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Molecular Targets of Psychedelics and Their Role in Behavioral Models of Hallucinogenic Action

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A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Physiology and Biophysics at Virginia Commonwealth University

by

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List of Abbreviations

5-HT	Serotonin
5-HT_{2A}	Serotonin receptor 2A
DOI	2,5-dimethoxy-4-iodoamphetamine
DOM	2,5-dimethoxy-4-methylamphetamine
GPCR	G protein-coupled receptor
Grm2$^{-/-}$	mGluR2 knockout
HTR	Head-twitch response
Htr2a$^{-/-}$	5-HT_{2A} knockout
ISI	Interstimulus interval
LSD	Lysergic acid diethylamide
M100,907	Volinanserin
mGluR2	Metabotropic glutamate receptor subtype 2
mGluR3	Metabotropic glutamate receptor subtype 3
PPI	Prepulse inhibition

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Abstract

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Psychedelics are a subset of hallucinogenic drugs that exert their characteristic effects through agonist activity at the serotonin receptor 2A (5-HT_{2A}). In this study, I aimed to characterize the modulatory role of the metabotropic glutamate subtype 2 receptor (mGluR2) in the 5-HT_{2A}-specific rodent model of hallucinogenic action, head-twitch response (HTR). Secondly, I aimed to explore if 5-HT_{2A} agonist-induced deficits in prepulse inhibition (PPI) of the startle response, an additional model of hallucinogenic action, could be produced in mice. Though 5-HT_{2A} agonist-induced PPI deficits, which represent interruptions in normal sensorimotor gating, have been described in both rats and humans, attempts to translate this behavior to mice are rare. In contrast to prior gene knockout studies suggesting the mGluR2 is necessary for 5-HT_{2A} agonist-induced HTR, mGluR2 knockout (*Grm2*$^{-/-}$) mice still displayed HTR upon administration of the psychedelic 2,5-dimethoxy-4-iodoamphetamine (DOI). Additionally, DOI and lysergic acid diethylamide (LSD) produced unexpected improvements in PPI in male 126S6/Sv wild-type mice, depending on the experimental protocol used and the origin of the animals. Sex differences were observed as DOI-induced improvements in PPI were present in female 129S6/Sv mice of the same origin and tested with the same protocol as their

male counterparts; this effect in females was absent in 5-HT_{2A} knockout (*Htr2a*$^{-/-}$) mice. The results of this study shed light on issues with replicability and reproducibility in science, the importance of highlighting the origin and background of animal subjects, and potential sex differences in hallucinogenic drug action.

Chapter 1: Introduction

Psychedelics

Psychedelics are psychoactive substances that alter perception, emotion, and cognition, exerting their effects through the serotonin receptor 2A (5-HT_{2A}). They are also known as classic serotonergic hallucinogens, a subset of hallucinogenic drugs that share a common mechanism of action in producing altered states of consciousness (Nichols, 2016). Psychedelics can be divided into three categories based on their structural and behavioral characteristics: ergolines, tryptamines, and phenethylamines (Winter, 2009). Lysergic acid diethylamide (LSD) is categorized as an ergoline (Nichols, 2004); however, it is important to note that its chemical structure contains components of both tryptamines and phenethylamines (Winter, 2009). Tryptamines include N,N-dimethyltryptamine (DMT), 5-methoxy-N,N-dimethyltryptamine (MDMT), psilocybin, and its derivative psilocin; phenethylamines include mescaline, 2,5-dimethoxy-4-methylamphetamine (DOM), and 2,5-dimethoxy-4-iodoamphetamine (DOI) (Shulgin & Shulgin, 1990; Winter, 2009).

Naturally sourced psychedelic compounds from plants and mushrooms have been consumed by humans for centuries for spiritual and religious purposes (Nichols, 2004), but the introduction of psychedelics to the modern era began with the accidental discovery of LSD's mind-altering effects by chemist Albert Hoffman in 1943. Below is his description of his experience:

> Last Friday, April 16, 1943, I was forced to interrupt my work in the laboratory in the middle of the afternoon and proceed home, being affected by a remarkable restlessness, combined with a slight dizziness. At home I lay down and sank into a not unpleasant intoxicated-like condition, characterized by an extremely stimulated imagination. In a

> dreamlike state, with eyes closed (I found the daylight to be unpleasantly glaring), I perceived an uninterrupted stream of fantastic pictures, extraordinary shapes with intense, kaleidoscopic play of colors. After some two hours this condition faded away. (Hofmann, 1980, p. 12).

This discovery was followed by years of research into the use of LSD and other psychedelic substances as tools for psychiatric investigation and as potential treatments for mental illnesses (Belouin & Henningfield, 2018).

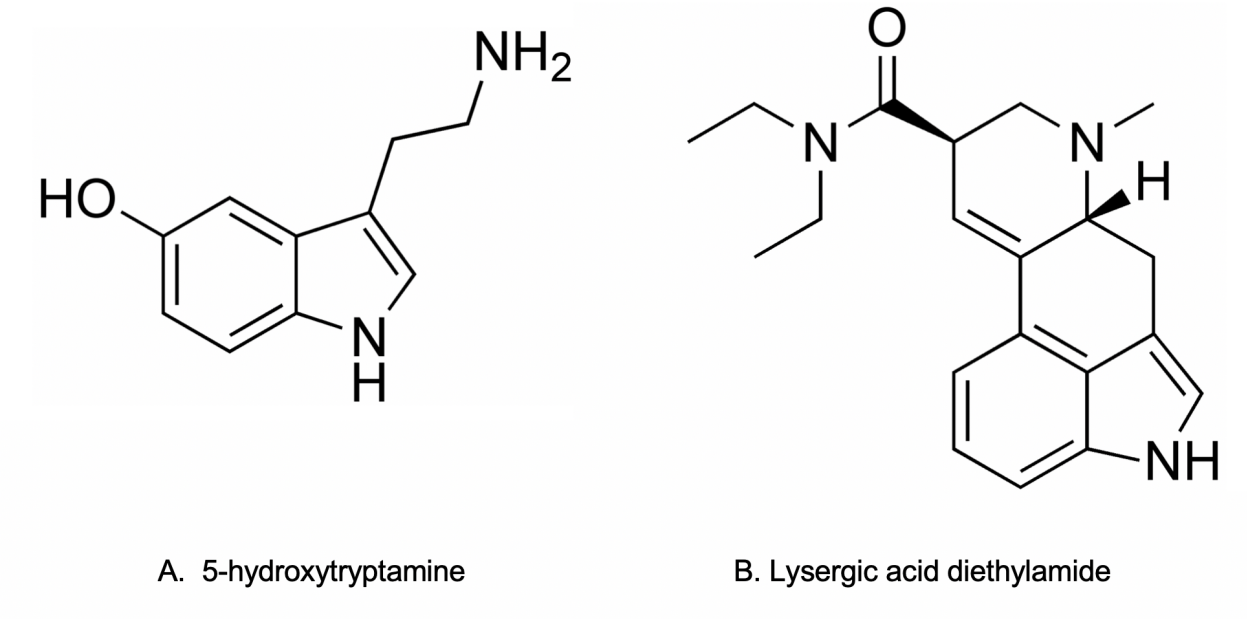

Figure 1: Comparison of the chemical structures of serotonin and LSD. **(A)** 5-hydroxytryptamine (serotonin) and **(B)** lysergic acid diethylamide (LSD) both contain tryptamine, suggesting LSD functions in the brain by interfering with the actions of serotonin. ChemDraw Professional 16.0.

Serotonin hypothesis of hallucinogenic action

A decade after Hoffman's discovery, serotonin (5-HT) activity was detected in rat and dog brains (Twarog & Page, 1953). Later that year, Gaddum (as cited in Nichols, 2016) found that LSD antagonized the effects of 5-HT in peripheral tissues. More specifically, it was

observed the following year that LSD abolished the vasoconstrictor effect of 5-HT in a rabbit ear and resulted in a small decrease of the effect of 5-HT on a rat uterus (Gaddum & Hameed, 1954). These pieces of evidence, along with the structural similarities between 5-HT and LSD observed (Figure 1), led to the hypothesis that LSD specifically functions to produce altered mental states by blocking 5-HT in the brain (Gaddum & Hameed, 1954; Woolley & Shaw, 1954). Woolley and Shaw expanded the hypothesis to suggest that if LSD induces altered states of consciousnesses and does so through interactions with serotonin, then perhaps serotonin plays a role in maintaining normal mental processes (1954).

However, two years later, LSD was shown to mimic the function of 5-HT on the heart of clam by producing a stimulatory response, suggesting it was a 5-HT receptor agonist (Shaw & Woolley, 1956). Eventually, it was concluded LSD primarily has an agonist effect on 5-HT (Aghajanian & Marek, 1999), particularly after additional evidence showed that LSD and 5-hydroxytryptophan (5-HTP), a precursor to 5-HT, produced similar functional effects on rat spinal reflexes (Andén et al., 1968). These findings led to the conclusion that stimulation of a 5-HT receptor may be responsible for the hallucinogenic effects of LSD (Andén et al., 1968). Later, this hypothesis was expanded to include that hallucinogens such as psilocin and mescaline also act similarly to LSD at the 5-HT receptor (Aghajanian & Marek, 1999).

5-HT_{2A} receptor as primary target of psychedelics

From early research in the 1980s and 1990s, seven distinct families of 5-HT receptors have been discovered comprising of 14 receptor subtypes in mammalian cells. Most of these receptor subtypes are G protein-coupled receptors (GPCRs), though one, the 5-HT_3 receptor, is a known ligand-gated ion channel (Barnes & Sharp, 1999). GPCRs are a family of receptors

characterized by a common motif of seven transmembrane alpha-helices comprising of hydrophobic amino acids and an association with intracellular heterotrimeric GTP-binding proteins, or G proteins. They can be divided into three classes: A, B, and C, which correspond to rhodopsin, secretin, and metabotropic glutamate receptor families, respectively. Upon binding of the appropriate ligand, intracellular signaling is mediated through the activation of G proteins made up of α, β and γ subunits. The type of G protein associated with the receptor determines its intracellular signaling cascade: G_s proteins are stimulatory, $G_{i/o}$ proteins are inhibitory, and $G_{q/11}$ proteins are also stimulatory and are known to cause downstream effects that lead to increased intracellular calcium concentration (Alexander et al., 2011).

Of the 5-HT receptors, the 5-HT_2 receptor family was more specifically implicated in mediating the hallucinogenic effects of psychedelics following several drug discrimination studies involving the use of 5-HT_2 receptor antagonists. In one study, pretreatment of rats with the 5-HT_2 antagonists ketanserin and pirenperone blocked the discriminative cue of the psychedelic DOM and also blocked DOM-stimulus generalization to mescaline and LSD (Glennon et al., 1983). Another study also showed a strong correlation between the binding of several psychedelics to 5-HT_2 in rat cortical tissue preparations and their human hallucinogenic potencies (Glennon et al., 1984). Later, the attention shifted to the 5-HT_{2A} receptor subtype as the necessary or central target for mediating the hallucinogenic drug effects after studies showed that antagonists blocking the action of psychedelics had a higher affinity for the 5-HT_{2A} receptor compared to the 5-HT_{2C} receptor (Fiorella et al., 1995).

The role of the 5-HT_{2A} receptor was also confirmed through behavioral studies in animals and humans. Head-twitch response (HTR), as will be described in a later section, is a behavior displayed by rodents upon administration of 5-HT agonists. Willins and Meltzer showed that

head-twitch response induced by direct injection of DOI into the medial prefrontal cortex of rats was inhibited by pretreatment with the 5-HT_{2A} antagonists ketanserin and M100,907, but not a selective 5-$HT_{2C/2B}$ antagonist (1997). This narrowed the essential receptor for mediating HTR to the 5-HT_{2A}. Additionally, DOI- and LSD-induced head-twitch response was blocked in 5-HT_{2A} knockout mice compared to wild-types, further supporting the role of this receptor in the action of psychedelics by using genetically modified animal models (González-Maeso et al., 2003). The first evidence in humans of the role of the 5-HT_{2A} receptor in mediating hallucinations was from a 1998 study showing that psilocybin-induced psychosis in human subjects was blocked by ketanserin and the atypical antipsychotic risperidone, another 5-HT_{2A} antagonist (F. Vollenweider et al., 1998).

The 5-HT_{2A} receptor is a class A GPCR that couples to the $G_{q/11}$ protein intracellularly. Though psychedelic drugs function through this receptor, not all 5-HT_{2A} agonists produce hallucinogenic effects. One study set out to distinguish the responses of hallucinogenic and non-hallucinogenic 5-HT_{2A} agonists in mouse somatosensory cortex to understand how the receptor specifically elicits this unique behavioral effect. Transcriptional responses to DOI, LSD, and the non-hallucinogenic 5-HT_{2A} agonist lisuride hydrogen maleate showed increased expression of the gene *c-fos*, whereas only DOI and LSD modulated the expression of the gene *egr-2*. These changes were also absent in 5-HT_{2A} knockout animals, confirming these effects were dependent on activation of this receptor (González-Maeso et al., 2003).

mGluR2 modulation of hallucinogenic action

The glutamate neurotransmitter system has been shown to play a role in 5-HT_{2A}-mediated hallucinogenic drug action, particularly through interaction with the class C $G_{i/o}$-coupled

metabotropic glutamate receptor subtype 2 (mGluR2). The characteristic induction of *c-fos* by 5-HT_{2A} agonists described previously was later shown to be a $G_{q/11}$-dependent mechanism, whereas LSD- and DOI-induced expression of *egr-2* was a $G_{i/o}$-dependent mechanism (González-Maeso et al., 2008). This hallucinogen-specific expression of *egr-2* was blocked by treatment with the metabotropic glutamate receptor 2/3 (mGluR2/3) agonist LY379268 in primary cortical cultures and in mouse cortex in *vivo*, whereas the induction of *c-fos* remained unaffected (González-Maeso et al., 2008). Additionally, DOI-induced expression of *egr-2* was lacking in mGluR2 knockout mice compared to wild-types, further supporting the involvement and necessity of the mGluR2 receptor for this pattern of hallucinogen-specific signaling (Moreno et al., 2011).

Though the mechanism through which mGluR2 modulates 5-HT_{2A}-specific behaviors is uncertain, there are several theories including interactions through synaptic mechanisms and functional crosstalk through a GPCR heterodimer. Marek et al. found through electrophysiological studies that activation of presynaptic glutamate receptors by mGluR2 agonists negatively modulated 5-HT_{2A} receptor-dependent glutamate release in the prefrontal cortex (2000). On the other hand, González-Maeso et al. proposed that the 5-HT_{2A} receptor and mGluR2 form a heterodimer through which hallucinogens function (2008). Regardless of the specific mechanism underlying this interaction, animal behavioral models of hallucinogenic action using pharmacological and genetic tools support the involvement of the mGluR2 in some capacity.

Behavioral models of hallucinogenic action

Two behavioral models of hallucinogenic drug action were the focus of this study: head-twitch response and prepulse inhibition of the startle reflex.

Head-twitch response

Head-twitch response (HTR) is a rhythmic, quick, rotational head movement observed in rodents in response to hallucinogenic 5-HT_{2A} agonist administration, as mentioned previously (Canal & Morgan, 2012). Though other receptor and neurotransmitter systems have been shown to modulate HTR, this behavior is linked specifically to the 5-HT_{2A} receptor (Halberstadt & Geyer, 2013b). Typically, head-twitch responses are measured by a trained observer watching video recordings of mice behavior. However, this method has the potential to produce inconsistent results due to observer biases. Recently, a magnetometer detection system was developed by Halberstadt and Geyer to make data collection more standardized (2013a). In this procedure, mice are surgically implanted with a small magnet on their scalp. After recovery, they are placed in a glass beaker surrounded by magnet wire. When amplification and Fourier analysis of the coil output was completed, and head-twitch responses were manually counted by identifying sinusoidal wavelets meeting certain criteria characteristic of the rapid side-to-side rotational head movement.

The involvement of the mGluR2 in this 5-HT_{2A} receptor-mediated behavior in rodents has been studied by using pharmacological tools in HTR. In Albino-Swiss mice, mGluR2/3 agonists LY379268 and LY354740 significantly decreased DOI-induced (2.5 mg/kg) HTR in a dose-dependent manner (Kłodzinska et al., 2002). A recent study by Halberstadt et al. using male C57BL/6J mice also showed that acute pretreatment with LY379268 (10 mg/kg), an mGluR2/3

agonist, significantly attenuated head-twitch induced by the 5-HT_{2A}-selective agonist 25CN-NBOH (1 mg/kg) (2019). On the other hand, administration of an mGluR2/3 antagonist, LY341495 (1 mg/kg), enhanced HTR in Sprague-Dawley rats (Gewirtz & Marek, 2000).

The interaction between these two receptors in HTR has also been modulated through chronic administration of 5-HT_{2A} and mGluR2 ligands as a means of inducing receptor downregulation or desensitization. In the same aforementioned study by Halberstadt et al., administration of the mGluR2/3 agonist LY379268 over a period of 21 days reduced 25CN-NBOH-induced head-twitch response compared to chronic vehicle-treated mice by roughly 50% (2019). Additionally, chronic treatment with the mGluR2/3 antagonist LY341495 decreased HTR in 129S6/Sv mice treated with LSD (Moreno et al., 2013). This result is in contrast to the enhanced HTR with acute treatment with the same mGluR2/3 antagonist observed by Gewirtz and Marek (2013). These differences can be explained by findings from Moreno et al. where reduced 5-HT_{2A} receptor binding was observed following chronic treatment with the mGluR2/3 antagonist LY341495 (2013).

Additionally, mGluR2 knockout mice have been used as experimental tools to further understand the role of this receptor in 5-HT_{2A}-mediated HTR. Moreno et al. showed that while DOI and LSD produced significant HTR in wild-type 129S6/Sv mice, no significant HTR was observed in mGluR2 knockout mice (2011). Another study by Benvenga et al. replicated these results, where DOI produced less head twitch responses in mGluR2 knockout mice compared to age-matched wild-type mice and mGluR3 knockout mice (2018). Furthermore, DOI-induced head twitches produced by both wild-type and mGluR3 knockout mice were blocked by administration of the mGluR2/3 agonist LY379268 and the mGluR2 positive allosteric modulator CBiPES (Benvenga et al., 2018). These results suggest the mGluR2 is specifically

involved in modulating head-twitch response in comparison with the closely associated mGluR3 receptor. Moreno et al. also confirmed that the expression of 5-HT_{2A} in the mGluR2 knockout mice used in their study was not significantly changed from wild-type mice, further supporting the role of mGluR2 in this behavior (2011).

Prepulse inhibition of the startle reflex

Prepulse inhibition, or PPI, refers to the reduction in startle response to a stimulus as a result of a low-intensity stimulus preceding it (Hanks & González-Maeso, 2012). It is commonly used as a measure of sensorimotor gating, a normal neural process in healthy humans that prevents overload of the senses. This attenuation of the startle response is shown to be deficient in patients suffering from different psychiatric illnesses; for example, it is considered an endophenotype of schizophrenia that models two different subsets of symptoms of the disease: psychosis and cognitive disruption (Braff et al., 2007). Hallucinogens are thought to disrupt normal sensorimotor gating behavior due to their ability to induce sensory overload and cognitive dysfunction; thus, psychedelics are often used as experimental tools in animals to model the sensory filtering deficits observed in mental illnesses such as schizophrenia (F. Vollenweider et al., 1998). More broadly, the use of psychedelics in animal models of PPI can help further our understanding of drug-induced hallucinations as a behavior in itself.

The 5-HT_{2A} receptor has been implicated in the mechanism of action of hallucinogen-induced deficits in PPI in several previous studies. In humans, the 5-HT_{2A} agonist psilocybin induces deficits in PPI (F. X. Vollenweider et al., 2007). Psychedelics have also been shown to disrupt PPI in rats. As reviewed by Halberstadt and Geyer, several drugs including DOI and LSD induce PPI deficits in a 5-HT_{2A}-dependent manner. On the other hand, 5-HT_{2A} receptor

activation does not consistently produce PPI deficits in mice, with some studies reporting that tryptamine psychedelics actually produce increases in PPI due to their action on the 5-HT_{1A} receptor as well (Halberstadt & Geyer, 2016).

Psychedelics as models of psychosis

In addition to expanding our understanding of how hallucinations manifest in the brain, psychedelic drugs have several potential applications in medical research. Based off of their action through the 5-HT_{2A} receptor as well as the involvement of this receptor in illnesses such as schizophrenia, psychedelics are used as models of psychosis (Halberstadt & Geyer, 2013b). Psychosis is defined by delusions, hallucinations, thought disorders, and catatonia (Howes & Murray, 2014). These models have helped us understand the role 5-HT and other neurotransmitter systems play in mediating this condition, leading to the development of new therapeutic drugs for treating the psychotic symptoms of schizophrenia.

Schizophrenia is a complex psychiatric disorder characterized by a combination of behavioral and cognitive symptoms with a lifetime morbid risk of 0.7% (McGrath et al., 2008). The core features of schizophrenia include positive or psychotic symptoms such as delusions and hallucinations as described previously; negative symptoms such as impaired motivation, reduction in spontaneous speech, and social withdrawal; and lastly, cognitive impairment (Owen et al., 2016). Though the lifetime morbid risk is relatively low, its effects are severe, as life-long symptoms lead to disability and problems with social function. People with schizophrenia have an estimated two- to threefold increased risk of dying compared to the general population (McGrath et al., 2008) due to comorbid conditions such as cardiovascular disease, lung cancer, chronic obstructive pulmonary disease, and substance abuse (Olfson et al., 2015). Side effects

produced by second-generation antipsychotic medications, such as metabolic syndrome and weight gain, also contribute to this higher mortality rate (McGrath et al., 2008). Additionally, it is estimated that 4.9% of people will schizophrenia will commit suicide during their lifetimes (Palmer et al., 2005).

As summarized by Owen et al., no specific neuroanatomical abnormalities can be attributed to the disorder despite the wealth of research surrounding this disease. This is potentially due to the fact that schizophrenia is a heterogeneous disorder comprising of a wide range of symptoms, and there is considerable overlap with the clinical manifestations of other psychiatric illnesses (2016). To address the issue of complexity, many approaches to understanding the pathophysiology of and improving treatment options for schizophrenia focus on a subset of behavioral dysfunction observed in the disorder. One area of focus is psychosis, a syndrome seen in many psychiatric disorders including schizophrenia as a positive symptom. Thus, research into the mechanisms of action of hallucinogenic drugs can prove useful for understanding the pathophysiology of a subset of symptoms of schizophrenia and potentially lead to improved treatment options for people diagnosed with this illness.

Rationale for this study

The differential effects of a mGluR2/3 antagonist in producing HTR compared to mGluR2 knockout mice begs the question of the role mGluR2 specifically plays in this behavior. It seems surprising that acute administration of antagonist of mGluR2/3 prior to DOI would enhance HTR, whereas in mGluR2 knockouts, arguably a full model of mGluR2 antagonism due to the receptor's absence, no significant HTR is seen. I aimed to take a closer look at the role of mGluR2 by measuring DOI-induced HTR in wild-type and mGluR2 knockout mice using lower

doses of DOI than used in existing literature. By doing this, I hoped to see whether head-twitch behavior is truly abolished in mGluR2 knockout mice or if this genotype has experienced a leftward shift in its inverted U-shaped dose-response curve. If a leftward shift has occurred, lower doses of DOI may fall on the descending arc this curve. Additionally, I set out to induce 5-HT_{2A} agonist-mediated PPI deficits in male and female mice using different psychedelics and time intervals between the stimulus and the preceding low-intensity stimulus. While the role of the 5-HT_{2A} receptor in PPI has been shown in rats and humans, the ability of mice to also show deficits in PPI has been uncertain. Finding a model will serve as the springboard for future studies using this animal model.

Chapter 2: Materials and Methods

Animals

Adult (10-20 weeks old) male C57BL/6 mice from Taconic Biosciences were used for the validation of the head-twitch detection system. Other head-twitch experiments involving mGluR2 knockout (*Grm2*$^{-/-}$) mice were performed on adult (13-24 weeks old) males, originally obtained from RIKEN BioResource Center in Japan and backcrossed into the inbred 129S6/Sv strain for over 10 generations. Wild-type mice, denoted as *Grm2*$^{+/+}$, from the same colony bred at Virginia Commonwealth University were used as controls. mGluR2 knockouts were previously described by Moreno et al. (2011).

All PPI experiments utilized 129S6/Sv mice, though from several sources. Experiments with only wild-type males either involved adult (12-14 weeks old) mice from Taconic or adult (37-43 weeks old) wild-type mice, denoted as *Htr2a*$^{+/+}$, from a 5-HT$_{2A}$ knockout colony originating in the 129S6/Sv strain and bred at Virginia Commonwealth University. Experiments with male and female 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice were performed on adult (11-19 weeks old) mice with *Htr2a*$^{+/+}$ mice used as controls. 5-HT$_{2A}$ knockouts were previously described by González-Maeso, et al. (2003).

All mice were housed in cages with up to 5 littermates on a 12-hour light/dark cycle at 23°C with food and water ad libitum, except during behavioral testing. Experiments were conducted in accordance with NIH guidelines and were approved by the Virginia Commonwealth University Institutional Animal Care and Use Committee (IACUC).

Drugs

The IUPAC names and sources of drugs used are as follows: 1-(4-iodo-2,5-dimethoxyphenyl)propan-2-amine (DOI) from Sigma Aldrich; (6*aR*,9*R*)-*N*,*N*-diethyl-7-methyl-6,6*a*,8,9-tetrahydro-4*H*-indolo[4,3-fg]quinoline-9-carboxamide (LSD) from LIPOMED, and (*R*)-(2,3-dimethoxyphenyl)-[1-[2-(4-fluorophenyl)ethyl]piperidin-4-yl]methanol (volinanserin or M100,907) from Tocris Bioscience. Drugs were dissolved in saline and injection volumes and concentrations were determined based on body weight (0.01 mL/gram). All drugs were administered intraperitoneally (i.p.). Any indication for use of vehicle refers to an equivalent volume of 0.9% NaCl administered to the animal.

Head-twitch response

Surgical implantation of the head-mounted magnet was conducted as previously described (Halberstadt & Geyer, 2013a). Attachment of magnet-implanted ear tags was performed by using standard mouse ear tags affixed with a small magnet, with the south pole glued to the tag and the north pole facing upwards. Ear tags were attached to the lower half of both ears, close to the head and not obstructing the ear canal.

Full details on data collection are described in another study (de la Fuente Revenga et al., manuscript submitted). Data collection for automated head-twitch detection was performed in non-overlapping ~500-turn enameled wire (30 AWG) coils supported in plastic cylinders (inner dimensions, 11 cm diameter x 14 cm tall) with both terminals of each coil connected to a phono preamplifier (Pyle PP444). The amplified signal output was recorded at a 1000 Hz sampling rate using a myDAQ (National Instruments) data acquisition system controlled through Matlab version R2018b (Mathworks) along with an additional NI myDAQ support package. For the

validation experiment, data was then processed through a high bandpass filter between 70-110 Hz and transformed into absolute values. After double local maxima processing, each original wavelet is transformed into a unipolar peak that is automatically identified as a head-twitch event by a script when meeting certain criteria regarding frequency, duration, and intensity of the peak. For the dose-response experiment, data was processed through low and high bandpass filters, with the advanced script also including an individual event detector and a Fourier transform analysis detector to better exclude non-head-twitch events.

Visual detection of head-twitch for the validation experiment was performed by recording mice with a video camera at close range and watching the recordings in 0.75x speed. This was also aided by identification of subjective, time-stamped peaks viewed through graphing and processing of simultaneous recorded data through a simple high bandpass filter between 70-100 Hz. These peaks helped to ensure no potential head-twitch behavior was missed while viewing the videos.

Western blot

Plasma membrane preparations of mouse somatosensory cortex were performed as previously described for the Western blot analysis (Moreno et al., 2016). The amount of protein in the membrane preparations was estimated through a Bradford assay, and protein was denatured with heat and ß-mercaptoethanol for 5 minutes. The samples were then resolved by 10% SDS-PAGE and transferred to a nitrocellulose membrane by electroblotting overnight. The membrane was blocked with blocking buffer, after which it was incubated overnight with the primary antibody anti-mGluR2 (Abcam; 1:1000). This antibody was shown to be specific for mGluR2 in a previous study (Fribourg et al., 2011). Then, the membrane was incubated with the

horseradish peroxidase-conjugated secondary antibody sheep anti-mouse IgG (Abcam, 1:5000), after which immunoreactivity was detected using the SuperSignal West Pico #34080 chemiluminescent substrate from ThermoFisher.

Prepulse inhibition of the startle reflex

The SR-LAB™ Startle Response System from San Diego Instruments was used to record startle magnitude in response to acoustic stimuli. Mice were placed in the chambers after which a series of trials were presented. The magnitude of the pulse, or stimulus, was 119 dB, and of the prepulses were 73 dB, 77 dB, and 85 dB. The background noise was set to 69 dB. After a five-minute acclimation period set to the background noise, the protocol utilized was as follows:

5 stimulus-only trials

65 trials with the following trial-types presented randomly to prevent habituation

13 stimulus-only trials

13 no stimulus trials

13 73 dB prepulse and stimulus trials

13 77 dB prepulse and stimulus trials

13 85 dB prepulse and stimulus trials

5 stimulus-only trials

Each trial lasted either for a total of 200 ms, 170 ms, or 140 ms depending on the length of the pulse, prepulse, and the interval between the two stimuli, or interstimulus interval (ISI). One of the following PPI protocols was used for each experiment, with the trial length noted:

20/80/20: 20 ms prepulse, 80 ms ISI, 20 ms pulse; each trial was 200 ms

10/60/40: 10 ms prepulse, 60 ms ISI, 40 ms pulse; each trial was 170 ms

10/30/40: 10 ms prepulse, 30 ms ISI, 40 ms pulse; each trial was 140 ms

Each session lasted about a total of 30 minutes. The 10 stimulus-only trials were excluded during data analysis. %PPI for each prepulse and stimulus trial was determined by averaging the peak startle amplitude (V_{max}) for the stimulus only and the appropriate prepulse and stimulus trials and using the following equation:

$$\%PPI = \frac{(average\ Vmax\ of\ stimulus\ only\ trials - average\ Vmax\ of\ prepulse\ and\ stimulus\ trials)}{average\ Vmax\ of\ stimulus\ only} \times 100$$

Statistical analysis

Statistical analyses were performed with GraphPad Prism software version 8. Animals were randomly allocated into the different experimental groups. Correlation between visual identification vs. automated detection of HTR was assessed by linear regression. Statistical significance of experiments was assessed by a two-way ANOVA as each experiment had two independent variables. Variables assessed for the dose-response HTR experiment were treatment and genotype, whereas variables assessed for PPI were prepulse and treatment or genotype. Sex analysis involved measuring the effects of sex and prepulse. Sidak's multiple comparisons test was used for *post hoc* analysis as it has more power, a select set of means was chosen for comparison, and the mean comparisons were independent of each other. The level of significance was chosen at $p < 0.05$. All data are presented as mean ± standard error of the mean (S.E.M).

Chapter 3: Results

Validation of automated head-twitch response detection system

Though the recently developed magnetometer-based HTR detection system addresses observer biases, the process of identifying events is still lengthy (Halberstadt & Geyer, 2013a). A newer system based on Halberstadt and Geyer's work has been developed by de la Fuente Revenga et al. in which the number of sinusoidal wavelets corresponding to head-twitch events are detected automatically (manuscript submitted). In order to validate this new automated detection system, three wild-type C57BL/6 male mice with surgically implanted head magnets were recorded using a video camera for a total of eight 10-minute increments while head-twitch behavior was simultaneously automatically measured. In addition to basal head-twitch response, head-twitches induced with administration of DOI (0.2 mg/kg and 1 mg/kg) after the second and fifth increments, respectively, were also measured. There was a strong correlation observed between the automated and visual detection systems ($r = 0.9957$; slope = 0.9923) (Figure 2), with the automated detection system producing a false positive rate of 0% and a false negative rate of 1.39%.

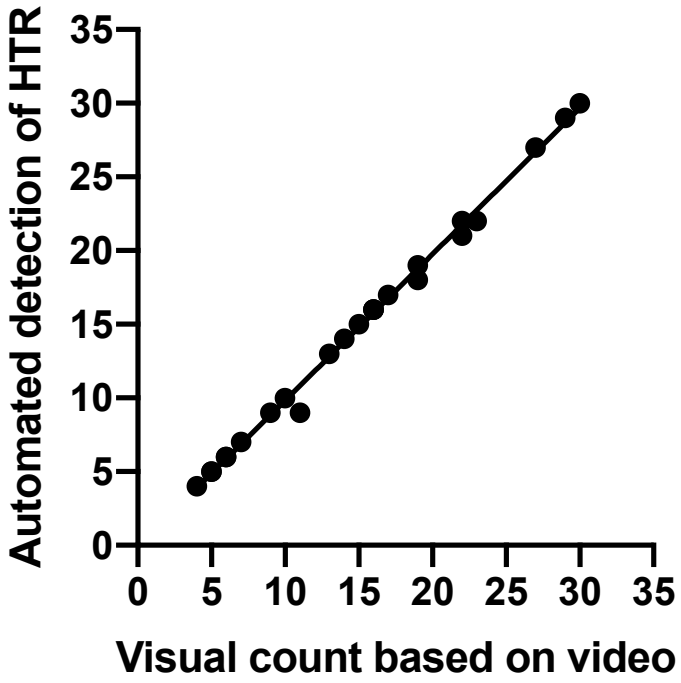

Figure 2: Correlation between visual and automated detection systems for measuring head-twitch response. Each point represents one 10-minute video set and corresponding automated recording of a single mouse (n = 24). Mice were either given no treatment or DOI (0.2 mg/kg and 1 mg/kg) to generate a range of HTR counts.

DOI dose-dependent head-twitch response in male 129S6/Sv wild-type (*Grm2*$^{+/+}$) and mGluR2 knockout (*Grm2*$^{-/-}$) mice

Wild-type (*Grm2*$^{+/+}$) and mGluR2 knockout (*Grm2*$^{-/-}$) male mice of the 129S6/Sv strain were prepared for head-twitch experiments by attachment of magnet-implanted ear tags. After a week-long recovery process, mice were allowed to habituate to the magnetometer coils for 1.5 hours. They were then measured a few days later for basal head-twitch response. Each mouse received different doses of DOI in the following order with a week-long washout period between each round: 2 mg/kg, 0.5 mg/kg, 0.05 mg/kg. This was followed by 0.9% saline as vehicle. Data were collected for 60 minutes, and analysis was completed for a 30-minute block following an initial 15-minute habituation period.

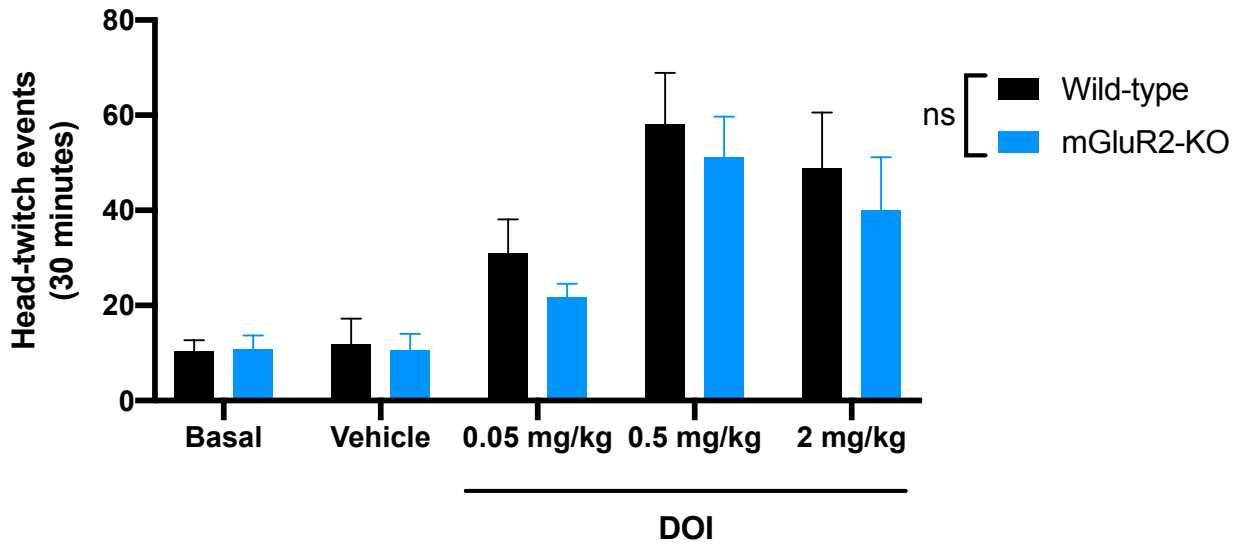

Figure 3: DOI dose-dependent HTR in 129S6/Sv wild-type (*Grm2*$^{+/+}$) and mGluR2 knockout (*Grm2*$^{-/-}$) mice. Head-twitch events were measured by automated detection system developed by de la Fuente Revenga et al. using mice (n = 4-6) with magnet-implanted bilateral ear tags. Data are presented as group mean ± S.E.M. Two-way ANOVA (n.s. = not significant).

The vehicle was administered last to check for any residual effects of DOI following the final one-week washout period. The lack of difference between head-twitch counts during the initial basal round and final vehicle round for both groups (two-way ANOVA, $F(1, 18) = 0.02853$, $p = 0.8678$) suggested that the washout period prescribed was sufficient, and any effects of DOI observed for each round were reliable. Concern for 5-HT_{2A} receptor downregulation following repeated administration of DOI has also been unfounded in a previous study showing that rats treated every seven days for four consecutive weeks with DOI (1.25 mg/kg, i.p.) showed a lack of significant decrease in HTR between the first and last weeks (Gewirtz & Marek, 2000). Contrary to previously published data, no effect of genotype was observed for each treatment round (two-way ANOVA, $F(1, 42) = 1.040$, $p = 0.3137$) (Figure 3), even at the same doses of DOI used in other studies showing a lack of response in mGluR2 knockouts.

Confirmation of lack of mGluR2 expression in mGluR2 knockout (*Grm2*$^{-/-}$) mice

I wanted to confirm whether our *Grm2*$^{-/-}$ mice truly lacked mGluR2 expression by using membrane preparations of mice somatosensory cortex in a Western blot analysis. The results showed show that these knockouts indeed did not express this receptor in comparison to *Grm2*$^{+/+}$ mice (Figure 4).

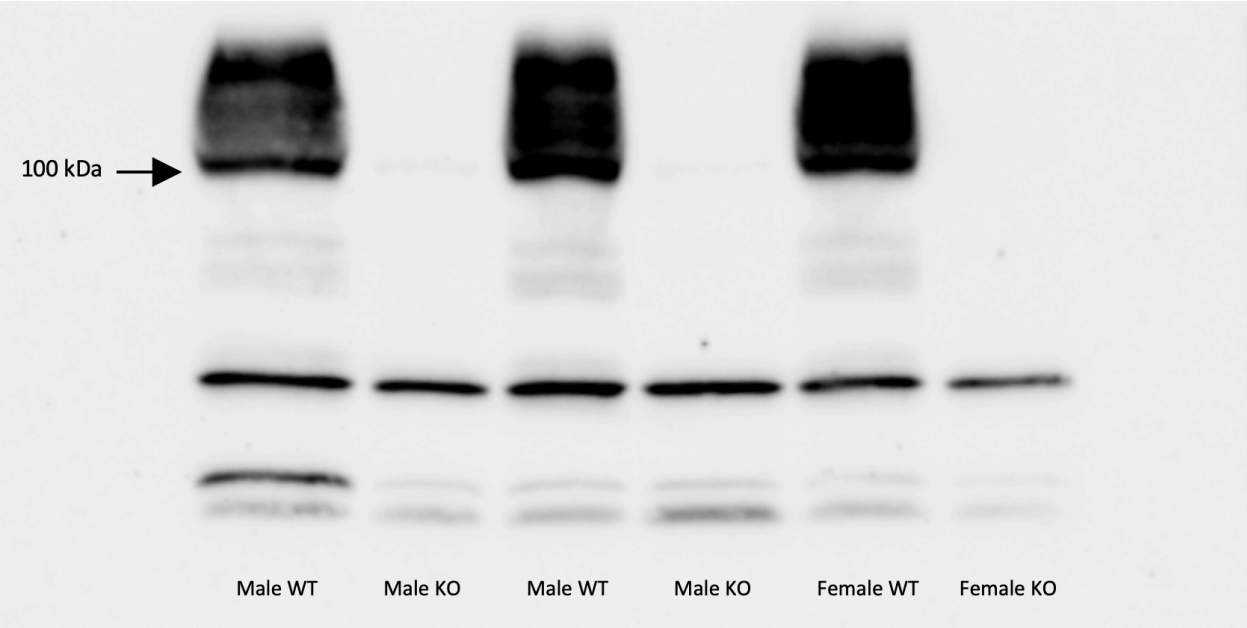

Figure 4: Western blot of mGluR2 expression in mice somatosensory cortex membrane preparation. Monomeric mGluR2 bands are visible for both male and female wild-type (*Grm2*$^{+/+}$) mice at 100 kDa, but not mGluR2 knockout (*Grm2*$^{-/-}$) mice. This confirms the lack of mGluR2 in our knockout colony.

Effect of DOI on PPI in male 129S6/Sv wild-type mice

In an effort to induce PPI deficits, male 129S6/Sv wild-type mice from Taconic and *Htr2a*$^{+/+}$ control mice from the 5-HT$_{2A}$ knockout colony were treated with four different doses of DOI (0.5, 1, 2, and 5 mg/kg i.p.). The PPI protocol used was 20/80/20. Prepulse intensity produced significant differences in PPI (two-way ANOVA: $F(2, 18) = 22.30$, $p < 0.0001$). 0.5 mg/kg DOI had an effect on PPI in *Htr2a*$^{+/+}$ control mice (two-way ANOVA: $F(1, 24) = 8.691$,

p = 0.0070; *post hoc*: (73 dB prepulse; vehicle vs. DOI) p = 0.0060). Also, 1 mg/kg DOI had an effect on PPI in wild-type mice from Taconic (two-way ANOVA: F (1, 18) = 6.860, p = 0.0174; *post hoc*: (73 dB; vehicle vs. DOI), p = 0.0036) with a significant prepulse x treatment interaction (p = 0.0281). However, surprisingly these two doses of DOI improved PPI in wild-type and *Htr2a*$^{+/+}$ control mice instead of producing deficits. Using 2 mg/kg and 5 mg/kg of DOI did not result in the same improvement in PPI, though a slight trend was still observed (Figure 5).

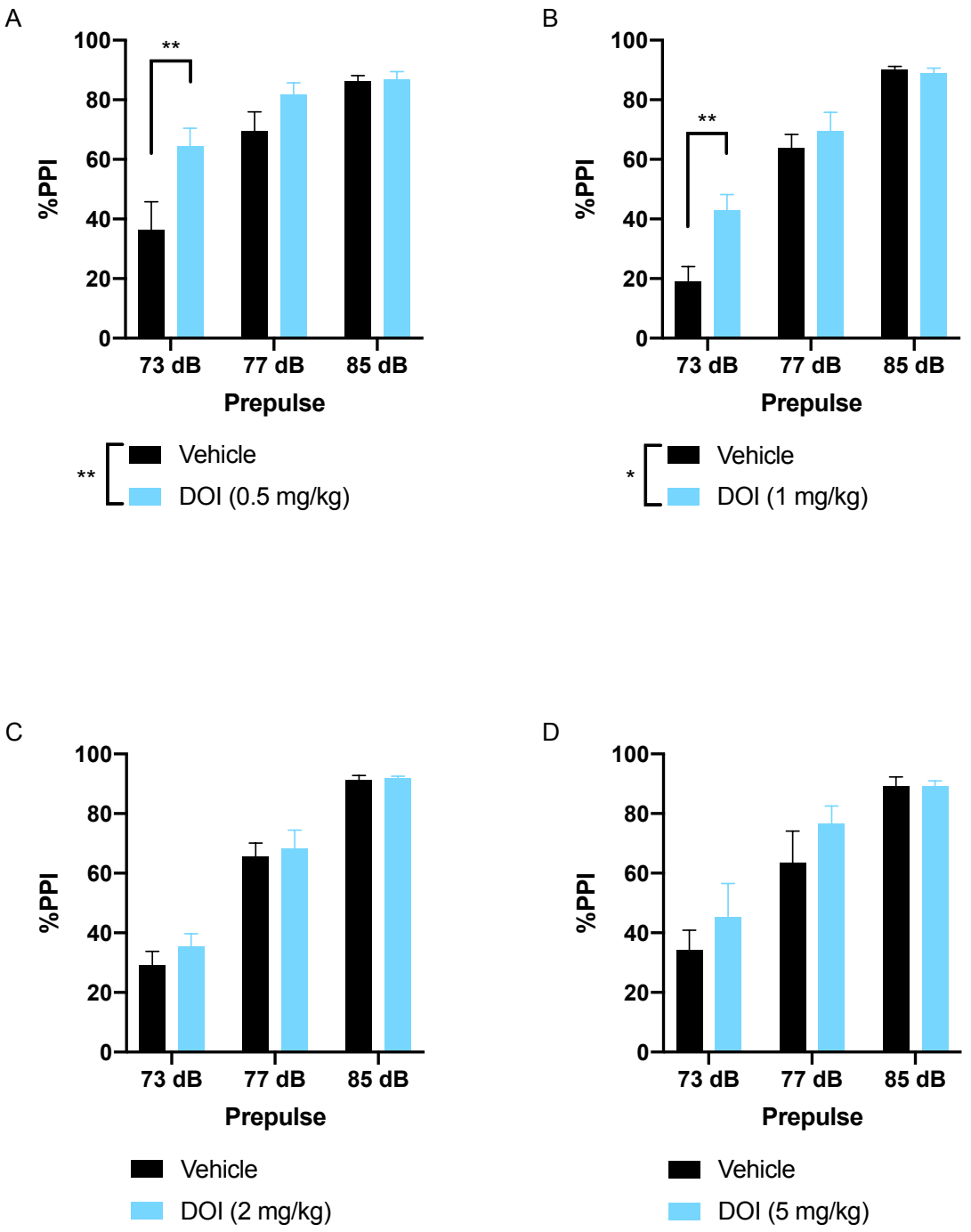

Figure 5: Dose-dependent effects of DOI on PPI in male 129S6/Sv wild-type mice. **A** *Htr2a*$^{+/+}$ from 5-HT$_{2A}$ knockout colony, **B-D** Taconic. PPI results are shown in mice administered vehicle or DOI (0.5 mg/kg) immediately prior to testing (**A**), DOI (1 mg/kg) 15 minutes prior to testing (**B**), DOI (2 mg/kg) 15 minutes prior to testing (**C**), and DOI (5 mg/kg) immediately prior to testing (**D**). n = 5 for each group in A, and n = 4 for each group in B, C, and D. Data are presented as group mean ± S.E.M. Two-way ANOVA with Sidak's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$).

Effect of LSD on PPI in male 129S6/Sv wild-type mice

In order to determine whether DOI's improvement of PPI was a behavior specific to the drug or was a result of psychedelic action at the 5-HT_{2A} receptor, I repeated the experiment using LSD (0.24 mg/kg) in male 129S6/Sv wild-type mice from Taconic. The same protocol as above, 20/80/20, was used. LSD also showed an enhancing effect on PPI (two-way ANOVA: F (1, 18) = 9.000, p = 0.0077; *post hoc*: (73 dB prepulse; vehicle vs. LSD), p = 0.0489) (Figure 6).

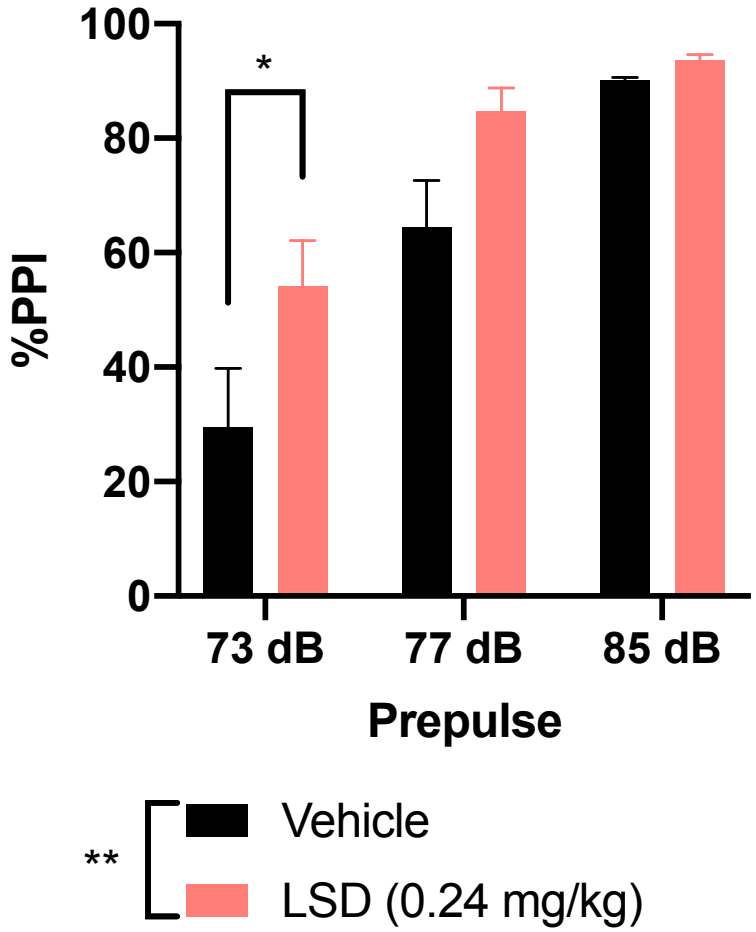

Figure 6: Effect of LSD on PPI in male 129S6/Sv wild-type mice. Mice were from Taconic. PPI results are shown in mice administered vehicle (n = 4) or DOI (0.5 mg/kg) (n = 4) immediately prior to testing. Data are presented as group mean ± S.E.M. Two-way ANOVA with Sidak's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$).

Effect of M100,907 on DOI-induced PPI improvement in male 129S6/Sv wild-type mice

I hypothesized that the lack of 5-HT_{2A} agonist-induced PPI deficits in the previous two experiments may be due to the durations of the prepulse, ISI, and pulse used. Aubert et al. showed that slight differences in intensities and durations of acoustic stimulus could affect PPI results (2006). In a strain comparison study, peak PPI was reached with prepulses 5 and 10 dB above baseline at a duration of 15 ms, and at a duration of 10 ms for a prepulse 15 dB above baseline in 129S2 mice. Additionally, a 10 ms long prepulse produced peak PPI with ISIs of 100, 50, and 50 ms at intensities of 5, 10, and 15 dB above baseline, respectively, while utilizing a 40 ms pulse (Aubert et al., 2006). As our PPI protocol utilizes prepulses 4, 8, and 16 dB above baseline, and mice with a 129S6/Sv background, I felt that I was able to make a relatively fair comparison and used the data from this paper to adjust our protocol accordingly, lowering our prepulse duration and ISI. I chose a 10 ms prepulse, 60 ms ISI, and 40 ms pulse (10/60/40) to use moving forward. This decision was supported by the observation that in another study using CD-1 mice, PPI deficits were successfully induced by DOI (0.5 mg/kg, i.p.) using the same pulse and interval durations (Ibarra-Lecue et al., 2018).

Mice were randomly assigned to receive a pretreatment of 0.9% saline or M100,907 (1 mg/kg) and a treatment of 0.9% saline or DOI (0.5 mg/kg). Despite the adjustments in protocol, I continued to observe improvements in PPI as a result of DOI administration in 129S6/Sv male wild-type mice (two-way ANOVA: $F(1, 54) = 11.31$, $p = 0.0014$; *post hoc*: (73 dB; saline and saline vs. saline and DOI), $p = 0.0003$), with a significant prepulse x treatment interaction (two-way ANOVA: $F(2, 54) = 5.195$, $p = 0.0086$). Here, I was also able to show that DOI's effects are mediated through the 5-HT_{2A} receptor as I observed a significant effect of the 5-HT_{2A} antagonist M100,907 on PPI in DOI-treated animals (two-way ANOVA: $F(1, 51) = 10.43$, $p =$

0.0022; *post hoc* analysis (saline and DOI vs. M100,907 and DOI at 73 dB), p = 0.0001) with significant prepulse x treatment interaction (two-way ANOVA: F (2, 51) = 5.961, P=0.0047). By pharmacologically blocking the 5-HT_{2A} receptor with M100,907, DOI's improvement in PPI was absent compared to DOI only-treated group. M100,907 alone had no effect on PPI in comparison to the saline only-treated group (Figure 7).

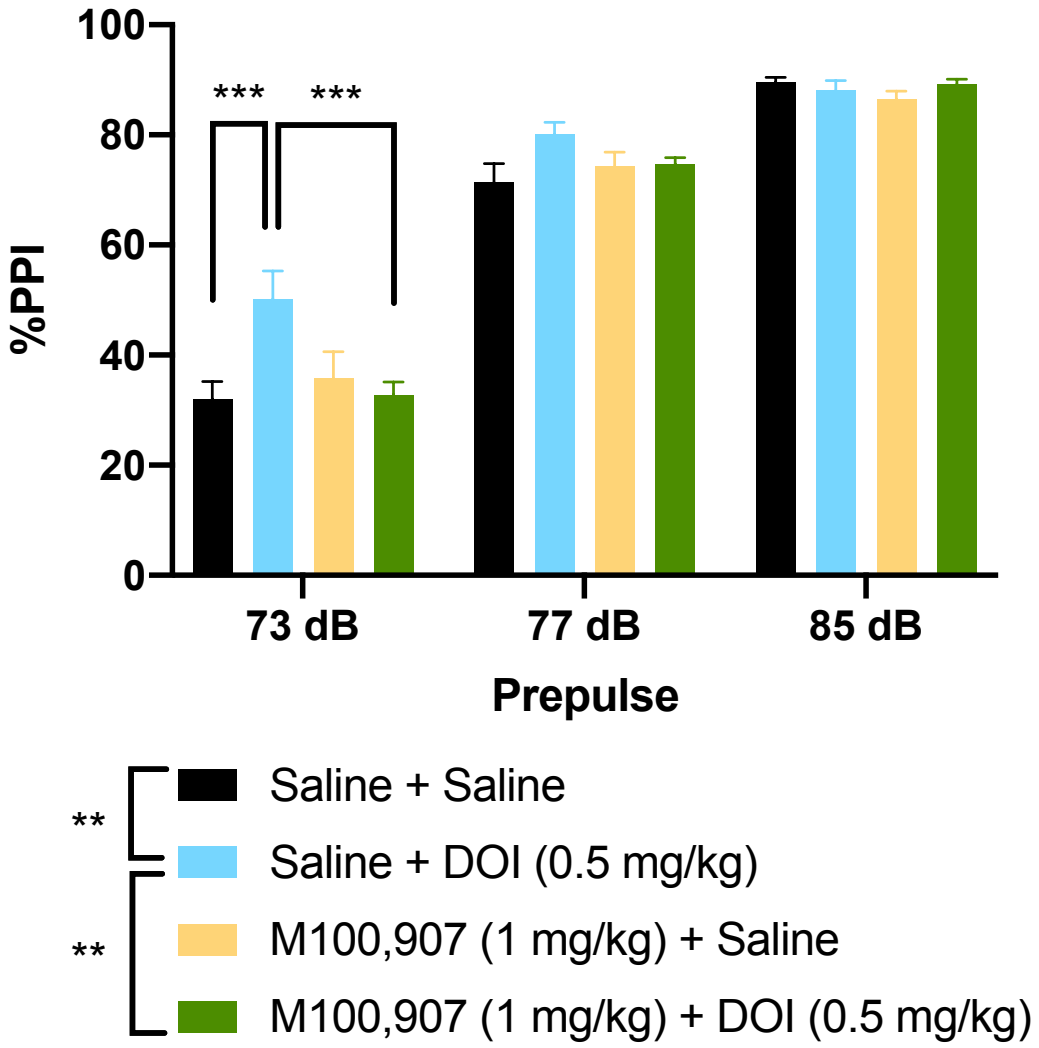

Figure 7: Effect of M100,907 on DOI-induced PPI improvement in male 129S6/Sv wild-type mice. Mice were from Taconic. PPI results are shown in mice administered saline and saline, saline and DOI (0.5 mg/kg), M100,907 (1 mg/kg) and saline, and M100,907 (1 mg/kg) and DOI (0.5 mg/kg). Time between pretreatment and treatment was 15 minutes. n = 9-10 for each group. Data are presented as group mean ± S.E.M. Two-way ANOVA with Sidak's multiple comparisons test (** $p < 0.01$, *** $p < 0.001$).

Effect of DOI on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice

To further confirm the dependency of DOI on the 5-HT$_{2A}$ receptor for facilitating improvements in PPI, I used a cohort of male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice with the 10/60/40 PPI protocol. Interestingly, I was unable to replicate the DOI-induced enhancement of PPI in male *Htr2a*$^{+/+}$(Figure 8) despite using the same protocol and dose of DOI as in Figure 7 and the same dose of DOI and mice as in Figure 5A. No variation based on treatment was observed in *Htr2a*$^{-/-}$ male mice. On the other hand, female *Htr2a*$^{+/+}$mice did display an effect of DOI on PPI (two-way ANOVA: $F(1, 63) = 12.69$, $p = 0.0007$; *post hoc*: (73 dB prepulse; saline vs. DOI) $p = 0.0086$, (77 dB prepulse; saline vs. DOI) $p = 0.0305$) (Figure 8). Similar to males, no effect of treatment was seen in *Htr2a*$^{-/-}$ mice.

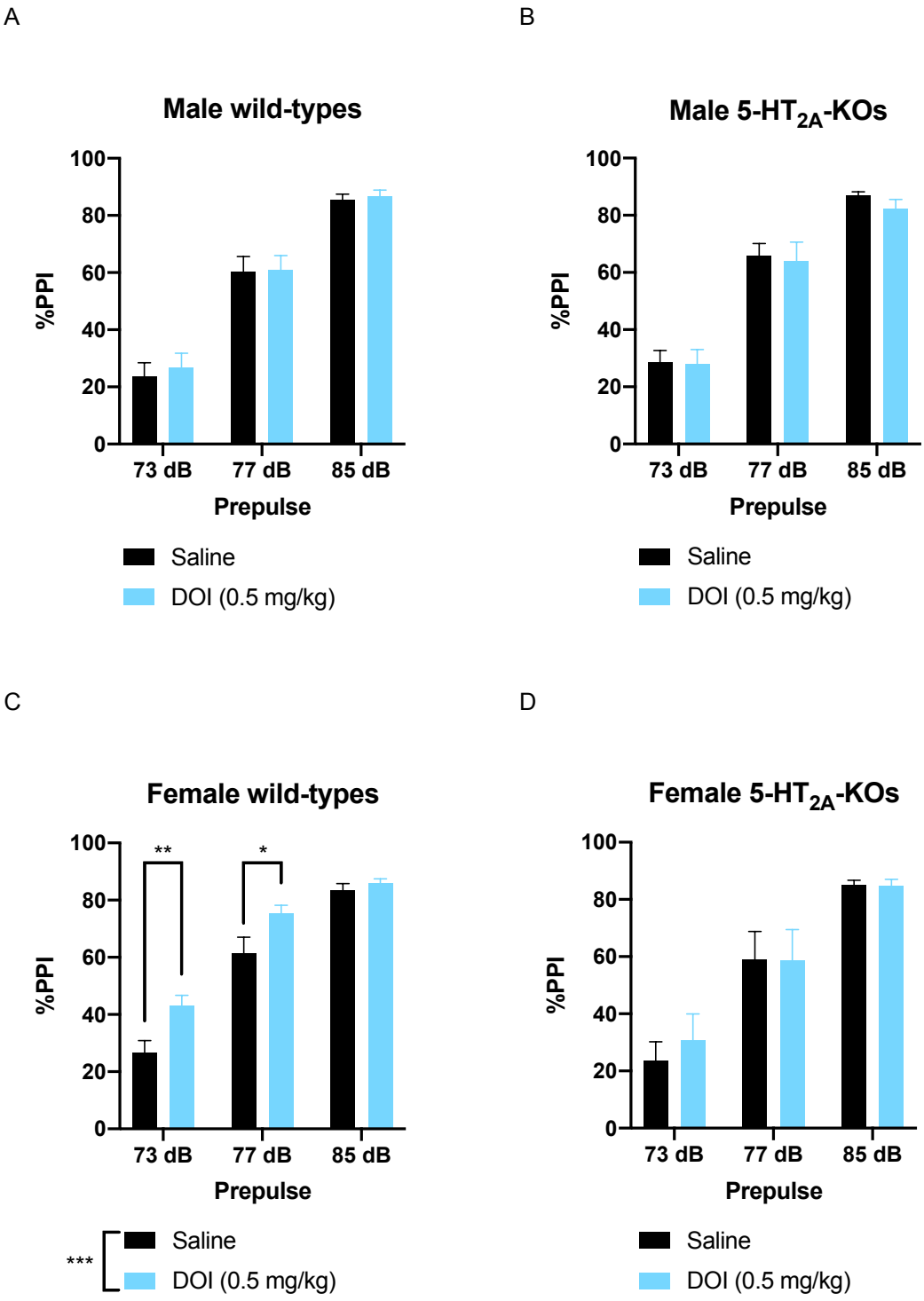

Figure 8: Effect of DOI on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice. PPI results are shown in mice administered saline or DOI (0.5 mg/kg). n = 8-9 for each group of males and n = 7-12 for each group of females. Data are presented as group mean ± S.E.M. Two-way ANOVA with Sidak's multiple comparisons test (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Effect of genotype and sex on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice

There was no effect of genotype on PPI found in either male or female mice (Figure 9) when comparing the saline groups from Figure 8. Additionally, there was no effect of sex observed on baseline PPI.

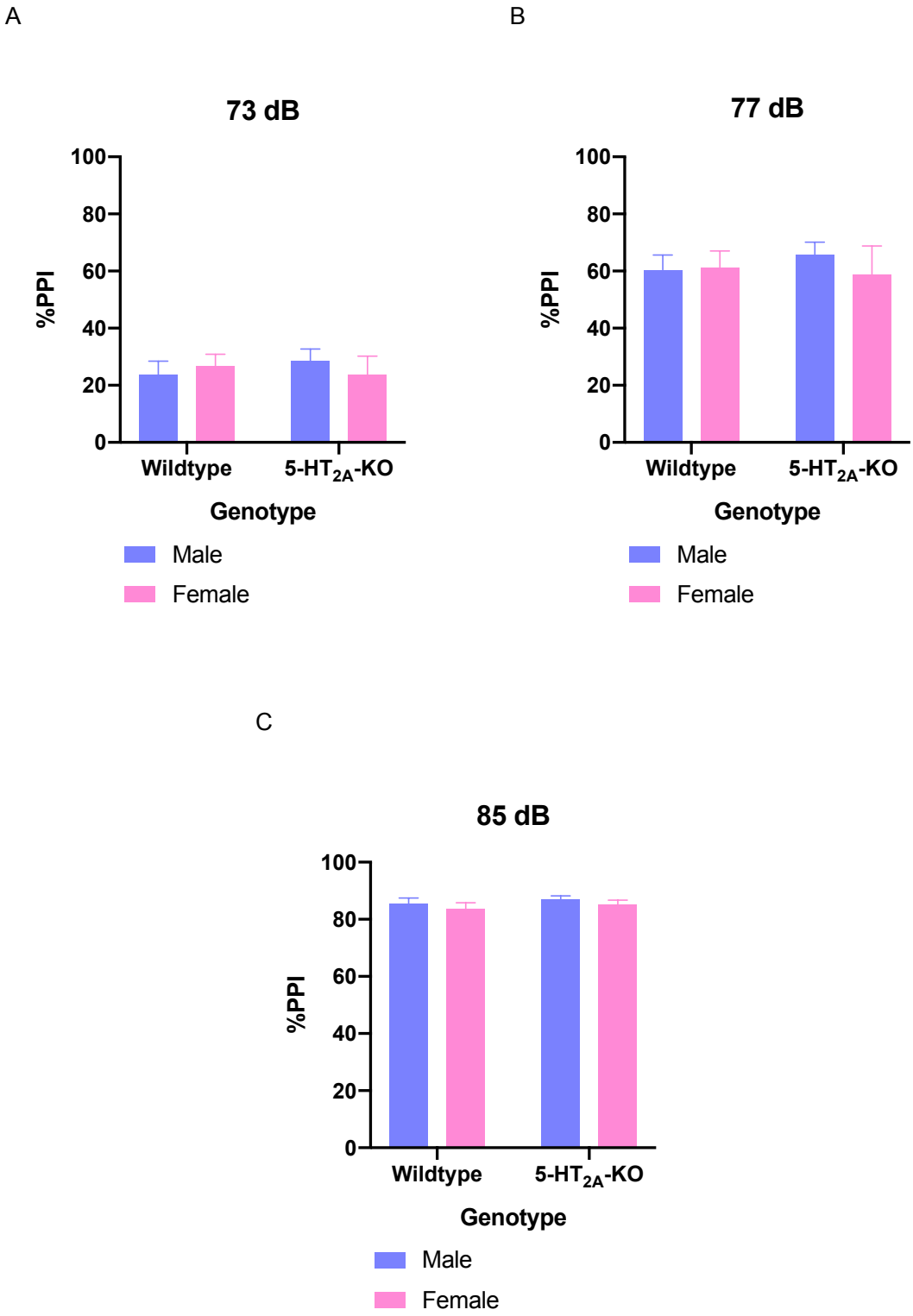

Figure 9: Effect of genotype and sex on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice. PPI results are separated by prepulse intensity. n = 8-9 for each group of males and n = 7-12 for each group of females. Data are presented as group mean ± S.E.M.

Effect of shorter ISI on DOI's effect on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice

I then hypothesized that shortening the ISI would produce DOI-induced PPI deficits in males and females. A study in healthy human volunteers described that psilocybin, a 5-HT$_{2A}$ receptor agonist, produced vastly different effects on PPI depending on the ISI used. While psilocybin had no effect on PPI with a 60 ms ISI, it increased PPI at ISIs above 120 ms and reduced PPI at an ISI of 30 ms (F. X. Vollenweider et al., 2007). Based off of these findings, I decided to repeated our experiments in male and female wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice after reducing our ISI to 30 ms; the protocol used was 10/30/40.

However, even with a shorter ISI, DOI did not produce an effect on PPI in male *Htr2a*$^{+/+}$ mice. Additionally, DOI did not have an effect on *Htr2a*$^{-/-}$. However, female *Htr2a*$^{+/+}$ mice continued to display improved PPI in response to DOI (two-way ANOVA: $F(1, 60) = 5.471$, $p = 0.0227$) (Figure 10). This effect was again shown to be dependent on the 5-HT$_{2A}$ receptor as it was absent in *Htr2a*$^{-/-}$ mice.

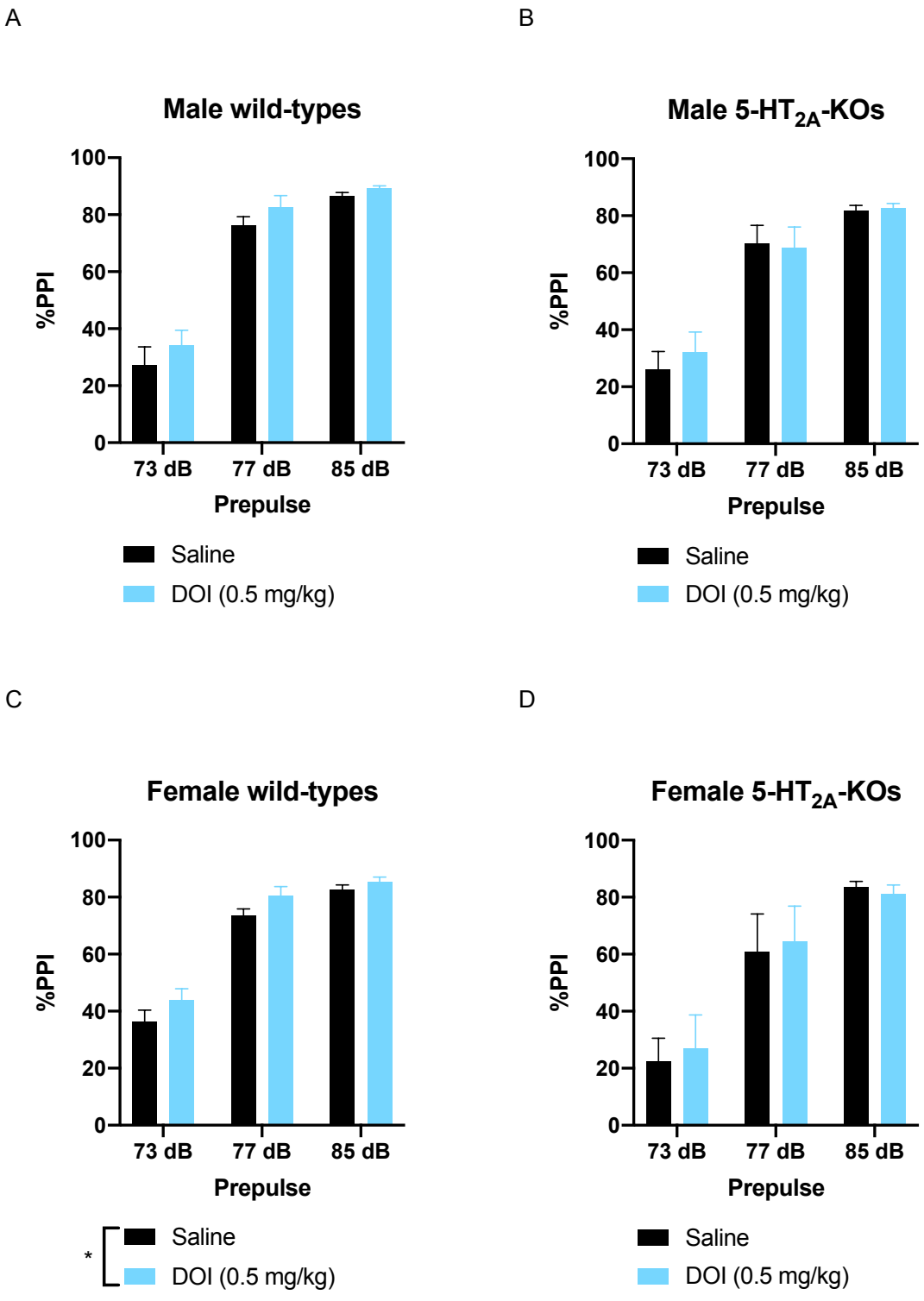

Figure 10: Effect of shorter ISI on DOI's effects on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice. PPI results are shown in mice administered saline or DOI (0.5 mg/kg). n = 7 for each group of males and n = 7-11 for each group of females. Data are presented as group mean ± S.E.M. Two-way ANOVA (* $p < 0.05$).

Effect of genotype and sex on DOI's effect on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice using a shorter ISI

No effect of genotype was observed on baseline PPI with the shorter ISI in both males and females (Figure 11) when comparing the saline groups from Figure 10. Additionally, there was no effect of sex on baseline PPI as well.

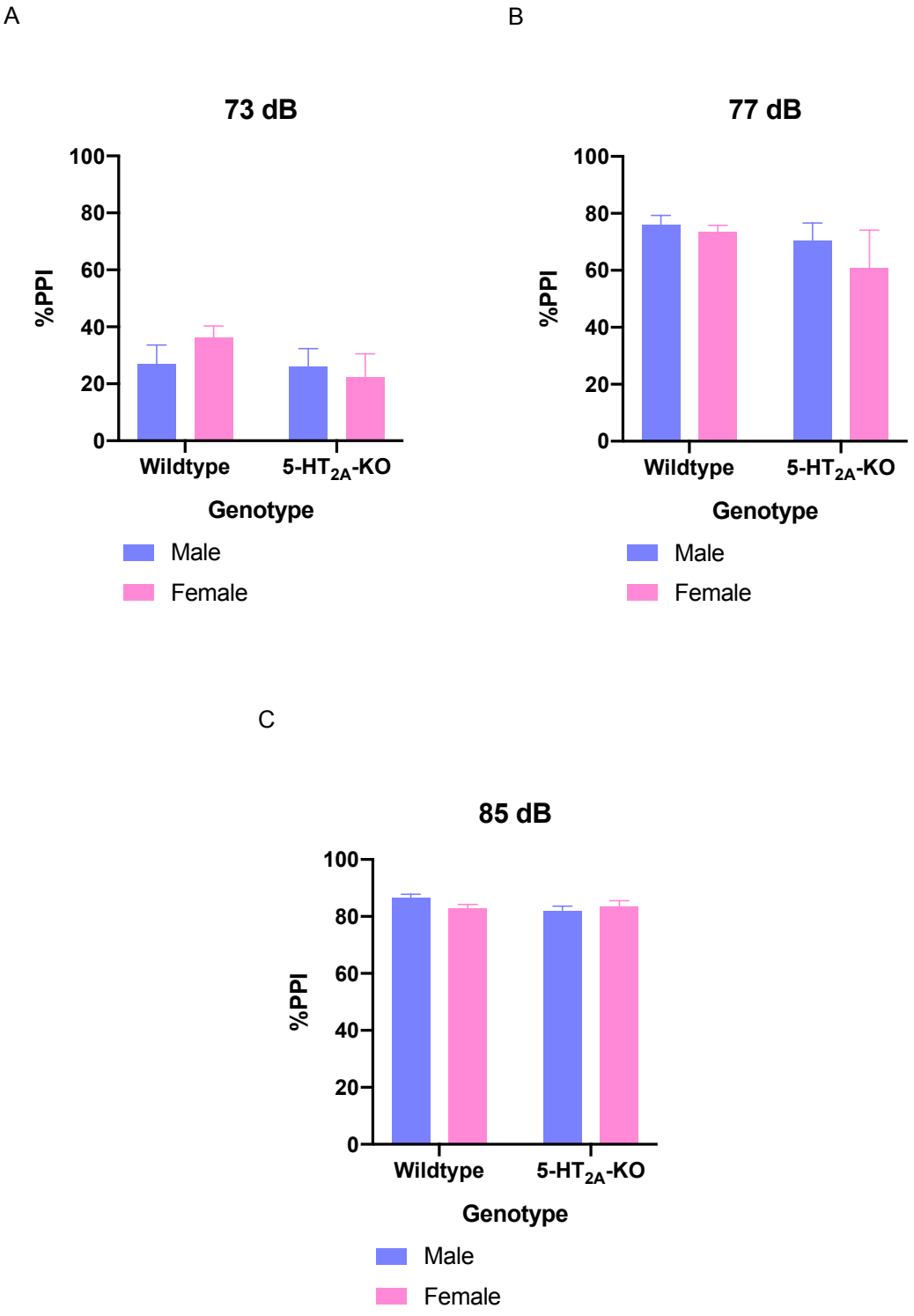

Figure 11: Effect of genotype and sex on DOI's effect on PPI in male and female 129S6/Sv wild-type (*Htr2a*$^{+/+}$) and 5-HT$_{2A}$ knockout (*Htr2a*$^{-/-}$) mice using a shorter ISI. PPI results are separated by prepulse intensity. n = 7 for each group of males and n = 7-11 for each group of females. Data are presented as group mean ± S.E.M.

Chapter 4: Discussion

In contrast to previously published literature suggesting the mGluR2 is necessary for DOI-induced HTR, *Grm2*$^{-/-}$ mice still responded to the DOI and exhibited HTR at multiple low doses, despite using mice from the same mutant colony as reported by others (Moreno et al., 2011). Additionally, two doses of DOI, 0.5 mg/kg and 1 mg/kg, produced unexpected improvements in PPI in male 126S6/Sv wild-type controls (*Htr2a*$^{+/+}$) and 129S6/Sv wild-type mice from Taconic, respectively, using the 20/80/20 protocol. The same effect was observed using another psychedelic, LSD (0.24 mg/kg), in male 129S6/Sv wild-type mice from Taconic also using the 20/80/20 protocol. Using a 10/60/40 protocol, the DOI (0.5 mg/kg) enhancing effect was replicated in 129S6/Sv wild-type mice from Taconic; thus, I continued my experiments using this protocol as a baseline. DOI's effect was absent following pretreatment with the 5-HT$_{2A}$ antagonist M100,907 (1 mg/kg), suggesting this improvement is 5-HT$_{2A}$-dependent. Surprisingly, when using this adjusted 10/60/40 protocol in male 129S6/Sv *Htr2a*$^{+/+}$ mice, DOI produced no effect. DOI-induced improvements in PPI were still present in female 129S6/Sv *Htr2a*$^{+/+}$ mice, and this effect was absent in *Htr2a*$^{-/-}$ mice, again suggesting the 5-HT$_{2A}$ receptor mediates this increase in PPI. DOI also produced PPI increases in female 129S6/Sv *Htr2a*$^{+/+}$ mice using a shorter ISI with the 10/30/40 protocol, but again, no response was seen in males.

The HTR results could be explained by the differences in genetic background of the mice used. Our mGluR2 knockout colony originated in a C57BL/6 background and was backcrossed into the 129S6/Sv strain. The difference in genetic makeup between the colony's current generation and the generation used in our lab's previous publications could account for the differences in head-twitch results. A closer look at the data published by Moreno et al. shows

that the *Grm2*$^{-/-}$ mice used had been backcrossed for at least 10 generations, meaning that no more than 0.09% of the mouse genetic makeup was of the C57BL/6 strain (2011). Though this is small, currently these mice contain even less of a C57BL/6 background as breeding has been ongoing for several years since. Additionally, there could be compensatory mechanisms at play after generations of mGluR2 knockout mice breeding, where another receptor could be overexpressed to support the functions of the missing mGluR2. Further studies should analyze mRNA levels of different GPCRs in these knockout mice to assess whether there is an overcompensation compared to wild-types. Both *Grm2*$^{+/+}$ and *Grm2*$^{-/-}$ should also be compared to wild-type 129S6/Sv mice from Taconic, as an inbred substrain could have been developed as well if mouse breeding was not carried out appropriately. Overall, while these results are surprising, it's important to remember that DOI's primary target, the 5-HT_{2A} receptor, was still present in these mice.

While PPI is a cross-species measure of sensorimotor gating, there have been reported differences in the neural mechanisms of PPI between rats and mice (Geyer et al., 2002; van den Buuse, 2010). For instance, 5-HT_{1A} receptor agonists have been shown to disrupt PPI in rats, but display the opposite effect in mice (Geyer et al., 2001; Gogos et al., 2008). However, DOI is not known to act at the 5-HT_{1A} receptor, so our results cannot be explained by this reasoning (Canal & Morgan, 2012). I also showed that blocking the 5-HT_{2A} receptor pharmacologically in males and using female receptor gene knockout models resulted in the absence of DOI's increase in PPI, suggesting this is a 5-HT_{2A}-specific behavior.

Since it is understood that different strains of mice have varying baseline PPI levels and responses to drugs (van den Buuse, 2010), I used 129S6/Sv mice for all experiments. However, there are other sources of potential differences between the mice from the 5-HT_{2A} knockout

colony and newly acquired mice from Taconic. Though our 5-HT_{2A} knockout colony originated in the 129S6/Sv strain from Taconic, it has been maintained at a different facility for years. Environmental differences between Taconic and Virginia Commonwealth University's facilities could have led to epigenetic changes causing genetic drift in our 5-HT_{2A} knockout colony. This could explain the discrepancy between Figures 7 and 8A, in which the same dose of DOI and the same PPI protocol (10/60/40) resulted in different findings. This theory is supported by a recent analysis that assessed mice of the same substrain obtained from three different suppliers in a multitude of behavioral tests; the study found that the origin of the mice had an effect on test results (Åhlgren & Voikar, 2019).

Additionally, a review by van de Buuse emphasized how different interstimulus intervals could produce alternate results in PPI experiments (van den Buuse, 2010). This could explain the discrepancy between our results in Figures 5A, 8A, and 10A, in which the same dose of DOI was administered and the same mice (*Htr2a*$^{+/+}$) were used, but three different PPI protocols were tested. For this same reason, future studies should incorporate a longer ISI to test whether this protocol change results in altered PPI findings. If multiple PPI protocols continue to produce improvements in PPI, these results could support the use of psychedelics as therapeutic drugs.

Lastly, the sex differences observed in response to DOI-induced changes in PPI follows previous work on sex differences in behavioral tests, including PPI, using LSD; however, this study uses rats and shows that males and females in one phase of the oestrus cycle produce PPI deficits with LSD in comparison to females in a different phase (Pálenícek et al., 2010). Still, it is interesting to know our results are not in isolation. Future studies should also continue to incorporate data from both male and female mice to help identify the mechanisms underlying these differences in hallucinogenic behavior.

This study reveals the important effect that mice genetic and environmental background can have on the results of behavioral experiments. It also touches on the difficulties in reproducing results across different labs as well as translating behavioral results across species, suggesting that the generalizability of results from scientific research is limited. Lastly, it brings to light the potential for sex differences in hallucinogenic drug action.

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