ABSTRACT

Title of Thesis: A SYSTEMATIC INVESTIGATION ON THE MEDICINAL USE OF LYSERGIC ACID DIETHYLAMIDE

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Animal and Avian Science

Evidence points to several mechanistic relationships where lysergic acid diethylamide (LSD) alters the serotonergic system in autism spectrum disorders (ASD) and positively impacts ASD-related clinical outcomes. Clinically relevant endpoint measurements resulting from the interaction of LSD with various psychiatric disorders and the etiology of ASD were selected and analyzed for a review. Peer reviewed and publically available original scientific studies in humans, animal models, or cell cultures with LSD as the primary treatment and a reasonable sample size were included for review. The endpoint measurements selected for the review fall into the following categories: changes in neurotransmitters, physiological markers, metabolites and other intermediates, impact on brain connectivity, brain morphology and histology, receptor activity, and gene expression. The review intends to elucidate a promising mechanism of action through which LSD could be interacting with the factors responsible for the etiology of ASD. The overarching goal of the review is to illustrate the potential for the therapeutic use of LSD and its analogues towards the management of various psychological and neurodevelopmental disorders, including ASD. This review could

reveal a refined hypothesis for future research in order to identify specific molecular targets of LSD or its analogues for the treatment and management of ASD.

A SYSTEMATIC INVESTIGATION ON THE MEDICINAL USE OF LYSERGIC ACID DIETHYLAMIDE

by

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Nomenclature

1P-LSD	1-propionyl lysergic acid diethylamide, an analogue of LSD
Agonist	a substance that initiates a physiological response when combined with a receptor
AMPA	2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate
Analogue	a compound with a molecular structure similar to that of another
ASD	autism spectrum disorder
cAMP	cyclic adenosine monophosphate
Comorbidity	when two diseases exist in one patient
Hallucinogen	a psychoactive agent which causes hallucinations and changes in emotion
NMDA	N-methyl-D-aspartate
PFC	Prefrontal cortex
PKA	Protein Kinase A
PKC	Protein Kinase C
LSD	lysergic acid diethylamide, a synthetic hallucinogen
Psilocybin	natural psychedelic compound
Serotonergic	associated with the action of serotonin
Serotonin	a neurotransmitter, or chemical messenger that transmits signals across a synapse

Introduction

Evolution of LSD: A Historical Perspective

Lysergic acid diethylamide (LSD) is a hallucinogenic drug with prominent psychoactive effects. First synthesized in 1938 and ingested in 1943, Swiss chemist Albert Hoffman is credited with both the creation of the substance and the discovery of its hallucinogenic effects (Ulrich & Patten, 1991). Since then, LSD has been closely studied in an effort to better understand its mechanism of action. The discovery of LSD was born out of a desire to use the natural compound, lysergic acid, in medicinal ways to help treat illness and disease. Early research on the use of LSD, conducted from the late 1940s to the mid-1970s, aligned with the motivation behind its discovery and investigated its potential use in treating anxiety, addiction, headaches, depression, and even autism spectrum disorders (ASD) (Sigafoos, 2007). With the rise of LSD use, the identification of its potent hallucinogenic side effects and its consequent abuse in recreational settings, the compound was eventually categorized as a Schedule 1 substance. With newfound stigma surrounding this once promising therapeutic agent, LSD research came to a halt and experienced a 30-year hiatus. Upon the dawn of the 2000s, renewed interest in transpersonal psychology and the potential therapeutic use of psychedelics in medicine experienced a renaissance. Although the early studies of LSD have faced severe criticism, the fundamental ideas underlying the studies are worth revisiting through a reformed framework of experimental design and execution. In this review article, we seek to reevaluate and present the findings from several studies that support LSD's therapeutic potential in addressing symptoms of various mental and

neurodevelopmental disorders. We specifically focus on LSD's potential to interact with molecular mechanisms towards mitigating symptoms associated with ASD.

Benefits and Deficiencies of LSD

The story surrounding LSD has been one of controversy, as scientists have spent decades debating if the compound's potential for substantive therapeutic benefits mitigates its potent hallucinogenic effects. Research has shown that LSD binds to and activates the human serotonin (5-hydroxytryptamine or 5-HT) 5-HT_{1A}, 5-HT_{2A}, and 5-HT_{2C} receptors, and that LSD is only a partial agonist at 5-HT_{2A} receptors (Rickli et al., 2016). The ability of LSD to bind with 5-HT_{2A} receptors in a partial agonistic manner has been shown to be primarily responsible for the hallucinogenic effects of LSD (Preller et al., 2017). These hallucinogenic effects associated with LSD caused it to rise in popularity as a recreational psychedelic drug. The government sought to regulate the access to and use of the drug as its hallucinogenic properties were deemed to be a danger to the population due to its potential for abuse and psychological/physical dependence ("Drug Scheduling"). This regulation and scheduling of LSD as a Schedule 1 drug prevented further scientific exploration of its beneficial effects for several decades.

Despite the potent hallucinogenic responses, significant evidence also points towards the fact that, when used in a defined and controlled manner, the medicinal and therapeutic properties of LSD can be significant and warrant further exploration. LSD research has documented a variety of therapeutic potentials for LSD, towards a number of disorders, in turn motivating further research into LSD assisted therapies. Recent studies suggest that LSD has potential medicinal uses, such as managing alcoholism and supplementing psychotherapy techniques in terminal cancer patients. A research study

conducted by Gasser et al. (2014) has shown that LSD can be successfully used to reduce anxiety for an extended period in patients with anxiety associated with life-threatening disease. Studies comparing pre- and post-treatment conditions of terminal cancer patients after LSD-assisted therapies have illustrated dramatic improvements in the degrees of emotional and physical distress (Grof, 1973). Recent studies corroborate these findings where, when LSD was implemented into psychotherapies for patients with terminal illness, 77.9% of patients reported to have less fear of death and 66.7% reported an improved quality of life, both of which persisted 8 months after the LSD assisted psychotherapy (Gasser et al., 2015).

Strategies and Continued Efforts towards Investigating the Benefits of LSD

Gaining access to utilize LSD, even in a research setting, has many barriers due to its Schedule 1 status. The use of analogues, molecules similar to major compounds with minute variations in chemical structure (Coney et al., 2017), has proven to be a viable method by which to carry out LSD research without utilizing the actual drug. For example, 1P-LSD is an analogue of LSD that has been shown to have similar physiological effects as LSD in mice. The pharmacological similarities between LSD and 1P-LSD are likely due to their similar chemical structures and mechanisms of action, as indicated by the shared indole functional group. The only structural difference between LSD and 1P-LSD is the substitution of the hydrogen bonded to the indole nitrogen in LSD with a propionyl group in 1P-LSD (Brandt et al., 2016).

A research study by Brandt et al. (2016) has shown that the hallucinogenic effects of LSD and 1P-LSD can be measured in mice and rats using the head-twitch response (HTR), as it can distinguish between hallucinogenic and non-hallucinogenic 5-HT_{2A}

agonists. When given up to 100 μg/kg of 1P-LSD, mice showed no significant difference in HTR when compared to the control group over the span of 30 minutes post-administration. This indicates the potential of employing microdosing techniques (defined as a dosage below that which would result in hallucinogenic effects) in the study and implementation of LSD and 1P-LSD as a therapeutic drug. Additionally, 1P-LSD was found to have about 38% the potency of LSD based on results from the study. Further research is necessary, but the reduced potency of 1P-LSD may mitigate the hallucinogenic impacts of the drug. As a result, 1P-LSD may be an effective alternative to LSD.

In recent years LSD research in humans has made a resurgence since its initial decline in the 1970s. There have been a wide variety of studies seeking to understand the impact of LSD on human behavior, physiology, and brain function. Several positive clinical outcomes from human studies following LSD treatment illustrate the promise of LSD as a management strategy for various psychiatric disorders (see Table 1). In human behavior studies, LSD was shown to reduce anxiety (Gasser, 2014; Gasser, 2015). Additionally, LSD has been shown to enhance empathy and prosocial behavior (Dolder, 2016).

Physiologically, doses of LSD between 100 μg and 200 μg cause increased blood pressure, heart rate, body temperature, and pupil size (Dolder, 2015; Dolder, 2016; Dolder, 2017; Schmid, 2015). Studies that utilized lower doses or microdoses of LSD did not find any effect on blood pressure (Family, 2020), heart rate (Bershad, 2020; Family, 2020), and body temperature (Bershad, 2020). Research has shown that single microdoses of LSD can produce orderly dose-related subjective effects in healthy

individuals and that a threshold dose of 13 μg might be used safely in an investigation of repeated administrations (Bershad, 2019). Research has also shown that low doses of LSD (below 20 μg) enhance sustained attention, reduce speed of information processing, affect mood states in positive directions, and increase anxiety and confusion (Hutten, 2020). Furthermore, most apparent effects are present at doses above 20 μg (Hutten, 2020). More serious complications such as precipitation of mania or psychosis are possible with the administration of LSD, however these effects are unlikely at low doses and for people with ASD.

Functional MRI studies have shown that LSD can increase thalamic connectivity (Muller, 2017; Muller 2019; Preller, 2018; Preller, 2019), as well as brain wide connectivity (Preller, 2018), and amygdala seed-based connectivity (Bershad, 2020). Magnetoencephalography (MEG) imaging shows that LSD increases cerebral blood flow (CBF) in the visual cortex (Carhart-Harris, 2016). MEG imaging also indicates that LSD decreases alpha power (Carhart-Harris, 2016; Pallavicini, 2019), delta power (Barnett, 2020; Carhart-Harris, 2016), and theta power (Pallavicini, 2019). Overall, there is a wealth of information pointing to several beneficial effects of LSD on a variety of outcome measures.

Mechanisms of action of LSD

Research has identified strong connections between the activity of 5-HT_{1A} receptors with anxiolytic and depressive disorders. As the serotonin system is known to regulate mood and the resultant physiological changes expressed (Heisler, 1998), research unrelated to LSD has shifted focus to examining the mechanisms and downstream effects of 5-HT_{1A} receptors, including post-synaptic N-methyl-D-aspartate

(NMDA) receptor and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor hyperactivity. The underlying pathologies in these mechanisms have been linked to many different psychiatric and neurodevelopmental disorders including ASD (Rossignol & Frye, 2012).

LSD's various mechanisms of action have been extensively explored and published. Mechanistically, LSD is a well-established serotonin agonist. The ergoline is known to bind with high affinities to most serotonin receptors in the brain, most notably the 5-HT_1 and 5-HT_2 receptor families (Backstrom, 1999). The hallucinogenic aspect of the drug upon binding to the 5-HT_{2A} receptor garners the majority of attention that it receives, often excluding much of the complete mechanistic effects of the drug as a whole. In fact, a 2016 experiment by Rickli, Moning, Hoener, & Liechti has deduced that LSD was equally potent at the 5-HT_{1A} and 5-HT_{2A} receptors. In addition, LSD was the only tested hallucinogen to demonstrate affinity to the 5-HT_{1A} receptor at 1-digit nanomolar concentrations. Thus, it is imperative to consider the mechanistic effects of LSD at other major binding receptors, such as the 5-HT_{1A} receptor. Similar to the commonly prescribed anxiolytic drug buspirone, LSD shows partial agonist character at the 5-HT_{1A} autoreceptor (Aghajanian, 1999). Desensitization of the autoreceptor and activation of the postsynaptic 5-HT_{1A} receptor is accomplished through the general increase in serotonin levels by serotonin precursor supplementation. Inhibition resulting from the autoreceptor activity is hindered by dislocating the inhibitory G-protein signaling cascade of the 5-HT_{1A} autoreceptor. This activity is implicated in rebalancing hippocampal 5-HT_2/5-HT_{1A} signaling by decreasing and increasing respective receptor activity, a mechanism seen in established antidepressants (Buchborn, 2015). The

metabolic implications of the consumption of LSD includes the ability to upregulate phosphoinositide hydrolysis through the promotion of protein kinase C translocation, increased endogenous levels of creatine, tryptophan, and tyrosine (Backstrom, 1999; Berg, 1998; Dumuis, 1988; Halaris, 1975; Lewis, 1965; Smith, 1975). LSD also stimulates the metabolism of 5-HT in the nucleus accumbens (Antkiewicz-Michaluk, 1997).

The potential roles of the 5-HT_{2A} receptor in ASD and other neurodevelopmental disorder pathology have also been extensively explored. For example, ASD has been associated with increased platelet serotonin and decreased 5-HT_{2A} receptor binding in the brain's cortical regions (Raote et al., 2007). Similarly to implications made from LSD's mechanisms of action with the 5-HT_{1A} receptor, research demonstrates promising indications of LSD administration, as it functions as a 5-HT_{2A} agonist. However, much has yet to be discovered. It is fully necessary for additional future research of the 5-HT_{2A} receptor in regards to ASD. Potential research may consider the analysis of 5-HT_{2A} receptor heteromers, such as the mGlu2-5-HT_{2A} heteromer (DelaCuesta-Barrutia et al., 2020).

LSD has also been shown to affect the glutamatergic receptors N-methyl-D-aspartate (NMDA) and 2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl) propionate (AMPA). LSD, acting as a 5-HT_{1A} receptor agonist, can inhibit glutamate release into the synapse (De Gregorio et al., 2018). This prevents the over-excitation of NMDA receptors, which in turn prevents the overload of calcium that causes the accumulation of reactive oxygen species (ROS) which signal for apoptosis and neuronal death (Dong, 2009). It has also been suggested that LSD can disrupt the assembly of NMDA receptor

subunits, limiting the number of functional NMDA receptors. AMPA receptors are affected in a similar way, as the reduced glutamate also prevents over-excitation (De Gregorio et al., 2018). However, excitatory transmission involving AMPA currents is potentiated by LSD in the medial prefrontal cortex (mPFC) through mTORC1 signaling, whereas NMDA-mediated synaptic responses were reduced in the same brain region (Arvanov, 1999; De Gregorio, 2021).

Additionally, administration of LSD affects the dopaminergic system. LSD acts as an agonist to D2 receptors, and a partial agonist to D1 receptors (Giacomelli et al., 1998). It is not fully clear if or how this activity contributes to the psychoactive effects of LSD, but it has been suggested that interaction between the serotonin and dopamine systems that causes differences in LSD's effects between subjects (Passie, 2008). Furthermore, LSD's modulatory effect on the endogenous dopamine (DA) system has been the subject of recent investigations. LSD has been shown to decrease metabolic DA urinary output, suggesting a therapeutic mechanism for phenotypes of ASD with upregulated DA activity (Messiha, 1973; Nakamura, 2010). However, it has recently been shown that low doses of LSD do not significantly alter DA firing involved in the ventral tegmental area (VTA)-dependent addiction mechanism, supporting the hypothesis of a low chance of addiction existing from the chronic administration of microdoses of LSD (De Gregorio, 2016).

As a result of collective, auspicious human studies, LSD has definitively manifested its promise as a potential therapeutic for a variety of psychiatric disorders (see Table 1). As further research continues to delve into the widespread application of its mechanistic potentials, the drug's clinical capacity undoubtedly advances.

TABLE 1. Evidence of beneficial effects of LSD from in vivo human studies.

Outcome Measures	Findings	Dosage
Behavioral		
State-Trait Anxiety Inventory (STAI)	Significant reduction in generalized anxiety for 12 months post drug administration[a] and reduction in anxiety with increased quality of life[b]	20 µg (placebo), 200 µg (experimental)[a,b]
Multifaceted Empathy Test (MET) and Social Value Orientation (SVO)	Enhanced implicit and explicit emotional empathy and increased prosocial behavior in patients with anxiety and life-threatening illnesses[c]	100 µg, (experimental), 200 µg (experimental)[c]
Physiological		
Blood Pressure	Significant increase in healthy patient,[d] increase in healthy patients,[c,e,f] increase in microdosed healthy patient,[g] and no significant change in low dose healthy patient[h]	200 µg (experimental)[d]
Heart Rate	Significant increase in healthy patient[d] increase in healthy patients[c,e,f] no significant change in low/micro dosed healthy patients[g,h]	0 µg (placebo), 5 µg, 10 µg, or 20 µg (experimental)[h]
Body Temperature	Significant increase in healthy patient [d] increase in healthy patients[c,e,f] no significant change in microdosed healthy patient[g]	6.5 µg, 13 µg, and 26 µg (experimental)[g]
Pupil Size	Significant increase in healthy patient[d, f] increase in healthy patient[c]	200 µg (experimental)[c,f]
Brain		
Resting State fMRI, BOLD	Increased thalamic connectivity[i,j,k,l] decreased connectivity from ventral striatum to the thalamus[j] increased cerebral blood flow [m] increased amygdala seed-based connectivity [g] increased fractal dimension of BOLD signals in dorsal-attention network[n]	100 µg (experimental)[i,j], 75 µg and 100 µg (experimental)[k], 100 µg (experimental)[l], 75 µg (experimental)[n]
Magento Encephalography (MEG)	associations between ego dissolution and decreased delta and alpha power; associations between hallucinations and decreased alpha power[m] occipital, parietal and frontal decreases in the low alpha and theta bands[o] general decrease in spectral power δ–β bands, significant increases in MI[p]	75 µg (experimental)[m], 75 µg (experimental)[o], 75 µg (experimental)[p]

Note: Data are from Gasser et al. 2014[a], Gasser et al. 2015[b], Dolder et al. 2016[c], Schmid et al. 2015[d], Dolder et al. 2017[e], Dolder et al. 2015[f], Bershad et al. 2020[g], and Family et al. 2019[h], Preller et al. 2018[i], Preller et al. 2019[j], Muller et al. 2019[k], Muller et al. 2017[l], Carhart-Harris et al. 2016[m], Varley et al. 2020[n], Pallavicini et al. 2019[o], Barnett et al. 2020[p].

TABLE 2. Evidence of beneficial effects of LSD from in vivo animal models and in vitro experimental systems.

Outcome Measures	Findings	Dosage
Metabolites		
Creatine	Increase[a]	0-1 mg/kg (experimental)[a]
Tryptophan	Increase[b]	Unavailable[b]
Tyrosine	Slight increase[c]	520 mug/kg or 1040 mug/kg[c]
cAMP	Significant decrease[d]	Unavailable[d]
Phosphoinositide hydrolysis, PKC, Ca2+ release	LSD and endogenous 5-HT promote phosphoinositide hydrolysis and translocation of PKC, but LSD cannot promote calcium release[e]	At 1 µM concentration (experimental)[e]
Arachidonic acid release, PLC-inositol phosphate, calcium	LSD is a more effective agonist than endo5-HT at phospholipase A2-mediated arachidonic acid release. LSD is not more effective in PLC-inositol phosphate accumulation or calcium mobilization[f]	At 0.3 µM (concentration)[f]
Neurotransmitters		
Dopamine	Significant increase[g], reduced urinary dopamine excretion[g], high doses increase dopamine turnover[h]	200-300 µg (experimental)[g], 0.1 and 2.0 mg/kg (experimental)[h]
Norepinephrine	Significant decrease[c]	520 mug/kg or 1040 mug/kg[c]
Glutamate	Increase[i,j], decrease in frontal cortex[h]	0.1 mg/kg (experimental)[i], 0.1 mg/kg (experimental)[j]
Serotonin	Decrease in nucleus accumbens[h, k]	25 µg/kg (experimental)[k]
Receptor Expression/Activity		
NMDA currents	LSD inhibits N-methyl-d-aspartate (NMDA)-induced inward current and NMDA receptor-mediated synaptic responses evoked by electrical stimulation of the forceps minor in	0.5-2 µM (experimental)[l]

	pyramidal cells of the prefrontal cortex[l]	
AMPA currents	LSD potentiates the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) synaptic responses in the mPFC.[p]	30 µg/kg (experimental)[p]
5-HT receptor activity	Potentiating mPFC excitatory transmission through 5-HT_{2A}/ AMPAR and mTORC 1 signaling.[p] 5-HT_{1A} plays a modulatory role in the stimulus effects of LSD[n]. LSD may rebalance hippocampal 5-HT signaling[o].	0.1 mg/kg (experimental)[n], 0.13 mg/kg/d (experimental)[o]
5-HT/DA receptor activity	At low doses, decrease in dorsal raphe nucleus 5-HT firing through 5-HT_{2A} and D2 receptors. Does not alter VTA DA firing activity[p]. Similar partial agonist activity of 5-HT_{1A} autoreceptor to buspirone[q,r] .	5-20 µg/kg or 30-120 µg/kg (experimental)[p], 10 nM concentration (experimental)[q], 0.025 mg/kg (experimental) [r]
Brain Morphology, Histology, and Electrophysiology		
Formation of neurons, dendritic spines, and synapses	LSD increases neuritogenesis, spinogenesis, and synaptogenesis both in vitro and in vivo[s]	10 nM (experimental)[s]
Purkinje neuron morphology	LSD causes formation of cisternal stacks in the dendrites of Purkinje neurons[t]	1700 µg/kg (experimental)[t]
Neuron firing	Reticular thalamic neuron burst-firing and spontaneous firing activity decreased in 50% at 10µg/kg of LSD and firing increased at 40µg/kg LSD. LSD doses also resulted in increased spontaneous firing and burst-firing in thalamocortical neurons[u]	5-160 µg/kg (experimental)[u]
Gene Expression		
RNA sequencing and QPCR	Expression of genes involving neurotransmissions, synaptic plasticity, energy metabolism and neuropeptide signaling increased[v]	0.16 mg/kg (experimental)[v]
Immunohistochemistry and light microscopy of c-Fos cells	Increases in the number of c-Fos-positive cells were observed with d-LSD doses[w]	0.95 nM (experimental)[w]
RNase protection assays	In PFC, there was no change in p65/RelA expression, but homer1a expression increased[x]	0.5 or 1 mg/kg (experimental)[x]

Note: Data are from Lewis et al. 1965[a], Halaris et al. 1975[b], Smith et al. 1975[c], Dumuis et al. 1988[d], Backstrom et al. 1999[e], Berg et al. 1998[f], Messiha, Grof 1973[g], Antkiewicz-Michaluk et al. 1997[h], Winter et al. 2004[i], Muschamo et al. 2004[j], Aghajanian, Foote, Sheard 1968[k], Arvanov et al. 2001[l], Gregorio et al. 2021[m], Reissig et al. 2005[n], Buchborn et

al. 2014[o], Gregorio et al. 2016[p], Aghajanian, Marek 1999[q], Buchborn et al. 2015[r], Ly et al. 2018[s], Hendelman et al. 1983[t], Inserra et al., 2021[u], Martin et al. 2014[v], Fantegrossi et al. 2008[w], Nichols et al. 2003[x]

Inaugural research regarding the mechanistic effects of LSD have highlighted many downstream responses in the brain (see Table 2). First, fluctuations in metabolite and neurotransmitter levels, including creatinine, tryptophan, dopamine, and norepinephrine, have been illustrated after administration of LSD at varying doses. Initial trials exploring the brain's responses to LSD hold potential limitations as the drug was not as comprehensively understood as it is today. Yet, these trials demonstrate promising results, such as an increase in brain dopamine after LSD administration (Messiha & Grof 1973; Antkiewicz-Michaluk et al., 1997). Such positive initial findings strengthened the therapeutic possibilities of the drug in regards to clinical implementation and certainly generated more implications to follow.

Subsequently, with the use of increasingly modern technology, contemporary research has been able to examine the specific intricacies of LSD's mechanisms and downstream effects. Implementation of advanced imaging techniques, prevalent assays, and electrophysiological techniques have further unraveled the drug's possibilities, highlighting key features such as the relevance of glutamate receptors (Gregorio et al., 2021; Reissig et al., 2005) and improvements in brain morphology after administration of the drug (Ly et al. 2018). As experimental techniques continue to advance, the understanding of LSD's mechanistic properties and clinical presence will only continue to expand correspondingly.

Pathology of Autism Spectrum Disorder

TABLE 3. Proposed mechanisms which contribute to the etiology of ASD.

Outcome Measures	Findings
Receptor Expression/Activity	
5-HT and DA transporter binding	Low 5-HT, high DA activity in human patients with high functioning ASD[a]. In Asperger's syndrome, reduced receptor binding of the 5-HT_{2A} receptor correlates with abnormal social communication, suggesting abnormalities in the cortical receptor density may underlie social deficits.[b]
Cell Signaling	
Cytokine	Possible that cytokine dysregulation contributes directly to neural dysfunction in ASD[c]
Tumour necrosis factor-α	Findings looking at the serum of children with ASD indicate elevated tumour necrosis factor-α[d]
Lipid metabolism	Findings indicate that various biomarkers of fatty acid elongation and desaturation were statistically elevated in all autistic subjects indicating pervasive mitochondrial stress[e]
Metabolites	
cAMP	In autistic children, the plasma cyclic AMP levels were higher than in normal children and were positively correlated with the WWPAS score[f]
Tryptophan	Finding of decreased tryptophan metabolism appears to provide a unifying biochemical basis for ASDs and perhaps an initial step in the development of a diagnostic assay for ASD[g]
Kynurenine pathway	Findings also suggest the increased quinolinic acid may be linked to 16p11.2 mutations leading to abnormal glutamatergic activity associated with ASD pathogenesis[h]
Urinary metabonomic profiling	An increase in the levels of tryptophan, hippurate, glycine, and creatine, and a decrease in trigonelline, melatonin, pantothenate, serotonin, and taurine were observed compared to the control group[i]
Neurotransmitters	
Dopamine	Impaired striatal DA neurotransmission and altered DA-dependent behaviors that correspond with some of the behavioral phenotypes observed in ASD[j]
Glutamate	Glutamate reuptake is inhibited through immune system abnormalities causing increased tumour necrosis factor-α release in conjunction with microglial activation consequently resulting in excitotoxicity[k] and findings indicate elevated serum glutamate levels in patients with ASD compared to control groups[k]
Amino acids	Autistic children showed elevated levels of glutamic acid and asparagine; lower levels of phenylalanine, tryptophan, methionine and histidine. A low molar ratio of (tryptophan/large neutral amino acids) x 100 was observed in autism, indicating lesser

	availability of tryptophan for neurotransmitter serotonin synthesis. Elevated levels of excitatory amino acids (glutamate and asparagine), decreased essential amino acids (phenylalanine, tryptophan and methionine) and decreased precursors of neurotransmitters (tyrosine and tryptophan) are the distinct characteristics of plasma amino acid profile of autistic children[m]
Brain Morphology, Histology, and Electrophysiology	
Dendritic spine density	ASD genes contribute or regulate the pruning and turnover of dendritic spines during development[n]
fMRI and connectivity	Individuals with ASD have been found to display disrupted intra-thalamic connectivity and dysregulated thalamocortical networks as well as decreased thalamic local functional connectivity density[o] and there may be impairment of thalamocortical connectivity that contributes to the symptoms of autism, and while findings are not casual they do indicate an association[p]
Behavioral	
Comorbid mental illness	Adults with ASD were significantly more likely to have psychiatric disorders including major depressive disorder and multiple anxiety disorders than those without ASD[q]
Social activity	Children with ASD participate in less social activity and experience more obstacles to participation in social events than children without ASD[r]

Note: Data are from Nakamura et al., 2010[a], Murphy et al., 2006[b], Goines & Ashwood, 2013[c], Cohly & Panja, 2005[d], Pastural et al., 2009[e], Hoshino et al., 1980[f], Boccuto et al., 2013[g], Lim et al 2016[h], Liang et al., 2020[i], DiCarlo et al., 2019[j], Essa et al., 2013[k], Shinohe et al., 2007[l], Naushad et al., 2013[m], Varghese et al., 2017[n], Tomasi and Volkow, 2017[o], Nair et al., 2013[p], Joshi, 2013[q], Shattuck, 2011[r].

ASD, a series of neurodevelopmental disorders, are very complex traits that have many physiological manifestations (Goines & Ashwood, 2013). Due to the heterogeneity of ASD, the true etiology and pathways of mechanisms leading to the development of ASD and its symptoms remain unclear and require more research (Rossignol & Frye, 2012; Watts, 2008). Despite the complexity of ASD, there are many physiological traits that seem to be consistently prevalent in many people with ASD (see Table 3). While some may consider ASD primarily a genetic disorder caused by gene or chromosomal mutations, there is increasing evidence that metabolic and physiological dysfunctions play a major role in ASD phenotypes (Rossignol & Frye, 2012). Many studies point towards immune dysregulation and neural activity differences as major reasons for the

various phenotypes of ASD (Goines & Ashwood, 2013; Rossignol & Frye, 2012; Watts, 2008).

In a study of ASD research trends from 1971-2010, it was found that immune dysregulation/inflammation and oxidative stress was one of the most studied physiological mechanisms leading to the development of ASD. Known to be present in a variety of other psychiatric disorders, inflammation, specifically of the neurons, is also prevalent in ASD (Rossignol & Frye, 2012). In ASD, neuro-inflammation damages the brain mainly through pro-inflammatory cytokines release, and this has been found in various studies to be a cause of autism (Rossignol & Frye, 2012; Schaefer, 2008). Cytokines are proteins that are central to the regulation of immune response in the body and serve as a liaison between the immune and nervous systems (Goines & Ashwood, 2013). The release of cytokines in response to such neuro-inflammation can result from over-excitation of NMDA receptors in neuronal cells. Under normal conditions, NMDA receptor activity modulates the influx of calcium into neural cells by the binding of glutamate (Chaparro-Huerta et al., 2005). However, in ASD, hyperactivity of NMDA receptors from glutamate toxicity often induces excitotoxicity, or the injury and death of neurons from glutamate overexposure (Dong, 2009).

There are also metabolic disorders associated with the development of ASD. Mitochondrial dysfunction is commonly associated with a multitude of disorders such as bipolar disorder and depression as well as ASD. Mitochondria produce adenosine triphosphate, the cell's energy currency, and dysfunctions in this organelle have been proven to impact calcium homeostasis and synaptic plasticity in addition to causing irregularity in the central nervous system and gastrointestinal tract (Rossignol & Frye,

2012). Additionally, mitochondrial stress in individuals with ASD is indicated by elevated biomarkers of fatty acid elongation and desaturation (Pastural et al., 2009). Furthermore, irregularity in the kynurenine pathway metabolism, the breakdown of tryptophan to serotonin and melatonin has also been associated with the development of ASD. Studies have suggested that an immune response causes the irregularity of metabolism in the Kynurenine Pathway which has been linked to excitotoxicity and brain damage (Lim et al., 2016).

Individuals with ASD have also been shown to have impaired neurotransmission of dopamine (DiCarlo, 2019). Altered levels of many amino acids including increasing glutamic acid and asparagine and decreased phenylalanine, tryptophan, methionine and histidine has been shown in people with ASD (Boccuto, 2013; Naushad, 2013). In another study through urinary metabolomic profiling, researchers found increased tryptophan, hippurate, glycine and creatine with decreased trigonelline, melatonin, pantothenate, serotonin and taurine in individuals with ASD (Liang, 2020). In high functioning individuals with ASD, lower 5-HT activity and higher DA activity has been observed (Nakamura, 2010). A study of adults diagnosed with Asperger's syndrome using single photon emission computed tomography with a 5-HT_{2A} receptor ligand showed that reduced receptor binding resulting from abnormally low cortical receptor density correlated with abnormal social communication (Murphy, 2006). Genes associated with ASD contribute to the pruning and turnover of dendritic spines during development (Varghese, 2017). It has been suggested that cytokine dysregulation contributes directly to neural dysfunction in ASD (Goines & Ashwood, 2013). Increased quinolinic acid may be linked to mutations associated with ASD (Lim, 2016).

Current Methods of Managing ASD Symptoms

Various medications can be prescribed to manage symptoms of ASD. Although many of these drugs have not been approved by the FDA, the comorbid nature of ASD with other disorders allows these drugs to be prescribed. Risperidone is the only FDA-approved drug to treat autism symptoms specifically. This drug focuses on decreasing irritation within children with ASD who are 5-16 years old (Eunice Kennedy Shriver National Institute of Child Health and Human Development, 2017). Other drug-related treatment options for ASD include selective serotonin reuptake inhibitors (SSRIs), tricyclics, psychoactive medications, antipsychotic medications, stimulants, anti-anxiety medications, and anticonvulsants. Many of these drugs seek to reduce the severity of symptoms associated with ASD, and their prescription depends on the specific presentations on the spectrum and individuals' needs.

A novel method for managing symptoms of ASD involves modulation of the neurotransmitter serotonin within the body, which has been closely linked to many of the pathological and behavioral symptoms of ASD (Siemann et al., 2017). This research study has shown that serotonin levels play a role in neocortical excitation/inhibition, correct sensory stimulus tuning, and social behavior. It was shown that low serotonin levels in 15q11-13 CNV mice (a rodent model for ASD) resulted in impairment of social behavior, and that restoration of normal serotonin levels reversed these effects. As a result, recent research indicates that there is a potential therapeutic benefit for the use of serotonin and serotonin receptor agonists in the treatment of ASD symptoms, including lack of social behavior associated with social anxiety.

SSRIs are the class of drugs that have been traditionally used to treat anxiety disorders and have been used to manage anxiety related symptoms of ASD. One of the main criticisms of SSRIs is that their effects diminish over time, as the result of a negative feedback loop facilitated by the indirect activation of presynaptic 5-HT_{1A} receptors (Celada et al., 2013). LSD has been proposed as an effective alternative treatment for the symptoms of anxiety disorders including those comorbid with ASD. This is because LSD also activates presynaptic 5-HT_{1A} receptors and inhibits serotonin release while activating and preventing downregulation of postsynaptic 5-HT_{1A} receptors (Passie, 2008). As a result, LSD may be more effective in managing anxiety related symptoms of ASD than SSRIs.Anecdotal evidence suggests the therapeutic potential of the daily administration of microdoses of LSD in order to support social behavior in individuals with ASD. This rationale is reasonable based on the existing literature linking modulations in 5-hydroxytryptamine (5-HT) receptors with social behavior. As discussed in the following section, LSD shows potential of addressing many ASD symptoms beyond behavioral concerns to target pathology as well.

Proposed Beneficial Actions of LSD towards the Management of ASD

In human behavior studies, LSD was shown to reduce anxiety (Gasser, 2014). Anxiety is a common comorbidity of ASD (Joshi, 2013), and therefore this effect may provide a significant benefit over a significant period of time. Multiple studies have shown that the anxiety reducing effects of LSD treatment persist for at least one year after receiving only a single dose (Gasser, 2014; Gasser, 2015). This effect of LSD has been largely studied in patients with life-threatening diseases, and it has shown enough promise in such situations that it warrants further investigation in other populations.

People with ASD experience major obstacles to full social participation, especially at young ages (Shattuck, 2011). This can contribute to decreased social activity, which may worsen existing comorbid mental illnesses such as social anxiety and depression. LSD has been shown to enhance empathy and prosocial behavior (Dolder, 2016). This effect may allow people with ASD to more fully participate in social situations, and in general more easily connect with other people.

Physiologically, doses of LSD between 100ug and 200ug cause increased blood pressure, heart rate, body temperature, and pupil size (Dolder, 2015; Dolder, 2016; Dolder, 2017; Schmid, 2015). Though in certain populations any increase in these measures has the potential to be dangerous, safety evaluations in every study examined in this review concluded that there were no significant safety concerns for the general public at these dosage levels. Studies that utilized lower doses or microdoses of LSD did not find any effect on blood pressure (Family, 2020), heart rate (Family, 2020; Bershad, 2020), and body temperature (Bershad, 2020). This indicates that a lower dosage level may be a treatment option for those more at risk from the changes produced from a higher or more "standard" dose while still experiencing the therapeutic effects (Family, 2020; Bershad, 2020). All literature reviewed here indicated that while LSD can affect physiological measures such as heart rate and blood pressure, it presents little danger to the general population when used as a therapeutic. Additionally, lower doses may be a viable option for providing the therapeutic effects of LSD to other, more vulnerable populations.

LSD has also been found to impact the brain by altering connectivity between regions including those found to be hypo-connected in people with ASD. The function of

the thalamus region is traditionally associated with managing sensory inputs and transmitting it to the the cerebral cortex, but studies have also found that it may play a role in the emergence of emotional and cognitive disorders (Nair et al., 2013; Carrera & Bogousslavsky, 2006). Studies utilizing resting state fMRI to analyze the impact of ingesting 75 μg and 100 μg of LSD indicate that increased thalamic connectivity was a common finding of analyses. This finding shows promise in addressing the dysregulated and impaired thalamocortical networks that have been found among adults and adolescents with ASD (Tomasi & Volkow, 2017; Nair et al., 2013). The ability of LSD to increase thalamocortical connectivity in non-ASD patients indicates the potential for LSD to impact the connectivity of thalamocortical networks among those with ASD.

In terms of neurotransmitters, treatment with LSD reverses the negative trends seen in ASD. These negative trends include decreased dopamine signaling (DiCarlo et al., 2019) and increased levels of glutamate (an excitatory amino acid) (Shinohe et al., 2007). LSD results in a significant increase in dopamine levels (Smith, 1975). LSD results in a decrease in glutamate levels in the frontal cortex (Antkiewicz-Michaluk et al., 1997), leading to decreased glutamate neurotoxicity (Nakatsu et al., 2006).

In terms of metabolites, treatment with LSD also reverses the negative trends seen in ASD. These negative trends include decreased levels of essential amino acids and precursors of neurotransmitters (Naushad et al., 2013). LSD increases levels of essential amino acids (tryptophan) (Halaris, 1975) and precursors of neurotransmitters (tyrosine and tryptophan) (Halaris, 1975; Smith, 1975). Treatment with LSD has also been shown to significantly decrease levels of cAMP (Dumuis, 1988).

In terms of receptor interactions, treatment with LSD increases the activity of 5-HT receptors (Rickli et al., 2016) and AMPA receptors (De Gregorio, 2021), and decreases the activity of NMDA receptors (Arvanov, 1999). In terms of gene expression, treatment with LSD increases the expression of genes involving neurotransmissions, synaptic plasticity, energy metabolism and neuropeptide signaling (Martin et al., 2014). LSD also results in the formation of neurons, synapses, and dendritic spines (Ly et al., 2018). This increases brain connectivity, which is traditionally lacking in ASD due to the pruning of dendritic spines (Varghese et al., 2017).

Targeted Mechanisms of Interaction between LSD and ASD

Although there are many pathways that could lend to the pathology of ASD, more recent findings suggest the excitotoxicity of neurons as one of the mechanisms of autism (Essa et al., 2013). Excitotoxicity is characterized by neuronal hyper-excitation and possible cell death due to an excess of excitatory neurotransmitters at the synapse (Farooqui & Horrocks, 1994; Olney, 1969). An accumulation of excitatory neurotransmitters (i.e. glutamate and aspartate) typically over-excites NMDA and AMPA receptors, leading to an aggregation of ROS (Essa et al., 2013). The ROS then disrupt energy production within a cell and consequently induce neurodegeneration, or the loss of neurons (Eliasson et al., 1999). ASD patients often display an elevated serum level of glutamate, indicating over-activation of synaptic glutamate receptors, including NMDA and AMPA receptors (Essa et al., 2013).

Although excitotoxicity is widely accepted as a factor in neurological disorders, the specific mechanism behind excitotoxicity in ASD is ambiguous. One suggested pathway is the inhibition of glutamate release through selective agonists of the 5-HT_{1A}

receptors. When glutamate binds to NMDA receptors, an influx of calcium enters the neuron. This calcium is taken and buffered by the endoplasmic reticulum (ER), a process which is induced by energy from the mitochondria. However, in excitotoxic conditions, an overload of calcium induces excessive generation of ROS, ultimately signaling cell apoptosis and neuronal death (Dong, 2009). Excitotoxicity is present in many neurological conditions, including ASD. The targeting of 5-HT_{1A} receptors could prove to be an effective method of alleviating excitotoxicity. When activated, 5-HT_{1A} is responsible for the inhibition of glutamate release and stops the accumulation of calcium that leads to excitotoxicity (Ramos et al., 2004). As a serotonin receptor agonist, lysergic acid diethylamide (LSD) is hypothesized to downregulate glutamate by binding to 5-HT_{1A} receptors, thus decreasing the prevalence of excitotoxicity (Oosterink et al., 1998).

Another potential pathway that involves the activation of the 5-HT_{1A} receptor results in negative distortions on the assembly of NMDA receptors in neuronal cells. Through this specific mechanism, activation of 5-HT_{1A} ultimately causes disruptions in the synthesis of NMDA receptors in the dendrites of neurons. First, this intricate, multi-step process begins with the stimulation of 5-HT_{1A}, which directly prompts the coupling of G_i/G_o proteins. Effectively, the coupling of these proteins decreases levels of cyclic adenosine monophosphate (cAMP) through the adenylyl cyclase pathway (Alberts et al., 2016). In turn, the decreased levels of cAMP within cells directly affect the functionality of Protein Kinase A (PKA). PKA requires the binding of cAMP to operate and phosphorylates, or activates, other proteins. Thus, PKA is rendered mostly inactive. Under these conditions, PKA cannot phosphorylate and activate two other extremely important downstream protein kinases, CaMKII and ERK. These two protein kinases are

tasked with the phosphorylation of Microtubule Associated Protein 2 (MAP2), which plays a large role in the stabilization of the neural network that is used for transportation of molecules around and between cells. More specifically, MAP2 assists in the linking of microtubules, which are essentially the roadways for motor proteins that transport molecules. When not phosphorylated, MAP2 cannot assist in the linking of microtubules, causing the formation of degenerate microtubules. Certain motor proteins that utilize these microtubules, such as KIF17, carry subunits, or parts, of the NMDA receptor. To highlight such an effect, an increase in free tubulin was demonstrated within the brain (Yuen et al., 2005). In total, the NMDA receptor has 7 different subunits (Cull-Candy et al., 2001). With substandard microtubules, motor proteins cannot transport all required subunits to their destinations, causing the formation of incomplete, inefficient NMDA receptors.

As another glutamatergic receptor heavily influenced by the presence of glutamate in the brain, AMPA receptors and their associated mechanisms have been extensively explored and linked with the 5-HT_{1A} receptor in regards to ASD pathology. Autistic subjects have been shown to express greater concentrations of glutamate, larger imbalances between brain glutamate and gamma aminobutyric acid (GABA), and resulting hyperactive AMPA receptors. GABA, as an amino acid consequent of glutamate, is often tasked with many developmental processes, including cell proliferation, differentiation, and apoptosis. It also works as an inhibitory neurotransmitter, often used to balance the excitatory effects of glutamate. Thus, a higher concentration of glutamate, as well as an imbalance in the regulatory abilities of these excitatory/inhibitory neurotransmitters provides a scope for possible further insight

regarding the pathologies and social deficits recognized in ASD (El-Ansary & Al-Ayadhi, 2014).

Studies linking glutamate, AMPA receptors, and the 5-HT_{1A} receptor have suggested possible implications for the application of LSD as a potential remedy for social deficits associated with ASD. Through the initial inhibition of Ca^{2+}/Calmodulin-dependent Kinase 2 (CaMKII), the amplitude of AMPA receptor currents have been shown to decrease after application of serotonin and activation of the 5-HT_{1A} receptor. Similar effects were demonstrated upon application of other serotonin agonists and blocked by serotonin antagonists. The 5-HT_{1A} receptor was concluded to downregulate glutamatergic signaling as well (Cai et al., 2002). As an agonist for the 5-HT_{1A} receptor that would ultimately downregulate the release of glutamate, LSD exhibits promise as a method for addressing deficits associated with ASD.

A 2019 comprehensive study performed by Kim et al. explored potential roles of AMPA receptors in ASD using acclaimed mouse models and an AMPA receptor-antagonist (Kim, 2019). The utero valproic acid-exposed (VPA) mouse model, which was shown to demonstrate increased glutamate receptor expression and transmission through Western Blotting and electrophysiology, most accurately simulates the mechanism in question. Through evaluation of rodent mannerism using social interaction tests and juvenile play, the VPA mouse exhibited well-known ASD-associated social deficits as well. CP465022, an AMPA receptor antagonist, was then intraperitoneally administered to the VPA mice at differing doses. Thirty minutes after administration of the antagonist, behavioral tests were once again performed. Social deficits and behavior evaluated prior to administration were rescued in multiple tests. To further strengthen the indication of

AMPA receptor involvement, Kim et al. hypothesized that disruption of AMPA-derived neuronal transmission in control mice could induce the social deficits found in autistic rodent models. Administration of either AMPA receptor antagonist and agonist to the control mouse model generated similar impaired behaviors found in the ASD models used in the study. Thus, such a study once again substantiates the hypothesis that LSD, as a 5-HT_{1A} agonist that would function similarly to an AMPA receptor antagonist through the downregulation of glutamate release, could potentially be implemented as an approach for addressing social deficits associated with ASD (Kim et al., 2019).

As a result, prior research regarding such intricate mechanisms suggest that a treatment model targeting the activity of the 5-HT_{1A} receptor could assist in the downregulation of glutamatergic receptors such as NMDA and AMPA. Ultimately, LSD could assist in the confrontation of resulting excitotoxicity of neurons in ASD. Therefore, the effects of LSD, as a serotonin agonist that demonstrates a high affinity for the 5-HT_{1A} receptor, could potentially be examined as a method to ameliorate the social deficits seen in ASD brought about by NMDA and AMPA receptor hyperactivity and the excitotoxicity that results.

Conclusion

A thorough literature review was conducted to evaluate the potential beneficial effects of LSD as a management strategy for psychiatric disorders. Several promising mechanisms through which LSD could exert its beneficial effects were identified which include:

- The inhibition of glutamate release upon the binding of LSD to presynaptic $5HT_{1A}$ receptors.

- The modulation of excitotoxicity through a multi-step mechanism that downregulates the construction of NMDA receptors.
- The regulation of imbalance between brain glutamate and GABA, ultimately modulating hyperactive AMPA receptor currents.

While these mechanisms hold promise, controlled studies need to be conducted in experimental animal models and humans to highlight the projected benefits of these mechanisms and weigh them against the well-known hallucinogenic effects of LSD. Due to the heterogeneity of ASD, LSD may not be beneficial in managing symptoms for everyone with ASD (Rossignol & Frye, 2012; Watts, 2008). For example, psychosis may be comorbid with a specific subtype of ASD, and because of this, microdosing LSD may have negative impacts in that domain (Larson et al., 2017). With this in mind, research is needed to determine where LSD would be most beneficial in symptom management plans and for who. Future researchers using rodent models may use mini-endoscope visualization to observe calcium release from endogenous storage at the single neuronal level, an event downstream of the targeted pathway. Researchers may also further probe the binding characteristics of LSD in vivo, particularly at the 5-HT_{1A} receptor site, using positron emission tomography imaging. Behavioral assays must be used in conjunction with any imaging data in order to gain a more holistic understanding of LSD's therapeutic potential. With the many established rodent models for ASD, one can evaluate modulations in sociability using the three-chambered approach. Here, the experimental animal has the choice to approach a novel conspecific animal or the empty chamber, and the researcher may quantify the time spent in each defined area. The potential anxiolytic properties of LSD may also be evaluated by an elevated plus/zero

maze, measuring the competing drives for an animal to explore and to avoid openly exposed areas. The serotonergic mechanism of action for LSD also suggests that other serotonergic psychedelics could serve as a treatment for ASD pathology. The use of LSD and other serotonergic psychedelics as a treatment for ASD pathology would have to be managed in a manner that mitigates the negative hallucinogenic effects. To this end, microdosing has been found to be a suitable delivery technique for LSD and other serotonergic hallucinogens that can provide the benefits of serotonergic action, but without the negative hallucinogenic effects. The technique of microdosing produces a sub-hallucinogenic effect while still having the potential of receptor activity at a therapeutic level. Microdosing studies have been conducted with human subjects using psilocybin and LSD (Johnstad, 2018). Overall, our findings indicate that LSD has potential as a treatment for managing the etiology of ASD and other neurodevelopmental disorders.

Equity Impact Report

After LSD was categorized as a Schedule 1 substance, research into its possible beneficial effects for its management of symptoms of psychiatric disorders was halted for many years. In the 2000s, research into LSD started up again, but there remains a stigma to this day surrounding the use of LSD. Presenting information and knowledge on the mechanistic functions of the drug and the proposed benefits that it may have is actively contributing to the knowledge that is already out there and working towards destigmatizing its use. People with ASD are also stigmatized, and their marginalization has led to a decreased emphasis on research into ASD and potential treatments for the etiology and symptoms of ASD. ASD has also been primarily associated with white men,

which has led to the marginalization of other people with ASD. Our research attempts to find a treatment that can help all people with ASD.

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Appendix A: Experimental Design from IACUC

The objective of our experiment is to monitor NMDA receptor activity in response to microdoses of 1P-LSD. In this project, we will administer microdoses of 1P-LSD to a previously established and validated mice model of ASD. Our goal is to determine whether treatment with 1P-LSD is an effective way to improve dysfunction in ASD, specifically by attenuating the over excitation of the NMDA receptors, a commonly observed pathology of ASD. The **Phase 1** of this project will consist of four different microdoses of 1P-LSD in order to establish two final doses for the second phase. The goal of **Phase 1** is to select the two doses for the **Phase 2**, which does not produce any hallucinogenic effects in the normal mice. **Phase 2** will then be used to observe the changes in NMDA receptors in the mutated mice models. Two strains of mice will be utilized in this experiment:

1. C57BL/6J (control) (https://www.jax.org/strain/000664)
    - Normal mice with no abnormalities/mutations
    - Recommended control for chosen transgenic mouse model
2. SLC6A4 (experimental) (https://www.jax.org/strain/008355)
    - Autistic rodent models with genetic mutations (knockout of Slc6a4 gene)
    - Our rationale for the selection of this particular mouse model is based on the fact that the SLC6A4 mutation results in dysfunctional serotonin transport and impaired NMDA activity. We expect the treatment with 1P-LSD to alleviate this impairment.

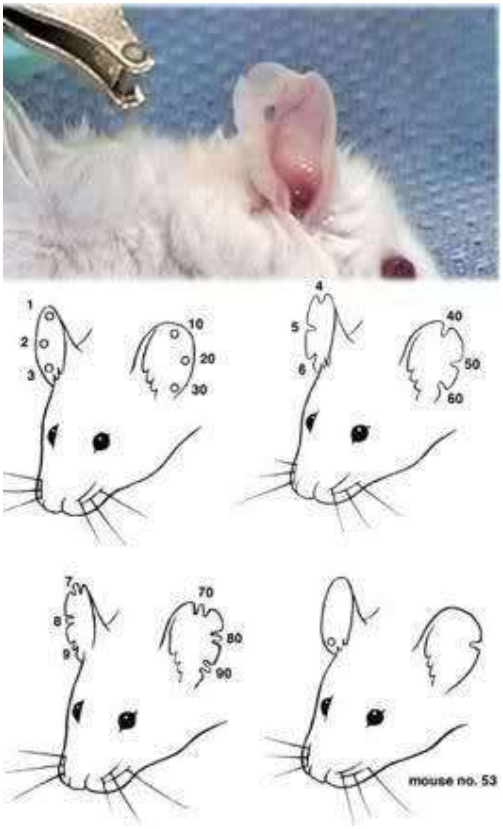
Both strains will be purchased directly from an approved vendor (Jackson Laboratory).

Animal Husbandry

The mice will be purchased at 6-8 weeks old and given a two week period to acclimatize to the housing facility, University of Maryland building and room number ANSC 0354, prior to the start of experimental trials at 8-10 weeks of age. An ear punching scheme, detailed in the chart below, will be used to identify the mice, and will be performed upon arrival to the housing facility. The mice will be housed in groups of 4, and will be monitored daily by lab personnel. The cages will contain an enrichment scratch pad and bedding, which will be changed weekly. Mice will be fed ad libitum on a normal chow

diet. Thach-Vu Nguyen, under the supervision of Nishanth Sunny will be in charge of ear punching the mice, as indicated in his respective Personnel Qualification Forms.

Figure 1: Ear Punch Code



The general experimental protocol is presented in **Figures 2 and 3**.

Figure 2: Experimental Design: Phase 1

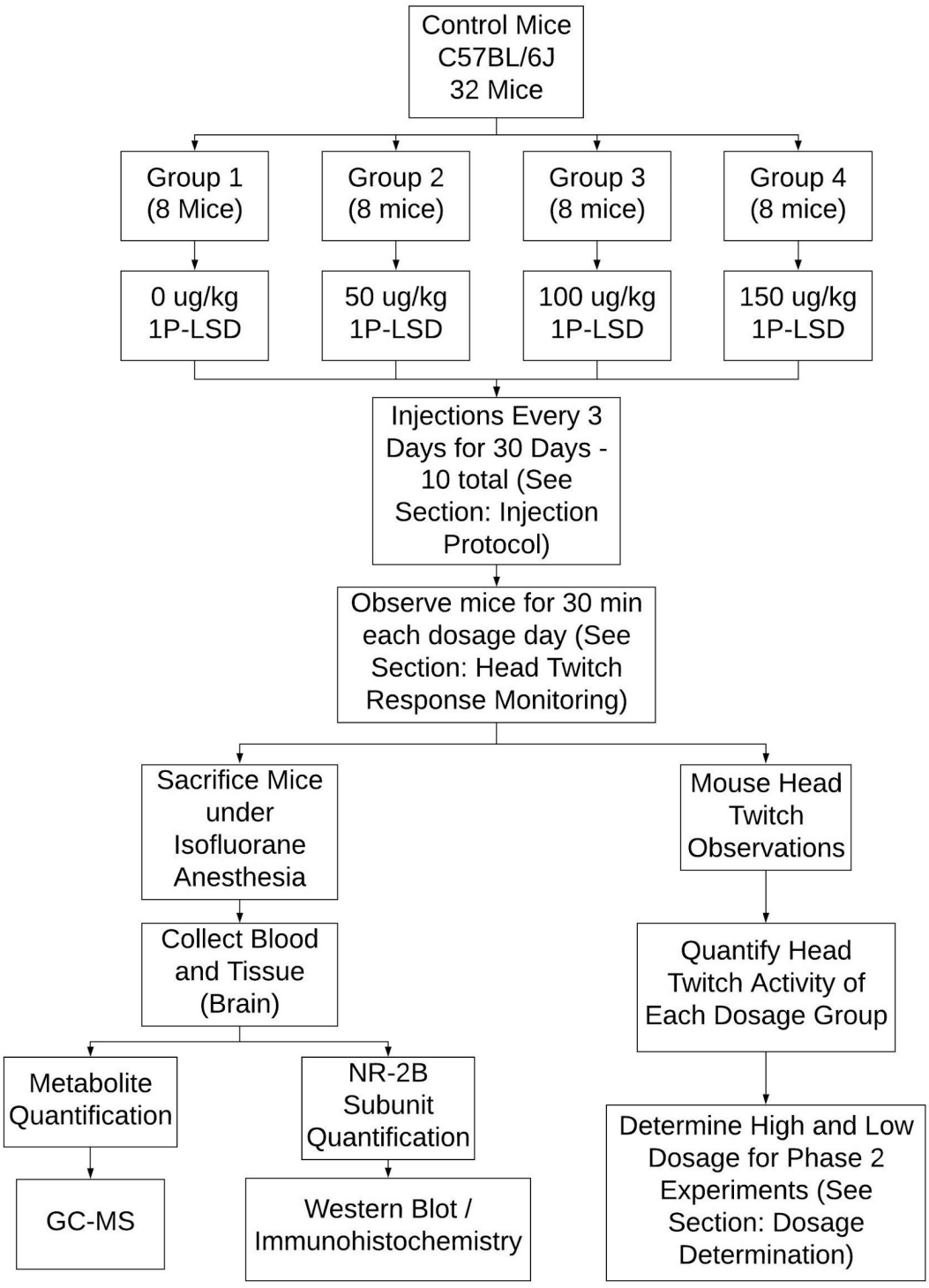
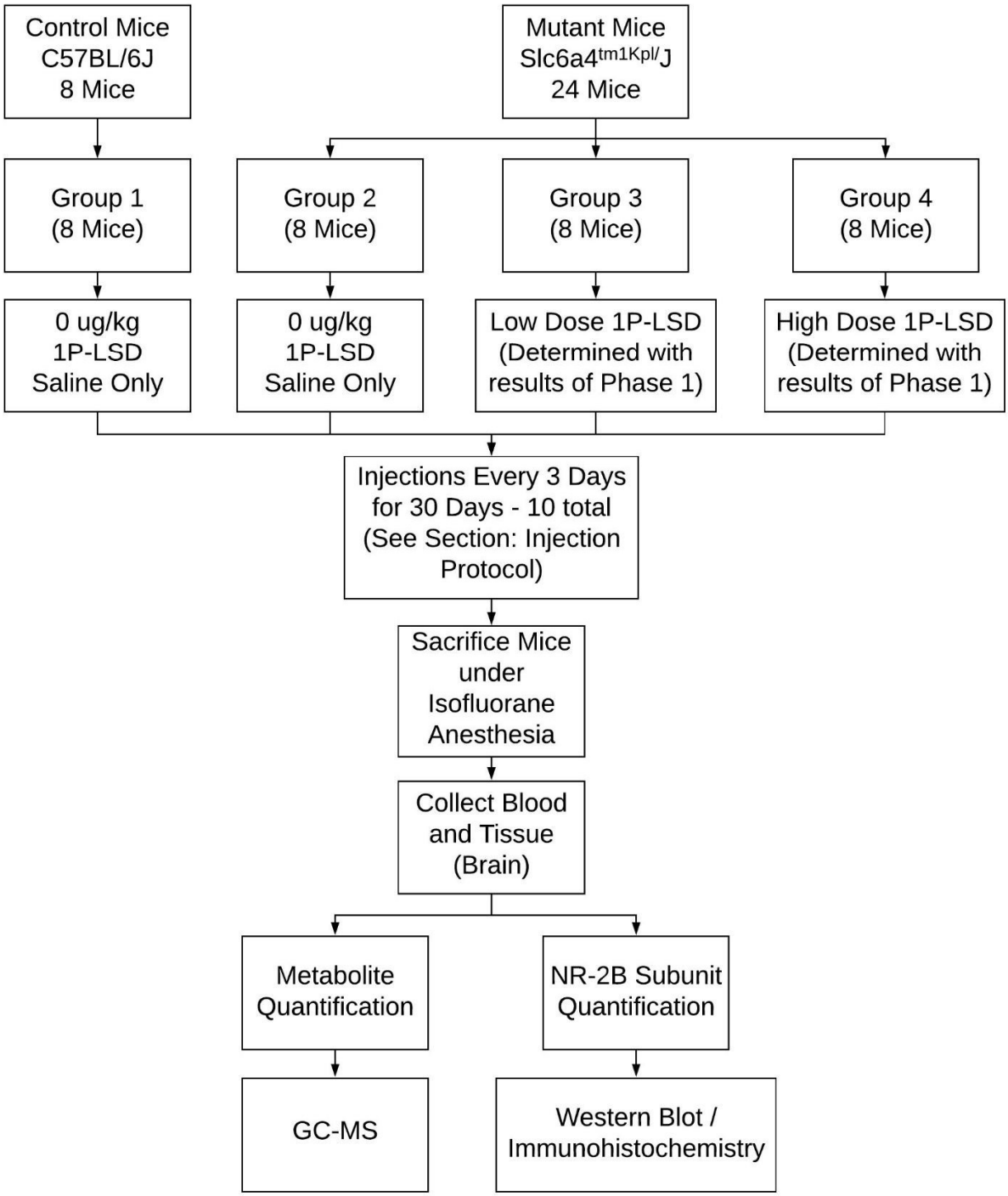


Figure 3: Experimental Design: Phase 2



Experimental Protocol

Phase 1: The goal of the first phase is to determine the highest dosage of 1P-LSD that does not produce hallucinogenic effects in the mice. This information will be used to determine the two dosages to be utilized in phase 2. Four groups of eight C57BL/6J mice will be randomly assigned to receive a dose of 1P-LSD, 0 ug/kg, 50 ug/kg, 100 ug/kg, and 150 ug/kg respectively. From the available literature, all these doses are thought to be below the required dosage which can cause hallucinogenic response in normal mice (Brandt et al., 2016). However, we have decided to validate the ideal dosage for our phase 2 study as detailed below.

1. The entire phase 1 and the injection protocol will be conducted over a 30 day period. During this period, once every three days, groups of normal mice (n =8) will be assigned to a 1P-LSD dosage (Figure 1) and will be injected intraperitoneally with their respective 1P-LSD dosage. Before each injection, mice will be anesthetized using isoflurane (5% to induce and 1% to sustain anesthesia). Anesthesia will act as chemical restraint that will allow for easier IP injection for novice undergraduate students. A 1 inch 29 gauge needle will be used to deliver 0.2 mL of drug mixture as an intraperitoneal injection (See Section: 1P-LSD).
2. Thirty minutes after injection, each mouse will be observed for another 30 minutes to count the number of head twitch responses displayed by each mouse. The number of head twitch responses (an index of hallucinogenic response) will be analyzed compared to the control (the 0 ug/kg 1P-LSD group) to determine if the higher doses of 1P-LSD are associated with the onset of hallucinogenic responses. This protocol will be implemented every time following the intraperitoneal injections. From this data, a high and low dose (maximum without hallucinations and 50% of the maximum) will be determined for phase 2.
3. After 30 days of injection, mice will be sacrificed under isoflurane anesthesia for blood and tissue collection and storage. (see section: Sample Analysis).
4. Blood and tissue will be collected from the sacrificed mice.
    a. Blood – centrifuged at 4 degree C at 8000 RPM, plasma collected and frozen at -80°C for later analysis

b. Tissue (brain and liver) will be collected and flash frozen in liquid nitrogen and stored at -80°C for later analysis

Phase 2: The goal of the second phase is to investigate the effects of 1P-LSD dosage on glutamate metabolism and NMDA receptor assembly in mice with ASD symptoms. One group of eight C57BL/6J mice will be assigned to receive 0 ug/kg of 1P-LSD, and another three groups of eight Slc6a4$^{tm1Kpl/}$J mice will be randomly assigned to receive 0 ug/kg, low dose, and high dose of 1P-LSD.

1. The entire injection protocol will be conducted over a 30 day period as detailed for phase one. Once every three days during the 30 day study period, mice will be injected intraperitoneally with their respective 1P-LSD dosage. Before each injection, mice will be anesthetized under isoflurane (5% to induce and 1% to sustain anesthesia). A 1 inch 29 gauge needle will be used to deliver 0.2 mL of drug mixture (See Section: 1P-LSD).
2. After 30 days of injection, mice will be sacrificed under isoflurane anesthesia (see section: Sample Analysis).
3. Blood and tissue will be collected from the sacrificed mice.
    a. Blood – centrifuged at 4 degree C at 8000 RPM, plasma collected and frozen at -80°C for later analysis
    b. Tissue (brain and liver) will be collected and flash frozen in liquid nitrogen and stored at -80°C for later analysis

1P-LSD and Injection Protocol

Ampules containing 100 µg of 1P-LSD dissolved in 1 mL of acetonitrile solvent will be obtained from Cayman Chemicals (https://www.caymanchem.com/product/27727/1p-lsd-(solution)). The 1P-LSD in powder form will be isolated from the acetonitrile solvent using inert nitrogen gas blowdown evaporation. This apparatus allows for the drying of 1P-LSD under an inert gas, leading to a sterile powder form of 1P-LSD. This dried 1P-LSD powder will be reconstituted into a stock concentration using the vehicle as and when required. The vehicle will be prepared with a ratio of 1 emulphor: 1 absolute ethanol : 18 physiological saline. The stock solution in the reconstituted vehicle can be diluted to the correct concentration for injection into each experimental group (Table 1). The concentration of the drug to be injected into each cohort of mice will be calculated

based on the average body weight of the particular cohort. The intraperitoneal injection volume will be held constant at or around 200 microliters. The dried 1P-LSD will be stored in a locked container located in a locked room only accessible only by the Principal Investigator (University building ANSC 4147).

Dosage group	Mass of 1P-LSD (µg) /200 uL	Concentration (µg/mL)
Dosage group 1: 0 µg 1P-LSD per kg of body weight	0 µg	0 µg/mL
Dosage group 2: 50 µg 1P-LSD per kg of body weight	1.25 µg	6.25 µg/mL
Dosage group 3: 100 µg 1P-LSD per kg of body weight	2.5 µg	12.5 µg/mL
Dosage group 4 (reconstituted stock): 150 µg 1P-LSD per kg of body weight	3.75 µg	18.75 µg/mL

Table 1. Mass of 1P-LSD to be dissolved in vehicle and concentration of 1P-LSD for each dosage group. Dosage group 4 will be the same concentration as the reconstituted stock solution that will be securely stored in the Principal Investigator's laboratory. The stock will be diluted to obtain the concentrations for lower dosage groups. Calculations were done using an average 25 g mice and a 0.2 mL injection volume per mouse.

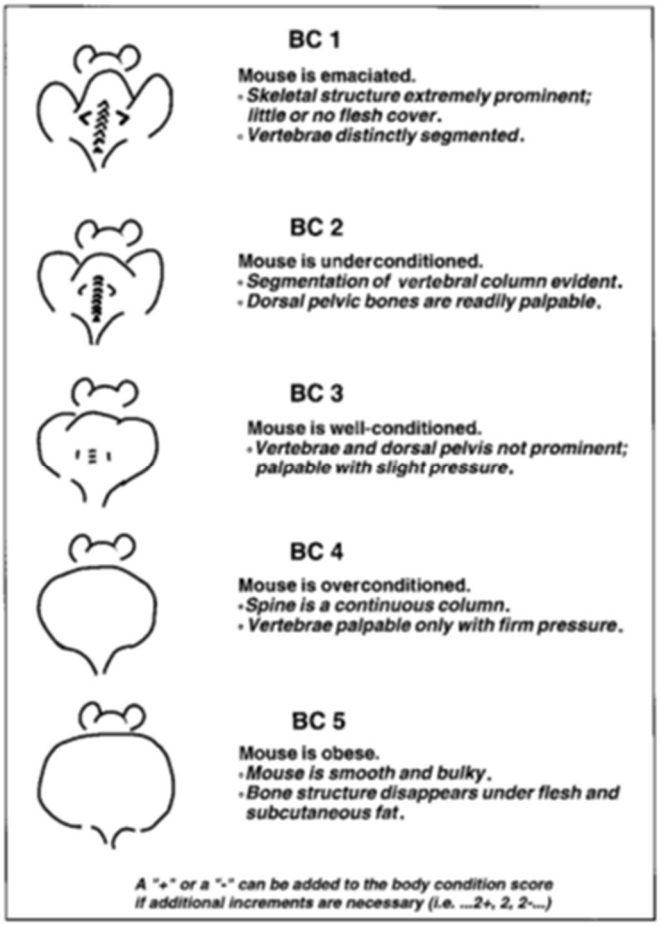
Before each injection, mice will be anesthetized through an isoflurane induction chamber set at 5%. The induction chamber will be reduced to 1% once the mouse loses consciousness. The chamber will be held at 1% during the injection. A 1 inch 29 gauge needle will be used to deliver 0.2 mL of the drug mixture intraperitoneally, ensuring that the bladder or liver is not punctured. After drug delivery, the mouse will be removed from the isoflurane induction chamber and returned to its cage.

Sample Analysis

Mice will be sacrificed through an isoflurane induction chamber set at 5%. Whole brain, blood plasma, and liver samples will be harvested in an aseptic, sterile manner and flash frozen in liquid nitrogen at -80°C. Gas chromatography–mass spectrometry (GS-MS) will be performed on collected blood plasma and homogenate samples of the prefrontal cortex to measure glutamate levels. A western blot will be performed on collected samples in order to quantify NR-2B subunit expression in the prefrontal cortex

Endpoints due to illness:

In both phases of the study, we will monitor each mouse's body weight, food intake, and activity daily. We do not expect rapid fluctuation of body weight by more than 10 to 15% from the baseline weight within 3 days. We will also monitor each mouse's body condition to ensure that the mice do not become emaciated. A decrease in ambulation or a decrease in food intake by 20% will serve as additional endpoints due to illness. Any animal which loses over 15% of its body weight in three days will be excluded and euthanized.



A scoring rubric will be used for humane endpoint assessment. A total score between 1-3 will require no interventions. A total score between 4-8 will require monitoring, notification to the PI, and necessary interventions (e.g. wet pellets, hydrogel) from the animal care or veterinary staff. If no improvement is observed within 3-4 days of

interventions, the animal will be removed from the study and euthanized. An animal with a total score of 9 or greater will be excluded from the study and euthanized.

Body weight changes		**Score**
0	Normal	
1	<10 percent weight loss in 3 days	
2	10-15 percent weight loss in 3 days	
3	>20 percent weight loss in 3 days	
Body condition score (BCS diagram above)		**Score**
0	BCS >3	
1	BCS >2 and <3	
2	BCS >1 and <2	
3	BCS <1	
Physical appearance		**Score**
0	Normal	
1	Lack of grooming	
2	Rough coat, nasal/ocular discharge	
3	Very rough coat, abnormal posture, enlarged pupils	
Unprovoked behavior		**Score**
0	Normal	
1	Minor changes	
2	Abnormal, reduced mobility, decreased alertness, inactive	
3	Self-mutilation, Restlessness or immobile	

Total score