamp mode to measure changes in membrane voltage and 2) voltage clamp mode to measure changes in membrane current. This technique allows us to determine the effect of MA on cell excitability, activity of ion channels, and the mechanisms behind those effects. With the MADR and CRISPR-*Cas9*-generated mice with differential TAAR1 function, this technique serves as an ideal tool for assessing whether TAAR1 functionality itself can dictate DR 5-HT neuron activity and glutamatergic input. The technique does have several limitations: Recordings are usually performed on one cell at a time making these experiments low throughput. Additionally, we are constrained by the small size of the DR in mice. Slices prepared at a thickness of 230-250 μm yield only 2-3 slices with sizable DR subregions. Since the DR is located on the midline, we cannot hemi-sect the slices to increase yield. Furthermore, the effects of MA are irreversible; once MA is applied to the slice, it cannot be reused to make pre- and post-MA comparisons.

Electrophysiology Methods to Characterize Properties of Dorsal Raphe Serotonin Neurons

Whole-cell patch-clamp electrophysiology can be performed on acute brain slices encompassing the DR to assess whether there are baseline differences in intrinsic membrane properties, action potential dynamics, and glutamatergic inputs onto DR 5-HT neurons. This *in vitro* approach allows us to evaluate physiological properties such as spontaneous firing frequency, response to hyperpolarizing or depolarizing current injections, resting membrane

potential (RMP), firing pattern features, as well as AMPA and NMDA receptor mediated currents of DR 5-HT neurons. We can then apply MA to DR-containing slices to determine whether there are MA-induced changes in the properties mentioned above. By performing these experiments with the MADR and CRISPR-*Cas9*-generated mouse lines, we can then determine whether the basal characteristics and/or observed effects of MA on DR 5-HT neuron activity are influenced by TAAR1 receptor functionality and the TAAR1-dependent processes.

Hypotheses and Experimental Aims

The overarching goal of this dissertation was to investigate how TAAR1 functionality influences the intrinsic activity of DR 5-HT neurons in response to MA and glutamatergic synaptic transmission onto these neurons in the absence and presence of MA. Importantly, any observed differences in basal characteristics between the lines suggests an effect of selective breeding and may relate to differential genetic risk for MA intake and reward/aversion sensitivity. DR 5-HT neurons are involved in the perception of rewarding stimuli (Li et al., 2016; Nagai et al., 2020; Ren et al., 2018; Wang et al., 2019) and TAAR1 is widely expressed within this region (Borowsky et al., 2001; Lindemann et al., 2008; Rutigliano et al., 2017). Therefore, studying the activity of DR 5-HT neurons from the MADR and CRISPR-*Cas9*-generated mice with differential TAAR1 function, MA reward sensitivity, and voluntary MA intake enables us to investigate these goals.

The first aim was designed to 1) characterize the effect of MA on DR 5-HT neuron excitability, 2) determine the influence of *Taar1* genotype and 3) investigate the TAAR1-dependent mechanisms underlying these effects. TAAR1 agonists reduce excitability of DR 5-HT neurons expressing or overexpressing TAAR1, an effect that is absent in TAAR1 KO DR 5-HT neurons (Revel et al., 2012; Revel et al., 2011). However, AMPHs increase extracellular 5-HT concentrations (Ferre et al., 1994; Rothman et al., 2001; Schmidt et al., 1987; Sprouse et al.,

1989; Sulzer et al., 2005; Underhill & Amara, 2020), potentially inducing feedback inhibition of DR 5-HT neurons through activation of 5-HT inhibitory autoreceptors (Aghajanian & Lakoski, 1984; Dedic et al., 2019; Sprouse et al., 1989). In fact, the activation of 5-HT_{1A} or 5-HT_{1B} inhibitory autoreceptors has been shown to attenuate TAAR1-induced cAMP production (Xie et al., 2008). I expanded on this by evaluating the effect of MA in the absence and presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists in the MADR mice. To determine whether the effect of MA is dependent on the absence of TAAR1 function, I repeated experiments with the 5-HT autoreceptor antagonists in the CRISPR-*Cas9*-generated mice. Additionally, MA is a substrate of SERT (Han & Gu, 2006) and its expression at the membrane is modulated by TAAR1 (Underhill & Amara, 2020). Therefore, I evaluated whether SERT is necessary for the observed effects of MA. Finally, to determine whether the absence of TAAR1 function is critical for heightened MA consumption and reward sensitivity I included an experiment that evaluated whether KI of functional TAAR1 attenuated binge-level MA intake.

The second aim was designed to 1) characterize the influence of TAAR1 functionality on baseline glutamatergic synaptic transmission onto DR 5-HT neurons and 2) how differences in TAAR1 function influenced glutamatergic synaptic transmission onto these neurons in response to MA. The MADR lines differ in their *Taar1* genotypes, which are linked to the observed differences in voluntary MA intake and reward/aversion sensitivities (Hitzemann et al., 2019; Phillips & Shabani, 2015; Shabani et al., 2011; Wheeler et al., 2009). These mice also exhibit baseline region-specific differences in glutamate receptor, EAAT3, and scaffolding protein expression (Lominac et al., 2016; Szumlinski et al., 2017). Therefore, I first wanted to explore whether *Taar1* genotype and its associated TAAR1 function influenced baseline spontaneous (sEPSCs) and the paired pulse ratio (PPR) of DR 5-HT neurons. However, for my studies, I used the CRISPR-*Cas9*-generated mice, which serve as a causal model for a *Taar1* SNP effect on voluntary MA intake and MA reward/aversion sensitivity (Phillips et al., 2021; Stafford et al.,

2019). The MADR mice also exhibit region-specific changes in extracellular glutamate concentrations following MA exposure (Lominac et al., 2016; Szumlinski et al., 2017). Therefore, I also evaluated whether MA influenced glutamatergic synaptic transmission onto DR 5-HT neurons by recording sEPSCs and PPRs following administration of MA. Finally, ample evidence demonstrates that TAAR1 activation potentiates NMDA receptor-mediated glutamatergic synaptic activity onto DA neurons (Li et al., 2017; Underhill et al., 2019; Underhill et al., 2014). Thus, I expanded on the work from Chapter 2, to determine whether MA-induced depolarization and potentiation of DR 5-HT neurons in mice lacking functional TAAR1 is dependent on NMDA receptors.

Chapter 2: Absence of TAAR1 function increases methamphetamine-induced excitability of dorsal raphe serotonin neurons and drives binge-level methamphetamine intake

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Rios, S. M., Mootz, J. R. K., Phillips, T. J., & Ingram, S. L. (2025). Absence of TAAR1 function increases methamphetamine-induced excitability of dorsal raphe serotonin neurons and drives binge-level methamphetamine intake. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-025-02063-w

Acknowledgements: These studies were designed by Samantha Rios in collaboration with Drs. Tamara Phillips and Susan Ingram. Acquisition of all electrophysiology data was performed by Samantha Rios. The behavioral experiment was performed by John R.K. Mootz. Analysis of electrophysiology and behavioral data was completed by Samantha Rios. The manuscript was written by Samantha Rios in consultation with Drs. Tamara Phillips and Susan Ingram.

Abstract

Methamphetamine (MA) is a potent psychostimulant capable of exerting both rewarding and aversive effects, the balance of which likely drives variation in voluntary MA intake. Understanding the genetic factors underlying sensitivity to these effects of MA is critical for developing effective treatments. The activity of dorsal raphe serotonin neurons is linked to reward processing. Here, we performed whole-cell patch-clamp electrophysiology in dorsal raphe serotonin neurons from mice with high or low MA intake corresponding with high or low MA reward sensitivity. The MA drinking (MADR) mice consist of the MA reward sensitive MA high drinking (MAHDR) and the MA reward insensitive MA low drinking (MALDR) lines. MA is a trace amine-associated receptor 1 (TAAR1) agonist, and MAHDR mice are homozygous for a mutation in the *Taar1* gene, *Taar1*^{m1J}, that encodes non-functional TAAR1, whereas MALDR mice possess at least one copy of the reference *Taar1*$^{+}$ allele that encodes functional TAAR1. Our previous research using CRISPR-*Cas9*-generated MAHDR-*Taar1*$^{+/+}$ knock-in mice in which *Taar1*^{m1J} was replaced with *Taar1*$^{+}$, and non-edited MAHDR-*Taar1*$^{m1J/m1J}$ controls demonstrated that lack of TAAR1 function is critical for heightened MA consumption and MA reward sensitivity. Here, electrophysiological recordings in the MADR lines demonstrate a MA-induced decrease in dorsal raphe serotonin neuron activity from MALDR, but not MAHDR mice. However, in the presence of serotonin autoreceptor antagonists, MA potentiates dorsal raphe serotonin neuron activity of MAHDR, but not MALDR mice. Importantly, potentiation in the presence of the antagonists is abolished in knock-in mice expressing functional TAAR1. The knock-in mice did not display binge-level MA intake, consistent with the loss of MA-reward sensitivity previously reported in mice with functional TAAR1. Finally, because MA is a substrate of the serotonin transporter, we evaluated whether the serotonin transporter is necessary for MA-induced potentiation of dorsal raphe serotonin neuron activity in mice with non-functional TAAR1. The serotonin transporter antagonist fluoxetine blocks MA-induced potentiation for both

MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice. Thus, TAAR1 function directly impacts MA reward sensitivity and MA intake and serves as a critical regulator of MA-induced activity of dorsal raphe serotonin neurons through its interaction with the serotonin transporter.

Introduction

The incidence of methamphetamine (MA) use disorders has dramatically risen during the past decade (Han et al., 2021). No effective treatments for MA use disorder exist. Further investigation into mechanisms by which MA influences neurotransmission in brain regions associated with reward and aversion processing is necessary for understanding cellular mechanisms underlying addiction and for development of MA use disorder therapies. A vigorous genetic tool for examining the impact of initial sensitivity to rewarding and aversive effects of MA on subsequent MA use is mice selectively bred for differential voluntary MA consumption. Our lab employed a two-bottle choice voluntary MA consumption procedure to generate the MA drinking (MADR) selected lines, consisting of mice bred for high and low MA intake; the MA high drinking (MAHDR) and MA low drinking (MALDR) lines, respectively (Wheeler et al., 2009). Behaviorally, MAHDR mice exhibit high sensitivity to rewarding effects of MA, while MALDR mice exhibit insensitivity. High reward sensitivity in MAHDR mice is associated with diminished sensitivity to aversive effects of MA, compared to MALDR mice, which exhibit high aversion sensitivity. Selection response for high vs. low MA consumption and differential sensitivity to rewarding and aversive effects of MA have been confirmed across five replicate sets of MADR lines (Hitzemann et al., 2019; Phillips & Shabani, 2015; Shabani et al., 2011; Wheeler et al., 2009).

Whole genome mapping identified a location on mouse chromosome 10 accounting for 60% of the genetic variance in MA intake between the MADR lines (Belknap et al., 2013), which was traced to a single nucleotide polymorphism within the coding sequence of the trace amine associated receptor 1 (*Taar1)* gene (Harkness et al., 2015; Stafford et al., 2019). MA is a full agonist at the intracellular G protein-coupled receptor encoded by the *Taar1* gene, TAAR1 (Bunzow et al., 2001). Within 1-2 generations of selective breeding, all MAHDR mice are homozygous for the spontaneously mutated *Taar1* allele, denoted as $Taar1^{m1J}$, that encodes non-

functional TAAR1 (Reed et al., 2018). Conversely, MALDR mice possess at least one copy of the reference *Taar1*$^{+}$ allele that encodes functional TAAR1 (Reed et al., 2018). Using a CRISPR-*Cas9*-generated MAHDR-*Taar1*$^{+/+}$ knock-in (KI) line, compared to a MAHDR-*Taar1*$^{m1J/m1J}$ line that served as a control for the KI, TAAR1 functionality was determined to be critical for differential MA intake, and sensitivity to rewarding, aversive, and physiological effects of MA (Phillips et al., 2021; Stafford et al., 2019). Herein, we used the MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice to examine whether replacement with functional TAAR1 would attenuate binge-level MA intake, as it does for MA intake at low concentrations (Stafford et al., 2019). Mice were tested using a two-bottle choice procedure with increasing MA concentrations previously used to demonstrate binge-level MA intake in MAHDR mice (Shabani et al., 2016).

Overall, the pronounced imbalance between MA-induced reward and aversion sensitivity in the MADR mice, linked to TAAR1 functionality, makes them ideal for determining whether effects of MA on neural activity (1) correspond with perception of MA reward or aversion and (2) are dependent on TAAR1 functionality. Our studies focus on the dorsal raphe (DR) which contains serotonin (5-HT) neurons. DR 5-HT neurons are involved in the perception of rewarding stimuli, including sucrose, food, and social interaction (Li et al., 2016; Liu et al., 2014; Nagai et al., 2020; Ren et al., 2018; Wang et al., 2019). DR 5-HT neuron activity increases in response to rewarding stimuli (Li et al., 2016; Liu et al., 2014; Ren et al., 2018) and optogenetic stimulation of DR 5-HT neurons induces reward-motivated behaviors (Liu et al., 2014; Nagai et al., 2020; Wang et al., 2019). In contrast, optogenetic inhibition of DR 5-HT neurons reduces reward-motivated behavior (Nagai et al., 2020). Amphetamines induce 5-HT release in the DR (Ferre et al., 1994; Sprouse et al., 1989) and TAAR1 is widely expressed within this region (Borowsky et al., 2001; Lindemann et al., 2008; Rutigliano et al., 2017). Activation of $G\alpha_{13}$-coupled TAAR1 by the psychostimulant MDMA stimulates RhoA, leading to the internalization of the serotonin transporter (SERT) and a decrease in 5-HT uptake in a TAAR1 function dependent manner

(Underhill & Amara, 2020). In response to the TAAR1 agonist, RO5166017, DR 5-HT neurons exhibit a reduction in firing, an effect that is absent in DR 5-HT TAAR1 KO cells (Revel et al., 2011). These studies demonstrate that TAAR1 modulates 5-HT concentrations and DR 5-HT neuron activity.

To investigate the relationship between TAAR1 function and DR 5-HT neuron activity in mice with differential MA intake and sensitivity to MA-induced reward, we performed whole-cell patch clamp electrophysiology experiments on DR 5-HT neurons from MADR and CRISPR-*Cas9* generated mice. By examining the electrophysiological properties of these neurons, we determined that the effects of MA on the intrinsic properties of DR 5-HT neurons are influenced by TAAR1 functionality and its underlying mechanisms.

Materials and Methods

Animal maintenance and housing

All mice were born within the VA Portland Health Care System (VAPORHCS) veterinary medical unit. After weaning, mice were maintained in standard acrylic plastic shoebox cages on corncob bedding with wire lids and filter tops. Mice were maintained in climate-controlled rooms under a standard 12:12 h light:dark cycle with lights on at 0600 h and *ad libitum* access to water and rodent block food (5LOD PicoLab Rodent Diet; Animal Specialties, Woodburn, Oregon). All animal care and testing procedures were approved by the VAPORHCS Animal Care and Use Committee and were conducted in compliance with the National Institutes of Health Guidelines for Care and Use of Laboratory Animals.

Methamphetamine drinking selected mouse lines

MA-naïve male and female MAHDR and MALDR mice 28-45 days of age were used for electrophysiological studies. MADR mice were selectively bred from a reciprocal F2 cross of

C57BL/6J and DBA/2J inbred strains, based on voluntary MA intake during a two-bottle choice procedure. Details of the selective breeding procedures and responses to selection of multiple replicate sets of MADR lines have been fully described in previous publications (Hitzemann et al., 2019; Shabani et al., 2011; Wheeler et al., 2009). Briefly, mice were provided a water bottle versus 20 mg/L MA in water for 18 h/day for 4 days and then 40 mg/L MA in water for an additional 4 days. Mice used for selective breeding were chosen based on average MA consumed in mg/kg, during access to the 40 mg/L MA solution.

CRISPR-Cas9 knock-in of Taar1^{+}

Male and female MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice 82-85 days of age were tested in an escalating MA concentration two-bottle choice procedure and mice 29-43 days of age were used for electrophysiological study. The MAHDR-*Taar1*$^{+/+}$ KI mice were created at Oregon Health & Science University's Transgenic Mouse Models Shared Resource Core, utilizing CRISPR-*Cas9* technology to exchange the *Taar1*^{m1J} allele with the *Taar1*$^{+}$ reference allele. The MAHDR-*Taar1*$^{m1J/m1J}$ line that served as a control for the KI was derived from mice in which the *Taar1*^{m1J} allele was not successfully excised and exchanged, thus retaining the *Taar1*$^{m1J/m1J}$ genotype. Further details can be found in Stafford et al. (2019).

Drugs

(+) MA hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). D-APV, bicuculline, and fluoxetine were purchased from HelloBio (Princeton, NJ, USA). SB 216641 and WAY 100635 were purchased from Cayman Chemical (Ann Arbor, MI, USA). Serotonin hydrochloride was purchased from Sigma Aldrich (Burlington, MA, USA). All drugs were dissolved in double distilled water, except when MA was used for drinking, when it was dissolved in tap water.

Two-bottle choice drinking of escalating MA concentrations

Methods were consistent with our previous study (Shabani et al., 2016). Voluntary MA consumption was measured from 20 to 140 mg/L MA concentrations, with concentration increasing in 20 mg/L increments every 4 days. Forty mice (10 per MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ line per sex) were weighed and individually housed in plastic shoe box cages with stainless steel wire tops. For the first 48 hours, mice acclimated to consuming fluid from the novel drinking bottles, 25-ml graduated cylinders fitted with stoppers and stainless-steel sippers placed between bars of the cage tops. Food and one water bottle were provided *ad libitum* during this period. On day 3, mice were weighed, and MA-containing bottles were added onto the cage tops for an 18-h period 3-h before the dark cycle started and removed 3-h into the light phase. Fluid consumption was determined for both the 18-h (water vs. MA) and 6-h (water only) periods. To account for position bias, the position of water and MA bottles was alternated every 2 days. Body weight data were collected every 2 days. Fluid consumption and body weight data were used to determine mg/kg of MA consumed daily. Consistent with selection and previous studies, mg/kg consumed during days 2 and 4 (the second day after a water vs. MA bottle position switch) of each MA concentration were averaged to represent drinking for each concentration.

Brain slice preparation and electrophysiological recordings

Mice were deeply anesthetized with isoflurane for brain removal. Brains were immersed in ice-cold sucrose aCSF containing the following (in mM): 80 NaCl, 2.7 KCl, 0.1 $CaCl_2$, 6.5 $MgSO_4$, 1.3 NaH_2PO_4, 24 $NaHCO_3$, 2.8 dextrose, and 82 sucrose with 87.5 µM D-APV, equilibrated with 95.0% O_2/5% CO_2. Coronal slices containing the DR were cut 230-250 µm thick with a vibratome (Leica Microsystems) and placed in oxygenated aCSF containing the following (in mM): 123.5 NaCl, 21 $NaHCO_3$, 19 dextrose, 2.45 KCl, 2.55 $CaCl_2$, 1.2 $MgSO_4$, and 1.2 NaH_2PO_4, and equilibrated with 95% O_2/5% CO_2 at 34 °C until the start of recording. Brain slices were placed onto the recording chamber on an upright Olympus BX51WI microscope and

superfused with 31-33°C aCSF. Electrophysiological recordings were made using the Sutter Instruments Integrated Patch Clamp Amplifier and data acquisition system (Sutter Instruments, Novato, CA, USA). Data were acquired at 5 kHz and low pass filtered at 2 kHz.

Whole-cell recordings in current clamp mode were conducted with glass electrodes with resistances of 3 - 6 MΩ and filled with potassium gluconate internal solution containing the following (in mM): 127 D-gluconic acid potassium salt, 10 HEPES, 1 EGTA, 10 KCl, 1 $MgCl_2$, 0.3 $CaCl_2$, 2 MgATP, and 0.5 NaGTP, pH 7.3-7.4, and 285-295 mOsm. A junction potential of 15 mV was corrected at the start of experiments and for all reported resting membrane potentials (RMPs). During whole-cell current clamp experiments, no holding current was applied. Only neurons with stable RMPs that exhibited action potentials crossing 0 mV when depolarized by current step protocols were used for analysis. In current clamp mode, 2s long depolarizing steps (-40 pA to +60 pA in 20 pA increments, every 10s) were used to evaluate firing patterns of DR 5HT neurons. RMP was measured during the 100 ms before the current injection.

Putative serotonergic DR neurons were selected initially by their reversable inhibitory response to bath application of serotonin hydrochloride (10 μM). Neurons with a capacitance exceeding 50 pF were confirmed as serotonergic and subsequently used for electrophysiological studies.

Experimental Design and Statistical Analysis

All firing frequency data are expressed as mean ± SEM. Data were analyzed using Statistica 13.3 software (TIBCO Software, Inc, Palo Alto, CA, USA). Each cell is considered an independent observation; numbers of cells and mice are given in the figure legends. Differences in firing frequency were assessed using repeated measures ANOVA, followed by Tukey HSD when appropriate. Differences in RMPs between the same cells were assessed using paired t-tests, while comparisons between different cells were analyzed using unpaired t-tests. Differences in

MA and total consumption were assessed using repeated measures ANOVA, followed by within-subjects or between mouse line contrasts of means when appropriate. The level of significance for all statistical tests was set at ≤ 0.05.

Results

MA hyperpolarizes and inhibits firing of MALDR DR 5-HT neurons

TAAR1 agonists inhibit monoamine neurons, including DR 5-HT neurons from C57BL/6J mice (Revel et al., 2012; Revel et al., 2011), a MADR progenitor strain with the *Taar1* gene variant that encodes functional TAAR1 (Harkness et al., 2015). In contrast, TAAR1 agonists have no effect on DR 5-HT neuron activity in TAAR1 KO mice (Revel et al., 2011). To determine the effects of MA on DR 5-HT neuron activity in brain slices from the MADR mouse lines, we measured spontaneous firing frequency and firing frequency across a series of current injections pre- and post-MA application (Fig. 1). MA superfusion alone did not affect the mean firing frequency of DR 5-HT neurons of MAHDR mice, which possess non-functional TAAR1 (Fig. 1A; treatment: $F_{(1,6)}$=0.05, p=0.83). MA had no effect on the RMPs of MAHDR DR 5-HT neurons (Fig.1B; $t_{(6)}$=0.22, p=0.84). However, MA superfusion significantly decreased mean firing frequency of DR 5-HT neurons of MALDR mice, which possess functional TAAR1 (Fig. 1C; treatment: $F_{(1,7)}$=12.46, p=0.0096; treatment x current injected: $F_{(5,35)}$=8.01, p=<0.001). Furthermore, MA significantly hyperpolarized MALDR DR 5-HT neurons (Fig. 1D; $t_{(7)}$=2.57, p=0.037).

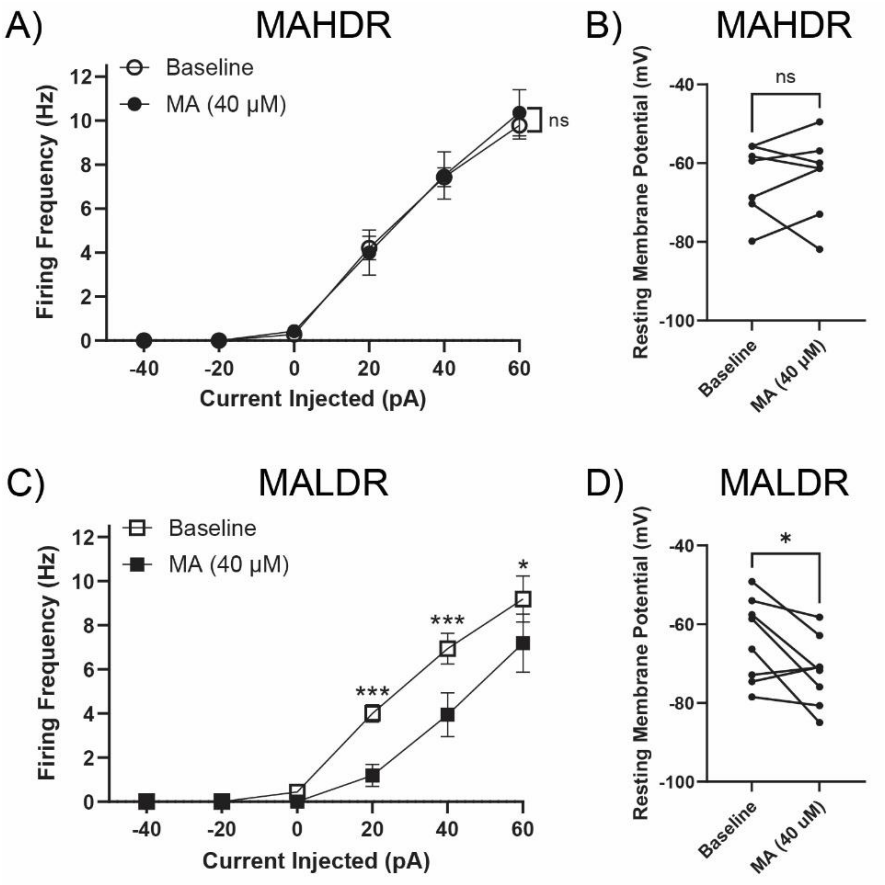

Figure 2.1. MA hyperpolarizes and inhibits firing of DR 5-HT neurons from MALDR mice. A: Mean firing frequency of MAHDR DR 5-HT neurons pre- and post-MA application [7 recordings (7 mice: Male=3, Female=4). Means ± SEM are presented collapsed on sex.]. B. RMPs of MAHDR DR 5-HT neurons were not significantly changed by MA. Each set of symbols represents a recording in the absence and presence of MA. C: Mean firing frequency of MALDR DR 5-HT neurons pre- and post-MA application [8 recordings (8 mice: Male=4, Female=4); Tukey's HSD post hoc test, *p<0.05, ***p<0.001 for baseline compared to MA at a given current. Means ± SEM are presented collapsed on sex.] D. RMPs of MALDR DR 5-HT neurons were significantly hyperpolarized by MA, *p<0.05). Each set of symbols represents a recording in the absence and presence of MA.

MA potentiates DR 5-HT neuron activity of MAHDR but not MALDR mice in the presence of 5HT autoreceptor antagonists

MA is a substrate of SERT (Han & Gu, 2006) and increases extracellular 5-HT concentrations by competing for intracellular transport. Increased extracellular 5-HT can induce feedback inhibition of DR 5-HT neurons through activation of 5-HT_{1A} and 5-HT_{1B} autoreceptors (Aghajanian & Lakoski, 1984; Dedic et al., 2019; Sprouse et al., 1989). To determine whether MA alters intrinsic activity of DR 5-HT neurons, spontaneous firing, and firing frequency across a series of current injections were recorded pre- and post-MA application in the presence of $5HT_{1A}$ and $5HT_{1B}$ autoreceptor antagonists. MA significantly increased the overall firing frequency of MAHDR DR 5-HT neurons (Fig. 2A; treatment: $F_{(1,8)}$=29.70, p=<0.001; treatment x current injected: $F_{(5,40)}$=7.03, p=<0.001). Representative traces demonstrate that MA potentiated MAHDR DR 5-HT neuron activity when recording spontaneous activity and when injecting +40 pA of current (Fig. 2B). MA depolarized the RMPs of MAHDR DR 5-HT neurons (Fig. 2C; $t_{(8)}$=4.36, p=0.0024). We observed a different profile in MALDR mice, where MA had no effect in the presence of 5-HT autoreceptor inhibitors (Fig. 2D; treatment: $F_{(1,6)}$=0.26, p=0.63). Representative traces demonstrate that MA had no effect on spontaneous firing or firing during the +40 pA current injection (Fig. 2E) and no effect on RMPs (Fig. 2F; $t_{(6)}$=2.21, p=0.07) of MALDR DR 5-HT neurons. These data indicate that the MA-induced reduction in firing and hyperpolarization observed in MALDR mice (Fig. 1C,D) is due to activation of 5-HT autoreceptors.

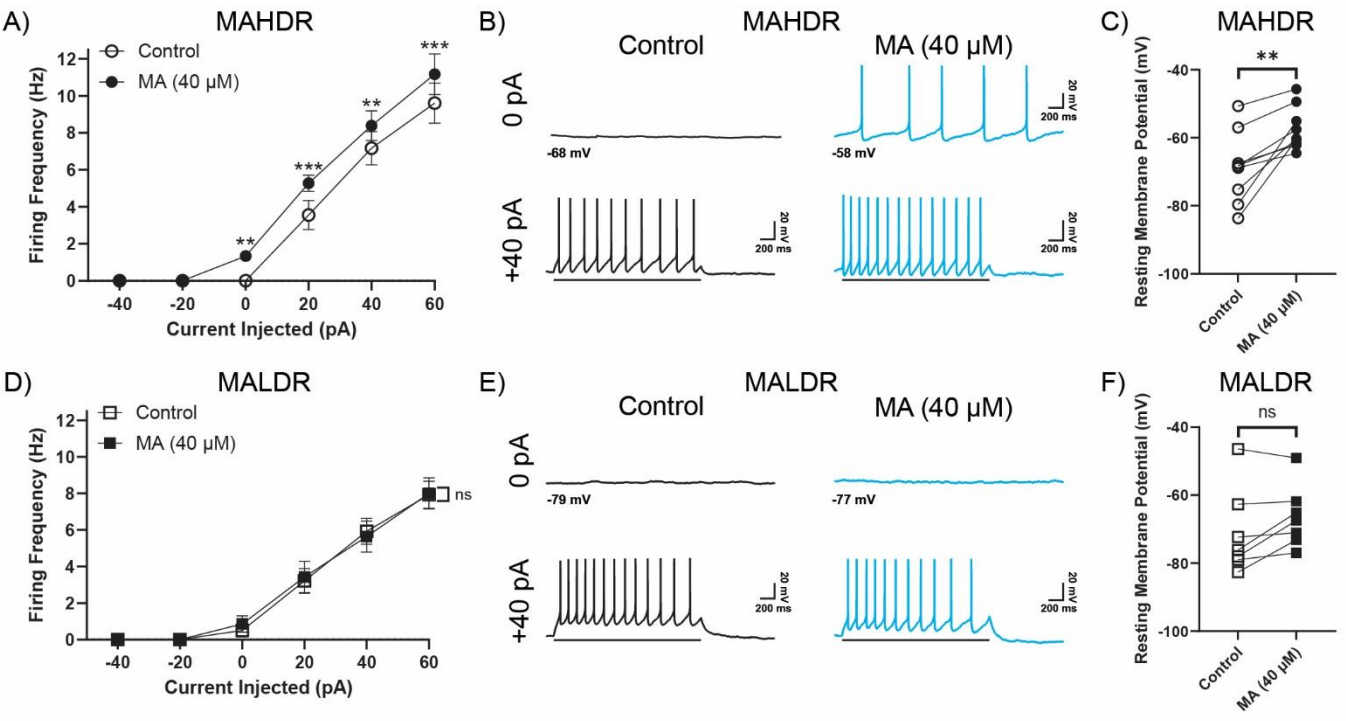

Figure 2.2. MA potentiates DR 5-HT neuron activity of MAHDR but not MALDR mice in the presence of $5HT_{1A}$ and $5HT_{1B}$ autoreceptor antagonists. Control refers to the firing frequency, activity, and RMP in the presence of antagonists for $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptors. A: Mean firing frequency of MAHDR DR 5-HT neurons pre- and post-MA application [9 recordings (8 mice: Male=5, Female=4); Tukey's HSD post hoc test, **p<0.01, ***p<0.001 for control compared to MA at a given current. Means ± SEM are presented collapsed on sex.] B: Representative traces of MAHDR DR 5-HT recordings at 0 pA and +40 pA current injection in the absence (black) and presence of MA (teal). C: RMPs of MAHDR DR 5-HT neurons were significantly depolarized by MA, $^{**}p<0.01$). Each set of symbols represents a recording in the absence and presence of MA. D: Mean firing frequency of MALDR DR 5-HT neurons pre- and post-MA application [7 recordings (7 mice: Male=3, Female=4). Means ± SEM are presented collapsed on sex.] E: Representative traces of MALDR DR 5-HT recordings at 0 pA and +40 pA current injection in the absence (black) and presence of MA (teal). F: RMPs of MALDR DR 5-HT neurons were not significantly changed by MA. Each set of symbols represents a recording in the absence and presence of MA.

Binge-level MA consumption is dependent on *Taar1*^{m1J} encoding non-functional TAAR1

Our previous work first linked (Harkness et al., 2015; Reed et al., 2018), then determined a causal role (Phillips et al., 2021; Stafford et al., 2019), for TAAR1 functionality in determining differential MA intake, and sensitivity to rewarding, aversive, and physiological effects of MA. To further evaluate the causal role of TAAR1 in MA intake, we determined whether replacement of the *Taar1*^{m1J} allele with the *Taar1*$^{+}$ allele on the MAHDR background, producing MAHDR mice with functional TAAR1, attenuated binge-level MA intake, as it did for MA intake at low concentrations (Stafford et al., 2019).

No significant effects involving sex for mg/kg MA consumption were found in the initial repeated measures ANOVA, therefore, data were collapsed on sex and reanalyzed for effects of line and MA concentration. There was a significant concentration x line interaction (Fig. 3A; concentration x line: $F_{(6,192)}$=22.25, p<0.001). MAHDR-*Taar1*$^{m1J/m1J}$ mice consumed significantly more MA at all concentrations compared to MAHDR-*Taar1*$^{+/+}$ KI mice. Within-subjects contrasts of means between previous and subsequent MA concentration revealed significant increases in MA intake at 40, 60, and 80 mg/L MA concentrations in MAHDR-*Taar1*$^{m1J/m1J}$ mice (concentration: $F_{(6,102)}$=28.18, p=<0.001). MAHDR-*Taar1*$^{+/+}$ KI mice consumed low and comparable levels of MA at all concentrations offered.

In the initial repeated measures ANOVA for total fluid consumption (ml; MA and water; Fig. 3B) during the 18-h MA access period, there was a significant concentration x sex interaction ($F_{(6,198)}$=4.27, p=<0.001). Total volume consumed by males was significantly greater than females at 140mg/L. Because there was no interaction of sex with line, data were collapsed on sex and reanalyzed for effects of line and MA concentration. There was a significant concentration x line interaction (concentration x line: $F_{(6,198)}$=8.15, p=<0.001). Total volume consumed by MAHDR-*Taar1*$^{m1J/m1J}$ mice was significantly greater than MAHDR-*Taar1*$^{+/+}$ KI mice at each concentration between 80 and 140 mg/L. Within-subjects contrasts of means between previous and subsequent

MA concentration revealed a significant increase in total volume at 40 mg/L in MAHDR-*Taar1*$^{+/+}$ KI mice (concentration: $F_{(6,108)}$=3.31, p=0.005) and at 40 and 60 mg/L in MAHDR-*Taar1*$^{m1J/m1J}$ mice (concentration: $F_{(6,102)}$=16.69, p=<0.001).

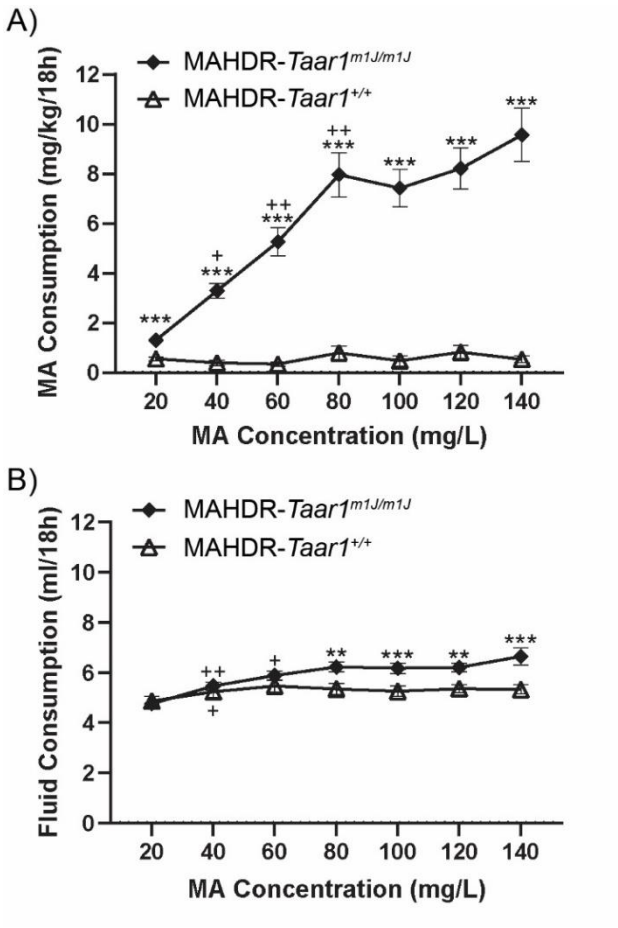

Figure 2.3. Binge-level MA consumption is dependent on non-functional TAAR1. A: Total MA (mean ± SEM) consumption in mg/kg/18 h (day 2 and 4 average at each concentration) for each line at each MA concentration offered. ***p*<0.01, ****p*<0.001 for the difference in MA consumed between the MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$ KI mice. Further analysis revealed a significant effect of MA concentration for MAHDR-*Taar1*$^{m1J/m1J}$ mice only. Within-subjects contrasts of means, ^{+}p <0.05, ^{++}p <0.01 for the difference in MA consumed compared to the next lower MA concentration. B: Total fluid (mean ± SEM) consumed by each line during the same 18 h period when each MA concentration was offered. ***p*<0.01, ****p*<0.001 for the difference in total volume consumed between the MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$ KI lines. Further analysis revealed a significant effect of MA concentration for both the MAHDR-*Taar1*$^{m1J/m1J}$ line and MAHDR-*Taar1*$^{+/+}$ KI line. Within-subjects contrasts of means, ^{+}p <0.05, ^{++}p <0.01 for the difference in total volume at a given concentration compared to the previous MA concentration.) n=40 mice (10 mice per line per sex).

MA-induced potentiation of DR 5-HT neurons is dependent on non-functional TAAR1

To determine whether the acute effects of MA on firing frequency of DR 5-HT neurons are dependent on *Taar1* genotype, we compared the effects of MA on DR 5-HT neuron activity of MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice. DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ mice exhibited a significant increase in overall mean firing frequency in response to MA (Fig. 4A; treatment: $F_{(1,6)}$=28.13, p=0.0018; treatment x current injected: $F_{(5,30)}$=12.02, p=<0.001), similar to MAHDR mice. Representative traces demonstrate the MA-induced potentiation of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neuron activity when recording spontaneous activity and when injecting +40 pA of current (Fig. 4B). MA depolarized the RMPs of DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ mice (Fig. 4C; $t_{(6)}$=3.62, p=0.011). In contrast, mean firing frequency of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons did not change in response to MA across the range of current injections (Fig. 4D, E; $F_{(1,5)}$=0.029 p=0.87). MA had no effect on RMPs of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons (Fig. 4F; $t_{(5)}$=1.87, p=0.12).

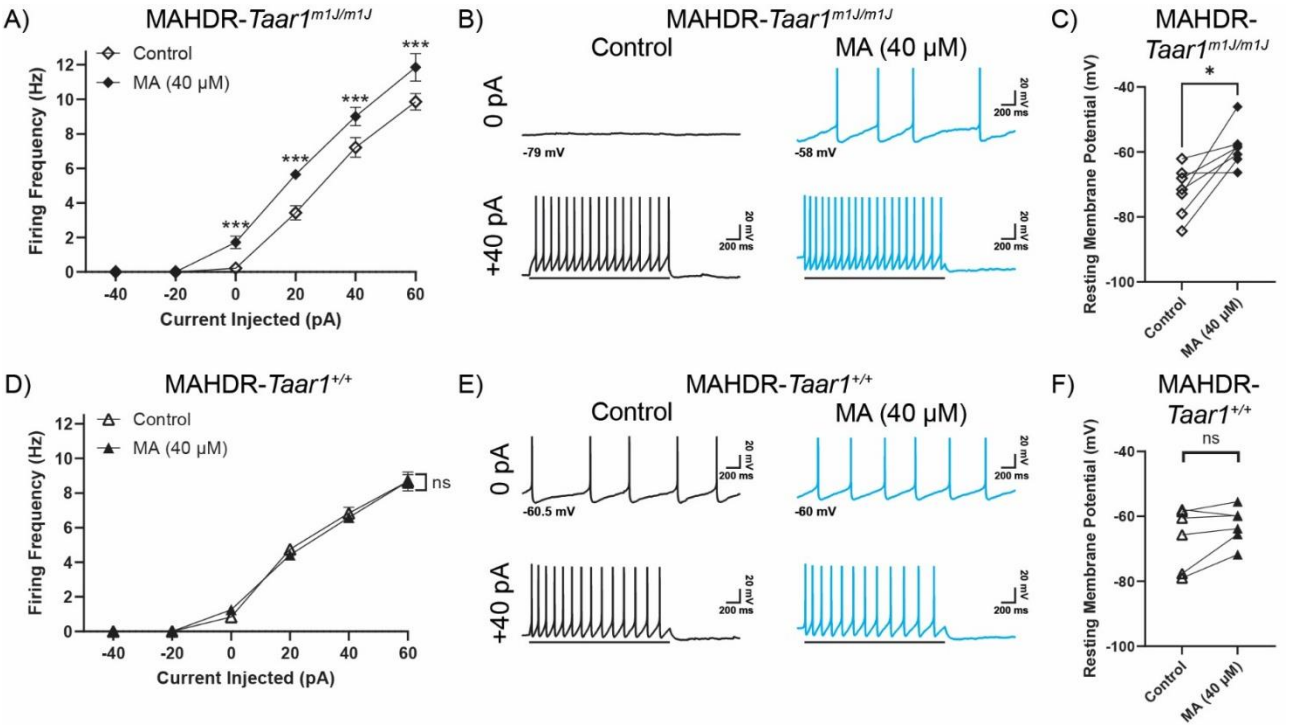

Figure 2.4. MA-induced potentiation of DR 5-HT neurons is dependent on non-functional TAAR1. Control refers to the firing frequency, activity, and RMP in the presence of antagonists for $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptors. A: Mean firing frequency of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons pre- and post-MA application [7 recordings (7 mice: Male=4, Female=3); Tukey's HSD post hoc test, ***p<0.001 for control compared to MA at a given current. Means ± SEM are presented collapsed on sex.] B: Representative traces of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT recordings at 0 pA and +40 pA current injections in the absence (black) and presence of MA (teal). C: RMPs of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons were significantly depolarized by MA, *p<0.05. Each set of symbols represents a recording in the absence and presence of MA. D: Mean firing frequency of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons pre- and post-MA application [6 recordings (6 mice: Male=3, Female=3). Means ± SEM are presented collapsed on sex] E: Representative traces of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT recordings at 0 pA and +40 pA current injections in the absence (black) and presence of MA (teal). F: RMPs of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons were unchanged by MA. Each set of symbols represents a recording in the absence and presence of MA.

MA-induced potentiation of DR 5-HT neuron expressing non-functional TAAR1 is SERT dependent.

To determine whether the TAAR1-dependent MA-induced potentiation observed in DR 5-HT neurons of MAHDR mice is due to synaptic inputs onto DR 5-HT neurons, the AMPA receptor antagonist NBQX (10 μM) and the $GABA_A$ receptor antagonist, bicuculline (10 μM) were included in the recording solution, in addition to 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists. The MA-induced increase in overall mean firing frequency of DR 5-HT neurons was not blocked (Fig. 5A; treatment: $F_{(1,6)}$=73.00, p=<0.001; treatment x current injected: $F_{(5,30)}$=12.93, p=<0.001). MA significantly increased mean firing frequency throughout the range of injected currents. Additionally, MA depolarized the RMPs of DR 5-HT neurons from MAHDR mice in the presence of NBQX and bicuculline (Fig. 5B; $t_{(6)}$=3.06, p=0.022). Thus, these synaptic inputs are unlikely to contribute to the MA-induced potentiation and depolarization of DR 5-HT neurons.

Because MA is a substrate of the SERT transporter, we evaluated whether SERT is necessary to observe MA-induced potentiation of DR 5-HT neuron firing in *Taar1*$^{m1J/m1J}$ mice. RMPs in the presence of fluoxetine and autoreceptor antagonists were not statistically different compared to RMPs in the presence of autoreceptor antagonists only (MAHDR, $t_{(14)}$=0.87, p=0.40; N=7, 9 respectively; MAHDR -*Taar1*$^{m1J/m1J}$ controls, $t_{(12)}$=0.93, p=0.37; N=7 for both conditions). However, the MA-induced effects on firing frequency and RMP were blocked in the presence of fluoxetine. MA had no effect on mean firing frequency over a range of current injections for MAHDR or MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons (MAHDR, Fig. 5C; treatment: $F_{(1,6)}$=2.74, p=0.15; MAHDR-*Taar1*$^{m1J/m1J}$, Fig. 5E; treatment: $F_{(1,6)}$=0.55, p=0.49), indicating SERT is necessary for MA-induced potentiation of DR 5-HT neuron firing. In the presence of fluoxetine, MA had no effect on the RMPs of DR 5-HT neurons of either mouse line (MAHDR, Fig. 5D; $t_{(6)}$=0.20, p=0.85; MAHDR-*Taar1*$^{m1J/m1J}$, Fig. 5F; $t_{(6)}$=1.92, p=0.10), indicating a role for SERT in

MA-induced depolarization. Figure 5G illustrates the hypothesized differences between mice with non-functional TAAR1 (left panel) and functional TAAR1 (right panel).

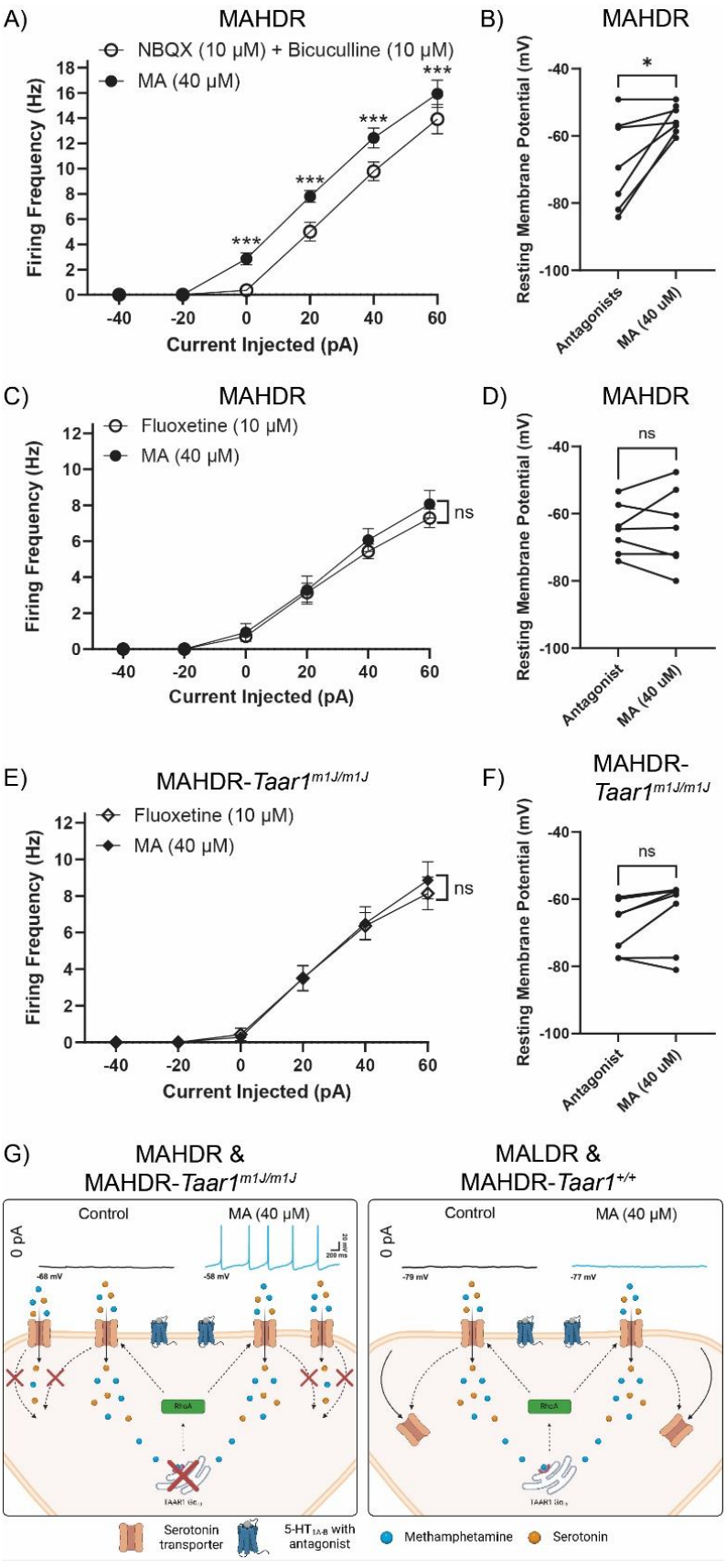
A)
MAHDR
NBQX (10 μM) + Bicuculline (10 μM)
MA (40 μM)
Firing Frequency (Hz)
Current Injected (pA)
***
B)
MAHDR
Resting Membrane Potential (mV)
*
Antagonists
MA (40 uM)
C)
MAHDR
Fluoxetine (10 μM)
MA (40 μM)
ns
D)
MAHDR
E)
MAHDR-Taar1m1J/m1J
F)
MAHDR-Taar1m1J/m1J
Antagonist
G)
MAHDR &
MAHDR-Taar1m1J/m1J
MALDR &
MAHDR-Taar1+/+
Control
MA (40 μM)
0 pA
-68 mV
-58 mV
-79 mV
-77 mV
RhoA
Serotonin transporter
5-HT2A-E with antagonist
Methamphetamine
Serotonin

Figure 2.5. MA-induced potentiation of DR 5-HT neurons expressing non-functional TAAR1 is SERT dependent. All experiments were performed in the presence of antagonists for $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptors. A: Mean firing frequency of MAHDR DR 5-HT neurons pre- and post-MA application in the presence of the AMPA receptor antagonist NBQX (10 μM) and $GABA_A$ receptor antagonist bicuculline (10 μM) [7 recordings (7 mice: Male=4, Female=3). Means ± SEM are presented collapsed on sex] B: RMPs of MAHDR DR 5-HT neurons depolarized in response to MA in the presence of NBQX and bicuculline. Each set of symbols represents a recording in the absence and presence of MA. C: The effect of MA is blocked in the presence of fluoxetine in recordings from MAHDR DR 5-HT neurons [7 recordings (6 mice: Male=3, Female=4). Means ± SEM are presented collapsed on sex]. D: RMPs of MAHDR DR 5-HT neurons were not significantly changed by MA in the presence of fluoxetine. Each set of symbols represents a recording in the absence and presence of MA. E: Blockade of the MA effect by fluoxetine is confirmed in DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control mice [7 recordings (6 mice: Male=4, Female=3). Means ± SEM are presented collapsed on sex]. F: RMPs of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons were not significantly changed by MA in the presence of fluoxetine. Each set of symbols represents a recording in the absence and presence of MA. G. Schematic of TAAR1 differences between lines. *Left panel:* MA enters DR 5-HT neurons of MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice through SERT. Due to non-functional TAAR1, MA cannot activate TAAR1 signaling pathways, preventing the internalization of SERT from the membrane (denoted by the red Xs). In these mice, MA depolarizes DR 5-HT neurons resulting in increased activity in the presence of autoreceptor antagonists. The effect of MA can be blocked by the SERT antagonist fluoxetine. *Right panel*: MA enters DR 5-HT neurons of MALDR and MAHDR-*Taar1*$^{+/+}$ KI mice through SERT. MA activates TAAR1 signaling pathways leading to internalization of SERT from the membrane. As a result, MA does not depolarize DR 5-HT neurons, and their activity remains unchanged in the presence of 5-HT autoreceptor antagonists. Created in BioRender. Rios, S. (2025) https://BioRender.com/t93y794.

Discussion

The data presented show MA-induced depolarization, increases in DR 5-HT neuron excitability, and binge-level MA consumption in mice lacking functional TAAR1. Moreover, MA enhanced DR 5-HT neuron excitability through a SERT-dependent mechanism. These findings highlight the role of TAAR1 as a regulator of DR 5-HT neuron excitability and MA intake.

KI of functional TAAR1 on the MAHDR background converts binge level MA intake to low intake

Previously, using MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice, our lab confirmed a *causal* relationship between *Taar1* genotype and sensitivity to rewarding and aversive effects of MA (Phillips et al., 2021), as well as MA consumption at low MA concentrations (Stafford et al., 2019), which we had *linked* to *Taar1* genotype and TAAR1 function in MADR mice (Harkness et al., 2015; Reed et al., 2018). Building on this, we used the MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice to determine whether TAAR1 function is critical for binge-level MA consumption, as observed in MAHDR mice (Shabani et al., 2016). Binge-level MA consumption was found in MAHDR-*Taar1*$^{m1J/m1J}$, but not MAHDR-*Taar1*$^{+/+}$ KI mice. Therefore, KI of *Taar1*$^{+}$, which expresses functional TAAR1, converted binge-level MA intake to low MA intake, confirming that *Taar1 causally* regulates MA consumption and the presence of non-functional TAAR1 drives binge-level MA intake. Given TAAR1's critical role in determining MA consumption and sensitivity to MA reward, we explored whether TAAR1 function determines the effects of MA on DR 5-HT neurons, which are involved in reward signaling.

TAAR1 functionality mediates MA-induced changes in DR 5-HT neuron activity

MA inhibited firing of MALDR DR 5-HT neurons that have functional TAAR1. This inhibition is consistent with studies using other TAAR1 agonists (Grinchii et al., 2022; Revel et

al., 2011). MA and other psychostimulants increase extracellular 5-HT concentrations which activates inhibitory 5-HT autoreceptors (Ferre et al., 1994; Kuczenski et al., 1995; Schmidt et al., 1987; Sprouse et al., 1989; Underhill & Amara, 2020). Our findings support this mechanism, as MA alone hyperpolarized MALDR DR 5-HT neurons, and both the hyperpolarization and inhibition of firing were reversed by 5-HT autoreceptor antagonists. In contrast, MA depolarized and increased the firing of DR 5-HT neurons in the presence of 5-HT autoreceptor antagonists in recordings from mice with non-functional TAAR1 (MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$). Importantly, the MA-induced depolarization and increase in excitability were absent in MAHDR-*Taar1*$^{+/+}$ KI which are genetically matched to the MAHDR-*Taar1*$^{m1J/m1J}$ mice except for the single point mutation in TAAR1. This indicates that lack of TAAR1 functionality is crucial for MA-induced depolarization and excitability of DR 5-HT neurons, likely contributing to heightened reward sensitivity. The effect of MA on the firing rate and RMP of MAHDR DR 5-HT neurons was maintained in the presence of AMPA and $GABA_A$ receptor antagonists, suggesting these synaptic inputs do not mediate the effect of MA. This does not rule our other neuromodulators that may be present in our slices that could mediate the depolarization, such as norepinephrine (Han & Gu, 2006; Khamma et al., 2022).

MA-induced depolarization and excitability of DR 5-HT neurons expressing non-functional TAAR1 is SERT dependent

The next set of experiments identified SERT as the cellular mechanism underlying the MA-induced increase in DR 5-HT neuron excitability. Fluoxetine completely blocked the effects of MA on RMP and excitability of DR 5-HT neurons from MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice. MA, as a substrate of SERT (Han & Gu, 2006), is transported into DR 5-HT neurons, where it can interact with TAAR1 as a potent agonist (Bunzow et al., 2001). Notably, our previous research described amphetamine-induced internalization of DAT in midbrain dopamine neurons (Li et al., 2017; Wheeler et al., 2015) via TAAR1-dependent $G\alpha_{13}$-mediated RhoA

signaling (Underhill et al., 2019). Similarly, TAAR1-mediated internalization of SERT has also been described which is significant because SERT expression on the membrane tightly regulates extracellular 5-HT levels (Blakely et al., 1998). In DR 5-HT cultures expressing functional TAAR1, the psychostimulant MDMA activates TAAR1 leading to increased $G\alpha_{13}$-coupled RhoA signaling. This cascade drives SERT internalization, reducing serotonin reuptake (Underhill & Amara, 2020). Importantly, MDMA has no effect on surface expression of SERT in TAAR1 KO cells (Underhill & Amara, 2020). These results indicate that MDMA, a derivative of MA, interacts with both SERT and TAAR1. Since we observed MA-induced increases in firing in DR 5-HT neurons from mice that have non-functional TAAR1, it is likely that TAAR1 is unable to traffic SERT from the membrane, allowing for the continued transport of MA. One possible mechanism explaining the depolarization and increase in excitability of DR 5-HT neurons is an increase in SERT-dependent ion currents. Amphetamine and MA stimulate DAT-dependent currents that are uncoupled from electrogenic transport and increase the excitability of DA neurons (Branch & Beckstead, 2012; Ingram et al., 2002). SERT expressed in cell lines also display coupled and uncoupled ion currents (Buchmayer et al., 2013; Ni et al., 2001; Schicker et al., 2012; Sucic et al., 2010). The SERT-dependent increase in excitability of DR 5-HT neurons from mice with non-functional TAAR1 suggests that impaired internalization of SERT may reveal MA-induced SERT currents, which would be responsible for the observed MA-induced depolarization that is blocked in the presence of fluoxetine. We cannot rule out the possibility that maintaining SERT on the membrane also allows MA transport into the neurons to affect ion channels from the intracellular space or activate ion channels downstream of TAAR1 signaling. Further experiments are necessary to determine the discrete site where MA interacts to increase excitability.

In summary, we observed MA-induced depolarization and an increase in firing frequency of DR 5-HT neurons in MA reward-sensitive MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice and

observed binge-level MA intake by MAHDR-*Taar1*$^{m1J/m1J}$ mice but not MA reward-insensitive MAHDR-*Taar1*$^{+/+}$ mice. These data further support a critical link between DR 5-HT neuron activity and reward-related behaviors. The dual dependence of MA-induced changes in RMP and firing rate of DR 5-HT neurons on both TAAR1 functionality and SERT activity further underscores a complex regulatory mechanism. This intricate relationship highlights the necessity for further investigation into how TAAR1 and SERT interact to modulate DR 5-HT neuron activity in response to MA, particularly considering the dramatic effect the *Taar1* mutation has on MA consumption (Harkness et al., 2015; Phillips et al., 2021; Stafford et al., 2019). Exploring this interplay will provide insights into the genetic and molecular mechanisms influencing MA use disorders and uncover novel therapeutic targets.

Chapter 3: TAAR1 functionality as a determinant of baseline and methamphetamine-induced glutamatergic transmission onto dorsal raphe serotonin neurons

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Acknowledgements: These studies were designed by Samantha Rios in collaboration with Drs. Tamara Phillips and Susan Ingram. Acquisition and analysis of all electrophysiology data were performed by Samantha Rios. The manuscript was written by Samantha Rios in consultation with Drs. Tamara Phillips and Susan Ingram.

Abstract

Our laboratory identified a region on mouse chromosome 10 that accounts for 60% of the genetic variance in methamphetamine (MA) intake in mice selectively bred for high versus low MA consumption. We previously used CRISPR-Ca9-generated mice to demonstrate that variants at a specific sequence location of the trace amine-associated receptor 1 (*Taar1)* gene have a causal role in MA intake and MA-reward sensitivity. In this study, we used these mice to explore whether a single nucleotide polymorphism in the *Taar1* gene differentially affects glutamatergic transmission onto dorsal raphe serotonin (5-HT) neurons at baseline and in response to MA. Both glutamate and 5-HT are linked to reward processing that may influence MA intake. Briefly, the mutant *Taar1*^{m1J} allele was replaced with the reference *Taar1*$^{+}$ allele on the MA high drinking (MAHDR) mouse line background to create the MAHDR-*Taar1*$^{+/+}$ knock-in line. MAHDR mice are homozygous for the *Taar1*^{m1J} gene variant and have consistently exhibited high voluntary MA intake, high sensitivity to MA-induced reward, and low sensitivity to MA-induced aversion. The *Taar1*^{m1J} gene variant encodes a non-functional TAAR1 receptor, whereas the reference *Taar1*$^{+}$ gene variant encodes a functional TAAR1 receptor. Previous studies demonstrated that knock-in of the *Taar1*$^{+}$ gene variant on the MAHDR background reduced voluntary MA intake, converted sensitivity from high to low for MA-induced reward, and increased sensitivity to MA-induced aversion, compared to the MAHDR-*Taar1*$^{m1J/m1J}$ control line . Here, we perform whole-cell patch clamp electrophysiology experiments of glutamatergic synaptic transmission onto dorsal raphe 5-HT neurons. We demonstrate that MAHDR-*Taar1*$^{+/+}$ knock-in mice exhibit significantly higher baseline frequencies of spontaneous excitatory post-synaptic currents (sEPSC) onto dorsal raphe 5-HT neurons compared to MAHDR-*Taar1*$^{m1J/m1J}$ control mice. No differences in baseline sEPSC amplitudes were found. In response to MA, sEPSC frequencies onto dorsal raphe 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice increased but decreased in recordings from MAHDR-*Taar1*$^{+/+}$ knock-in mice. Additionally, we measured and observed no baseline differences in

paired pulse ratio (PPR). However, MA significantly increased the PPR of dorsal raphe 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control mice but decreased the PPR in recordings from MAHDR-*Taar1*$^{+/+}$ knock-in mice. Expanding on previous findings, we found that the MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice in the presence of autoreceptor antagonists is dependent on NMDA receptors. These findings highlight the importance of *Taar1* gene variants in regulating the interplay between serotonergic and glutamatergic systems.

Introduction

In recent years, the number of individuals reporting frequent methamphetamine (MA) use (>100d/year) increased by 66% (Han et al., 2021). The likelihood of continued and frequent use may be influenced by an individual's sensitivity to the rewarding and aversive effects of MA (Chait, 1993; de Wit et al., 1986; Shabani et al., 2011). The MA drinking (MADR) mouse lines were selectively bred to investigate the genetic influences on high and low voluntary MA intake using a two-bottle choice procedure. This resulted in the establishment of the MA high drinking (MAHDR) and MA low drinking (MALDR) lines (Wheeler et al., 2009). Bidirectional selective breeding for MA intake resulted in differences in sensitivity to the rewarding and aversive effects of MA, indicating shared genetic influence. Thus, MAHDR mice exhibit high sensitivity to the rewarding effects of MA and diminished sensitivity to its aversive effects. In contrast, MALDR mice are insensitive to the rewarding effects of MA and highly sensitive to its aversive effects. Consistent selection response to voluntary MA consumption and differential sensitivity to MA-induced reward and aversion have been confirmed across 5 replicate sets of MADR lines (Hitzemann et al., 2019; Phillips & Shabani, 2015; Shabani et al., 2011; Wheeler et al., 2009).

Quantitative trait locus analysis identified a region on mouse chromosome 10 that accounts for 60% of the genetic variance in MA intake between the MADR lines (Belknap et al., 2013; Harkness et al., 2015). Using publicly available sequencing data, a spontaneously arising single nucleotide polymorphism (SNP) was identified within the coding region of the trace amine-associated receptor 1 (*Taar1*) at this location that differentiated the DBA/2J and C57BL/6J progenitors of the MADR lines. Importantly, this gene is responsible for encoding the TAAR1 receptor at which MA acts as a full agonist (Bunzow et al., 2001). Subsequent research determined that MAHDR mice are homozygous for the mutated variant of the *Taar1* allele, denoted as $Taar1^{m1J}$, which encodes a non-functional receptor (Harkness et al., 2015). In contrast, MALDR mice possess at least one copy of the $Taar1^{+}$ allele which encodes a functional TAAR1

receptor. Using CRISPR-*Cas9* technology to generate the MAHDR-*Taar1*$^{+/+}$ knock-in (KI) line and a MAHDR-*Taar1*$^{m1J/m1J}$ control line, the laboratory confirmed *Taar1* genotype causally influence MA intake and sensitivity to the rewarding and aversive effects of MA. KI of the *Taar1*$^{+}$ gene encoding a functional TAAR1 receptor on the MAHDR background converted high MA intake and reward sensitivity, along with low MA-induced aversion to low MA intake and reward sensitivity, and high MA-induced aversion (Phillips et al., 2021; Rios et al., 2025; Stafford et al., 2019). In contrast, the absence of TAAR1 function in MAHDR-*Taar1*$^{m1J/m1J}$ control mice resulted in high voluntary and binge-level MA intake, and high sensitivity to the rewarding properties of MA.

We previously examined how TAAR1 functionality plays a causal role in regulating the activity of dorsal raphe (DR) serotonin (5-HT) neurons in response to MA. The absence of TAAR1 function in the MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control lines resulted in MA-induced depolarization and potentiation of DR 5-HT neurons in the presence of 5-HT$_{1A}$ and 5-HT$_{1B}$ antagonists. Conversely, functional TAAR1 blocks these MA-induced effects in MALDR and MAHDR-*Taar1*$^{+/+}$ KI lines (Rios et al., 2025). Here, our goal was to further investigate the TAAR1-mediated mechanisms underlying the effects of MA on the activity of DR 5-HT neurons. Given that TAAR1 also influences glutamatergic signaling machinery in DA neurons, as well as glutamatergic signaling onto DA neurons following AMPH administration (Li et al., 2017; Lominac et al., 2016; Szumlinski et al., 2017; Underhill et al., 2019; Underhill et al., 2014) we focused our attention on glutamatergic inputs. This focus was further supported by data indicating glutamate is a critical neurotransmitter involved in addiction, influencing synaptic plasticity and adaptations in neural circuits associated with substance use which may contribute to different components of addictive behavior (Kalivas et al., 2009; Kauer & Malenka, 2007; van Huijstee & Mansvelder, 2014; Wolf, 2025). Psychostimulants modulate plasma expression of membrane glutamate transporters in both DA and 5-HT neurons in a TAAR1-dependent manner (Underhill

& Amara, 2020; Underhill et al., 2019; Underhill et al., 2014) indicating that MA may modulate glutamatergic synaptic transmission onto 5-HT neurons as measured in midbrain DA neurons (Li et al., 2017; Underhill et al., 2019; Underhill et al., 2014)

To investigate the relationship between TAAR1 functionality and glutamatergic synaptic transmission onto DR 5-HT neurons, pre- and post-MA, we performed whole-cell patch-clamp electrophysiology experiments on DR 5-HT neurons from MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice.

Materials and Methods

Animal maintenance and housing

All mice were born within the VA Portland Health Care System (VAPORHCS) medical unit. Mice were housed in standard acrylic plastic shoebox cages lined with corncob bedding, with wire lids and filter tops. Rooms were climate-controlled on a 12:12 h light dark cycle (lights on at 0600 h). Food (5LOD PicoLab; Animal Specialties, Woodburn, OR) and water were available *ad libitum.* All animal care and testing methods were approved by the VAPORHCS Animal Care and Use Committee and adhered to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

Methamphetamine drinking selected mouse lines

MA-naïve male and female MAHDR mice over 28 days of age were used for electrophysiological study. MAHDR mice were selectively bred from a reciprocal F2 cross of C57BL/6J and DBA/2J inbred strains, based on high voluntary MA intake during a two-bottle choice procedure. Details of the selective breeding procedures and responses to selection of multiple replicate sets of MAHDR lines have been fully described in previous publications (Hitzemann et al., 2019; Shabani et al., 2011; Wheeler et al., 2009). Briefly, mice were provided

with a water bottle versus 20 mg/l MA in water for 18 h/day for 4 days and then 40 mg/l MA in water for an additional 4 days. Mice used for selective breeding were chosen based on higher average MA consumed in mg/kg within the distribution of mice tested, during access to the 40 mg/l MA solution.

CRISPR-Cas9 knock-in of Taar1^{+}

MA-naïve male and female MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ mice over 28 days of age were used for electrophysiological study. The MAHDR-*Taar1*$^{+/+}$ KI mice were created by the Oregon Health & Science University Transgenic Mouse Models Shared Resource, utilizing CRISPR-*Cas9* technology to exchange the *Taar1*^{m1J} allele with the *Taar1*$^{+}$ reference allele. The MAHDR-*Taar1*$^{m1J/m1J}$ line that served as a control for the KI was derived from mice in which the *Taar1*^{m1J} allele was not successfully excised and exchanged, thus retaining the *Taar1*$^{m1J/m1J}$ genotype. Further details can be found in Stafford et al. (2019).

Drugs

(+) MA hydrochloride was purchased from Sigma-Aldrich (St. Louis, MO, USA). MK-801, D-APV, QX-314, and SR-95531 hydrobromide (gabazine) were purchased from HelloBio (Princeton, NJ, USA). SB 216641 and WAY 100635 were purchased from Cayman Chemical (Ann Arbor, MI, USA). All drugs were dissolved in double distilled water.

Brain slice preparation and electrophysiological recordings

Mice were deeply anesthetized with isoflurane, followed by decapitation and brain removal. Brains were immersed in ice-cold sucrose aCSF containing the following (in mM): 80 NaCl, 2.7 KCl, 0.1 $CaCl_2$, 6.5 $MgSO_4$, 1.3 NaH_2PO_4, 24 $NaHCO_3$, 2.8 dextrose, and 82 sucrose with 87.5-100 µM D-APV or 10 µM MK-801, equilibrated with 95.0% O_2/CO_2. Coronal slices containing the DR were cut 230-250 µm thick with a vibratome (Leica Microsystems). Slices were placed in a holding chamber of oxygenated aCSF containing the following (in mM): 123.5

NaCl, 21 $NaHCO_3$, 19 dextrose, 2.45 KCl, 2.55 $CaCl_2$, 1.2 $MgSO_4$, and 1.2 NaH_2PO_4, and equilibrated with 95% O_2 /5% CO_2 at 34 °C until the start of recording. Brain slices were placed onto the recording chamber on an upright Olympus BX51WI microscope and superfused with 31-33°C aCSF. Electrophysiological recordings were made using the Sutter Instruments Integrated Patch Clamp Amplifier and data acquisition system (Sutter Instruments, Novato, CA, USA). Data were acquired at 5 kHz and low pass filtered at 2 kHz.

Whole-cell recordings in voltage clamp mode were conducted with glass electrodes with resistances of 1.5- 4 MΩ and filled with a cesium chloride internal solution containing the following (in mM): 149 cesium chloride, 10 HEPES, 1.1 EGTA, 2 $MgCl_2$, 0.1 $CaCl_2$, 4 MgATP, and 1 NaGTP; pH was adjusted to 7.3-7.4 with CsOH and osmolarity to 285-295 mOsm. A junction potential of 5 mV was corrected at the start of each recording. QX-314 (100 µM) was added to the internal solution for spontaneous EPSC (sEPSC) and paired pulse ratio (PPR) experiments to reduce action potentials in the recording cell. Glutamatergic events were isolated in the presence of the $GABA_A$ receptor antagonist gabazine (10µM). All experiments were performed in the presence of 5-HT_{1A} (WAY 100635, 100 nM) and 5-HT_{1B} (SB 216641, 200 nM) autoreceptor antagonists. sEPSC and PPR were measured at a holding potential of -55 mV. PPR experiments were elicited using bipolar stimulating electrodes (two 2 ms stimulations, 100 ms apart). PPR was measured by dividing the amplitude of pulse 2 by the amplitude of pulse 1. Access resistance was continuously monitored and recordings in which access changed by >20% during the experiment were excluded from data analysis.

Whole-cell recordings in current clamp mode were conducted using glass electrodes with resistances of 3 - 6 MΩ, filled with a potassium gluconate internal solution containing the following (in mM): 127 D-gluconic acid potassium salt, 10 HEPES, 1 EGTA, 10 KCl, 1 $MgCl_2$, 0.3 $CaCl_2$, 2 MgATP, and 0.5 NaGTP; pH was adjusted to 7.3-7.4 with KOH and osmolarity to 285-295 mOsm. A junction potential of 15 mV was corrected at the start of each recording and for

all reported resting membrane potentials (RMP). All experiments were performed in the presence of 5-HT_{1A} (WAY 100635, 100 nM) and 5-HT_{1B} (SB 216641, 200 nM) autoreceptor antagonists. During whole-cell current clamp experiments, no holding current was applied. Only neurons with a stable RMP that exhibited action potentials crossing 0 mV when depolarized by current step protocols were used for analysis. In current-clamp, 2s long depolarizing steps from –40 pA to +60 pA in 20 pA increments, with a 10s delay between steps, were used to evaluate firing patterns of DR 5-HT neurons. Firing frequency during current steps was determined using the total number of action potentials elicited over the current step. RMP was measured during the 100 ms before the current injection.

Putative serotonergic DR neurons were selected initially by their reversable inhibitory response to bath application of serotonin hydrochloride (10 μM; data not shown). Neurons with a capacitance exceeding 50 pF were confirmed as serotonergic and subsequently used for electrophysiological studies (Rios et al., 2025).

Experimental Design and Statistical Analysis

Data were analyzed using Prism 9 (GraphPad software, version 10.3; San Diego, CA). Each cell is considered an independent observation. Comparisons of baseline sEPSC frequencies, sEPSC amplitude, and PPR between lines were made using unpaired t-tests. In all summary bar graphs, each dot represents an individual cell while numbers within or above the bars represent the number of cells and mice (number of cells/total mice). In one instance where the numbers did not fit within the bar, they are displayed above the bar. Comparisons of sEPSC frequencies, sEPSC amplitude, and PPR pre- and post-MA were made using paired t-tests. Comparisons of firing frequencies in current clamp mode were made using repeated measures ANOVA. Studies were underpowered for analysis of sex differences. The level of significance for all statistical tests was set at ≤ 0.05.

Results

DR 5-HT neurons with differential TAAR1 function display distinct baseline sEPSC frequency but not sEPSC amplitude

To determine whether TAAR1 function influences spontaneous baseline glutamatergic activity onto DR 5-HT neurons, we compared the mean frequencies and amplitude of sEPSCs between MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$mice. MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons exhibited significantly greater mean baseline sEPSC frequency compared to DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ mice (Fig. 3.1A). We found no significant difference in average baseline sEPSC amplitude between the lines (Fig. 3.1B).

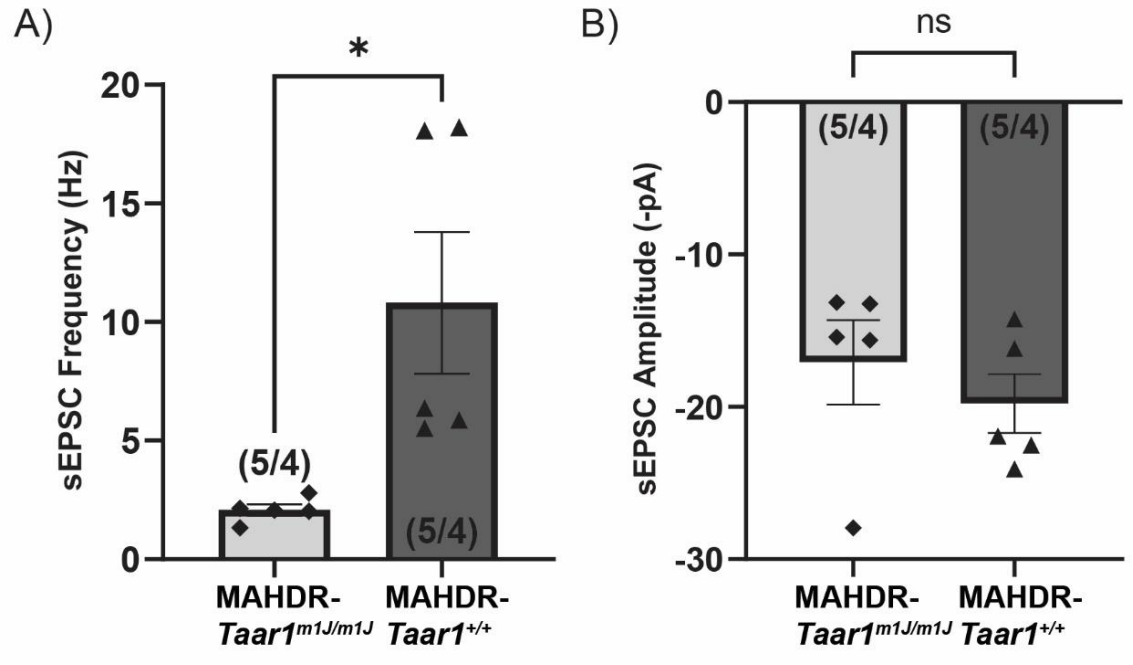

Figure 3.1. Average baseline sEPSC frequency, but not sEPSC amplitude, is greater in mice with functional TAAR1. All experiments were performed in the presence of the $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptor antagonists and the $GABA_AR$ antagonist gabazine (25 μM). Each symbol represents 1 recording. A: MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons exhibit lower mean baseline sEPSC frequencies compared to MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons (unpaired *t*-test; $t_{(8)} = 2.91$, $*p = 0.02$). B: There is no difference in average baseline sEPSC amplitudes between the lines (unpaired *t*-test; $t_{(8)} = 0.80$, $p = 0.44$). Means are ± SEM for data from both sexes; (cells/mice).

MA effects on sEPSC frequencies but not sEPSC amplitude are dependent on TAAR1 functionality

To determine whether MA modulates spontaneous glutamatergic activity onto DR 5-HT neurons, we compared the sEPSC frequencies and amplitudes pre- and post-MA for each line. MA significantly increased sEPSC frequencies in recordings from DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ mice (Fig. 3.2A) but had no effect on sEPSC amplitudes (Fig. 3.2B). Representative traces demonstrate the MA-induced increase in sEPSC frequencies in Fig. 3.2C. MA significantly decreased the sEPSC frequencies onto DR 5-HT neurons in slices from MAHDR-*Taar1*$^{+/+}$ mice (Fig. 3.2D) but had no effect on sEPSC amplitudes (Fig. 3.2E). Representative traces demonstrate the MA-induced decrease in sEPSC frequencies in Fig. 3.2F.

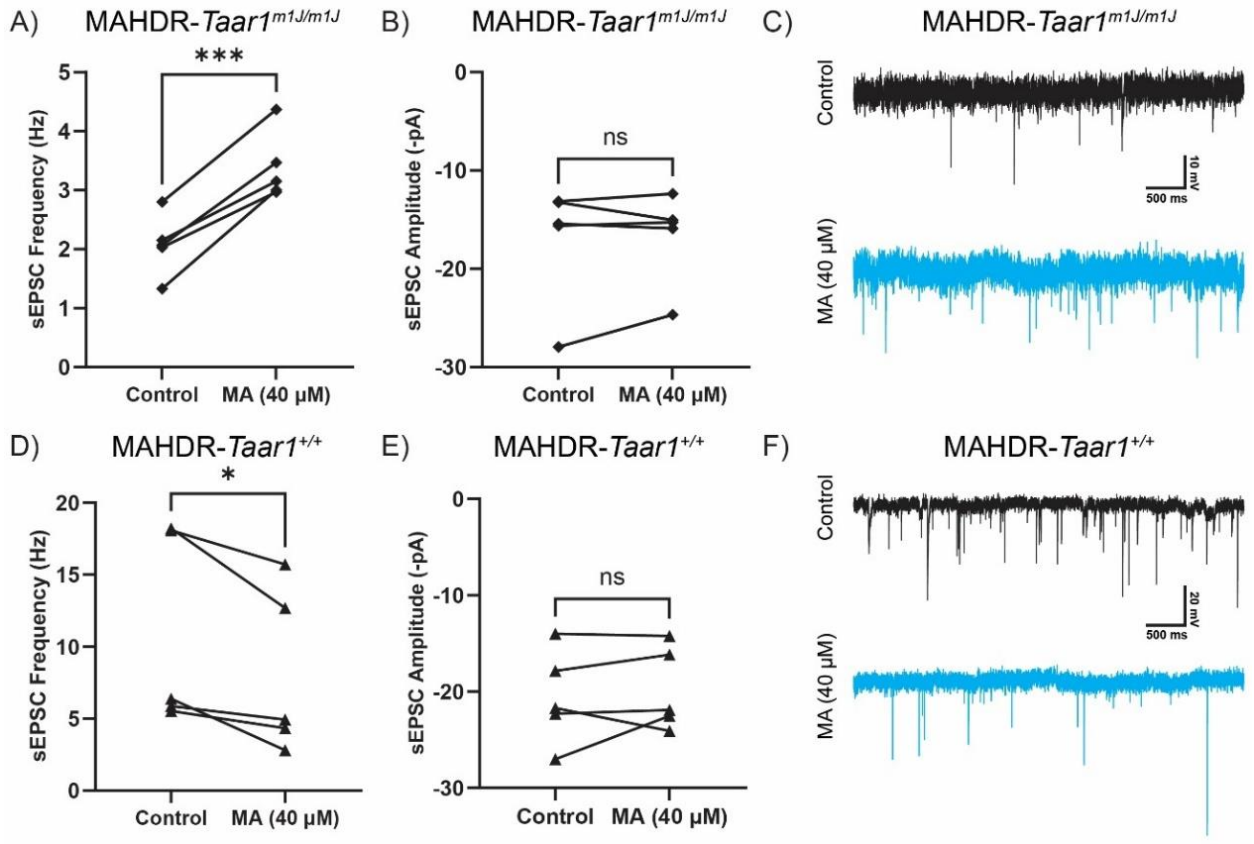

Figure 3.2. MA induces opposite effects on sEPSC frequencies but not sEPSC amplitudes in recordings from DR 5-HT neurons from mice with differential TAAR1 function. Control refers to sEPSC firing frequency and amplitude potential in the presence of $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptor antagonists and the $GABA_AR$ antagonist gabazine (25 µM). Each line represents 1 recording pre- and post-MA; data are not differentiated by sex. A: MA increases sEPSC frequencies of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons (5 cells/4 mice; paired *t*-test; $t_{(4)} = 8.89$, ***$p = <0.001$). B: MA had no effect on sEPSC amplitudes of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons (5 cells/4 mice) (paired *t*-test; $t_{(4)} = 0.518$, $p = 0.632$). C: Representative sEPSC traces from MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons in the absence (black) and presence (teal) of MA. D: MA decreases sEPSC frequencies of MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons (5 cells/4 mice; paired *t*-test; $t_{(4)} = 3.23$, **p* = 0.03). E: MA had no effect on sEPSC

amplitudes of MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons (5 cells/4 mice; paired *t*-test ; $t_{(4)} = 0.70$, $p = 0.52$). F: Representative sEPSC traces from MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons in the absence (black) and presence (teal) of MA.

Differential TAAR1 function drives MA-induced modulation of PPRs in recordings from DR 5-HT neurons despite similar baseline ratios

To determine whether TAAR1 function influences baseline evoked glutamatergic currents onto DR 5-HT neurons, we compared baseline PPR between MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$mice. No significant difference in mean baseline PPR was observed between DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$ (Fig. 3.3A).

Next, to evaluate whether TAAR1 function influences evoked glutamatergic activity onto DR 5-HT neurons following application of MA, we compared the PRR pre- and post-MA for each line. MA significantly increased mean PPR of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ mice (Fig. 3.3B), while having the opposite effect on average PPR of DR 5-HT neurons from MAHDR-*Taar1*$^{+/+}$ mice (Fig. 3.3C).

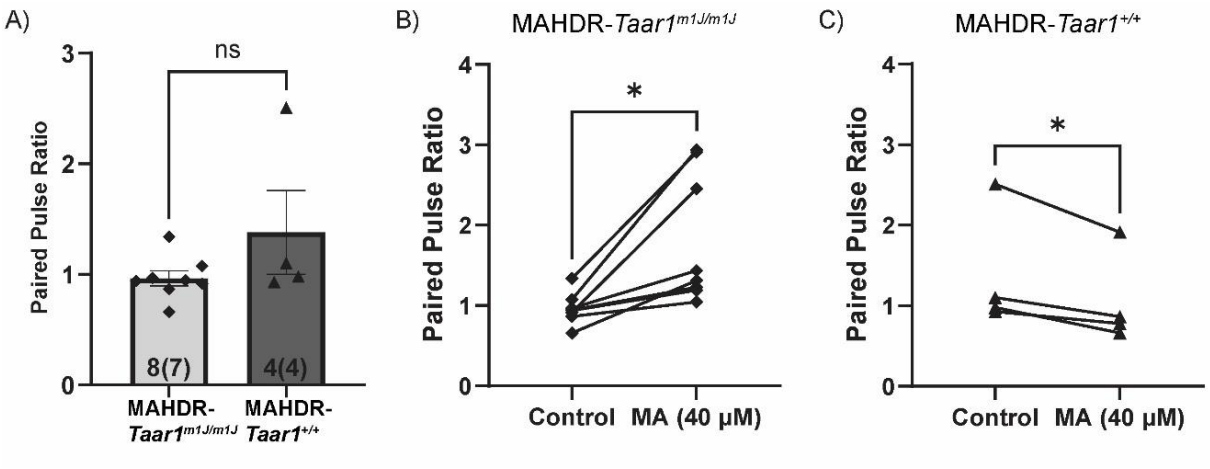

Figure 3.3. DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ and MAHDR-*Taar1*$^{+/+}$ mice show no differences in baseline PPR; however, MA-induces opposing effects on PPR in these lines. All experiments were performed in the presence of the $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptor antagonists and the $GABA_AR$ antagonist gabazine (25 µM). A: There is no difference in mean baseline PPR between the lines (unpaired *t*-test; $t_{(10)}$=1.53, p=0.157). (cells/mice) depicted within graph. Each symbol represents 1 cell. Means are ±SEM for data from both sexes. B: MA increases the average PPR of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons (paired *t*-tests; $t_{(7)}$=3.49, p=0.01). C: MA decreases the average PPR of MAHDR-*Taar1*$^{+/+}$ DR 5-HT neurons (paired *t*-tests; $t_{(4)}$=3.38, p=0.043). *p<0.05. Each line represents 1 cell pre- and post-MA.

MA-induced potentiation of DR 5-HT neuron activity in mice lacking functional TAAR1 is NMDA receptor dependent

The MA-mediated increase in sEPSC frequencies observed in DR 5-HT neurons lacking functional TAAR1 suggests that MA increases extracellular glutamate concentrations in these mice and can activate glutamate receptors on 5-HT neurons. My previous studies found that MA depolarized and potentiated the activity of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ mice, but this effect was not dependent on the activity of AMPA receptors [Dissertation Chapter 2; (Rios et al., 2025)]. Here, we evaluate whether MA stimulates the activity of NMDA glutamate receptors.

To determine whether the effects of MA are NMDA receptor-dependent, the NMDA receptor antagonist D-APV (50 μM) was included in the extracellular solution in addition to the 5-HT$_{1A}$ and 5-HT$_{1B}$ autoreceptor antagonists. With the addition of D-APV, MA had no effect on mean firing frequencies over a range of current injections for DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ mice (Fig. 3.4A) and no effect on the RMP (Fig. 3.4B). We observed a similar outcome for DR 5-HT neurons of MAHDR mice (Fig. 3.4C,D). These data indicate that NMDA receptor activation is necessary for the MA-induced potentiation observed in DR 5-HT neurons that lack TAAR1 function.

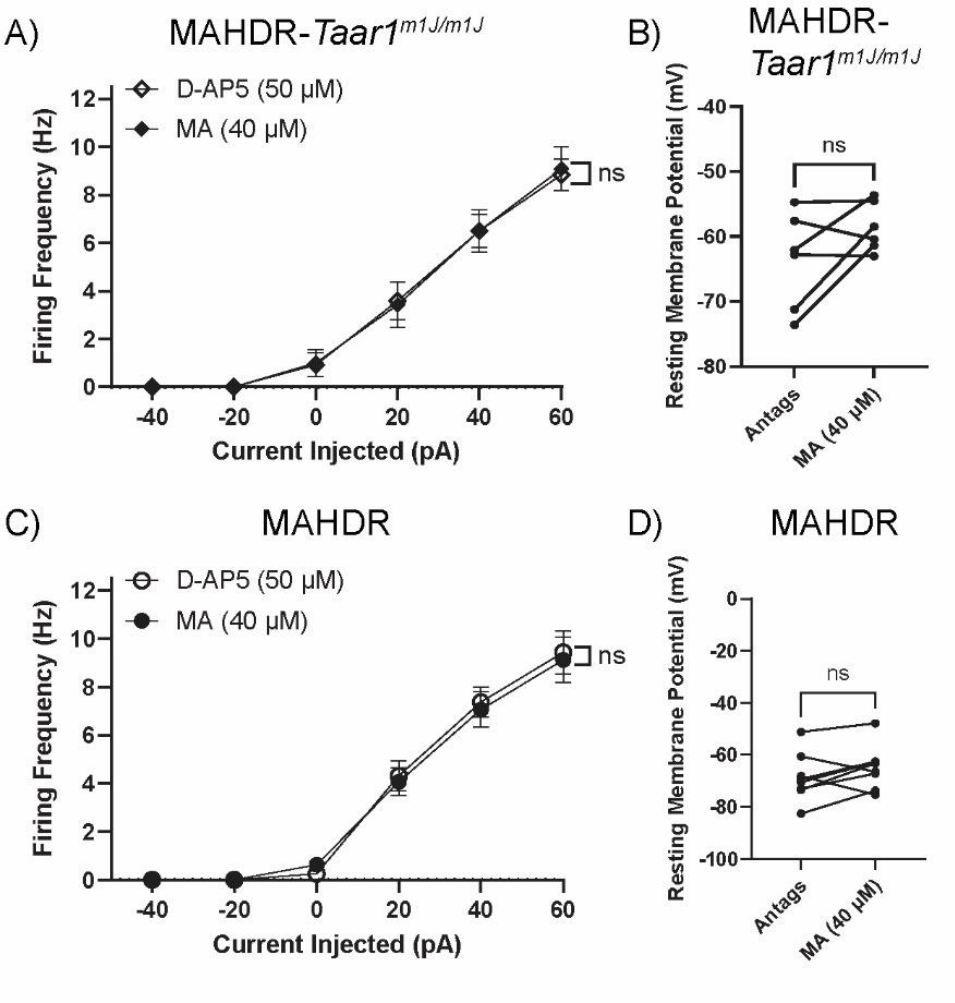

Figure 3.4. MA-induced potentiation of DR 5-HT neurons expressing non-functional TAAR1 is NMDA receptor dependent. Data shown are means ± SEM for data collapsed on sex. All experiments were performed in the presence of $5HT_{1A}$ (WAY 100635, 100 nM) and $5HT_{1B}$ (SB 216641, 200 nM) autoreceptor antagonists and NMDA receptor antagonist D-APV (50 µM). A: Mean firing frequencies of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons pre- and post-MA application (6 cells/6 mice); Repeated measures ANOVA, treatment: $F_{(1,5)}$=0.04, p=0.846]. B: RMP of MAHDR-*Taar1*$^{m1J/m1J}$ DR 5-HT neurons were not significantly changed by MA (paired *t*-test; $t_{(5)}$=1.81, p= 0.13). Each set of symbols represents a recording in the absence and presence of MA. C: Mean firing frequencies of MAHDR DR 5-HT neurons pre- and post-MA application (8

cells/8 mice) Repeated measures ANOVA, treatment: $F_{(1,7)}$=0.06, p=0.817]. D: RMP of MAHDR DR 5-HT neurons were not significantly changed by MA. (paired t-test; $t_{(7)}$=1.57, p= 0.161)). Each set of symbols represents a recording in the absence and presence of MA.

Discussion

Our preliminary findings suggest that TAAR1 function plays a critical role in modulating glutamatergic synaptic transmission onto DR 5-HT neurons at baseline and in response to MA. These findings underscore the need for further investigation to better understand the TAAR1-mediated mechanisms underlying these effects.

Previously, the laboratory found that MAHDR mice (genotype matched to MAHDR-*Taar1*$^{m1J/m1J}$ controls) display higher basal glutamate concentrations in the nucleus accumbens (NAc) and medial prefrontal cortex (mPFC) compared to MALDR (genotype matched to MAHDR-*Taar1*$^{+/+}$ KI) mice (Lominac et al., 2016; Szumlinski et al., 2017). These increases in basal glutamate levels do not appear to be the result of changes in glutamate transport at least in the NAc as there was no difference in glutamate clearance or reuptake (Szumlinski et al., 2017). In response to MA, MAHDR mice exhibit a significant increase in extracellular glutamate concentrations in the NAc, but a significant decrease in the mPFC relative to MALDR mice. However, in the MALDR mice, MA exhibited no significant change in extracellular glutamate in the NAc or mPFC (Lominac et al., 2016; Szumlinski et al., 2017). These discrepancies suggest that TAAR1 functionality may differentially regulate extracellular glutamate concentrations across brain regions at baseline and following MA, which in turn, would differentially regulate neuronal firing across brain regions. Therefore, we examined whether TAAR1 functionality alters glutamatergic synaptic activity onto DR 5-HT neurons pre- and post-MA administration.

In the first experiment, we examined basal glutamate synaptic activity in the different mouse lines. Spontaneous release of glutamate is a measure of excitatory synaptic currents (sEPSCs) elicited by neuronal activity in the slice. DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ controls displayed lower mean sEPSC frequencies compared to MAHDR-*Taar1*$^{+/+}$ KI mice. The observed differences in sEPSC frequencies suggest that TAAR1 functionality contributes to greater basal glutamate concentrations in the DR of MAHDR-*Taar1*$^{+/+}$ KI mice compared to

MAHDR-*Taar1*$^{m1J/m1J}$ control mice. Additionally, in DR 5-HT neurons from the MAHDR-*Taar1*$^{+/+}$ KI mice, we observed 2 distinct populations of sEPSC frequencies, one firing in the 5.5 - 6.5 Hz range and the other around ~18 Hz (Figure 3.1A). This may indicate potential DR subregion-specific heterogeneity in the glutamatergic synaptic input onto DR 5-HT neurons. More recordings are necessary to confirm the existence of two subpopulations of responses. We found no statistically significant difference in baseline sEPSC amplitude between the lines suggesting that TAAR1 functionality does not alter post-synaptic receptor properties under baseline conditions.

The finding that MAHDR-*Taar1*$^{+/+}$ KI mice exhibit greater sEPSC frequencies compared to MAHDR-*Taar1*$^{m1J/m1J}$ control mice was unexpected given the higher basal glutamate concentrations reported in other brain areas of MAHDR mice relative to MALDR mice. Further studies are necessary to understand how components of the glutamatergic synaptic activity (pre- and postsynaptic receptors, transporters, etc.) are altered between the two CRISPR lines.

We next examined the effect of MA on sEPSC measures in recordings of DR 5-HT neurons. In MAHDR-*Taar1*$^{m1J/m1J}$ control mice, MA increased sEPSC frequencies onto DR 5-HT neurons, suggesting a MA-induced increase in extracellular glutamate. This effect on sEPSC frequencies could be a MA-induced increase in presynaptic glutamate release or due to the MA-induced increase in 5-HT neuron excitability in the MAHDR-*Taar1*$^{m1J/m1J}$ control mice [Dissertation Chapter 2; (Rios et al., 2025)]. To discern between these two possible interpretations, these studies should also be performed with the inclusion of the sodium channel blocker tetrodotoxin (TTX) which inhibits voltage-gated Na^+ channels involved in action potentials. These experiments, referred to as miniature EPSCs (mEPSCs), would block MA-induced excitation of DR 5-HT neurons which are known to co-release glutamate and form autaptic connections (Johnson, 1994; Liu et al., 2014; Wang et al., 2019). Thus, mEPSCs would allow us to determine if MA increases the probability of presynaptic release in either line. If MA

increases mEPSC frequencies in either line, it suggests that there are additional effects of MA on presynaptic release. If there is no effect of MA on mEPSCs, then the increase in sEPSCs is due to release of glutamate from cells in the slice that are activated by MA, probably 5-HT neurons given our results in Chapter 2, (Rios et al., 2025). We found no statistically significant difference in sEPSC amplitude following MA suggesting that lack of TAAR1 function does not alter post-synaptic receptor properties.

In contrast to the MAHDR-*Taar1*$^{m1J/m1J}$ control, in MAHDR-*Taar1*$^{+/+}$ KI mice, MA decreased sEPSC frequencies onto DR 5-HT neurons. Interestingly, despite the putative two populations of sEPSC frequencies in the MAHDR-*Taar1*$^{+/+}$ KI mice, both cells with low and high sEPSC frequencies showed a decrease in sEPSC frequencies following MA, which may indicate a shared mechanism in response to MA. This effect may be the result of indirect activation of GABAergic neurons in the slice. Again, these experiments would be easier to interpret if they were performed in the presence of TTX. We also found no statistically significant difference in sEPSC amplitude following MA suggesting that TAAR1 function does not alter post-synaptic receptor properties.

We also preliminarily examined whether TAAR1 influences evoked AMPA-mediated synaptic currents. We used a paired pulse ratio (PPR) protocol to examine the short-term plasticity of glutamate synaptic transmission between the lines. We observed no difference in baseline PPR between the lines. This would suggest that despite the potential influence of TAAR1 function on basal glutamate concentrations between the lines within the DR, at baseline, TAAR1 function does not appear to influence the presynaptic release probability or the short-term plasticity of glutamatergic synapses onto DR 5-HT neurons.

When we examined the effect of MA, we found that MA significantly increased the PPR of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control mice, while having the opposite effect in DR 5-HT neurons from MAHDR-*Taar1*$^{+/+}$ KI mice. This would suggest that MA modulates the

glutamate release probability in a TAAR1 function-dependent manner. However, there are 2 caveats, 1) if DR 5-HT neurons are co-releasing glutamate, it makes it challenging to interpret whether the PPR is measuring a pre- or post-synaptic effect and 2) without antagonists for AMPA receptor desensitization either differences in baseline glutamate or changes in glutamate in response to MA could be masking any effects mediated by TAAR1 functionality or MA. Specifically, in the MAHDR-*Taar1*$^{m1J/m1J}$ control mice, MA likely induces a significant release of glutamate which in turn facilitates synaptic transmission. In contrast, in the MAHDR-*Taar1*$^{+/+}$ KI mice, where basal glutamate concentrations appear to already be elevated, MA may induce AMPA receptor desensitization, thereby depressing the synaptic response. Supporting this, studies from VTA DA neurons with functional TAAR1 found that MA induced an increase in AMPA-mediated evoked post-synaptic currents. However, these studies utilized AMPA receptor desensitization inhibitors to mitigate maximal activation by synaptic glutamate (Underhill et al., 2014). Therefore, there are several factors which warrant caution when interpreting these findings and more detailed studies are needed before any conclusions can be made.

We also explored whether MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR mice was mediated by NMDA receptors, given the potential line differences in extracellular glutamate following bath application of MA. We have previously determined that the effects of MA in mice lacking functional TAAR1 is not mediated by AMPA receptors [Dissertation Chapter 2; (Rios et al., 2025)]. Here, we found that NMDA receptor antagonism blocked both MA-induced depolarization and potentiation of DR 5-HT neurons, highlighting the critical role of NMDA receptors in mediating the depolarization necessary for MA-induced excitation. Similarly, blocking the serotonin transporter (SERT) with fluoxetine also blocked both MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR mice [Dissertation Chapter 2; (Rios et al., 2025)]. Based on these findings, we

hypothesize, as shown in Figure 3.4 below, that MA induces a SERT-mediated uncoupled current, depolarizing the cell sufficiently to remove the magnesium block from NMDA receptors. This would permit NMDA receptor activation, permitting inward calcium and sodium currents, further depolarizing DR 5-HT neurons, and increasing excitability of cells with non-functional TAAR1. If either of these pathways are disrupted, then the cell will not depolarize, and MA-induced excitation cannot occur.

In summary, we observed line differences in baseline sEPSC measures but not PPR. The mean sEPSC frequencies onto DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control mice was significantly lower compared to MAHDR-*Taar1*$^{+/+}$ KI mice at baseline. This supports a critical link between TAAR1 functionality and glutamatergic synaptic transmission. Following administration of MA, both sEPSC and PPR demonstrated opposite effects between these mouse lines. sEPSC frequencies and PPR both increased for DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice and decreased in those of MAHDR-*Taar1*$^{+/+}$ KI mice. These effects could potentially be due to differences in extracellular glutamate concentrations, receptor expression, transporter expression, protein expression, and even receptor desensitization. Finally, we found that MA-induced depolarization and potentiation of DR 5-HT neurons from mice with non-functional TAAR1 (MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice) is dependent on NMDA receptors. Further investigation is needed to better understand the complexity of the role of TAAR1 function in modulating glutamatergic synaptic plasticity onto DR 5-HT neurons pre- and post-MA. Proposed experiments will be discussed in greater detail in Chapter 4.

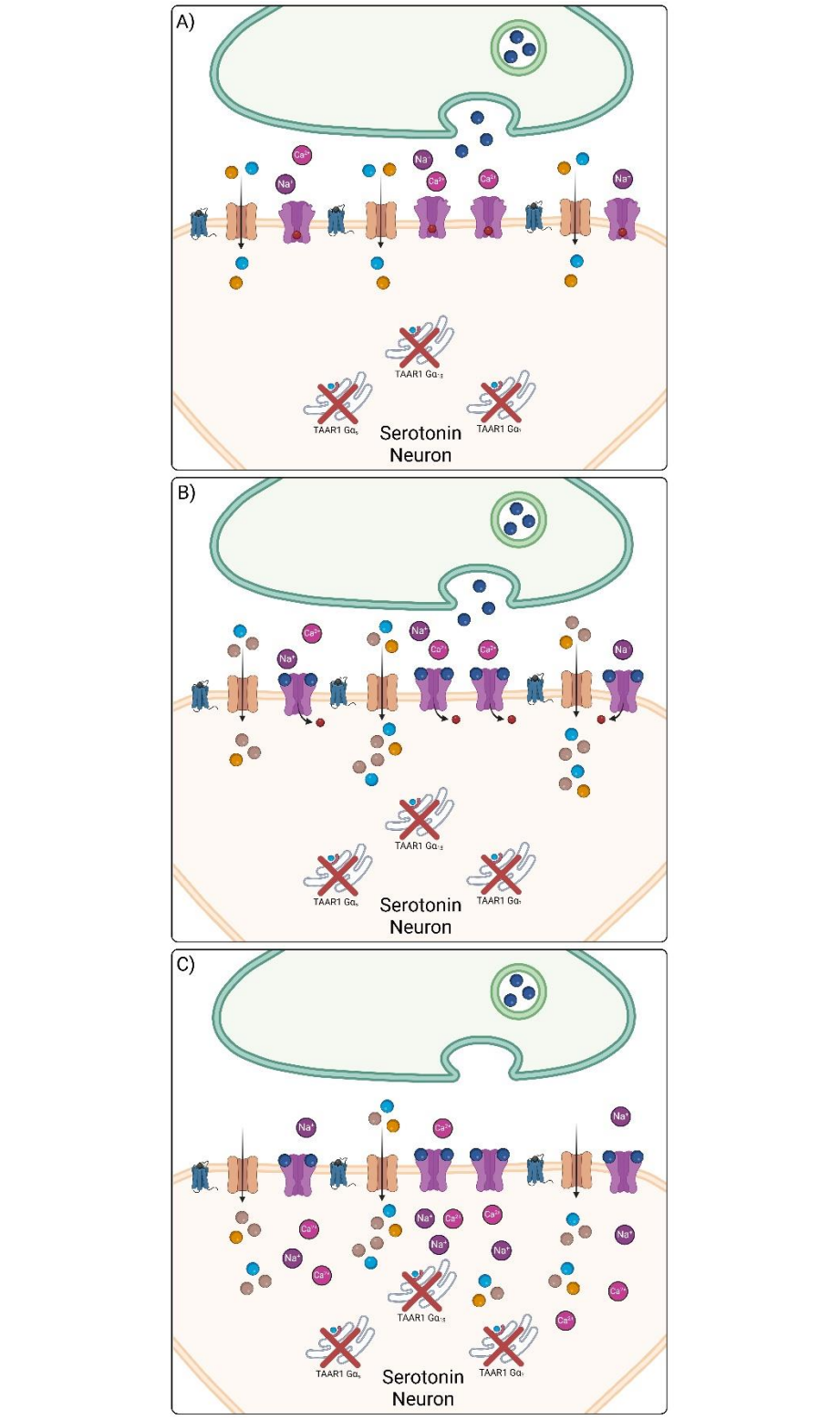
A)
TAAR1 Gα-s
TAAR1 Gαs
TAAR1 Gαi
Serotonin Neuron
B)
TAAR1 Gα-s
TAAR1 Gαs
TAAR1 Gαi
Serotonin Neuron
C)
TAAR1 Gα-s
TAAR1 Gαs
TAAR1 Gαi
Serotonin Neuron
NMDA receptor
5-HT1A-B with antagonist
Serotonin
Calcium
Serotonin transporter
Methamphetamine
Ions
Glutamate
Sodium
Magnesium

Figure 3.5. Proposed mechanism of MA-induced depolarization of DR 5-HT neurons from MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice. A) MA and 5-HT enter DR 5-HT neurons through SERT. However, because TAAR1 is non-functional, MA does not activate the $G\alpha_{13}$- or $G\alpha_s$-coupled TAAR1 signaling pathways, allowing for SERT to remain expressed on the membrane. B) With SERT maintained on the membrane, MA potentiates an unidentified uncoupled ionic current through the transporter. The uncoupled ionic current depolarizes the neuron sufficiently to remove the magnesium block from NMDA receptors on the post-synaptic membrane. C) The removal of the magnesium block enables NMDA receptor-mediated calcium and sodium currents into the post-synaptic cell resulting in an increase in cell excitability. *Created in BioRender.*

Chapter 4: General Discussion

Goals and Main Findings

Selective breeding for differential voluntary MA intake incidentally resulted in differential sensitivity to the rewarding and aversive effects of MA. QTL analysis revealed a region on mouse chromosome 10 that explained 60% of the genetic variance in voluntary MA intake between the MADR mouse lines (Belknap et al., 2013). The *Taar1* gene, which encodes the TAAR1 receptor at which MA acts as an agonist (Bunzow et al., 2001), was identified as a major contributor. *Taar1* genotype corresponds with both MA intake and MA-induced reward and aversion sensitivities. High sensitivity to the rewarding properties of MA has been consistently found in the MAHDR mice. Conversely, low sensitivity to the rewarding properties of MA has been consistently found in the MALDR mice (Harkness et al., 2015; Reed et al., 2018; Shabani, Dobbs, et al., 2012; Shabani et al., 2016; Shabani, McKinnon, et al., 2012; Shabani et al., 2011). Using the CRISPR-Cas9-derived MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines that differ only in their *Taar1* genotype and resulting TAAR1 functionality, the laboratory was able to confirm that voluntary MA intake and sensitivity to its rewarding or aversive effects are causally linked to TAAR1 function (Phillips et al., 2021; Stafford et al., 2019).

The goal of this dissertation was to investigate whether TAAR1 functionality mediates the intrinsic and extrinsic effects of DR 5-HT neurons in the absence and presence of MA. I chose to study DR 5-HT neurons for their role in reward processing which is understudied in the context of drug reward compared to the known actions of midbrain DA neurons. Using the MADR and CRISPR-*Cas9*-generated MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines, I characterized how MA differentially affects DR 5-HT neuron excitability and glutamatergic synaptic inputs onto these neurons in mice with functional and non-functional TAAR1. My findings are summarized in Table 4.1.

In Chapter 2, I report results from whole-cell patch-clamp electrophysiology experiments and a binge-level MA consumption study. I found that MA has different effects on 5-HT neuron RMP and excitability in the absence and presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists depending on TAAR1 function. In response to MA, DR 5-HT neurons of MALDR mice significantly hyperpolarized and exhibited an inhibition of firing while those of MAHDR mice exhibited no change in RMP or activity. However, in the presence of the 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists, MA-induced hyperpolarization and inhibition of MALDR DR 5-HT neurons were blocked, whereas MA significantly depolarized and potentiated the activity of DR 5-HT neurons of MAHDR mice. Using the CRISPR-Cas9-generated MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines, I confirmed that the observed MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR mice in the presence of these antagonists was dependent on the absence of TAAR1 function, as KI of functional TAAR1 on the MAHDR background blocked both these effects. Additionally, the MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ controls was dependent on SERT, as both effects of MA were blocked by the SERT antagonist fluoxetine. Regarding binge-level MA consumption, in Chapter 2 I report that KI of functional TAAR1 on the MAHDR background converts binge-level MA intake into low MA intake.

In Chapter 3, I report whole-cell patch clamp electrophysiology recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines. I found that baseline sEPSC frequencies but not amplitudes were significantly different between the lines; recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice displayed significantly higher sEPSC frequencies compared to recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice. In response to MA, sEPSC frequencies recorded from DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice increased, whereas recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice decreased. sEPSC amplitude was unaffected by MA in either line. I

also demonstrate that the MA-induced depolarization and potentiation of DR 5-HT neurons from mice lacking functional TAAR1 (MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR mice), is dependent on NMDA receptors. Additionally, I began preliminary investigations into the role of TAAR1 function on evoked currents. I found no difference in baseline PPR between the lines. However, I did find that MA significantly altered the PPR ratio in a TAAR1-dependent manner. The PPR of DR 5-HT neurons from MAHDR-*Taar1*$^{m1J/m1J}$ control mice significantly increased in response to MA but decreased in neurons from MAHDR-*Taar1*$^{+/+}$ KI mice.

Table 4.1: Summary of Results Presented

Chapter	Model	Method	Experiment	Result
2	MADR mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of MA alone	No effect of MA on MAHDR DR-5-HT neuron activity; MA-induced inhibition of MALDR DR 5-HT neuron activity
2	MADR mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of MA alone	No effect of MA on RMP of MAHDR DR 5-HT neurons; MA-induced hyperpolarization of MALDR DR 5-HT neurons
2	MADR mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of MA in the presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists	MA-induced excitation of MAHDR DR 5-HT neuron activity; MA-induced inhibition of MALDR DR 5-HT neuron activity was blocked

Chapter	Model	Method	Experiment	Result
2	MADR mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of MA in the presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists	MA-induced depolarization of MAHDR DR 5-HT neurons; No effect of MA on RMP of MALDR DR 5-HT neurons
2	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Voluntary MA Consumption	Escalating MA concentrations to test binge-level MA consumption	MAHDR-*Taar1*$^{m1J/m1J}$ control > MAHDR-*Taar1*$^{+/+}$ KI Only MAHDR-*Taar1*$^{m1J/m1J}$ control mice exhibited binge-level MA intake
2	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Total Fluid Consumption	Escalating MA concentrations to test binge-level MA consumption	MAHDR-*Taar1*$^{m1J/m1J}$ control > MAHDR-*Taar1*$^{+/+}$ KI at each concentration between 80 and 140 mg/L

Chapter	Model	Method	Experiment	Result
2	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of MA in the presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists	MA-induced excitation of MAHDR-*Taar1*$^{m1J/m1J}$ control DR 5-HT neuron activity; No effect of MA on MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neuron activity
2	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of MA in the presence of 5-HT_{1A} and 5-HT_{1B} autoreceptor antagonists	MA-induced depolarization of MAHDR-*Taar1*$^{m1J/m1J}$ control DR 5-HT neurons; MA had no effect on RMP of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons
2	MAHDR mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of AMPA and GABA Receptor Antagonists, NBQX and Bicuculline	MA-induced potentiation of DR 5-HT neuron activity persisted in the presence of NBQX and bicuculline

Chapter	Model	Method	Experiment	Result
2	MAHDR mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of AMPA and GABA Receptor Antagonists, NBQX and Bicuculline	MA-induced depolarization of DR 5-HT neurons persisted in the presence of NBQX and bicuculline
2	MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of SERT Antagonist Fluoxetine	MA-induced excitability of DR 5-HT neurons was blocked by fluoxetine
2	MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of SERT Antagonist Fluoxetine	MA-induced depolarization of DR 5-HT neurons was blocked by fluoxetine
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	Baseline sEPSC Frequencies	MAHDR-*Taar1*$^{m1J/m1J}$ control < MAHDR-*Taar1*$^{+/+}$ KI
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	Baseline sEPSC Amplitudes	MAHDR-*Taar1*$^{m1J/m1J}$ control = MAHDR-*Taar1*$^{+/+}$ KI

Chapter	Model	Method	Experiment	Result
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	MA-Induced Changes in sEPSC Frequencies	MA increased MAHDR-*Taar1*$^{m1J/m1J}$ control sEPSC frequencies; MA had no effect on MAHDR-*Taar1*$^{+/+}$ KI sEPSC frequencies.
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	MA-Induced Changes in sEPSC Amplitudes	MA had no effect on sEPSC amplitude in either line
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	Baseline PPR	No line difference in baseline PPR

Chapter	Model	Method	Experiment	Result
3	MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; Glutamatergic Synaptic Transmission	MA-Induced Changes in PPR	MA increased the PPR of MAHDR-*Taar1*$^{m1J/m1J}$ control DR 5-HT neurons; MA had no effect on PPR of MAHDR-*Taar1*$^{+/+}$ KI DR 5-HT neurons
3	MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron Excitability	Effect of NMDA receptor antagonist D-APV	MA-induced excitability of DR 5-HT neuron activity was blocked by D-APV
3	MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice	Electrophysiology; DR 5-HT Neuron RMP	Effect of NMDA receptor antagonist D-APV	MA-induced depolarization was blocked by D-APV

5-HT: serotonin; DR: dorsal raphe; KI: knock-in; MA: methamphetamine; MADR: methamphetamine drinking mice; PPR: paired pulse ratio; RMP: resting membrane potential; sEPSC: spontaneous excitatory post synaptic current; SERT: serotonin transporter; *Taar1*: gene encoding trace amine-associated receptor 1; TAAR1, trace amine-associated receptor 1

Intrinsic Activity of Dorsal Raphe Serotonin Neurons in Response to Methamphetamine is Dependent on TAAR1 Function

The SNP rs33645709 encodes a non-synonymous proline to threonine mutation at amino-acid position 77 (P77T) in DBA/2J but not C57BL/6J mice (Harkness et al., 2015), which occurs near a predicted binding pocket stabilizing residue (V75) in the second transmembrane loop of TAAR1 (Reese et al., 2014). The proline-to-threonine mutation is significant as proline is necessary for proper receptor function (Mazna et al., 2008; Reiersen & Rees, 2001; Sansom & Weinstein, 2000; T. W. Schwartz et al., 2006; Van Arnam et al., 2011). This raised the question of whether the *Taar1*^{m1J} variant results in a nonfunctional TAAR1 receptor, which the laboratory confirmed. In HEK293 cells transfected with the DBA/2J-like *Taar1*^{m1J} isoform, TAAR1-mediated stimulation of cAMP by MA did not occur. In contrast, cells transfected with the C57BL/6J- like *Taar1*$^{+}$ isoform produced a significant cAMP response following application of MA (Harkness et al., 2015). Unpublished data from the laboratory suggests that the DBA/2J-like *Taar1*^{m1J} isoform, results in a TAAR1 receptor with a drastically reduced capacity to bind TAAR1 agonists compared to the C57BL/6J- like *Taar1*$^{+}$ isoform. This is consistent with the predicted functional importance of proline near the binding pocket. Therefore, in the context of my studies, MA is most likely unable to bind with sufficient affinity for TAAR1 in MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice to exert effects, including activating a cAMP response.

In Chapter 2, I demonstrate that MA hyperpolarized and inhibited the activity of DR 5-HT neurons from MALDR mice expressing functional TAAR1. This finding aligns with previous studies showing that the activity of DR 5-HT neurons is inhibited by the exogenous TAAR1 agonist RO5166017 in cells with functional TAAR1 (Revel et al., 2012; Revel et al., 2011). Similarly, the psychostimulant MDMA has been shown to inhibit the activity of DR 5-HT neurons (Sprouse et al., 1989); however, whether the effect of MDMA is dependent on TAAR1 was not investigated. In addition, I demonstrate that MA had no effect on the RMP or activity of

DR 5-HT neurons from MAHDR mice expressing non-functional TAAR1 in the absence of 5-HT autoreceptor antagonists, also consistent with previous observations in DR 5-HT neurons where TAAR1 was knocked out (Revel et al., 2011). Therefore, in Chapter 2 I begin to provide direct evidence MA modulates DR 5-HT neuron activity through TAAR1-dependent mechanisms.

As mentioned in Chapter 1, TAAR1 activation results in increased extracellular monoamine levels, suggesting that the decrease in neural activity in MALDR mice may be due to inhibitory autoreceptor activation. Although some evidence supports this hypothesis (Bradaia et al., 2009; Revel et al., 2011; Sprouse & Aghajanian, 1987; Xie et al., 2008), earlier studies focused on TAAR1 did not use inhibitors of 5-HT autoreceptors (Revel et al., 2012; Revel et al., 2011). In Chapter 2 I demonstrate that the MA-induced hyperpolarization and inhibition of MALDR DR 5-HT neurons is dependent on the activation of 5-HT_{1A} and 5-HT_{1B} inhibitory autoreceptors. This is consistent with previous findings that demonstrated 2 mg/kg MA released a significantly higher amount of 5-HT in the mPFC of MALDR mice compared to MAHDR mice (Lominac et al., 2014). This suggests that the MA-induced increase in extracellular 5-HT levels following TAAR1 activation allows for 5-HT to activate these inhibitory autoreceptors sufficiently to hyperpolarize DR 5-HT neurons and suppress their firing. In contrast, MA depolarized and potentiated DR 5-HT neurons of MAHDR mice in the presence of autoreceptor antagonists. This would suggest that either basal or MA-induced changes in extracellular 5-HT concentrations are sufficient to prevent any changes in DR 5-HT neuron RMP and firing in response to MA in MAHDR mice. The addition of autoreceptor antagonists appears to unmask a depolarizing, MA-dependent current specifically in mice with non-functional TAAR1. These findings provide direct evidence that TAAR1-mediated effects of MA on DR 5-HT neuron RMP and activity are influenced by activation of 5-HT_{1A} and 5-HT_{1B} inhibitory autoreceptors. These data underscore the complex interplay between TAAR1 functionality and autoreceptor regulation in shaping monoaminergic neuron activity.

My research in the MADR lines demonstrates a potential relationship between TAAR1 functionality and DR 5-HT neuron RMP/activity in response to MA. To establish a causal link, I repeated the experiments using the autoreceptor antagonists in CRISPR-*Cas9*-generated MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control lines. The depolarization and potentiation of DR 5-HT neuron activity persisted in response to MA in mice lacking functional TAAR1, the MAHDR-*Taar1*$^{m1J/m1J}$ controls. Notably, KI of functional TAAR1 on the MAHDR background abolished MA-induced depolarization and potentiation of DR 5-HT neurons, indicating that the absence of TAAR1 function is crucial for their excitatory response to MA. These results suggest that TAAR1 plays a regulatory role on the RMP and activity of DR 5-HT neurons in the presence of agonists. In the absence of functional TAAR1, MA appears to engage an alternative signaling pathway(s) that leads to an increase in neuron excitability. Alternatively, the presence of functional TAAR1 may more effectively inhibit these alternative pathways.

Given the potential for MA to engage alternative signaling pathways, we next explored whether one of these pathways might involve SERT. Previous research has shown that MDMA triggers internalization of SERT in DR 5-HT neurons expressing functional TAAR1, an effect that is absent in TAAR1 KO cells (Underhill & Amara, 2020), suggesting an interaction between SERT and TAAR1. Therefore, we hypothesized that the effect of MA in mice expressing non-functional TAAR1 (MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control lines), is driven by SERT remaining on the membrane. In Chapter 2, I report that the SERT antagonist fluoxetine blocks MA-induced depolarization and potentiation of DR 5-HT neurons in these mice. There are several ways that SERT could be mediating these effects. First, as a substrate of SERT, MA may continue to enter the cell as long as SERT remains active on the membrane, allowing MA to affect the activity of ion channels or some other intracellular target aside from TAAR1. A second possibility is that MA stimulates SERT-dependent currents similar to how AMPHs stimulate DAT-dependent currents that are uncoupled from electrogenic transport and increase excitability of DA neurons

(Branch & Beckstead, 2012; Ingram et al., 2002). Further studies to explore the role of SERT in mediating MA-induced depolarization and potentiation, with detailed mechanisms and implications, are described in the future directions section later in this chapter.

Glutamatergic Synaptic Transmission onto Dorsal Raphe Serotonin Neurons is Dependent on TAAR1 Function

Glutamatergic transmission and related machinery such as receptors, transporters, and proteins are proposed to play a role in the initiation and expression of addiction related behaviors (Gass & Olive, 2008; Kalivas et al., 2009; Lominac et al., 2016; Szumlinski et al., 2017; Tzschentke & Schmidt, 2003). Considering the pronounced differences in MA intake and MA reward/aversion sensitivities between the MADR mice, the laboratory has prioritized investigation of glutamatergic systems in these mice.

It is important to note previous studies examining glutamatergic systems were performed using the MADR mice, whereas the work I performed utilized the CRISPR*Cas9*-generated MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR-*Taar1*$^{+/+}$ KI mice. For clarity, both MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice are homozygous for the *Taar1*$^{m1J/m1J}$ allele, which encodes non-functional TAAR1 receptors, whereas MALDR and MAHDR-*Taar1*$^{+/+}$ KI mice are homozygous or heterozygous for the reference *Taar1*$^{+}$ allele, which encodes functional TAAR1 receptors (Harkness et al., 2015; Shi et al., 2016). In the following section, I relate my findings to those previously published by the laboratory, however, there is unpublished data from the laboratory which contradicts the findings reported in Szumlinski et al., 2017. While Szumlinski et al. 2017 reported higher levels of the metabotropic glutamate receptor 5 and Homer2 and lower levels of EAAT3 in the NAc, these findings were not corroborated in a separate replicate of MADR mice (Mootz & Phillips, *unpublished*). In the following studies, I report results from the

CRISPR-*Cas9*-generated mice which will help rule out any unrelated effects of selective breeding between the replicates and emphasize TAAR1 specific effects on glutamatergic mechanisms.

In Chapter 3, I report a TAAR1-mediated baseline difference in sEPSC frequencies onto DR 5-HT neurons between lines. Recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ controls exhibited significantly lower baseline sEPSC frequencies compared to recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice. This finding would suggest that MAHDR-*Taar1*$^{+/+}$ KI mice possess greater basal extracellular glutamate concentrations in the DR compared to MAHDR-*Taar1*$^{m1J/m1J}$ control mice. A difference in baseline extracellular glutamate concentrations and sEPSC may relate to differential genetic risk for MA intake and reward/aversion sensitivity. However, this was an unexpected finding given previous reports of higher basal glutamate concentrations in the NAc and mPFC of MAHDR mice compared to MALDR mice (Lominac et al., 2016; Szumlinski et al., 2017). This discrepancy may be due to regional differences in expression of glutamate receptors, transporters, and related proteins, which have already been observed between the NAc and mPFC (Lominac et al., 2016; Szumlinski et al., 2017). If TAAR1 functionality regulates differences in expression of glutamate related machinery, baseline reuptake, clearance, and/or release of glutamate could be altered within the DR, especially if there are region-specific differences in TAAR1 expression. There may also be cell-type specific interactions, such as distinct interactions between glutamate and DA vs glutamate and 5-HT. For example, in the PFC, 5-HT release was significantly higher in MALDR mice compared to MAHDR mice while the opposite effect was found for DA release (Lominac et al., 2016). Additionally, I report no difference in sEPSC amplitude between the lines. This would suggest that at baseline, there is no difference in post-synaptic response to neurotransmitter release, therefore, receptor density or receptor sensitivity are likely not different between lines.

In response to MA, I report a significant increase in sEPSC frequencies in recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice. The simplest interpretation is that

MA is increasing pre-synaptic glutamate release in the DR of MAHDR-*Taar1*$^{m1J/m1J}$ control mice. However, this effect could be driven by MA-induced increase in excitability exhibited by DR 5-HT neurons [Dissertation Chapter 2; (Rios et al., 2025)]. Therefore, increases in glutamate could be coming from glutamatergic projections onto DR 5-HT neurons and/or local glutamate release from 5-HT neurons which have been shown to co-release glutamate and form autaptic connections ((Johnson, 1994; Liu et al., 2014; Wang et al., 2019) see Figure 4.1 below.) I report no effect of MA on sEPSC amplitude which would indicate lack of functional TAAR1 does not influence the effect of MA on post-synaptic response to neurotransmitter release, receptor density or receptor sensitivity in either line. These studies will need to be done in the presence of TTX to block action potential dependent glutamate release to distinguish between these two interpretations.

On the other hand, sEPSC frequencies decreased in recordings from DR 5-HT neurons of MAHDR-*Taar1*$^{+/+}$ KI mice. My first assumption was that MA decreased extracellular glutamate concentrations in the DR. However, this seems less likely because I observed no change in sEPSC amplitude, which would also suggest functional TAAR1 does not influence the post-synaptic response to neurotransmitter release, receptor density or receptor sensitivity in either line. This also did not fit what I had expected, which was an increase in extracellular glutamate concentrations because functional TAAR1 promotes the internalization of EAAT3 in DR 5-HT neurons (Underhill & Amara, 2020), which has been shown to significantly reduce glutamate uptake in midbrain DA neurons (Underhill et al., 2019; Underhill et al., 2014). One potential explanation for the decrease in sEPSC frequencies despite a possible increase in extracellular glutamate is the involvement of group II and III metabotropic glutamate receptors (mGluRs). Activation of group II and III mGluRs inhibits cAMP formation via G_i/G_o-protein signaling, thereby reducing pre-synaptic glutamate release (Cartmell & Schoepp, 2000; Conn & Pin, 1997; Pin & Duvoisin, 1995; Sugiyama et al., 1987). Activation of mGluRs has also been observed to

inhibit pre-synaptic voltage-dependent calcium channels contributing to the inhibition of EPSPs and induce changes in intracellular calcium (Glaum & Miller, 1995; Glaum et al., 1993; Maiese et al., 1999; Sahara & Westbrook, 1993; Sayer et al., 1992; Stefani et al., 1994; Swartz & Bean, 1992). A second potential explanation for the decrease in sEPSC frequencies despite a possible increase in extracellular glutamate is the activation of lateral habenula (LHb) glutamatergic projections onto GABAergic neurons contained within the slice (Ren et al., 2018; Zhou et al., 2017). The LHb is a significant contributor to the processing of aversive stimuli with previous reports demonstrating that activity of LHb neurons significantly increased in response to cues predicting aversive stimuli and the receipt of unpredicted aversive stimuli (Matsumoto & Hikosaka, 2007; Wang et al., 2017). Notably, stimulation of LHb neurons inhibits DR 5-HT neuron activity which can be blocked by the $GABA_A$ antagonist picrotoxin and bicuculline suggesting the LHb is also activating local GABAergic neurons (Ferraro et al., 1996; Wang & Aghajanian, 1977). In our laboratory, we previously examined the effect of acute MA exposure (2 mg/kg) on LHb activation. I found that the MA-aversion sensitive MALDR mice exhibited a significant MA-induced increase in cFos positive cells within the LHb compared to saline controls. MALDR mice demonstrated 1.5 times more cFos positive cells in the LHb compared to MA-reward sensitive MAHDR mice, with no difference between the lines after saline treatment (*unpublished)*. Therefore, in mice sensitive to MA-induced aversion, MA-induced activation of LHb glutamatergic terminals in the slice could be increasing extracellular glutamate, which, rather than acting on 5-HT neurons, activates local GABAergic inputs onto DR 5-HT neurons, resulting in decreased sEPSC frequencies.

Finally, I report that the MA-induced depolarization and potentiation observed in DR 5-HT neurons from mice lacking functional TAAR1 is mediated by activation of NMDA receptors. Given the potential for MA-induced increases in glutamate concentrations in the DR, similar to the NAc, and the observed MA-induced increase in sEPSC frequencies, I initially hypothesized

that the MA-induced depolarization and potentiation of DR 5-HT neurons was mediated by AMPA receptor activation. However, as reported in Chapter 2, this was not the case. Despite this, I evaluated whether the effect was instead mediated by NMDA receptor activation because the RMP of DR 5-HT neurons still depolarized in the presence of AMPA receptor antagonists. I originally hypothesized that perhaps DR 5-HT neurons possessed NMDA receptors with GluN3 subunits which are characterized as voltage independent (Zhu et al., 2020). Therefore, I tested the GluN3 antagonist, TK30, however, I could not complete these experiments because TK-30 itself reduced cell excitability and no stable baseline in firing frequencies could be established. Upon further investigation, I noticed that the MA-induced depolarization observed in DR 5-HT neurons from mice lacking functional TAAR1 was absent when antagonizing SERT. Therefore, my current hypothesis is that MA induces a SERT-mediated uncoupled current that depolarizes the neuron sufficiently to remove the magnesium block from NMDA receptors. This would permit inward calcium and sodium currents, further depolarization, and increase cell excitability of DR 5-HT neurons from mice lacking functional TAAR1. Experiments to tease apart this hypothesis are described below under Future Directions.

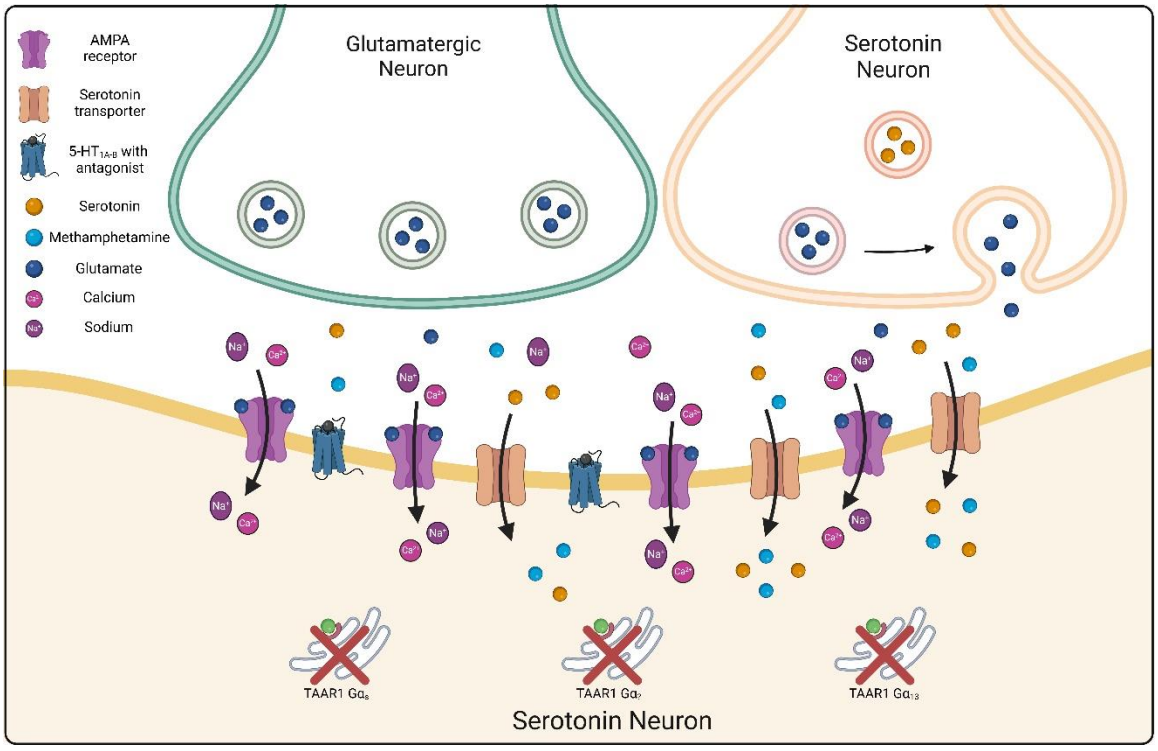

Figure 4.1. Proposed mechanism for the observed increase in sEPSC frequency following MA in MAHDR-*Taar1*$^{m1J/m1J}$ control mice. We hypothesize that MA causes DR 5-HT neurons to co-release glutamate, leading to the MA-induced depolarization and potentiation of DR 5-HT neurons in mice expressing non-functional TAAR1 in the presence of autoreceptor antagonists. In turn, increased glutamate release from DR 5-HT neurons likely drives the observed increase in sEPSC frequency. There is no effect of MA on sEPSC amplitude therefore, we hypothesize MA has no effect on the post-synaptic response to neurotransmitter release, receptor density, or receptor sensitivity. *Created in BioRender.*

TAAR1 Function Determines Binge-Level Methamphetamine Intake

As mentioned in Chapter 1, the laboratory established a causal link between TAAR1 function and MA consumption, as well as sensitivities to the rewarding and aversive effects of MA, using CRISPR-*Cas9* generated mice. The laboratory confirmed that replacement of the *Taar1*^{m1J} allele with the reference *Taar1*$^{+}$ allele allowing for functional TAAR1 expression on the MAHDR background resulted in significantly reduced voluntary MA consumption (Stafford et al., 2019). KI of functional TAAR1 also resulted in reduced sensitivity to MA-conditioned reward and increased sensitivity to MA-conditioned aversion (Phillips et al., 2021; Stafford et al., 2019). To further confirm a causal link between TAAR1 function and MA-related behaviors, the laboratory sought to investigate whether binge-level MA intake is dependent on the absence of TAAR1 function. The CRISPR-*Cas9*-generated mice were tested using a two-bottle choice procedure, where MA concentration increased every 4 days from 20 to 140 mg/l, a procedure previously used to demonstrate-binge level MA intake in MAHDR mice (Shabani et al., 2016). In Chapter 2, I report that KI of functional TAAR1 resulted in a complete reversal of binge-level MA intake, from 4-28 times more than the MALDR mice (Shabani et al., 2016) to intake similar to that of MALDR mice which also express functional TAAR1. These findings further validate the causal role of TAAR1 functionality in mediating high-level MA intake.

MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice which exhibit high sensitivity to the rewarding effects of MA, also engage in binge-level consumption of MA [Dissertation Chapter 2; (Rios et al., 2025; Shabani et al., 2016)]. Given that repeated exposure of AMPHs can intensify the rewarding effects of the drug (Lett, 1989), DR 5-HT neuron activity may play a key role in determining binge-level MA intake. As previously mentioned, DR 5-HT neuron activity increased in response to rewarding stimuli, including sucrose, food, and social interaction, while optogenetic stimulation of DR 5-HT neurons has been shown to induce reward-motivated behaviors (Li et al., 2016; Liu et al., 2014; Nagai et al., 2020; Ren et al., 2018; Wang et al., 2019).

Given that we observed a MA-induced increase in DR 5-HT neuron activity from our MA-reward sensitive MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ mice, it is plausible that this increase in excitability contributes to the expression of high sensitivity to the rewarding effects of MA, thereby increasing their MA intake, further supporting a critical link between the activity of these neurons and reward-related behaviors.

The activity of glutamate may also contribute to high sensitivity to the rewarding effects of MA and high voluntary MA intake. Based on previous findings in the MADR mice (Lominac et al., 2016; Szumlinski et al., 2017), the laboratory tested the effect of positive and negative allosteric modulators for metabotropic glutamate receptors 2 and 5, as well as a prodrug for the cystine-glutamate antiporter on acquisition and establishment of MA intake in the MAHDR mice. However, they found that all of the drugs tested did not alter MA intake (Mootz & Phillips, *unpublished*). While the MADR mice demonstrate no baseline difference in NMDA receptor subunit expression in the NAc or the mPFC (Lominac et al., 2016; Szumlinski et al., 2017), it is unknown whether acute MA exposure alters expression of NMDA receptor subunits in the MADR mice. The laboratory has not tested whether NMDA receptors mediate voluntary MA intake, although, in MALDR mice, MA-induced locomotor stimulation can be blocked by antagonizing NMDA-GluN2B receptors (Li et al., 2017). Psychostimulants broadly influence NMDA receptor expression and activity, in turn modulating psychostimulant-mediated behaviors, however no studies have examined the role of NMDA receptors within the DR. Different NMDA receptor antagonists have been found to block the establishment of AMPH-induced CPP and AMPH-induced increases in locomotion (Bespalov, 1996; Bespalov & Zvartau, 1996; Moroz et al., 1997; Papp et al., 2002). Administration of NMDAR antagonists also blocks the development of AMPH-induced behavioral sensitization (Karler et al., 1989; Wolf & Khansa, 1991; Wolf et al., 1994). Repeated exposure to MA reduced GluN2B expression in the NAc, caudate putamen, and the mPFC (Lominac et al., 2016; Mao et al., 2009). In the VTA, repeated MA exposure increased

NMDA receptor function, facilitating long term potentiation induction (Ahn et al., 2010). Building on these findings, as well as my own observation that MA-induced excitability of DR 5-HT neurons from mice lacking functional TAAR1 is dependent on NMDA receptor activation, a promising future experiment would be to test the effect of NMDA receptor antagonism on other MA-related behaviors in the MADR and CRISPR-*Cas9*-generated mice. However, it is important to note that even though the MA-induced excitability I observed was dependent on NMDA receptor activation, it was also dependent on antagonism of 5-HT_{1A} and 5-HT_{1B} receptors, which highlights an interplay between serotonergic inhibition and glutamatergic activation.

This dual dependency of 5-HT and glutamate on neural activity and reward-motivated behavior aligns with findings from studies examining the circuit between the DR-VTA. The DR sends serotonergic projections to VTA DA neurons, and several studies have demonstrated that activation of DR 5-HT projections elicited excitatory currents in VTA DA neurons, increased their firing, and promoted reward motivated behavior such as CPP (Liu et al., 2014; Wang et al., 2019). Importantly, 2/3^{rds} of DR 5-HT neurons express VGlut3 (Hioki et al., 2010) and have been shown to co-release glutamate (Liu et al., 2014; Wang et al., 2019). The ability of DR 5-HT projections to excite VTA DA neurons is dependent on glutamate release (Liu et al., 2014; Wang et al., 2019), and antagonizing AMPA and 5-HT_3 receptors blocks DR-VTA circuit activation-induced CPP (Wang et al., 2019). Furthermore, optical stimulation of DR VGluT3 projections to the VTA induced higher CPP scores compared to stimulation of DR SERT projections to the VTA (Wang et al., 2019). These findings highlight that the activity of the DR-VTA circuit is important for promoting reward and is heavily influenced by both 5-HT and glutamate. These findings underscore the functional significance of serotonergic neurons' ability to integrate and transmit signals through both serotonin and glutamate release in behaviorally relevant circuits. Further investigation of this circuit in relation to MA-induced reward is essential.

Additional Factors to Consider: Methamphetamine-Induced Aversion and *Oprm1*

Its critical to note that the *Taar1* SNP explains 60% of the genetic variance in voluntary MA consumption (Belknap et al., 2013; Harkness et al., 2015), meaning other factors also contribute to the observed differences between the MADR lines. However, because of our findings from the CRISPR-*Cas9*-generated mice, we can confidently say that *Taar1* is the primary determinant of differences in MA intake and MA-induced aversion sensitivity between the MADR lines. KI of the $Taar1^{+}$ allele on the MAHDR and DBA/2J progenitor background significantly reduced MA intake to levels comparable to MALDR mice, whereas KI of the $Taar1^{m1J}$ allele on the C57BL/6J progenitor background increased MA intake to levels comparable to MAHDR mice (Phillips et al., 2021; Stafford et al., 2019). KI of the functional $Taar1^{+}$ allele also profoundly increased sensitivity to MA-induced conditioned aversion, whereas KI of the $Taar1^{m1J}$ allele converted MA-induced hypothermia, a potential aversive physiological effect of MA, to hyperthermia (Phillips et al., 2021; Stafford et al., 2019). Additionally, the activation of TAAR1 with the agonist RO5256390 also induced aversion (Shabani et al., 2023). R05256390 is a more potent and selective TAAR1 agonist compared to MA (Berry et al., 2017; Galley et al., 2016; Reese et al., 2014). These findings provide compelling evidence that TAAR1 activation is directly linked to aversive responses. Importantly, MALDR mice not only consumed comparable amounts of the rewarding solution saccharin but even consumed significantly more morphine compared to MAHDR mice (Eastwood & Phillips, 2014; Shabani et al., 2011; Wheeler et al., 2009). MALDR mice also exhibited cocaine-induced CPP comparable to MAHDR mice (Gubner et al., 2013). These data would suggest that sensitivity to rewarding stimuli remains intact in mice expressing functional TAAR1. Thus, *Taar1* genotype likely impacts MA-induced aversion sensitivity specifically. Therefore, the laboratory hypothesized that TAAR1 activation likely masks or overrides the rewarding effects of MA, effectively reducing voluntary MA intake (Phillips et al., 2021).

Aside from *Taar1*, the μ-opioid receptor 1 gene*, Oprm1,* has been of interest as a potential quantitative trait gene for MA intake given its location on mouse chromosome 10 and the ability of μ-opioid receptor 1 (MOP-r) targeting drugs to modulate MA-related traits in both humans and rodents (Dlugos et al., 2011; Ide et al., 2004; Jayaram-Lindstrom et al., 2017; Jayaram-Lindstrom, Hammarberg, et al., 2008; Jayaram-Lindstrom, Konstenius, et al., 2008; Jayaram-Lindstrom et al., 2004). The laboratory found that *Oprm1* variants do not predict risk for MA intake but regulate a gene network underlying differential risk for MA intake (Belknap et al., 2013; Eastwood et al., 2018). Additionally, the MOP-r agonist morphine and partial agonist buprenorphine reduced MA intake in MAHDR mice (Eastwood et al., 2018; Eastwood & Phillips, 2014); however, effects of morphine may have been non-specific since morphine also reduced saccharin and total fluid intake (Eastwood et al., 2018). Notably, the laboratory found a genetic linkage between *Taar1* and *Oprm1*. MADR mice predominantly inherit the *Oprm1* allele from the progenitor strain from which they inherit the *Taar1* allele, MAHDR mice from DBA/2J (D2) mice and MALDR mice from C57BL/6J (B6) mice (Mootz et al., 2020). Upon further investigation, the laboratory found that *Oprm1* genotype differentially influenced MA consumption and hypothermia. *Oprm1* genotype influenced MA consumption in $Taar1^{m1J/m1J}$ mice but not in $Taar1^{+/+}$ mice, while the opposite pattern was observed for MA-induced hypothermia. Specifically, $Taar1^{m1J/m1J}/Oprm1^{D2/D2}$ mice consumed more MA, and $Taar1^{+/+}/Oprm1^{D2/D2}$ mice exhibited greater MA-induced hypothermia compared to their respective $Oprm1^{B6/B6}$ counterparts (Stafford et al., 2019). Therefore, this would suggest a potential epistatic interaction of *Taar1*/*Oprm1* genotypes on MA intake and hypothermic response to MA. Additionally, the combined effects of *Taar1* and *Oprm1* were non-additive, meaning *Oprm1* genotype only impacted the mice with the stronger MA trait; MA consumption in $Taar1^{m1J/m1J}$ mice and MA-induced hypothermia in $Taar1^{+/+}$ mice. For both traits, $Oprm1^{D2/D2}$ mice exhibited the strongest influence. $Taar1^{m1J/m1J}/Oprm1^{D2/D2}$ mice consumed more MA than their $Taar1^{m1J/m1J}/Oprm1^{B6/B6}$

counterparts and *Taar1*$^{+/+}$/*Oprm1*$^{D2/D2}$ mice exhibited great MA-induced hypothermia compared to their *Taar1*$^{+/+}$/*Oprm1*$^{B6/B6}$ counterparts (Stafford et al., 2019).

In summary, although excitability of DR 5-HT neurons and glutamatergic inputs may contribute to high MA intake, other systems that signal MA aversion are likely critical for inhibiting MA consumption of MALDR mice. These systems may also play a role in MA-avoidance in humans, though this remains to be explored. Additionally, these effects may be influenced by factors beyond *Taar1.* Notably, the QTL on chromosome 10 at the location of *Taar1* accounts for 60% of the genetic variance for MA intake between the MADR lines, leaving additional genetic factors to be discovered.

Future Directions

In Chapter 2, I confirmed that the MA-induced depolarization and potentiation of DR 5-HT neurons from MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice is dependent on SERT. As mentioned previously, further experiments need to be done to determine how SERT remaining on the membrane is responsible for these effects. SERT transport stoichiometry is observed as electroneutral which would suggest an uncoupled SERT current is driving the observed effect of MA (Keyes & Rudnick, 1982; Mager et al., 1994; Rudnick & Nelson, 1978). Therefore, I would first test whether MA elicits an inward current in MAHDR-*Taar1*$^{m1J/m1J}$ mice. If this is the case, I would next determine whether the MA-induced current is blocked by the SERT antagonist fluoxetine since fluoxetine blocked the MA-induced depolarization and potentiation observed in mice with non-functional TAAR1. If fluoxetine blocks the MA-mediated currents, that suggests these currents are SERT dependent. To follow up, I would also examine the current-voltage relationship elicited in the absence and presence of MA to determine whether MA alters the reversal potential of the neuron I am recording from and whether that effect can be blocked by fluoxetine.

I would then manipulate the ion concentrations of my internal recording solution to determine which ion is responsible for the inward current. For example, I could use a KREBs external with low chloride concentration and if MA no longer elicits an inward current, which suggests the MA-induced currents are chloride dependent. I could also substitute different anions with different permeabilities to test changes in reversal potential of the MA-induced current to further confirm whether it is carried by chloride. This finding would necessitate a series of follow-up experiments to further explore how the sustained presence of SERT on the membrane mediates MA-induced excitation of DR 5-HT neurons

As mentioned in Chapter 3, further experiments are needed to determine whether TAAR1 functionality differentially modulates pre- and post-synaptic mechanisms between MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice. First, given the potential TAAR1-mediated baseline differences in extracellular glutamate concentrations in the DR between the lines, I would repeat the sEPSC in the presence of tetrodotoxin (TTX) to block voltage-gated sodium channels involved in action potentials. I hypothesize that MA will have no effect on mEPSC frequencies onto DR 5-HT neurons of MAHDR-*Taar1*$^{m1J/m1J}$ control mice, as I suspect any glutamate release to originate from the 5-HT neurons themselves, given the MA-induced increases in firing frequencies observed. However, if MA does induce an increase in mEPSC frequencies that would suggest a pre-synaptic mechanism. Since the MA-induced potentiation of DR 5-HT neurons from mice lacking functional TAAR1 is NMDA receptor-dependent, there is a possibility that MA is acting on pre-synaptic receptors. Although traditionally known as post-synaptic receptors, there is evidence NMDA receptors are expressed pre-synaptically (Banerjee et al., 2016; Bouvier et al., 2015). To evaluate whether the effect of MA is driven by pre-synaptic NMDA receptors, I would record mEPSCs in the presence of an NMDA receptor antagonist. If the MA-induced increase in frequencies is blocked, that would suggest MA is acting on pre-synaptic NMDA receptors.

I would follow up these studies by recording the AMPA/NMDA ratio of DR 5-HT neurons in both lines, given that the MA-induced potentiation of DR 5-HT neurons in mice lacking functional TAAR1 (MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ controls) is dependent on NMDA receptor activation. Previously, no differences were observed in the expression of GluN1, GluN2a, or GluN2b NMDA receptor subunits within the NAc or mPFC pre-MA exposure of MADR mice. No data on AMPA receptor subunit expression was collected in either study (Lominac et al., 2016; Szumlinski et al., 2017). However, in TAAR1-KO mice, reduced GluN1 and GluN2b expression, but comparable GluA1 expression, was observed in the prefrontal cortex (PFC), resulting in a significantly lower NMDA/AMPA ratio in recordings from medial PFC neurons compared to wildtype mice (Espinoza et al., 2015). Given the opposite findings in the PFC, these findings potentially suggest that despite the absence of TAAR1 function in MAHDR mice, the expression of the receptors may still provide some baseline influence on NMDA receptor subunit expression while the complete absence of TAAR1 leads to a more pronounced effect on subunit expression. Therefore, it is difficult to hypothesize whether MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice demonstrate baseline differences in AMPA/NMDA ratio. Considering the more relevant data in the MADR mice, I would hypothesize that NMDA receptor subunit expression is similar between the MAHDR-*Taar1*$^{m1J/m1J}$ controls and MAHDR-*Taar1*$^{+/+}$ KI mice. Therefore, any differences in AMPA/NMDA ratio would be dependent on AMPA receptor subunit expression of which we have no data. An important caveat is that receptor subunit expression in these studies was measured by western blot, which does not provide functional data. To determine whether baseline differences in NMDA receptor expression exist, I could iontophorese N-Methyl-D-aspartic acid directly onto the cell I am recording from in both MAHDR-*Taar1*$^{+/+}$ KI and MAHDR-*Taar1*$^{m1J/m1J}$ control mice. This approach eliminates confounding factors such as differences in pre-synaptic glutamate release which would influence the NMDA-mediated current from the cell I am recording from, allowing for a direct comparison of NMDA-mediated currents between the lines. Then, to elucidate the functional composition of

AMPA and NMDA receptors on the membrane, I could test subunit-specific antagonists to better understand receptor subunit contributions to baseline activity and the effects of MA.

There are no studies directly examining the effect of TAAR1 functionality on AMPA and NMDA receptor subunit expression following acute MA exposure in the MADR or CRISPR-*Cas9*-generate mice. However, one study used the C57BL/6J mice, one of the progenitor strains of the MADR mice from which the wildtype *Taar1*$^{+}$ allele is inherited to examine the effect of MA withdrawal on receptor subunit expression. In the mPFC, they found a reduction in GluN1 subunit expression 1- and 21-days following MA withdrawal compared to saline controls. They also found a reduction in GluN2b receptor expression 1 day following MA withdrawal compared to saline controls. No effect of MA withdrawal on GluA1 expression was observed (Lominac et al., 2016). Forming hypotheses based on this work is challenging due to its focus on withdrawal-induced effects on subunit expression rather than acute MA administration. Nevertheless, I hypothesize that following acute administration of MA, NMDA receptor subunit expression decreases in MAHDR-*Taar1*$^{+/+}$ KI which would result in an increase in AMPA/NMDA ratio. With regard to MAHDR-*Taar1*$^{m1J/m1J}$ control mice, there are two possibilities: (1) MA has no effect on NMDA receptor subunit expression, leaving the AMPA/NMDA ratio unchanged, but their baseline expression on the membrane is sufficient to enable NMDA receptor-mediated depolarization of DR 5-HT neurons, or (2) MA increases NMDA receptor subunit expression on the post-synaptic membrane, reducing the AMPA/NMDA ratio and facilitating NMDA receptor-mediated depolarization. Either outcome could explain the MA-induced potentiation of DR 5-HT neurons we observed in mice lacking functional TAAR1. To evaluate this hypothesis, I could measure NMDA-mediated currents pre- and post-MA application. If current amplitudes or kinetics shift following MA, it would suggest an increase in receptor density.

It is also important to consider the broader role of 5-HT and glutamate on voluntary and binge-level MA consumption. Evidence supports the involvement 5-HT and NMDA receptor

subtypes in voluntary and binge-level alcohol intake (Belmer, Patkar, et al., 2018; Sabino et al., 2013; Tabbara et al., 2021). However, their involvement in MA intake remains underexplored despite the well documented effects of AMPHs on brain 5-HT and glutamate systems, with existing studies lacking specificity to the DR. There are some limitations to designing a study to determine whether DR 5-HT neurons and/or glutamate in the DR play a significant role in MA consumption. The procedures used for measuring voluntary MA intake and binge-level intake used by our lab requires 18-hour access to MA, making designer receptors exclusively activated by designer drugs (DREADDs) or systemic injection of a drug unsuitable choices. Also, the DR is located posterior to lambda, deep within the brainstem beneath the cerebral aqueduct, making cannulation difficult. We could take a more generalized approach and lesion the DR of MAHDR and/or MAHDR-*Taar1*$^{m1J/m1J}$ control mice to test whether MA consumption reduced.

To more specifically assess the role of 5-HT and glutamate within the DR on MA intake, we would have to decrease the amount of time mice have access to MA. I'm inclined to first test whether voluntary MA intake exhibited by the MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice can be attenuated by 5-HT$_{1B}$ agonists based on previous findings that AMPH self-administration can be attenuated by activation of 5-HT$_{1B}$ receptors (Fletcher & Korth, 1999; Miszkiel et al., 2012). Also, given that SERT potentially remains expressed on the membrane due to the lack of TAAR1 activation in MAHDR or MAHDR-*Taar1*$^{m1J/m1J}$ control mice (Underhill & Amara, 2020) and the reductions in AMPH-mediated behaviors by SERT antagonists, including AMPH self-administration, CPP, and locomotor sensitization (Porrino et al., 1989; Takamatsu et al., 2006; Yu et al., 1986), I would also like to test the whether the SERT antagonist fluoxetine attenuates voluntary MA intake in MAHDR and MAHDR-*Taar1*$^{m1J/m1J}$ control mice. Finally, as previously mentioned, I would like to assess whether NMDA receptor antagonists also attenuate voluntary MA intake.

If funding and technical limitations were not a concern, a compelling study would be to evaluate whether inhibiting or activating dual DR SERT-VGluT3 neurons projecting to the VTA modulates voluntary MA intake. This experiment is based on my findings that DR 5-HT neuron excitability in response to MA in mice with non-functional TAAR1 receptors is dependent on both SERT and NMDA receptor activity. Complementing this, Wang et al., 2019 demonstrated that DR-SERT projections to the VTA elicit glutamate-dependent excitatory currents and that optical stimulation of DR-VGluT3+ projections to the VTA induced CPP, highlighting a role for glutamate in promoting reward and 5-HT neuron-mediated excitation. Therefore, I would predict that inhibiting DR SERT-VGluT3+ projections to the VTA would reduce voluntary MA intake in the reward sensitive MAHDR-*Taar1*$^{m1J/m1J}$ control mice, while activating these inputs in the reward-insensitive MAHDR-*Taar1*$^{+/+}$ KI mice would induce voluntary MA intake. To tease apart this circuit's role in reward sensitivity we could also test whether inhibition or activation of this circuit in the MAHDR-*Taar1*$^{m1J/m1J}$ control and MAHDR-*Taar1*$^{+/+}$ KI mice alters expression of CPP. Such experiments would provide a novel understanding of the functional role of co-released 5-HT and glutamate in modulating MA intake and reward sensitivity. Furthermore, these experiments would provide new insights on the influence of TAAR1 on neurocircuitry underlying reward-motivated behaviors.

References

Abrams, J. K., Johnson, P. L., Hollis, J. H., & Lowry, C. A. (2004). Anatomic and functional topography of the dorsal raphe nucleus. *Ann N Y Acad Sci*, *1018*, 46-57. https://doi.org/10.1196/annals.1296.005

Adell, A., Celada, P., & Artigas, F. (2001). The role of 5-HT1B receptors in the regulation of serotonin cell firing and release in the rat brain. *J Neurochem*, *79*(1), 172-182. https://doi.org/10.1046/j.1471-4159.2001.00550.x

Aghajanian, G. K., & Lakoski, J. M. (1984). Hyperpolarization of serotonergic neurons by serotonin and LSD: studies in brain slices showing increased K+ conductance. *Brain Res*, *305*(1), 181-185. https://doi.org/10.1016/0006-8993(84)91137-5

Ago, Y., Nakamura, S., Uda, M., Kajii, Y., Abe, M., Baba, A., & Matsuda, T. (2006). Attenuation by the 5-HT1A receptor agonist osemozotan of the behavioral effects of single and repeated methamphetamine in mice. *Neuropharmacology*, *51*(4), 914-922. https://doi.org/10.1016/j.neuropharm.2006.06.001

Ahn, K. C., Bernier, B. E., Harnett, M. T., & Morikawa, H. (2010). IP3 receptor sensitization during in vivo amphetamine experience enhances NMDA receptor plasticity in dopamine neurons of the ventral tegmental area. *J Neurosci*, *30*(19), 6689-6699. https://doi.org/10.1523/JNEUROSCI.4453-09.2010

Albert, P. R., Zhou, Q. Y., Van Tol, H. H., Bunzow, J. R., & Civelli, O. (1990). Cloning, functional expression, and mRNA tissue distribution of the rat 5-hydroxytryptamine1A receptor gene. *J Biol Chem*, *265*(10), 5825-5832. https://www.ncbi.nlm.nih.gov/pubmed/2156831

Altieri, S. C., Garcia-Garcia, A. L., Leonardo, E. D., & Andrews, A. M. (2013). Rethinking 5-HT1A receptors: emerging modes of inhibitory feedback of relevance to emotion-related behavior. *ACS Chem Neurosci*, *4*(1), 72-83. https://doi.org/10.1021/cn3002174

Anglin, M. D., Burke, C., Perrochet, B., Stamper, E., & Dawud-Noursi, S. (2000). History of the methamphetamine problem. *J Psychoactive Drugs*, *32*(2), 137-141. https://doi.org/10.1080/02791072.2000.10400221

Atkins, A. L., Helms, M. L., O'Toole, L. A., & Belknap, J. K. (2001). Stereotypic behaviors in mice selectively bred for high and low methamphetamine-induced stereotypic chewing. *Psychopharmacology (Berl)*, *157*(1), 96-104. https://doi.org/10.1007/s002130100774

Banerjee, A., Larsen, R. S., Philpot, B. D., & Paulsen, O. (2016). Roles of Presynaptic NMDA Receptors in Neurotransmission and Plasticity. *Trends Neurosci*, *39*(1), 26-39. https://doi.org/10.1016/j.tins.2015.11.001

Bang, S. J., Jensen, P., Dymecki, S. M., & Commons, K. G. (2012). Projections and interconnections of genetically defined serotonin neurons in mice. *Eur J Neurosci*, *35*(1), 85-96. https://doi.org/10.1111/j.1460-9568.2011.07936.x

Barandier, C., Ming, X. F., Rusconi, S., & Yang, Z. (2003). PKC is required for activation of ROCK by RhoA in human endothelial cells. *Biochem Biophys Res Commun*, *304*(4), 714-719. https://doi.org/10.1016/s0006-291x(03)00668-5

Barkley-Levenson, A. M., & Crabbe, J. C. (2014). High drinking in the dark mice: a genetic model of drinking to intoxication. *Alcohol*, *48*(3), 217-223. https://doi.org/10.1016/j.alcohol.2013.10.007

Barr, A. M., Panenka, W. J., MacEwan, G. W., Thornton, A. E., Lang, D. J., Honer, W. G., & Lecomte, T. (2006). The need for speed: an update on methamphetamine addiction. *J Psychiatry Neurosci*, *31*(5), 301-313. https://www.ncbi.nlm.nih.gov/pubmed/16951733

Battaglia, G., & De Souza, E. B. (1989). Pharmacologic profile of amphetamine derivatives at various brain recognition sites: selective effects on serotonergic systems. *NIDA Res Monogr*, *94*, 240-258. https://www.ncbi.nlm.nih.gov/pubmed/2514364

Beaulieu, J. M., & Gainetdinov, R. R. (2011). The physiology, signaling, and pharmacology of dopamine receptors. *Pharmacol Rev*, *63*(1), 182-217. https://doi.org/10.1124/pr.110.002642

Belknap, J. K., Hitzemann, R., Crabbe, J. C., Phillips, T. J., Buck, K. J., & Williams, R. W. (2001). QTL analysis and genomewide mutagenesis in mice: complementary genetic approaches to the dissection of complex traits. *Behav Genet*, *31*(1), 5-15. https://doi.org/10.1023/a:1010249607128

Belknap, J. K., McWeeney, S., Reed, C., Burkhart-Kasch, S., McKinnon, C. S., Li, N., Baba, H., Scibelli, A. C., Hitzemann, R., & Phillips, T. J. (2013). Genetic factors involved in risk for methamphetamine intake and sensitization. *Mamm Genome*, *24*(11-12), 446-458. https://doi.org/10.1007/s00335-013-9484-9

Belknap, J. K., Richards, S. P., O'Toole, L. A., Helms, M. L., & Phillips, T. J. (1997). Short-term selective breeding as a tool for QTL mapping: ethanol preference drinking in mice. *Behav Genet*, *27*(1), 55-66. https://doi.org/10.1023/a:1025615409383

Belmer, A., Patkar, O. L., Lanoue, V., & Bartlett, S. E. (2018). 5-HT1A receptor-dependent modulation of emotional and neurogenic deficits elicited by prolonged consumption of alcohol. *Sci Rep*, *8*(1), 2099. https://doi.org/10.1038/s41598-018-20504-z

Belmer, A., Quentin, E., Diaz, S. L., Guiard, B. P., Fernandez, S. P., Doly, S., Banas, S. M., Pitychoutis, P. M., Moutkine, I., Muzerelle, A., Tchenio, A., Roumier, A., Mameli, M., & Maroteaux, L. (2018). Positive regulation of raphe serotonin neurons by serotonin 2B receptors. *Neuropsychopharmacology*, *43*(7), 1623-1632. https://doi.org/10.1038/s41386-018-0013-0

Berry, M. D. (2004). Mammalian central nervous system trace amines. Pharmacologic amphetamines, physiologic neuromodulators. *J Neurochem*, *90*(2), 257-271. https://doi.org/10.1111/j.1471-4159.2004.02501.x

Berry, M. D., Gainetdinov, R. R., Hoener, M. C., & Shahid, M. (2017). Pharmacology of human trace amine-associated receptors: Therapeutic opportunities and challenges. *Pharmacol Ther*, *180*, 161-180. https://doi.org/10.1016/j.pharmthera.2017.07.002

Bespalov, A. (1996). The expression of both amphetamine-conditioned place preference and pentylenetetrazol-conditioned place aversion is attenuated by the NMDA receptor antagonist (+/-)-CPP. *Drug Alcohol Depend*, *41*(1), 85-88. https://doi.org/10.1016/0376-8716(96)01227-6

Bespalov, A. Y., & Zvartau, E. E. (1996). Intraaccumbens administration of NMDA receptor antagonist (+/-)-CPP prevents locomotor activation conditioned by morphine and amphetamine in rats. *Pharmacol Biochem Behav*, *55*(2), 203-207. https://doi.org/10.1016/s0091-3057(96)00065-2

Bhattacharya, S. K., Bhattacharya, A., & Ghosal, S. (1998). Anxiogenic activity of methylenedioxymethamphetamine (Ecstasy) : An experimental study [Article]. *Biogenic Amines*, *14*(3), 217-237. https://www.scopus.com/inward/record.uri?eid=2-s2.0-0031842014&partnerID=40&md5=8daf0eea881e1bec24140c096cf8e546

Blakely, R. D., Ramamoorthy, S., Schroeter, S., Qian, Y., Apparsundaram, S., Galli, A., & DeFelice, L. J. (1998). Regulated phosphorylation and trafficking of antidepressant-sensitive serotonin transporter proteins. *Biol Psychiatry*, *44*(3), 169-178. https://doi.org/10.1016/s0006-3223(98)00124-3

Blakemore, E. (2017, October 27, 2017). A Speedy History of America's Addiction to Amphetamine [October 14, 2024]. *Smithsonian Magazine*. https://www.smithsonianmag.com/history/speedy-history-americas-addiction-amphetamine-180966989/

Borowsky, B., Adham, N., Jones, K. A., Raddatz, R., Artymyshyn, R., Ogozalek, K. L., Durkin, M. M., Lakhlani, P. P., Bonini, J. A., Pathirana, S., Boyle, N., Pu, X., Kouranova, E., Lichtblau, H., Ochoa, F. Y., Branchek, T. A., & Gerald, C. (2001). Trace amines:

identification of a family of mammalian G protein-coupled receptors. *Proc Natl Acad Sci U S A*, *98*(16), 8966-8971. https://doi.org/10.1073/pnas.151105198

Bouvier, G., Bidoret, C., Casado, M., & Paoletti, P. (2015). Presynaptic NMDA receptors: Roles and rules. *Neuroscience*, *311*, 322-340. https://doi.org/10.1016/j.neuroscience.2015.10.033

Bradaia, A., Trube, G., Stalder, H., Norcross, R. D., Ozmen, L., Wettstein, J. G., Pinard, A., Buchy, D., Gassmann, M., Hoener, M. C., & Bettler, B. (2009). The selective antagonist EPPTB reveals TAAR1-mediated regulatory mechanisms in dopaminergic neurons of the mesolimbic system. *Proc Natl Acad Sci U S A*, *106*(47), 20081-20086. https://doi.org/10.1073/pnas.0906522106

Branch, S. Y., & Beckstead, M. J. (2012). Methamphetamine produces bidirectional, concentration-dependent effects on dopamine neuron excitability and dopamine-mediated synaptic currents. *J Neurophysiol*, *108*(3), 802-809. https://doi.org/10.1152/jn.00094.2012

Buchmayer, F., Schicker, K., Steinkellner, T., Geier, P., Stubiger, G., Hamilton, P. J., Jurik, A., Stockner, T., Yang, J. W., Montgomery, T., Holy, M., Hofmaier, T., Kudlacek, O., Matthies, H. J., Ecker, G. F., Bochkov, V., Galli, A., Boehm, S., & Sitte, H. H. (2013). Amphetamine actions at the serotonin transporter rely on the availability of phosphatidylinositol-4,5-bisphosphate. *Proc Natl Acad Sci U S A*, *110*(28), 11642-11647. https://doi.org/10.1073/pnas.1220552110

Bunzow, J. R., Sonders, M. S., Arttamangkul, S., Harrison, L. M., Zhang, G., Quigley, D. I., Darland, T., Suchland, K. L., Pasumamula, S., Kennedy, J. L., Olson, S. B., Magenis, R. E., Amara, S. G., & Grandy, D. K. (2001). Amphetamine, 3,4-methylenedioxymethamphetamine, lysergic acid diethylamide, and metabolites of the catecholamine neurotransmitters are agonists of a rat trace amine receptor. *Mol Pharmacol*, *60*(6), 1181-1188. https://doi.org/10.1124/mol.60.6.1181

Caldwell, J., Dring, L. G., & Williams, R. T. (1972). Metabolism of (14 C)methamphetamine in man, the guinea pig and the rat. *Biochem J*, *129*(1), 11-22. https://doi.org/10.1042/bj1290011

Calizo, L. H., Akanwa, A., Ma, X., Pan, Y. Z., Lemos, J. C., Craige, C., Heemstra, L. A., & Beck, S. G. (2011). Raphe serotonin neurons are not homogenous: electrophysiological, morphological and neurochemical evidence. *Neuropharmacology*, *61*(3), 524-543. https://doi.org/10.1016/j.neuropharm.2011.04.008

Cartmell, J., & Schoepp, D. D. (2000). Regulation of neurotransmitter release by metabotropic glutamate receptors. *J Neurochem*, *75*(3), 889-907. https://doi.org/10.1046/j.1471-4159.2000.0750889.x

Chait, L. D. (1993). Factors influencing the reinforcing and subjective effects of d-amphetamine in humans. *Behav Pharmacol*, *4*(3), 191-199. https://www.ncbi.nlm.nih.gov/pubmed/11224186

Chiellini, G., Frascarelli, S., Ghelardoni, S., Carnicelli, V., Tobias, S. C., DeBarber, A., Brogioni, S., Ronca-Testoni, S., Cerbai, E., Grandy, D. K., Scanlan, T. S., & Zucchi, R. (2007). Cardiac effects of 3-iodothyronamine: a new aminergic system modulating cardiac function. *FASEB J*, *21*(7), 1597-1608. https://doi.org/10.1096/fj.06-7474com

Chiu, V. M., & Schenk, J. O. (2012). Mechanism of action of methamphetamine within the catecholamine and serotonin areas of the central nervous system. *Curr Drug Abuse Rev*, *5*(3), 227-242. https://doi.org/10.2174/1874473711205030227

Cohen, P. A., Goday, A., & Swann, J. P. (2012). The return of rainbow diet pills. *Am J Public Health*, *102*(9), 1676-1686. https://doi.org/10.2105/AJPH.2012.300655

Conn, P. J., & Pin, J. P. (1997). Pharmacology and functions of metabotropic glutamate receptors. *Annu Rev Pharmacol Toxicol*, *37*, 205-237. https://doi.org/10.1146/annurev.pharmtox.37.1.205

Conrad, K. L., Tseng, K. Y., Uejima, J. L., Reimers, J. M., Heng, L. J., Shaham, Y., Marinelli, M., & Wolf, M. E. (2008). Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature*, *454*(7200), 118-121. https://doi.org/10.1038/nature06995

Cook, C. E., Jeffcoat, A. R., Hill, J. M., Pugh, D. E., Patetta, P. K., Sadler, B. M., White, W. R., & Perez-Reyes, M. (1993). Pharmacokinetics of methamphetamine self-administered to human subjects by smoking S-(+)-methamphetamine hydrochloride. *Drug Metab Dispos*, *21*(4), 717-723. https://www.ncbi.nlm.nih.gov/pubmed/8104133

Cook, C. E., Jeffcoat, A. R., Sadler, B. M., Hill, J. M., Voyksner, R. D., Pugh, D. E., White, W. R., & Perez-Reyes, M. (1992). Pharmacokinetics of oral methamphetamine and effects of repeated daily dosing in humans. *Drug Metab Dispos*, *20*(6), 856-862. https://www.ncbi.nlm.nih.gov/pubmed/1362938

Crabbe, J. C. (1999). Animal Models in Neurobehavioral Genetics: Methods for Estimating Genetic Correlation.

Crabbe, J. C. (2002). Genetic contributions to addiction. *Annu Rev Psychol*, *53*, 435-462. https://doi.org/10.1146/annurev.psych.53.100901.135142

Crabbe, J. C., Kosobud, A., Tam, B. R., Young, E. R., & Deutsch, C. M. (1987). Genetic selection of mouse lines sensitive (cold) and resistant (hot) to acute ethanol hypothermia. *Alcohol Drug Res*, *7*(3), 163-174. https://www.ncbi.nlm.nih.gov/pubmed/3827997

Crabbe, J. C., Metten, P., Rhodes, J. S., Yu, C. H., Brown, L. L., Phillips, T. J., & Finn, D. A. (2009). A line of mice selected for high blood ethanol concentrations shows drinking in the dark to intoxication. *Biol Psychiatry*, *65*(8), 662-670. https://doi.org/10.1016/j.biopsych.2008.11.002

Cruickshank, C. C., & Dyer, K. R. (2009). A review of the clinical pharmacology of methamphetamine. *Addiction*, *104*(7), 1085-1099. https://doi.org/10.1111/j.1360-0443.2009.02564.x

Davidson, C., & Stamford, J. A. (1995). Evidence that 5-hydroxytryptamine release in rat dorsal raphe nucleus is controlled by 5-HT1A, 5-HT1B and 5-HT1D autoreceptors. *Br J Pharmacol*, *114*(6), 1107-1109. https://doi.org/10.1111/j.1476-5381.1995.tb13321.x

de Wit, H., Uhlenhuth, E. H., & Johanson, C. E. (1986). Individual differences in the reinforcing and subjective effects of amphetamine and diazepam. *Drug Alcohol Depend*, *16*(4), 341-360. https://doi.org/10.1016/0376-8716(86)90068-2

Dedic, N., Jones, P. G., Hopkins, S. C., Lew, R., Shao, L., Campbell, J. E., Spear, K. L., Large, T. H., Campbell, U. C., Hanania, T., Leahy, E., & Koblan, K. S. (2019). SEP-363856, a Novel Psychotropic Agent with a Unique, Non-D(2) Receptor Mechanism of Action. *J Pharmacol Exp Ther*, *371*(1), 1-14. https://doi.org/10.1124/jpet.119.260281

Dlugos, A. M., Hamidovic, A., Hodgkinson, C., Shen, P. H., Goldman, D., Palmer, A. A., & de Wit, H. (2011). OPRM1 gene variants modulate amphetamine-induced euphoria in humans. *Genes Brain Behav*, *10*(2), 199-209. https://doi.org/10.1111/j.1601-183X.2010.00655.x

Doly, S., Bertran-Gonzalez, J., Callebert, J., Bruneau, A., Banas, S. M., Belmer, A., Boutourlinsky, K., Herve, D., Launay, J. M., & Maroteaux, L. (2009). Role of serotonin via 5-HT2B receptors in the reinforcing effects of MDMA in mice. *PLoS One*, *4*(11), e7952. https://doi.org/10.1371/journal.pone.0007952

Doly, S., Valjent, E., Setola, V., Callebert, J., Herve, D., Launay, J. M., & Maroteaux, L. (2008). Serotonin 5-HT2B receptors are required for 3,4-methylenedioxymethamphetamine-induced hyperlocomotion and 5-HT release in vivo and in vitro. *J Neurosci*, *28*(11), 2933-2940. https://doi.org/10.1523/JNEUROSCI.5723-07.2008

Eastwood, E. C., Eshleman, A. J., Janowsky, A., & Phillips, T. J. (2018). Verification of a genetic locus for methamphetamine intake and the impact of morphine. *Mamm Genome*, *29*(3-4), 260-272. https://doi.org/10.1007/s00335-017-9724-5

Eastwood, E. C., & Phillips, T. J. (2014). Morphine intake and the effects of naltrexone and buprenorphine on the acquisition of methamphetamine intake. *Genes Brain Behav*, *13*(2), 226-235. https://doi.org/10.1111/gbb.12100

Edwards, R. H. (1992). The transport of neurotransmitters into synaptic vesicles. *Curr Opin Neurobiol*, *2*(5), 586-594. https://doi.org/10.1016/0959-4388(92)90023-e

Eiden, L. E., Schafer, M. K., Weihe, E., & Schutz, B. (2004). The vesicular amine transporter family (SLC18): amine/proton antiporters required for vesicular accumulation and regulated exocytotic secretion of monoamines and acetylcholine. *Pflugers Arch*, *447*(5), 636-640. https://doi.org/10.1007/s00424-003-1100-5

Espinoza, S., Lignani, G., Caffino, L., Maggi, S., Sukhanov, I., Leo, D., Mus, L., Emanuele, M., Ronzitti, G., Harmeier, A., Medrihan, L., Sotnikova, T. D., Chieregatti, E., Hoener, M. C., Benfenati, F., Tucci, V., Fumagalli, F., & Gainetdinov, R. R. (2015). TAAR1 Modulates Cortical Glutamate NMDA Receptor Function. *Neuropsychopharmacology*, *40*(9), 2217-2227. https://doi.org/10.1038/npp.2015.65

Falconer, D., & Mackay, T. (1996). Introduction to Quantitative Genetics (Harlow, UK: Longman Group Ltd.).

Feller, D. J., & Crabbe, J. C. (1991). Effect of alcohols and other hypnotics in mice selected for differential sensitivity to hypothermic actions of ethanol. *J Pharmacol Exp Ther*, *256*(3), 947-953. https://www.ncbi.nlm.nih.gov/pubmed/2005589

Ferraro, G., Montalbano, M. E., Sardo, P., & La Grutta, V. (1996). Lateral habenular influence on dorsal raphe neurons. *Brain Res Bull*, *41*(1), 47-52. https://doi.org/10.1016/0361-9230(96)00170-0

Ferre, S., Cortes, R., & Artigas, F. (1994). Dopaminergic regulation of the serotonergic raphe-striatal pathway: microdialysis studies in freely moving rats. *J Neurosci*, *14*(8), 4839-4846. https://doi.org/10.1523/JNEUROSCI.14-08-04839.1994

Fleckenstein, A. E., Volz, T. J., & Hanson, G. R. (2009). Psychostimulant-induced alterations in vesicular monoamine transporter-2 function: Neurotoxic and therapeutic implications. *Neuropharmacology*, *56*, 133-138. https://doi.org/10.1016/j.neuropharm.2008.07.002

Fleckenstein, A. E., Volz, T. J., Riddle, E. L., Gibb, J. W., & Hanson, G. R. (2007). New insights into the mechanism of action of amphetamines. *Annu Rev Pharmacol Toxicol*, *47*, 681-698. https://doi.org/10.1146/annurev.pharmtox.47.120505.105140

Fletcher, P. J., & Korth, K. M. (1999). RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT1B receptors. *Behav Pharmacol*, *10*(2), 183-193. https://doi.org/10.1097/00008877-199903000-00007

Floor, E., & Meng, L. (1996). Amphetamine releases dopamine from synaptic vesicles by dual mechanisms. *Neurosci Lett*, *215*(1), 53-56. https://doi.org/10.1016/s0304-3940(96)12963-3

Fremeau, R. T., Jr., Burman, J., Qureshi, T., Tran, C. H., Proctor, J., Johnson, J., Zhang, H., Sulzer, D., Copenhagen, D. R., Storm-Mathisen, J., Reimer, R. J., Chaudhry, F. A., & Edwards, R. H. (2002). The identification of vesicular glutamate transporter 3 suggests novel modes of signaling by glutamate. *Proc Natl Acad Sci U S A*, *99*(22), 14488-14493. https://doi.org/10.1073/pnas.222546799

Fu, W., Le Maitre, E., Fabre, V., Bernard, J. F., David Xu, Z. Q., & Hokfelt, T. (2010). Chemical neuroanatomy of the dorsal raphe nucleus and adjacent structures of the mouse brain. *J Comp Neurol*, *518*(17), 3464-3494. https://doi.org/10.1002/cne.22407

Galley, G., Beurier, A., Decoret, G., Goergler, A., Hutter, R., Mohr, S., Pahler, A., Schmid, P., Turck, D., Unger, R., Zbinden, K. G., Hoener, M. C., & Norcross, R. D. (2016). Discovery and Characterization of 2-Aminooxazolines as Highly Potent, Selective, and Orally Active TAAR1 Agonists. *ACS Med Chem Lett*, *7*(2), 192-197. https://doi.org/10.1021/acsmedchemlett.5b00449

Gass, J. T., & Olive, M. F. (2008). Glutamatergic substrates of drug addiction and alcoholism. *Biochem Pharmacol*, *75*(1), 218-265. https://doi.org/10.1016/j.bcp.2007.06.039

Glaum, S. R., & Miller, R. J. (1995). Presynaptic metabotropic glutamate receptors modulate omega-conotoxin-GVIA-insensitive calcium channels in the rat medulla. *Neuropharmacology*, *34*(8), 953-964. https://doi.org/10.1016/0028-3908(95)00076-i

Glaum, S. R., Sunter, D. C., Udvarhelyi, P. M., Watkins, J. C., & Miller, R. J. (1993). The actions of phenylglycine derived metabotropic glutamate receptor antagonists on multiple (1S,3R)-ACPD responses in the rat nucleus of the tractus solitarius. *Neuropharmacology*, *32*(12), 1419-1425. https://doi.org/10.1016/0028-3908(93)90039-6

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J., & Hamon, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: 3H-PAT. *Nature*, *305*(5930), 140-142. https://doi.org/10.1038/305140a0

Grahame, N. J., Li, T. K., & Lumeng, L. (1999). Selective breeding for high and low alcohol preference in mice. *Behav Genet*, *29*(1), 47-57. https://doi.org/10.1023/a:1021489922751

Gras, C., Herzog, E., Bellenchi, G. C., Bernard, V., Ravassard, P., Pohl, M., Gasnier, B., Giros, B., & El Mestikawy, S. (2002). A third vesicular glutamate transporter expressed by cholinergic and serotoninergic neurons. *J Neurosci*, *22*(13), 5442-5451. https://doi.org/10.1523/JNEUROSCI.22-13-05442.2002

Grinchii, D., Hoener, M. C., Khoury, T., Dekhtiarenko, R., Nejati Bervanlou, R., Jezova, D., & Dremencov, E. (2022). Effects of acute and chronic administration of trace amine-associated receptor 1 (TAAR1) ligands on in vivo excitability of central monoamine-secreting neurons in rats. *Mol Psychiatry*, *27*(12), 4861-4868. https://doi.org/10.1038/s41380-022-01739-9

Grisel, J. E., Belknap, J. K., O'Toole, L. A., Helms, M. L., Wenger, C. D., & Crabbe, J. C. (1997). Quantitative trait loci affecting methamphetamine responses in BXD recombinant inbred

mouse strains. *J Neurosci*, *17*(2), 745-754. https://doi.org/10.1523/JNEUROSCI.17-02-00745.1997

Gubner, N. R., Reed, C., McKinnon, C. S., & Phillips, T. J. (2013). Unique genetic factors influence sensitivity to the rewarding and aversive effects of methamphetamine versus cocaine. *Behav Brain Res*, *256*, 420-427. https://doi.org/10.1016/j.bbr.2013.08.035

Hall, M. D., el Mestikawy, S., Emerit, M. B., Pichat, L., Hamon, M., & Gozlan, H. (1985). [3H]8-hydroxy-2-(di-n-propylamino)tetralin binding to pre- and postsynaptic 5-hydroxytryptamine sites in various regions of the rat brain. *J Neurochem*, *44*(6), 1685-1696. https://doi.org/10.1111/j.1471-4159.1985.tb07155.x

Han, B., Compton, W. M., Jones, C. M., Einstein, E. B., & Volkow, N. D. (2021). Methamphetamine Use, Methamphetamine Use Disorder, and Associated Overdose Deaths Among US Adults. *JAMA Psychiatry*, *78*(12), 1329-1342. https://doi.org/10.1001/jamapsychiatry.2021.2588

Han, D. D., & Gu, H. H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. *BMC Pharmacol*, *6*, 6. https://doi.org/10.1186/1471-2210-6-6

Harkness, J. H., Shi, X., Janowsky, A., & Phillips, T. J. (2015). Trace Amine-Associated Receptor 1 Regulation of Methamphetamine Intake and Related Traits. *Neuropsychopharmacology*, *40*(9), 2175-2184. https://doi.org/10.1038/npp.2015.61

Harris, D. S., Boxenbaum, H., Everhart, E. T., Sequeira, G., Mendelson, J. E., & Jones, R. T. (2003). The bioavailability of intranasal and smoked methamphetamine. *Clin Pharmacol Ther*, *74*(5), 475-486. https://doi.org/10.1016/j.clpt.2003.08.002

Hart, C. L., Gunderson, E. W., Perez, A., Kirkpatrick, M. G., Thurmond, A., Comer, S. D., & Foltin, R. W. (2008). Acute physiological and behavioral effects of intranasal methamphetamine in humans. *Neuropsychopharmacology*, *33*(8), 1847-1855. https://doi.org/10.1038/sj.npp.1301578

Haughey, H. M., Brown, J. M., Wilkins, D. G., Hanson, G. R., & Fleckenstein, A. E. (2000). Differential effects of methamphetamine on Na(+)/Cl(-)-dependent transporters. *Brain Res*, *863*(1-2), 59-65. https://doi.org/10.1016/s0006-8993(00)02094-1

Haughey, H. M., Fleckenstein, A. E., Metzger, R. R., & Hanson, G. R. (2000). The effects of methamphetamine on serotonin transporter activity: role of dopamine and hyperthermia. *J Neurochem*, *75*(4), 1608-1617. https://doi.org/10.1046/j.1471-4159.2000.0751608.x

Hayashi, K., Nakao, K., & Nakamura, K. (2015). Appetitive and aversive information coding in the primate dorsal raphe nucleus. *J Neurosci*, *35*(15), 6195-6208. https://doi.org/10.1523/JNEUROSCI.2860-14.2015

Hendricks, T., Francis, N., Fyodorov, D., & Deneris, E. S. (1999). The ETS domain factor Pet-1 is an early and precise marker of central serotonin neurons and interacts with a conserved element in serotonergic genes. *J Neurosci*, *19*(23), 10348-10356. https://doi.org/10.1523/JNEUROSCI.19-23-10348.1999

Hioki, H., Nakamura, H., Ma, Y. F., Konno, M., Hayakawa, T., Nakamura, K. C., Fujiyama, F., & Kaneko, T. (2010). Vesicular glutamate transporter 3-expressing nonserotonergic projection neurons constitute a subregion in the rat midbrain raphe nuclei. *J Comp Neurol*, *518*(5), 668-686. https://doi.org/10.1002/cne.22237

Hitzemann, R., Iancu, O. D., Reed, C., Baba, H., Lockwood, D. R., & Phillips, T. J. (2019). Regional Analysis of the Brain Transcriptome in Mice Bred for High and Low Methamphetamine Consumption. *Brain Sci*, *9*(7). https://doi.org/10.3390/brainsci9070155

Hornung, J.-P. (2010). CHAPTER 1.3 - The Neuronatomy of the Serotonergic System. In C. P. Müller & B. L. Jacobs (Eds.), *Handbook of Behavioral Neuroscience* (Vol. 21, pp. 51-64). Elsevier. https://doi.org/https://doi.org/10.1016/S1569-7339(10)70071-0

Hoyer, D. (2007). 5-HT-2B Receptor. In S. J. Enna & D. B. Bylund (Eds.), *xPharm: The Comprehensive Pharmacology Reference* (pp. 1-9). Elsevier. https://doi.org/https://doi.org/10.1016/B978-008055232-3.60132-1

Huestis, M. A., & Cone, E. J. (2007). Methamphetamine disposition in oral fluid, plasma, and urine. *Ann N Y Acad Sci*, *1098*, 104-121. https://doi.org/10.1196/annals.1384.038

Ide, S., Kobayashi, H., Tanaka, K., Ujike, H., Sekine, Y., Ozaki, N., Inada, T., Harano, M., Komiyama, T., Yamada, M., Iyo, M., Ikeda, K., & Sora, I. (2004). Gene polymorphisms of the mu opioid receptor in methamphetamine abusers. *Ann N Y Acad Sci*, *1025*, 316-324. https://doi.org/10.1196/annals.1316.039

Ingram, S. L., Prasad, B. M., & Amara, S. G. (2002). Dopamine transporter-mediated conductances increase excitability of midbrain dopamine neurons. *Nat Neurosci*, *5*(10), 971-978. https://doi.org/10.1038/nn920

Ishimura, K., Takeuchi, Y., Fujiwara, K., Tominaga, M., Yoshioka, H., & Sawada, T. (1988). Quantitative analysis of the distribution of serotonin-immunoreactive cell bodies in the mouse brain. *Neurosci Lett*, *91*(3), 265-270. https://doi.org/10.1016/0304-3940(88)90691-x

Jayaram-Lindstrom, N., Guterstam, J., Haggkvist, J., Ericson, M., Malmlof, T., Schilstrom, B., Halldin, C., Cervenka, S., Saijo, T., Nordstrom, A. L., & Franck, J. (2017). Naltrexone modulates dopamine release following chronic, but not acute amphetamine administration: a translational study. *Transl Psychiatry*, *7*(4), e1104. https://doi.org/10.1038/tp.2017.79

Jayaram-Lindstrom, N., Hammarberg, A., Beck, O., & Franck, J. (2008). Naltrexone for the treatment of amphetamine dependence: a randomized, placebo-controlled trial. *Am J Psychiatry*, *165*(11), 1442-1448. https://doi.org/10.1176/appi.ajp.2008.08020304

Jayaram-Lindstrom, N., Konstenius, M., Eksborg, S., Beck, O., Hammarberg, A., & Franck, J. (2008). Naltrexone attenuates the subjective effects of amphetamine in patients with

amphetamine dependence. *Neuropsychopharmacology*, *33*(8), 1856-1863. https://doi.org/10.1038/sj.npp.1301572

Jayaram-Lindstrom, N., Wennberg, P., Hurd, Y. L., & Franck, J. (2004). Effects of naltrexone on the subjective response to amphetamine in healthy volunteers. *J Clin Psychopharmacol*, *24*(6), 665-669. https://doi.org/10.1097/01.jcp.0000144893.29987.e5

Johnson, M. D. (1994). Synaptic glutamate release by postnatal rat serotonergic neurons in microculture. *Neuron*, *12*(2), 433-442. https://doi.org/10.1016/0896-6273(94)90283-6

Kahlig, K. M., Binda, F., Khoshbouei, H., Blakely, R. D., McMahon, D. G., Javitch, J. A., & Galli, A. (2005). Amphetamine induces dopamine efflux through a dopamine transporter channel. *Proc Natl Acad Sci U S A*, *102*(9), 3495-3500. https://doi.org/10.1073/pnas.0407737102

Kalivas, P. W., Lalumiere, R. T., Knackstedt, L., & Shen, H. (2009). Glutamate transmission in addiction. *Neuropharmacology*, *56 Suppl 1*(Suppl 1), 169-173. https://doi.org/10.1016/j.neuropharm.2008.07.011

Kamens, H. M., Burkhart-Kasch, S., McKinnon, C. S., Li, N., Reed, C., & Phillips, T. J. (2005). Sensitivity to psychostimulants in mice bred for high and low stimulation to methamphetamine. *Genes Brain Behav*, *4*(2), 110-125. https://doi.org/10.1111/j.1601-183X.2004.00101.x

Karler, R., Calder, L. D., Chaudhry, I. A., & Turkanis, S. A. (1989). Blockade of "reverse tolerance" to cocaine and amphetamine by MK-801. *Life Sci*, *45*(7), 599-606. https://doi.org/10.1016/0024-3205(89)90045-3

Kauer, J. A., & Malenka, R. C. (2007). Synaptic plasticity and addiction. *Nat Rev Neurosci*, *8*(11), 844-858. https://doi.org/10.1038/nrn2234

Keyes, S. R., & Rudnick, G. (1982). Coupling of transmembrane proton gradients to platelet serotonin transport. *J Biol Chem*, *257*(3), 1172-1176. https://www.ncbi.nlm.nih.gov/pubmed/7056713

Khamma, J. K., Copeland, D. S., Hake, H. S., & Gantz, S. C. (2022). Spatiotemporal Control of Noradrenaline-Dependent Synaptic Transmission in Mouse Dorsal Raphe Serotonin Neurons. *J Neurosci*, *42*(6), 968-979. https://doi.org/10.1523/JNEUROSCI.1176-21.2021

Khan, J. I., Kennedy, T. J., & Christian, D. R. (2012). *Basic principles of forensic chemistry*. Springer.

Khawaja, X. (1995). Quantitative autoradiographic characterisation of the binding of [3H]WAY-100635, a selective 5-HT1A receptor antagonist. *Brain Res*, *673*(2), 217-225. https://doi.org/10.1016/0006-8993(94)01416-f

Kim, I., Oyler, J. M., Moolchan, E. T., Cone, E. J., & Huestis, M. A. (2004). Urinary pharmacokinetics of methamphetamine and its metabolite, amphetamine following controlled oral administration to humans. *Ther Drug Monit*, *26*(6), 664-672. https://doi.org/10.1097/00007691-200412000-00013

Kuczenski, R., Segal, D. S., Cho, A. K., & Melega, W. (1995). Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. *J Neurosci*, *15*(2), 1308-1317. https://doi.org/10.1523/JNEUROSCI.15-02-01308.1995

Lemos, J. C., Pan, Y. Z., Ma, X., Lamy, C., Akanwa, A. C., & Beck, S. G. (2006). Selective 5-HT receptor inhibition of glutamatergic and GABAergic synaptic activity in the rat dorsal and median raphe. *Eur J Neurosci*, *24*(12), 3415-3430. https://doi.org/10.1111/j.1460-9568.2006.05222.x

Lett, B. T. (1989). Repeated exposures intensify rather than diminish the rewarding effects of amphetamine, morphine, and cocaine. *Psychopharmacology (Berl)*, *98*(3), 357-362. https://doi.org/10.1007/BF00451687

Li, M. H., Underhill, S. M., Reed, C., Phillips, T. J., Amara, S. G., & Ingram, S. L. (2017). Amphetamine and Methamphetamine Increase NMDAR-GluN2B Synaptic Currents in

Midbrain Dopamine Neurons. *Neuropsychopharmacology*, *42*(7), 1539-1547. https://doi.org/10.1038/npp.2016.278

Li, Y., Zhong, W., Wang, D., Feng, Q., Liu, Z., Zhou, J., Jia, C., Hu, F., Zeng, J., Guo, Q., Fu, L., & Luo, M. (2016). Serotonin neurons in the dorsal raphe nucleus encode reward signals. *Nat Commun*, *7*, 10503. https://doi.org/10.1038/ncomms10503

Lindemann, L., Ebeling, M., Kratochwil, N. A., Bunzow, J. R., Grandy, D. K., & Hoener, M. C. (2005). Trace amine-associated receptors form structurally and functionally distinct subfamilies of novel G protein-coupled receptors. *Genomics*, *85*(3), 372-385. https://doi.org/10.1016/j.ygeno.2004.11.010

Lindemann, L., & Hoener, M. C. (2005). A renaissance in trace amines inspired by a novel GPCR family. *Trends Pharmacol Sci*, *26*(5), 274-281. https://doi.org/10.1016/j.tips.2005.03.007

Lindemann, L., Meyer, C. A., Jeanneau, K., Bradaia, A., Ozmen, L., Bluethmann, H., Bettler, B., Wettstein, J. G., Borroni, E., Moreau, J. L., & Hoener, M. C. (2008). Trace amine-associated receptor 1 modulates dopaminergic activity. *J Pharmacol Exp Ther*, *324*(3), 948-956. https://doi.org/10.1124/jpet.107.132647

Liu, R., Jolas, T., & Aghajanian, G. (2000). Serotonin 5-HT(2) receptors activate local GABA inhibitory inputs to serotonergic neurons of the dorsal raphe nucleus. *Brain Res*, *873*(1), 34-45. https://doi.org/10.1016/s0006-8993(00)02468-9

Liu, Z., Zhou, J., Li, Y., Hu, F., Lu, Y., Ma, M., Feng, Q., Zhang, J. E., Wang, D., Zeng, J., Bao, J., Kim, J. Y., Chen, Z. F., El Mestikawy, S., & Luo, M. (2014). Dorsal raphe neurons signal reward through 5-HT and glutamate. *Neuron*, *81*(6), 1360-1374. https://doi.org/10.1016/j.neuron.2014.02.010

Lominac, K. D., McKenna, C. L., Schwartz, L. M., Ruiz, P. N., Wroten, M. G., Miller, B. W., Holloway, J. J., Travis, K. O., Rajasekar, G., Maliniak, D., Thompson, A. B., Urman, L. E., Phillips, T. J., & Szumlinski, K. K. (2014). Mesocorticolimbic monoamine correlates

of methamphetamine sensitization and motivation. *Front Syst Neurosci*, *8*, 70. https://doi.org/10.3389/fnsys.2014.00070

Lominac, K. D., Quadir, S. G., Barrett, H. M., McKenna, C. L., Schwartz, L. M., Ruiz, P. N., Wroten, M. G., Campbell, R. R., Miller, B. W., Holloway, J. J., Travis, K. O., Rajasekar, G., Maliniak, D., Thompson, A. B., Urman, L. E., Kippin, T. E., Phillips, T. J., & Szumlinski, K. K. (2016). Prefrontal glutamate correlates of methamphetamine sensitization and preference. *Eur J Neurosci*, *43*(5), 689-702. https://doi.org/10.1111/ejn.13159

Mackay, T. F. (2001). The genetic architecture of quantitative traits. *Annu Rev Genet*, *35*, 303-339. https://doi.org/10.1146/annurev.genet.35.102401.090633

Madden, J. T., Reyna, N. C., & Pentkowski, N. S. (2020). Antagonizing serotonin 2A (5-HT(2A)) receptors attenuates methamphetamine-induced reward and blocks methamphetamine-induced anxiety-like behaviors in adult male rats. *Drug Alcohol Depend*, *215*, 108178. https://doi.org/10.1016/j.drugalcdep.2020.108178

Mager, S., Min, C., Henry, D. J., Chavkin, C., Hoffman, B. J., Davidson, N., & Lester, H. A. (1994). Conducting states of a mammalian serotonin transporter. *Neuron*, *12*(4), 845-859. https://doi.org/10.1016/0896-6273(94)90337-9

Maiese, K., Ahmad, I., TenBroeke, M., & Gallant, J. (1999). Metabotropic glutamate receptor subtypes independently modulate neuronal intracellular calcium. *J Neurosci Res*, *55*(4), 472-485. https://doi.org/10.1002/(SICI)1097-4547(19990215)55:4<472::AID-JNR7>3.0.CO;2-Q

Maj, J., Chojnacka-Wojcik, E., Tatarczynska, E., & Klodzinska, A. (1987). Central action of ipsapirone, a new anxiolytic drug, on serotoninergic, noradrenergic and dopaminergic functions. *J Neural Transm*, *70*(1-2), 1-17. https://doi.org/10.1007/BF01252505

Mao, L. M., Wang, W., Chu, X. P., Zhang, G. C., Liu, X. Y., Yang, Y. J., Haines, M., Papasian, C. J., Fibuch, E. E., Buch, S., Chen, J. G., & Wang, J. Q. (2009). Stability of surface NMDA

receptors controls synaptic and behavioral adaptations to amphetamine. *Nat Neurosci*, *12*(5), 602-610. https://doi.org/10.1038/nn.2300

Marcinkiewicz, M., Verge, D., Gozlan, H., Pichat, L., & Hamon, M. (1984). Autoradiographic evidence for the heterogeneity of 5-HT1 sites in the rat brain. *Brain Res*, *291*(1), 159-163. https://doi.org/10.1016/0006-8993(84)90664-4

Martin, L. P., Jackson, D. M., Wallsten, C., & Waszczak, B. L. (1999). Electrophysiological comparison of 5-Hydroxytryptamine1A receptor antagonists on dorsal raphe cell firing. *J Pharmacol Exp Ther*, *288*(2), 820-826. https://www.ncbi.nlm.nih.gov/pubmed/9918594

Matsumoto, M., & Hikosaka, O. (2007). Lateral habenula as a source of negative reward signals in dopamine neurons. *Nature*, *447*(7148), 1111-1115. https://doi.org/10.1038/nature05860

May, A. C., Aupperle, R. L., & Stewart, J. L. (2020). Dark Times: The Role of Negative Reinforcement in Methamphetamine Addiction. *Front Psychiatry*, *11*, 114. https://doi.org/10.3389/fpsyt.2020.00114

Mazna, P., Grycova, L., Balik, A., Zemkova, H., Friedlova, E., Obsilova, V., Obsil, T., & Teisinger, J. (2008). The role of proline residues in the structure and function of human MT2 melatonin receptor. *J Pineal Res*, *45*(4), 361-372. https://doi.org/10.1111/j.1600-079X.2008.00598.x

McDevitt, R. A., & Neumaier, J. F. (2011). Regulation of dorsal raphe nucleus function by serotonin autoreceptors: a behavioral perspective. *J Chem Neuroanat*, *41*(4), 234-246. https://doi.org/10.1016/j.jchemneu.2011.05.001

Meredith, C. W., Jaffe, C., Ang-Lee, K., & Saxon, A. J. (2005). Implications of chronic methamphetamine use: a literature review. *Harv Rev Psychiatry*, *13*(3), 141-154. https://doi.org/10.1080/10673220591003605

Millan, M. J., Schreiber, R., Dekeyne, A., Rivet, J. M., Bervoets, K., Mavridis, M., Sebban, C., Maurel-Remy, S., Newman-Tancredi, A., Spedding, M., Muller, O., Lavielle, G., & Brocco, M. (1998). S 16924 ((R)-2-[1-[2-(2,3-dihydro-benzo[1,4] dioxin-5-yloxy)-ethyl]-

pyrrolidin-3yl]-1-(4-fluoro-phenyl)-ethanone), a novel, potential antipsychotic with marked serotonin (5-HT)1A agonist properties: II. Functional profile in comparison to clozapine and haloperidol. *J Pharmacol Exp Ther*, *286*(3), 1356-1373. https://www.ncbi.nlm.nih.gov/pubmed/9732399

Miszkiel, J., Adamczyk, P., Filip, M., & Przegalinski, E. (2012). The effect of serotonin 5HT1B receptor ligands on amphetamine self-administration in rats. *Eur J Pharmacol*, *677*(1-3), 111-115. https://doi.org/10.1016/j.ejphar.2011.12.033

Miszkiel, J., & Przegalinski, E. (2013). Effects of serotonin (5-HT)1B receptor ligands on amphetamine-seeking behavior in rats. *Pharmacol Rep*, *65*(4), 813-822. https://doi.org/10.1016/s1734-1140(13)71062-4

Mootz, J. R. K., Miner, N. B., & Phillips, T. J. (2020). Differential genetic risk for methamphetamine intake confers differential sensitivity to the temperature-altering effects of other addictive drugs. *Genes Brain Behav*, *19*(5), e12640. https://doi.org/10.1111/gbb.12640

Mootz, J. R. K., & Phillips, T. J. (*unpublished*). *The glutamate system in a model of high genetic risk for methamphetamine intake*. Oregon Health and Science University.

Moroz, I., Parker, L. A., & Siegel, S. (1997). Ibogaine interferes with the establishment of amphetamine place preference learning. *Exp Clin Psychopharmacol*, *5*(2), 119-122. https://doi.org/10.1037//1064-1297.5.2.119

Moser, P. C., Moran, P. M., Frank, R. A., & Kehne, J. H. (1996). Reversal of amphetamine-induced behaviours by MDL 100,907, a selective 5-HT2A antagonist. *Behav Brain Res*, *73*(1-2), 163-167. https://doi.org/10.1016/0166-4328(96)00090-3

Muller, C. P., & Homberg, J. R. (2015). The role of serotonin in drug use and addiction. *Behav Brain Res*, *277*, 146-192. https://doi.org/10.1016/j.bbr.2014.04.007

Nagai, Y., Takayama, K., Nishitani, N., Andoh, C., Koda, M., Shirakawa, H., Nakagawa, T., Nagayasu, K., Yamanaka, A., & Kaneko, S. (2020). The Role of Dorsal Raphe Serotonin

Neurons in the Balance between Reward and Aversion. *Int J Mol Sci*, *21*(6). https://doi.org/10.3390/ijms21062160

Nakamura, K. (2013). The role of the dorsal raphe nucleus in reward-seeking behavior. *Front Integr Neurosci*, *7*, 60. https://doi.org/10.3389/fnint.2013.00060

Nakamura, K., Matsumoto, M., & Hikosaka, O. (2008). Reward-dependent modulation of neuronal activity in the primate dorsal raphe nucleus. *J Neurosci*, *28*(20), 5331-5343. https://doi.org/10.1523/JNEUROSCI.0021-08.2008

Neve, K. A., Seamans, J. K., & Trantham-Davidson, H. (2004). Dopamine receptor signaling. *J Recept Signal Transduct Res*, *24*(3), 165-205. https://doi.org/10.1081/rrs-200029981

Ni, Y. G., Chen, J. G., Androutsellis-Theotokis, A., Huang, C. J., Moczydlowski, E., & Rudnick, G. (2001). A lithium-induced conformational change in serotonin transporter alters cocaine binding, ion conductance, and reactivity of Cys-109. *J Biol Chem*, *276*(33), 30942-30947. https://doi.org/10.1074/jbc.M104653200

Nomikos, G. G., & Spyraki, C. (1988). Effects of ritanserin on the rewarding properties of d-amphetamine, morphine and diazepam revealed by conditioned place preference in rats. *Pharmacol Biochem Behav*, *30*(4), 853-858. https://doi.org/10.1016/0091-3057(88)90110-4

Nordahl, T. E., Salo, R., & Leamon, M. (2003). Neuropsychological effects of chronic methamphetamine use on neurotransmitters and cognition: a review. *J Neuropsychiatry Clin Neurosci*, *15*(3), 317-325. https://doi.org/10.1176/jnp.15.3.317

O'Neill, M. F., Heron-Maxwell, C. L., & Shaw, G. (1999). 5-HT2 receptor antagonism reduces hyperactivity induced by amphetamine, cocaine, and MK-801 but not D1 agonist C-APB. *Pharmacol Biochem Behav*, *63*(2), 237-243. https://doi.org/10.1016/s0091-3057(98)00240-8

Ogawa, S. K., Cohen, J. Y., Hwang, D., Uchida, N., & Watabe-Uchida, M. (2014). Organization of monosynaptic inputs to the serotonin and dopamine neuromodulatory systems. *Cell Rep*, *8*(4), 1105-1118. https://doi.org/10.1016/j.celrep.2014.06.042

Olausson, P., Engel, J. A., & Soderpalm, B. (2000). Effects of serotonergic manipulations on the behavioral sensitization and disinhibition associated with repeated amphetamine treatment. *Pharmacol Biochem Behav*, *66*(1), 211-220. https://doi.org/10.1016/s0091-3057(00)00228-8

Oyler, J. M., Cone, E. J., Joseph, R. E., Jr., Moolchan, E. T., & Huestis, M. A. (2002). Duration of detectable methamphetamine and amphetamine excretion in urine after controlled oral administration of methamphetamine to humans. *Clin Chem*, *48*(10), 1703-1714. https://www.ncbi.nlm.nih.gov/pubmed/12324487

Palmer, A., Phillips, T. (2002). *Quantitative trait locus (QTL) mapping in mice.* (L. D. M. Liu Y., Ed.). https://doi.org/https://doi.org/10.1201/9781420042092

Panenka, W. J., Procyshyn, R. M., Lecomte, T., MacEwan, G. W., Flynn, S. W., Honer, W. G., & Barr, A. M. (2013). Methamphetamine use: a comprehensive review of molecular, preclinical and clinical findings. *Drug Alcohol Depend*, *129*(3), 167-179. https://doi.org/10.1016/j.drugalcdep.2012.11.016

Papp, M., Gruca, P., & Willner, P. (2002). Selective blockade of drug-induced place preference conditioning by ACPC, a functional NDMA-receptor antagonist. *Neuropsychopharmacology*, *27*(5), 727-743. https://doi.org/10.1016/S0893-133X(02)00349-4

Parsons, N. L. (2013). Lynne Rienner Publishers. https://doi.org/doi:10.1515/9781626373877

Penington, N. J., & Kelly, J. S. (1990). Serotonin receptor activation reduces calcium current in an acutely dissociated adult central neuron. *Neuron*, *4*(5), 751-758. https://doi.org/10.1016/0896-6273(90)90201-p

Penington, N. J., Kelly, J. S., & Fox, A. P. (1993). Whole-cell recordings of inwardly rectifying K+ currents activated by 5-HT1A receptors on dorsal raphe neurones of the adult rat. *J Physiol*, *469*, 387-405. https://doi.org/10.1113/jphysiol.1993.sp019819

Perez-Reyes, M., White, W. R., McDonald, S. A., Hill, J. M., Jeffcoat, A. R., & Cook, C. E. (1991). Clinical effects of methamphetamine vapor inhalation. *Life Sci*, *49*(13), 953-959. https://doi.org/10.1016/0024-3205(91)90078-p

Peter, D., Jimenez, J., Liu, Y., Kim, J., & Edwards, R. H. (1994). The chromaffin granule and synaptic vesicle amine transporters differ in substrate recognition and sensitivity to inhibitors. *J Biol Chem*, *269*(10), 7231-7237. https://www.ncbi.nlm.nih.gov/pubmed/8125935

Peter, D., Liu, Y., Sternini, C., de Giorgio, R., Brecha, N., & Edwards, R. H. (1995). Differential expression of two vesicular monoamine transporters. *J Neurosci*, *15*(9), 6179-6188. https://doi.org/10.1523/JNEUROSCI.15-09-06179.1995

Phillips, T. J., Broadbent, J., Burkhart-Kasch, S., Henderson, C., Wenger, C. D., McMullin, C., McKinnon, C. S., & Cunningham, C. L. (2005). Genetic correlational analyses of ethanol reward and aversion phenotypes in short-term selected mouse lines bred for ethanol drinking or ethanol-induced conditioned taste aversion. *Behav Neurosci*, *119*(4), 892-910. https://doi.org/10.1037/0735-7044.119.4.892

Phillips, T. J., Roy, T., Aldrich, S. J., Baba, H., Erk, J., Mootz, J. R. K., Reed, C., & Chesler, E. J. (2021). Confirmation of a Causal Taar1 Allelic Variant in Addiction-Relevant Methamphetamine Behaviors. *Front Psychiatry*, *12*, 725839. https://doi.org/10.3389/fpsyt.2021.725839

Phillips, T. J., & Shabani, S. (2015). An animal model of differential genetic risk for methamphetamine intake. *Front Neurosci*, *9*, 327. https://doi.org/10.3389/fnins.2015.00327

Pin, J. P., & Duvoisin, R. (1995). The metabotropic glutamate receptors: structure and functions. *Neuropharmacology*, *34*(1), 1-26. https://doi.org/10.1016/0028-3908(94)00129-g

Pollak Dorocic, I., Furth, D., Xuan, Y., Johansson, Y., Pozzi, L., Silberberg, G., Carlen, M., & Meletis, K. (2014). A whole-brain atlas of inputs to serotonergic neurons of the dorsal and median raphe nuclei. *Neuron*, *83*(3), 663-678. https://doi.org/10.1016/j.neuron.2014.07.002

Polter, A. M., & Li, X. (2010). 5-HT1A receptor-regulated signal transduction pathways in brain. *Cell Signal*, *22*(10), 1406-1412. https://doi.org/10.1016/j.cellsig.2010.03.019

Pompeiano, M., Palacios, J. M., & Mengod, G. (1992). Distribution and cellular localization of mRNA coding for 5-HT1A receptor in the rat brain: correlation with receptor binding. *J Neurosci*, *12*(2), 440-453. https://doi.org/10.1523/JNEUROSCI.12-02-00440.1992

Porrino, L. J., Ritz, M. C., Goodman, N. L., Sharpe, L. G., Kuhar, M. J., & Goldberg, S. R. (1989). Differential effects of the pharmacological manipulation of serotonin systems on cocaine and amphetamine self-administration in rats. *Life Sci*, *45*(17), 1529-1535. https://doi.org/10.1016/0024-3205(89)90418-9

Przegalinski, E., & Filip, M. (1997). Stimulation of serotonin (5-HT)1A receptors attenuates the locomotor, but not the discriminative, effects of amphetamine and cocaine in rats. *Behav Pharmacol*, *8*(8), 699-706. https://doi.org/10.1097/00008877-199712000-00004

Przegalinski, E., Siwanowicz, J., Baran, L., & Filip, M. (2000). Activation of serotonin (5-HT)1A receptors inhibits amphetamine sensitization in mice. *Life Sci*, *66*(11), 1011-1019. https://doi.org/10.1016/s0024-3205(99)00666-9

Qi, J., Zhang, S., Wang, H. L., Wang, H., de Jesus Aceves Buendia, J., Hoffman, A. F., Lupica, C. R., Seal, R. P., & Morales, M. (2014). A glutamatergic reward input from the dorsal raphe to ventral tegmental area dopamine neurons. *Nat Commun*, *5*, 5390. https://doi.org/10.1038/ncomms6390

Quentin, E., Belmer, A., & Maroteaux, L. (2018). Somato-Dendritic Regulation of Raphe Serotonin Neurons; A Key to Antidepressant Action. *Front Neurosci*, *12*, 982. https://doi.org/10.3389/fnins.2018.00982

Reed, C., Baba, H., Zhu, Z., Erk, J., Mootz, J. R., Varra, N. M., Williams, R. W., & Phillips, T. J. (2018). A Spontaneous Mutation in Taar1 Impacts Methamphetamine-Related Traits Exclusively in DBA/2 Mice from a Single Vendor. *Front Pharmacol*, *8*, 993. https://doi.org/10.3389/fphar.2017.00993

Reese, E. A., Norimatsu, Y., Grandy, M. S., Suchland, K. L., Bunzow, J. R., & Grandy, D. K. (2014). Exploring the determinants of trace amine-associated receptor 1's functional selectivity for the stereoisomers of amphetamine and methamphetamine. *J Med Chem*, *57*(2), 378-390. https://doi.org/10.1021/jm401316v

Reiersen, H., & Rees, A. R. (2001). The hunchback and its neighbours: proline as an environmental modulator. *Trends Biochem Sci*, *26*(11), 679-684. https://doi.org/10.1016/s0968-0004(01)01957-0

Ren, J., Friedmann, D., Xiong, J., Liu, C. D., Ferguson, B. R., Weerakkody, T., DeLoach, K. E., Ran, C., Pun, A., Sun, Y., Weissbourd, B., Neve, R. L., Huguenard, J., Horowitz, M. A., & Luo, L. (2018). Anatomically Defined and Functionally Distinct Dorsal Raphe Serotonin Sub-systems. *Cell*, *175*(2), 472-487 e420. https://doi.org/10.1016/j.cell.2018.07.043

Revel, F. G., Meyer, C. A., Bradaia, A., Jeanneau, K., Calcagno, E., Andre, C. B., Haenggi, M., Miss, M. T., Galley, G., Norcross, R. D., Invernizzi, R. W., Wettstein, J. G., Moreau, J. L., & Hoener, M. C. (2012). Brain-specific overexpression of trace amine-associated receptor 1 alters monoaminergic neurotransmission and decreases sensitivity to amphetamine. *Neuropsychopharmacology*, *37*(12), 2580-2592. https://doi.org/10.1038/npp.2012.109

Revel, F. G., Moreau, J. L., Gainetdinov, R. R., Bradaia, A., Sotnikova, T. D., Mory, R., Durkin, S., Zbinden, K. G., Norcross, R., Meyer, C. A., Metzler, V., Chaboz, S., Ozmen, L., Trube, G., Pouzet, B., Bettler, B., Caron, M. G., Wettstein, J. G., & Hoener, M. C. (2011). TAAR1 activation modulates monoaminergic neurotransmission, preventing hyperdopaminergic and hypoglutamatergic activity. *Proc Natl Acad Sci U S A*, *108*(20), 8485-8490. https://doi.org/10.1073/pnas.1103029108

Riad, M., Garcia, S., Watkins, K. C., Jodoin, N., Doucet, E., Langlois, X., el Mestikawy, S., Hamon, M., & Descarries, L. (2000). Somatodendritic localization of 5-HT1A and preterminal axonal localization of 5-HT1B serotonin receptors in adult rat brain. *J Comp Neurol*, *417*(2), 181-194. https://www.ncbi.nlm.nih.gov/pubmed/10660896

Rios, S. M., Mootz, J. R. K., Phillips, T. J., & Ingram, S. L. (2025). Absence of TAAR1 function increases methamphetamine-induced excitability of dorsal raphe serotonin neurons and drives binge-level methamphetamine intake. *Neuropsychopharmacology*. https://doi.org/10.1038/s41386-025-02063-w

Rothman, R. B., & Baumann, M. H. (2003). Monoamine transporters and psychostimulant drugs. *Eur J Pharmacol*, *479*(1-3), 23-40. https://doi.org/10.1016/j.ejphar.2003.08.054

Rothman, R. B., Baumann, M. H., Dersch, C. M., Romero, D. V., Rice, K. C., Carroll, F. I., & Partilla, J. S. (2001). Amphetamine-type central nervous system stimulants release norepinephrine more potently than they release dopamine and serotonin. *Synapse*, *39*(1), 32-41. https://doi.org/10.1002/1098-2396(20010101)39:1<32::AID-SYN5>3.0.CO;2-3

Rudnick, G., & Nelson, P. J. (1978). Platelet 5-hydroxytryptamine transport, an electroneutral mechanism coupled to potassium. *Biochemistry*, *17*(22), 4739-4742. https://doi.org/10.1021/bi00615a021

Rutigliano, G., Accorroni, A., & Zucchi, R. (2017). The Case for TAAR1 as a Modulator of Central Nervous System Function. *Front Pharmacol*, *8*, 987. https://doi.org/10.3389/fphar.2017.00987

Sabino, V., Narayan, A. R., Zeric, T., Steardo, L., & Cottone, P. (2013). mTOR activation is required for the anti-alcohol effect of ketamine, but not memantine, in alcohol-preferring rats. *Behav Brain Res*, *247*, 9-16. https://doi.org/10.1016/j.bbr.2013.02.030

Sahara, Y., & Westbrook, G. L. (1993). Modulation of calcium currents by a metabotropic glutamate receptor involves fast and slow kinetic components in cultured hippocampal neurons. *J Neurosci*, *13*(7), 3041-3050. https://doi.org/10.1523/JNEUROSCI.13-07-03041.1993

Sansom, M. S., & Weinstein, H. (2000). Hinges, swivels and switches: the role of prolines in signalling via transmembrane alpha-helices. *Trends Pharmacol Sci*, *21*(11), 445-451. https://doi.org/10.1016/s0165-6147(00)01553-4

Sayer, R. J., Schwindt, P. C., & Crill, W. E. (1992). Metabotropic glutamate receptor-mediated suppression of L-type calcium current in acutely isolated neocortical neurons. *J Neurophysiol*, *68*(3), 833-842. https://doi.org/10.1152/jn.1992.68.3.833

Schafer, M. K., Varoqui, H., Defamie, N., Weihe, E., & Erickson, J. D. (2002). Molecular cloning and functional identification of mouse vesicular glutamate transporter 3 and its expression in subsets of novel excitatory neurons. *J Biol Chem*, *277*(52), 50734-50748. https://doi.org/10.1074/jbc.M206738200

Schep, L. J., Slaughter, R. J., & Beasley, D. M. (2010). The clinical toxicology of metamfetamine. *Clin Toxicol (Phila)*, *48*(7), 675-694. https://doi.org/10.3109/15563650.2010.516752

Schepers, R. J., Oyler, J. M., Joseph, R. E., Jr., Cone, E. J., Moolchan, E. T., & Huestis, M. A. (2003). Methamphetamine and amphetamine pharmacokinetics in oral fluid and plasma after controlled oral methamphetamine administration to human volunteers. *Clin Chem*, *49*(1), 121-132. https://doi.org/10.1373/49.1.121

Schicker, K., Uzelac, Z., Gesmonde, J., Bulling, S., Stockner, T., Freissmuth, M., Boehm, S., Rudnick, G., Sitte, H. H., & Sandtner, W. (2012). Unifying concept of serotonin

transporter-associated currents. *J Biol Chem*, *287*(1), 438-445. https://doi.org/10.1074/jbc.M111.304261

Schmidt, C. J., Levin, J. A., & Lovenberg, W. (1987). In vitro and in vivo neurochemical effects of methylenedioxymethamphetamine on striatal monoaminergic systems in the rat brain. *Biochem Pharmacol*, *36*(5), 747-755. https://doi.org/10.1016/0006-2952(87)90729-5

Schwartz, K., Weizman, A., & Rehavi, M. (2006). The effect of psychostimulants on [3H]dopamine uptake and release in rat brain synaptic vesicles. *J Neural Transm (Vienna)*, *113*(9), 1347-1352. https://doi.org/10.1007/s00702-005-0383-4

Schwartz, T. W., Frimurer, T. M., Holst, B., Rosenkilde, M. M., & Elling, C. E. (2006). Molecular mechanism of 7TM receptor activation--a global toggle switch model. *Annu Rev Pharmacol Toxicol*, *46*, 481-519. https://doi.org/10.1146/annurev.pharmtox.46.120604.141218

Scibelli, A. C., McKinnon, C. S., Reed, C., Burkhart-Kasch, S., Li, N., Baba, H., Wheeler, J. M., & Phillips, T. J. (2011). Selective breeding for magnitude of methamphetamine-induced sensitization alters methamphetamine consumption. *Psychopharmacology (Berl)*, *214*(4), 791-804. https://doi.org/10.1007/s00213-010-2086-2

Shabani, S., Dobbs, L. K., Ford, M. M., Mark, G. P., Finn, D. A., & Phillips, T. J. (2012). A genetic animal model of differential sensitivity to methamphetamine reinforcement. *Neuropharmacology*, *62*(7), 2169-2177. https://doi.org/10.1016/j.neuropharm.2012.01.002

Shabani, S., Houlton, S., Ghimire, B., Tonello, D., Reed, C., Baba, H., Aldrich, S., & Phillips, T. J. (2023). Robust aversive effects of trace amine-associated receptor 1 activation in mice. *Neuropsychopharmacology*, *48*(10), 1446-1454. https://doi.org/10.1038/s41386-023-01578-4

Shabani, S., Houlton, S. K., Hellmuth, L., Mojica, E., Mootz, J. R., Zhu, Z., Reed, C., & Phillips, T. J. (2016). A Mouse Model for Binge-Level Methamphetamine Use. *Front Neurosci*, *10*, 493. https://doi.org/10.3389/fnins.2016.00493

Shabani, S., McKinnon, C. S., Cunningham, C. L., & Phillips, T. J. (2012). Profound reduction in sensitivity to the aversive effects of methamphetamine in mice bred for high methamphetamine intake. *Neuropharmacology*, *62*(2), 1134-1141. https://doi.org/10.1016/j.neuropharm.2011.11.005

Shabani, S., McKinnon, C. S., Reed, C., Cunningham, C. L., & Phillips, T. J. (2011). Sensitivity to rewarding or aversive effects of methamphetamine determines methamphetamine intake. *Genes Brain Behav*, *10*(6), 625-636. https://doi.org/10.1111/j.1601-183X.2011.00700.x

Shi, X., Walter, N. A., Harkness, J. H., Neve, K. A., Williams, R. W., Lu, L., Belknap, J. K., Eshleman, A. J., Phillips, T. J., & Janowsky, A. (2016). Genetic Polymorphisms Affect Mouse and Human Trace Amine-Associated Receptor 1 Function. *PLoS One*, *11*(3), e0152581. https://doi.org/10.1371/journal.pone.0152581

Shikanai, H., Yoshida, T., Konno, K., Yamasaki, M., Izumi, T., Ohmura, Y., Watanabe, M., & Yoshioka, M. (2012). Distinct neurochemical and functional properties of GAD67-containing 5-HT neurons in the rat dorsal raphe nucleus. *J Neurosci*, *32*(41), 14415-14426. https://doi.org/10.1523/JNEUROSCI.5929-11.2012

Sitte, H. H., Huck, S., Reither, H., Boehm, S., Singer, E. A., & Pifl, C. (1998). Carrier-mediated release, transport rates, and charge transfer induced by amphetamine, tyramine, and dopamine in mammalian cells transfected with the human dopamine transporter. *J Neurochem*, *71*(3), 1289-1297. https://doi.org/10.1046/j.1471-4159.1998.71031289.x

Solinas, M., Belujon, P., Fernagut, P. O., Jaber, M., & Thiriet, N. (2019). Dopamine and addiction: what have we learned from 40 years of research. *J Neural Transm (Vienna)*, *126*(4), 481-516. https://doi.org/10.1007/s00702-018-1957-2

Sprouse, J. S., & Aghajanian, G. K. (1986). (-)-Propranolol blocks the inhibition of serotonergic dorsal raphe cell firing by 5-HT1A selective agonists. *Eur J Pharmacol*, *128*(3), 295-298. https://doi.org/10.1016/0014-2999(86)90782-x

Sprouse, J. S., & Aghajanian, G. K. (1987). Electrophysiological responses of serotoninergic dorsal raphe neurons to 5-HT1A and 5-HT1B agonists. *Synapse*, *1*(1), 3-9. https://doi.org/10.1002/syn.890010103

Sprouse, J. S., Bradberry, C. W., Roth, R. H., & Aghajanian, G. K. (1989). MDMA (3,4-methylenedioxymethamphetamine) inhibits the firing of dorsal raphe neurons in brain slices via release of serotonin. *Eur J Pharmacol*, *167*(3), 375-383. https://doi.org/10.1016/0014-2999(89)90446-9

Stafford, A. M., Reed, C., Baba, H., Walter, N. A., Mootz, J. R., Williams, R. W., Neve, K. A., Fedorov, L. M., Janowsky, A. J., & Phillips, T. J. (2019). Taar1 gene variants have a causal role in methamphetamine intake and response and interact with Oprm1. *Elife*, *8*. https://doi.org/10.7554/eLife.46472

Stefani, A., Pisani, A., Mercuri, N. B., Bernardi, G., & Calabresi, P. (1994). Activation of metabotropic glutamate receptors inhibits calcium currents and GABA-mediated synaptic potentials in striatal neurons. *J Neurosci*, *14*(11 Pt 1), 6734-6743. https://doi.org/10.1523/JNEUROSCI.14-11-06734.1994

Sucic, S., Dallinger, S., Zdrazil, B., Weissensteiner, R., Jorgensen, T. N., Holy, M., Kudlacek, O., Seidel, S., Cha, J. H., Gether, U., Newman, A. H., Ecker, G. F., Freissmuth, M., & Sitte, H. H. (2010). The N terminus of monoamine transporters is a lever required for the action of amphetamines. *J Biol Chem*, *285*(14), 10924-10938. https://doi.org/10.1074/jbc.M109.083154

Sugiyama, H., Ito, I., & Hirono, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature*, *325*(6104), 531-533. https://doi.org/10.1038/325531a0

Sulzer, D., & Rayport, S. (1990). Amphetamine and other psychostimulants reduce pH gradients in midbrain dopaminergic neurons and chromaffin granules: A mechanism of action. *Neuron*, *5*(6), 797-808. https://doi.org/10.1016/0896-6273(90)90339-h

Sulzer, D., Sonders, M. S., Poulsen, N. W., & Galli, A. (2005). Mechanisms of neurotransmitter release by amphetamines: a review. *Prog Neurobiol*, *75*(6), 406-433. https://doi.org/10.1016/j.pneurobio.2005.04.003

Swartz, K. J., & Bean, B. P. (1992). Inhibition of calcium channels in rat CA3 pyramidal neurons by a metabotropic glutamate receptor. *J Neurosci*, *12*(11), 4358-4371. https://doi.org/10.1523/JNEUROSCI.12-11-04358.1992

Szumlinski, K. K., Lominac, K. D., Campbell, R. R., Cohen, M., Fultz, E. K., Brown, C. N., Miller, B. W., Quadir, S. G., Martin, D., Thompson, A. B., von Jonquieres, G., Klugmann, M., Phillips, T. J., & Kippin, T. E. (2017). Methamphetamine Addiction Vulnerability: The Glutamate, the Bad, and the Ugly. *Biol Psychiatry*, *81*(11), 959-970. https://doi.org/10.1016/j.biopsych.2016.10.005

Tabbara, R. I., Li, Z., Fletcher, P. J., & Le, A. D. (2021). The serotonin 2C receptor agonist lorcaserin, alone and in combination with the opioid receptor antagonist naltrexone, attenuates binge-like ethanol drinking. *Addict Biol*, *26*(5), e13040. https://doi.org/10.1111/adb.13040

Takamatsu, Y., Yamamoto, H., Hagino, Y., Markou, A., & Ikeda, K. (2011). The Selective Serotonin Reuptake Inhibitor Paroxetine, but not Fluvoxamine, Decreases Methamphetamine Conditioned Place Preference in Mice. *Curr Neuropharmacol*, *9*(1), 68-72. https://doi.org/10.2174/157015911795017236

Takamatsu, Y., Yamamoto, H., Ogai, Y., Hagino, Y., Markou, A., & Ikeda, K. (2006). Fluoxetine as a potential pharmacotherapy for methamphetamine dependence: studies in mice. *Ann N Y Acad Sci*, *1074*, 295-302. https://doi.org/10.1196/annals.1369.026

Teitler, M., Leonhardt, S., Appel, N. M., De Souza, E. B., & Glennon, R. A. (1990). Receptor pharmacology of MDMA and related hallucinogens. *Ann N Y Acad Sci*, *600*, 626-638; discussion 638-629. https://doi.org/10.1111/j.1749-6632.1990.tb16915.x

Tiger, M., Varnas, K., Okubo, Y., & Lundberg, J. (2018). The 5-HT(1B) receptor - a potential target for antidepressant treatment. *Psychopharmacology (Berl)*, *235*(5), 1317-1334. https://doi.org/10.1007/s00213-018-4872-1

Torres, B., & Ruoho, A. E. (2014). N-terminus regulation of VMAT2 mediates methamphetamine-stimulated efflux. *Neuroscience*, *259*, 194-202. https://doi.org/10.1016/j.neuroscience.2013.11.059

Tuv, S. S., Bergh, M. S., Andersen, J. M., Steinsland, S., Vindenes, V., Baumann, M. H., Huestis, M. A., & Bogen, I. L. (2021). Comparative Neuropharmacology and Pharmacokinetics of Methamphetamine and Its Thiophene Analog Methiopropamine in Rodents. *Int J Mol Sci*, *22*(21). https://doi.org/10.3390/ijms222112002

Tzschentke, T. M., & Schmidt, W. J. (2003). Glutamatergic mechanisms in addiction. *Mol Psychiatry*, *8*(4), 373-382. https://doi.org/10.1038/sj.mp.4001269

Uhl, G. R., Drgon, T., Johnson, C., Li, C. Y., Contoreggi, C., Hess, J., Naiman, D., & Liu, Q. R. (2008). Molecular genetics of addiction and related heritable phenotypes: genome-wide association approaches identify "connectivity constellation" and drug target genes with pleiotropic effects. *Ann N Y Acad Sci*, *1141*, 318-381. https://doi.org/10.1196/annals.1441.018

Underhill, S., & Amara, S. (2020). MDMA and TAAR1-mediated RhoA Activation in Serotonin Neurons. *The FASEB Journal*, *34*(S1), 1-1. https://doi.org/https://doi.org/10.1096/fasebj.2020.34.s1.05856

Underhill, S. M., Colt, M. S., & Amara, S. G. (2020). Amphetamine Stimulates Endocytosis of the Norepinephrine and Neuronal Glutamate Transporters in Cultured Locus Coeruleus Neurons. *Neurochem Res*, *45*(6), 1410-1419. https://doi.org/10.1007/s11064-019-02939-6

Underhill, S. M., Hullihen, P. D., Chen, J., Fenollar-Ferrer, C., Rizzo, M. A., Ingram, S. L., & Amara, S. G. (2019). Amphetamines signal through intracellular TAAR1 receptors coupled to Galpha13 and GalphaS in discrete subcellular domains. *Mol Psychiatry*, *26*(4), 1208-1223. https://doi.org/10.1038/s41380-019-0469-2

Underhill, S. M., Wheeler, D. S., Li, M., Watts, S. D., Ingram, S. L., & Amara, S. G. (2014). Amphetamine modulates excitatory neurotransmission through endocytosis of the glutamate transporter EAAT3 in dopamine neurons. *Neuron*, *83*(2), 404-416. https://doi.org/10.1016/j.neuron.2014.05.043

Van Arnam, E. B., Lester, H. A., & Dougherty, D. A. (2011). Dissecting the functions of conserved prolines within transmembrane helices of the D2 dopamine receptor. *ACS Chem Biol*, *6*(10), 1063-1068. https://doi.org/10.1021/cb200153g

van Huijstee, A. N., & Mansvelder, H. D. (2014). Glutamatergic synaptic plasticity in the mesocorticolimbic system in addiction. *Front Cell Neurosci*, *8*, 466. https://doi.org/10.3389/fncel.2014.00466

Varnas, K., Hall, H., Bonaventure, P., & Sedvall, G. (2001). Autoradiographic mapping of 5-HT(1B) and 5-HT(1D) receptors in the post mortem human brain using [(3)H]GR 125743. *Brain Res*, *915*(1), 47-57. https://doi.org/10.1016/s0006-8993(01)02823-2

Verge, D., Daval, G., Marcinkiewicz, M., Patey, A., el Mestikawy, S., Gozlan, H., & Hamon, M. (1986). Quantitative autoradiography of multiple 5-HT1 receptor subtypes in the brain of control or 5,7-dihydroxytryptamine-treated rats. *J Neurosci*, *6*(12), 3474-3482. https://doi.org/10.1523/JNEUROSCI.06-12-03474.1986

Wagner, D. J., Shireman, L. M., Ahn, S., Shen, D. D., & Wang, J. (2018). Disposition of Methamphetamine and Major Metabolites in Mice: Role of Organic Cation Transporter 3 in Tissue-Selective Accumulation of Para-Hydroxymethamphetamine. *Drug Metab Dispos*, *46*(9), 1277-1284. https://doi.org/10.1124/dmd.118.082131

Wang, D., Li, Y., Feng, Q., Guo, Q., Zhou, J., & Luo, M. (2017). Learning shapes the aversion and reward responses of lateral habenula neurons. *Elife*, *6*. https://doi.org/10.7554/eLife.23045

Wang, H. L., Zhang, S., Qi, J., Wang, H., Cachope, R., Mejias-Aponte, C. A., Gomez, J. A., Mateo-Semidey, G. E., Beaudoin, G. M. J., Paladini, C. A., Cheer, J. F., & Morales, M. (2019). Dorsal Raphe Dual Serotonin-Glutamate Neurons Drive Reward by Establishing Excitatory Synapses on VTA Mesoaccumbens Dopamine Neurons. *Cell Rep*, *26*(5), 1128-1142 e1127. https://doi.org/10.1016/j.celrep.2019.01.014

Wang, R. Y., & Aghajanian, G. K. (1977). Physiological evidence for habenula as major link between forebrain and midbrain raphe. *Science*, *197*(4298), 89-91. https://doi.org/10.1126/science.194312

Weihe, E., Schafer, M. K., Erickson, J. D., & Eiden, L. E. (1994). Localization of vesicular monoamine transporter isoforms (VMAT1 and VMAT2) to endocrine cells and neurons in rat. *J Mol Neurosci*, *5*(3), 149-164. https://doi.org/10.1007/BF02736730

Wheeler, D. S., Underhill, S. M., Stolz, D. B., Murdoch, G. H., Thiels, E., Romero, G., & Amara, S. G. (2015). Amphetamine activates Rho GTPase signaling to mediate dopamine transporter internalization and acute behavioral effects of amphetamine. *Proc Natl Acad Sci U S A*, *112*(51), E7138-7147. https://doi.org/10.1073/pnas.1511670112

Wheeler, J. M., Reed, C., Burkhart-Kasch, S., Li, N., Cunningham, C. L., Janowsky, A., Franken, F. H., Wiren, K. M., Hashimoto, J. G., Scibelli, A. C., & Phillips, T. J. (2009). Genetically correlated effects of selective breeding for high and low methamphetamine consumption. *Genes Brain Behav*, *8*(8), 758-771. https://doi.org/10.1111/j.1601-183X.2009.00522.x

Williams, J. T., Colmers, W. F., & Pan, Z. Z. (1988). Voltage- and ligand-activated inwardly rectifying currents in dorsal raphe neurons in vitro. *J Neurosci*, *8*(9), 3499-3506. https://doi.org/10.1523/JNEUROSCI.08-09-03499.1988

Wolf, M. E. (2025). Targeting Neuroplasticity in Substance Use Disorders: Implications for Therapeutics. *Annu Rev Pharmacol Toxicol*, *65*(1), 259-280. https://doi.org/10.1146/annurev-pharmtox-061724-080548

Wolf, M. E., & Khansa, M. R. (1991). Repeated administration of MK-801 produces sensitization to its own locomotor stimulant effects but blocks sensitization to amphetamine. *Brain Res*, *562*(1), 164-168. https://doi.org/10.1016/0006-8993(91)91202-c

Wolf, M. E., Xue, C. J., White, F. J., & Dahlin, S. L. (1994). MK-801 does not prevent acute stimulatory effects of amphetamine or cocaine on locomotor activity or extracellular dopamine levels in rat nucleus accumbens. *Brain Res*, *666*(2), 223-231. https://doi.org/10.1016/0006-8993(94)90776-5

Wright, D. E., Seroogy, K. B., Lundgren, K. H., Davis, B. M., & Jennes, L. (1995). Comparative localization of serotonin1A, 1C, and 2 receptor subtype mRNAs in rat brain. *J Comp Neurol*, *351*(3), 357-373. https://doi.org/10.1002/cne.903510304

Xie, Z., & Miller, G. M. (2007). Trace amine-associated receptor 1 is a modulator of the dopamine transporter. *J Pharmacol Exp Ther*, *321*(1), 128-136. https://doi.org/10.1124/jpet.106.117382

Xie, Z., Westmoreland, S. V., & Miller, G. M. (2008). Modulation of monoamine transporters by common biogenic amines via trace amine-associated receptor 1 and monoamine autoreceptors in human embryonic kidney 293 cells and brain synaptosomes. *J Pharmacol Exp Ther*, *325*(2), 629-640. https://doi.org/10.1124/jpet.107.135079

Yamamoto, H., Takamatsu, Y., Imai, K., Kamegaya, E., Hagino, Y., Watanabe, M., Yamamoto, T., Sora, I., Koga, H., & Ikeda, K. (2011). MOP Reduction During Long-Term Methamphetamine Withdrawal was Restored by Chronic Post-Treatment with Fluoxetine. *Curr Neuropharmacol*, *9*(1), 73-78. https://doi.org/10.2174/157015911795017056

Yu, D. S., Smith, F. L., Smith, D. G., & Lyness, W. H. (1986). Fluoxetine-induced attenuation of amphetamine self-administration in rats. *Life Sci*, *39*(15), 1383-1388. https://doi.org/10.1016/0024-3205(86)90337-1

Zhou, L., Liu, M. Z., Li, Q., Deng, J., Mu, D., & Sun, Y. G. (2017). Organization of Functional Long-Range Circuits Controlling the Activity of Serotonergic Neurons in the Dorsal Raphe Nucleus. *Cell Rep*, *18*(12), 3018-3032. https://doi.org/10.1016/j.celrep.2017.02.077

Zhu, Z., Yi, F., Epplin, M. P., Liu, D., Summer, S. L., Mizu, R., Shaulsky, G., XiangWei, W., Tang, W., Burger, P. B., Menaldino, D. S., Myers, S. J., Liotta, D. C., Hansen, K. B., Yuan, H., & Traynelis, S. F. (2020). Negative allosteric modulation of GluN1/GluN3 NMDA receptors. *Neuropharmacology*, *176*, 108117. https://doi.org/10.1016/j.neuropharm.2020.108117