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The Effects of Low Dose Lysergic Acid Diethylamide Administration in a Rodent Model of Delay Discounting

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THE EFFECTS OF LOW DOSE LYSERGIC ACID DIETHYLAMIDE ADMINISTRATION IN A RODENT MODEL OF DELAY DISCOUNTING

by

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The resurgence of Lysergic Acid Diethylamide (LSD) as a therapeutic tool requires a revival in research, both basic and clinical, to bridge gaps in knowledge left from a previous generation of work. Currently, no study has been published with the intent of establishing optimal microdose concentrations of LSD in an animal model. In the present study, rats were administered a range of LSD doses to quantify potential augmentations in choice behavior in a rodent model of delay discounting. In the first experiment, rats were administered LSD (20 or 40 µg/kg, i.p.) or saline at the start of terminal baseline training to determine the drug's influence on choice responding for a large, delayed reward when a novel, impactful set of delays is introduced. For the second experiment, a within-subject design (n= 8) was utilized to quantify the effects of LSD (20, 40, 80, & 160 µg/kg, i.p.) on choice behavior in an ascending dose pattern across four weeks.

Results from Experiment I indicate once weekly administration of low dose LSD during exposure to a novel set of delays does not produce significant changes in choice responding for the larger reward. In Experiment II, rats receiving 40 or 160 µg/kg LSD displayed increases in response selection for the immediate smaller reward during the first two delay conditions (0 and 10 seconds) when compared to saline or non-injection sessions. In Experiment I, response latencies among rats administered 20 µg/kg were significantly reduced at the 0 and 10 second

delay conditions when compared to training-control and saline-treated rats, although no differences in overall response latency were observed among treatment groups. In Experiment II, 40 and 160 µg/kg LSD produced the slowest overall responding when compared to 20 or 80 µg/kg. Considered together, these results are consistent with recent reports that repeated dosing with low dose LSD does not produce cognitive impairments in humans. However, further research is necessary to determine the extent of LSD's effect on behavior at doses lower than those typically administered. Moreover, given the increase in discounting displayed by rats receiving the highest dose of LSD, and the fact that therapeutic doses are typically higher than those administered in the present study, further research is warranted to elucidate any potential negative impairments associated with higher doses typically administered in therapeutic settings.

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INTRODUCTION

Hallucinogens

Defense mechanisms are important for the survival of nearly every species on earth and often include physical alterations (e.g. active or passive camoflauge) or chemical intoxicants that have evolved over countless generations (Furstenberg-Hagg, Zagrobelny, and Bak, 2013). Interestingly, the same defenses used to deter enemies have been harnessed by *homo sapiens* for therapeutic benefits in medicine and religious spirituality. For example, coca leaves, from which the psychostimulant cocaine is isolated, have been used in South American spiritual and medicinal practices since 500 AD (Martin, 1970). The leaves were cited as reducing hunger, producing vigor, and reducing "unhappiness" but are considered insecticides at concentrations contained in coca leaves (Nathanson et al., 1993). In the mid-1800s, cocaine became one of the first isolated compounds from a plant and was used medicinally as an anesthetic by the end of the century (Goldstein et al., 2009). Similarly, *Papaver somniferum,* a pod-like plant known for its poppies containing opium, has documented use dating back 6000BCE where it was referred to as the "plant of joy". Around the early 1800s, the active compound was isolated and is now recognized as the opioid morphine (Brook et al., 2017). Advances in chemistry during this time provided scientists with the ability to explore the medicinal benefits of many other naturally occurring substances including those known for their hallucinogenic properties.

Hallucinogens, a diverse class of drugs, provide some of the oldest accounts of bypassing plant defense mechanisms for medicinal use. Of note is the shrub *Psychotria viridis*, a component of a popular South American decoction termed ayahuasca, which contains the psychoactive alkaloid N, N-dimethyltryptamine (DMT) (Frecska, Bokor, and Winkelman, 2016). Ayahuasca use in South America has been documented for thousands of years and has been

described as benefitting social, physical and spiritual aspects of life (Naranjo, 1986). Additionally, "sacred" mushroom use has been described in these areas as well and refers to entheogenic mushrooms, *Psilocybe,* containing the alkaloid psilocybin (Carod-Artal, 2011). While examples of naturally occurring hallucinogens are prevalent, the class of hallucinogenic drugs is diverse and can be subdivided into four classes, psychedelics, entactogens, dissociative, and atypical based on mechanism of action and psychoactive properties (Garcia-Romeu, Kersgaard, and Addy, 2016). A brief review of this work is presented below.

Atypical hallucinogens include the psychoactive component in cannabis, delta-9-tetrahydrocannabinol, among other lesser-known substances such as salvinorin A and ibogaine. Dissociative subtypes are almost entirely anesthetics, capable of producing "out of body experiences" and visual distortions. Examples of dissociative subtypes include ketamine and the gaseous anesthetic nitrous oxide. Entactogens are named because of their prosocial and interpersonal effects (Nichols. 1986) and include the popular club drug "ecstasy", 3,4-methylenedioxy-methamphetamine (MDMA), and its derivatives. Of the four subclasses of hallucinogens, psychedelics provide the richest history of use, documented above, and are most associated with typical hallucinogen "experiences" consisting of sensory distortions and ego dissolution. Psychedelics include both synthetically derived (lysergic acid diethylamide) and naturally occurring (psilocybin) compounds that interact preferentially with brain serotonergic receptors. Within the psychedelic subclass are two distinct chemical classes separated by their primary amino acid structure.

Phenethylamines. The primary, naturally occurring phenethylamine-based hallucinogen from which others are derived, is the alkaloid mescaline. Mescaline is found in the "buttons" of the peyote cactus, *Lophophora williamsii*, and has documented use dating back 5700 years in

areas of North America (El-Seedi et al. 2005). Phenethylamine's structure is based on the essential amino acid phenylalanine after enzymatic decarboxylation, a biochemical process that removes carboxyl groups (-COOH). Several synthetic phenethylamines have been developed for the purpose of understanding the mechanism of action of similar hallucinogens including 2,5-dimethoxy-4-iodoamphetamine (DOI) and 4-bromo-2,5-dimethoxyamphetamine (DOB) (Fantegrossi, Murnane, and Reissig, 2008). Unfortunately, recent survey data suggest phenethylamines are not well tolerated and may pose a substantial health risk in recreational users when compared to tryptamines (Sexton, Nichols, and Hendricks, 2019).

Tryptamines. Psychedelics with tryptamine base structures are derived from the essential amino acid tryptophan through a similar decarboxylation process described above and can be identified by their double indole rings (Fantegrossi, Murnane, and Reissig, 2008). Unlike phenethylamines, two distinct tryptamine-based psychedelics can be found naturally occurring, DMT and 4-hydroxy-N,N-dimethyltryptamine (psilocin). Psilocybin, the primary tryptamine found within *Psilocybe* mushrooms, is converted into the psychoactive derivative psilocin after ingestion, through dephosphorylation by hydrolysis (Tittarelli et al., 2015). Structurally, these compounds closely resemble the tryptamine-based monoamine serotonin which is unsurprising given their serotonergic actions.

Lysergic Acid Diethylamide (LSD) is a unique synthetic tryptamine classified separately from other simple tryptamines because of its ergoline properties (Nichols, 2001). Ergolines are derived from the ergot fungus *Claviceps* and have been used for other medicinal applications including Parkinson's disease (Reichmann et al., 2006). LSD's psychoactive properties are thought to be mediated, in part, by the 9,10-double bond position of the molecule. Several studies have demonstrated diminished, or abolished hallucinogenic activity when these bonds are

reduced (Stoll and Hofmann, 1955; Nichols, 2018). LSD's complex structure, and extraordinary potency, make it an optimal target for potential therapeutic utility.

Lysergic Acid Diethylamide

Discovery. The advancement of scientific understanding is marked not only by rigorous exploration but by serendipitous moments occasioned through controlled curiosity. Drug discovery has provided numerous examples of these moments throughout history (Ban, 2006). The most infamous example, occurring in 1938, began as a search for a synthetic compound that would stimulate both the cardiovascular and respiratory system. After synthesizing 25 variations of the synthetic ergoline LSD with little success, Hofmann ended the project. Five years later, however, Hofmann writes that he "could not forget about the relatively uninteresting [25th variation of LSD]. A peculiar presentiment – the feeling that this substance could possess properties other than those established" (Hofmann, 1979, p.11). This curiosity required Hofmann to synthesize the 25th variation of LSD (LSD-25) again, leading to a remarkable discovery.

During the final stages of the synthesis process, Hofmann began to notice feelings of restlessness and, eventually, "a not unpleasant intoxicated-like condition, characterized by extremely stimulated imagination" (Hofmann, 1979, p.12). When Hofmann realized exposure to small quantities of LSD-25 lead to profound psychoactive effects, he began testing the drug on himself and detailing his experience. From Hofmann's own accounts, it is clear he understood the potential for the drug in fields such as psychology and neuroscience but did not believe it could be used for a patient's benefit. Shortly after these initial experiments, Hofmann disseminated his findings in hopes others would find use for the novel, yet extraordinary compound he had synthesized.

Prevalence and legal status. The distribution of LSD for research purposes led to an increase in use among the general population. Counter-culture movements, musicians, and socio-political individuals such as Timothy Leary, promoted the drug for its creative and spiritual benefits. Soon after, in 1971, the United State government made LSD illegal for recreational use leading to a substantial reduction in research surrounding the drug. Today, LSD is classified as a schedule 1 drug by the Drug Enforcement Agency (DEA), suggesting no legally accepted medicinal value and a high abuse liability. DEA classifications are oftentimes controversial and not supported by substance abuse researchers because of the detrimental effect these classifications have on research (Nutt, King and Nichols, 2013). For example, government funding agencies forego the inclusion of projects involving substances with no cited medicinal value. Recently, several drugs have shown promise for medicinal applications despite their schedule 1 status including psilocybin, ecstasy, and cannabis.

In the United States, 22% of individuals 12 and older report having used LSD in their lifetime with 9.7% having using it in the last year (National Survey on Drug Use and Health. 2018). For comparison, lifetime hallucinogen use for the same individuals was 36%, indicating LSD use encompasses a majority of the documented hallucinogen use. Interestingly, a study utilizing data from 2010 found that LSD use was higher in older adults than in younger adults who used psilocybin more frequently (Krebs and Johansen, 2013). The authors believe this may be due to the ease of at-home cultivation given the broader access to information in recent decades.

Given the estimates provided above, it is assumed that over 30 million individuals have used LSD recreationally at some point in their life. Amazingly, no deaths have been reported due directly to an overdose of LSD (Hendricks et al., 2015) and only a few published case studies

have documented deaths related to “bad trips” including delirium requiring police restraint (O’Halloran and Lewman, 1993; Reay et al., 1992). Moreover, a case study detailing an account of eight individuals becoming comatose from the insufflation of “extreme” quantities of LSD, which they believed to be cocaine, revealed that the individuals fully recovered after two to three days of supportive therapy (Klock et al., 1975). The lack of fatalities from recreational LSD use suggests an extraordinary pharmacological profile with a wide effective dose range and minimal peripheral effects.

Pharmacokinetics. Recreational users of LSD typically orally ingest the substance in a liquid suspension after it has been absorbed by a secondary mechanism, sometimes “blotter” paper. Other routes of administration include insufflation and injection. While oral ingestion is most common, very little clinical evidence has been published assessing the pharmacokinetics of LSD administered orally. Dolder et al. (2015) found that oral administration (100 and 200 µg) follows first order-kinetics with a peak plasma concentration after 1.5 hours, a half-life of approximately 2.6 hours, and a duration of 12 hours. First-order kinetics are typical of most drugs and describe exponential elimination rates proportional to the plasma concentration (Borowy and Ashurst, 2019). Mean maximum concentration (C_{max}) for the 100 ug dose was 1.7 ng/mL whereas 200 ug produced a C_{max} almost two times as high (3.1 ng/mL). LSD detection in blood becomes more difficult at lower doses. In a study by Family et al. (2019), LSD administered orally at 10 and 20 ug produced detectable levels in blood plasma while 5 ug did not. Unsurprisingly, intravenous (I.V.) LSD administration produces peak plasma concentrations more rapidly albeit with a similar half-life. Aghajanian and Bing (1964) demonstrated doses of 2 ug/kg LSD administered I.V. reached peak plasma concentrations of 6 to 7 ng/mL 30 minutes after injection, with a half-life 2.4 hours.

LSD is rapidly distributed into tissue with the highest concentrations found in the liver, kidney, spleen, and brain (Boyd et al., 1955; Boyd, 1959). Dolder et al. (2015) reported that only 1% of an administered dose of LSD will be excreted as the parent compound, the remaining as metabolites. In humans, LSD is metabolized in the liver through demethylation and hydroxylation processes where it is converted into N-demethyl-LSD (Nor-LSD) and 2-oxo-3-hydroxy-LSD (oxo-HO-LSD), respectively (Steuer et al., 2017). The latter, oxo-HO-LSD, is found at its highest concentrations in urine specimens (Poch et al., 1999). Nor-LSD concentration is generally low and has only been detected recently in human plasma (Steuer et al., 2017). In brain specimens, LSD is more prevalent than its metabolites because of its hydrophobic properties allowing it to cross plasma membranes more readily (Mardal et al., 2017).

Psychopharmacology. In the decades following its discovery, documented experiences of LSD intoxication have revealed an immense scope of perceptual alterations across a wide range of doses. Typically, these alterations manifest in the form of visual and auditory hallucinations accompanied by a sense of derealization and depersonalization that can last up to 12 hours (Schmid, 2015). Another perceptual alteration includes the distortion of temporal processing in both quantitative and subjective assessments (Yanakieva et al., 2019; Kenna and Sedman, 1964; Aronson et al., 1959). Quantitative assessments for temporal processing utilize interval timing procedures where participants are asked to estimate the duration of a particular stimulus (Wittman, 2007). The effect of LSD on these assessments is mixed, demonstrating over-production (Yanakieva et al., 2019) as well as under-reproduction (Aronson et al.,1959) of intervals. Authors Yanakieva et al. (2019) remark the mixed results between these two published

studies may be due to methodological differences including interval lengths, number of trials, and measurement recording.

LSD use can produce significant alterations in subjective effects related to mood that are highly dependent on the setting in which the drug is administered and dose. In clinical settings, users report dose-dependent subjective effects related to "positive mood" and "drug liking" at doses between 100-200 ug (Dolder et al., 2016). For example, participants administered 200 µg LSD orally reported significantly more happiness, openness and trust than did those receiving 100 µg. Although outcomes for participants are generally positive in clinical environments, negative subjective effects have been reported. Dolder et al. (2016) found that half of the participants receiving a dose of 200 µg reported negative drug effects at some point during the session. In non-experimental settings, LSD has been reported to induce schizophrenic-like psychosis in vulnerable individuals (Vardy and Kay, 1983) and is used to model schizophrenia in rodents through repeated administration (Braff and Geyer, 1980; Marona-Lewicka, Nichols, and Nichols, 2011). Finally, although rare, the hallucinogenic effects of LSD may persist for several months to years in the form of Hallucinogen Persisting Perception Disorder (HPPD). Individuals diagnosed with HPPD present with two types of symptomologies in the form of brief "flashbacks" or chronic perceptual alternations, which may be linked to pre-existing anxiety and drug-use (Halpern, Lerner, and Passie, 2016).

As mentioned, the chronic, negative effects associated with LSD are related to its recreational use and do not manifest in clinical settings. Rather, LSD administered in controlled environments has revealed profound therapeutic benefits in several domains, including anxiety and substance abuse. Remarkably, a meta-analysis of six randomized controlled trials by Krebs and Johansen (2012) revealed that a single dose of LSD during treatment for alcohol misuse

produced a significant reduction in misuse up to six months following its administration. In addition, Gasser et al. (2014) found that individuals suffering from anxiety related to a life-threatening disease reported a significant reduction in "state" anxiety with no changes in "trait anxiety following two LSD-assisted psychotherapy sessions. State-anxiety was measured with the State-Trait Anxiety Inventory (STAI), which encompasses anxiety symptoms related to particular events, whereas trait anxiety is said to represent personality features. Interestingly, when participants returned for follow-up testing 12 months later, both state and trait anxiety were significantly reduced compared to baseline (Gasser et al., 2015). Although these controlled, double-blind studies have demonstrated promising results, few seeking to establish LSD's therapeutic profile have been published.

Microdosing. The resurgence of LSD as a therapeutic tool reflects the need for a revival in research, both basic and clinical, to bridge gaps in knowledge left from a previous generation of work. Recently, sub-threshold dosing ("microdosing") with LSD has garnered the attention of scientific researchers as well as the general public. Microdose concentrations are generally considered to be about one tenth the standard psychoactive dose and do not produce noticeable sensory alterations (Kuypers., 2019; Rosenbaum et al., 2020). In a recent online forum-based survey, users reported subjective feelings of "positive emotionality" and decreased "dysfunctional attitudes" without noticeable perception changes typical of higher doses (Anderson et al., 2019). Another survey revealed that individuals who microdose oftentimes do so to alleviate symptoms of anxiety and depression or to enhance everyday functioning (Johnstad, 2018). These accounts, while observational in nature, run in parallel with recent data suggesting high-dose, LSD assisted psychotherapy produces remarkable decreases in depression, anxiety, and addictive-like behaviors (dos Santos et al., 2016; Fuentes et al., 2019).

In light of these reports, several recently published studies assessed the safety and efficacy of microdosing in controlled environments. Bershad et al. (2019) found dose-dependent effects of low-dose LSD (6.5, 13, 26 µg) related to mood, emotional processing and cognition. More specifically, doses of 13 and 26 µg appeared to produce significant subjective effects related to "feeling high" and "liking" the drug while also increasing vigor as it relates to the Profile of Mood States (POMS) questionnaire. In another study, Yanakieva et al. (2019) found that doses of 5, 10, or 20 ug had no significant effect on self-report measures but produced distortions in time perception (> 2 seconds) during a temporal reproduction task in older adults. While time distortion after LSD administration is well-documented, as aforementioned, data from this study revealed changes in behavior in the absence of altered consciousness.

Discrepancies between the subjective reports of these studies may be due to the level of experimental control and age of the participants. For example, in the study reported by Bershad et al. (2019) participants were younger ($\mu = 25$ vs. 62.92) and selected with more strict inclusion criteria. Furthermore, participants were given escalating doses of LSD once per week utilizing a within-subject design, whereas participants in the study reported by Yanakieva et al. (2018) received six doses of LSD administered every three days.

Neurobiology. LSD's primary mechanism of action is serotonergic agonism through the 2A receptor subset (5-HT_{2A}). This finding is supported by work from Gonzalez-Maeso et al. (2007) who reported that mice lacking 5-HT_{2A} did not display biochemical or behavioral changes commonly seen after LSD administration. Furthermore, the 5-HT_{2A} antagonist, ketanserin fully inhibits both the neuronal and subjective effects of LSD in humans (Preller et al., 2018; Kraehenmann et al., 2017). Serotonin (5-HT) is a neurotransmitter synthesized from tryptophan within the dorsal raphe of the brainstem and is found throughout the central nervous system.

There are three main 5-HT receptor classes (5-HT_1, 5-HT_2, and 5-$HT_{4,6,7}$) with several subtypes within each (Frazer and Hensler, 1990). Both 5-HT_{2A} and 5-HT_{2C}, the predominant receptors involved in LSD's actions, are found throughout several brain areas, but are most densely distributed in the neocortex, hippocampus, amygdala, and thalamic nuclei (Lopez-Gimenez and Gonzalez-Maeso, 2018; Clemett et al., 2000). Other evidence implicates 5-HT_{2C} receptor involvement in LSDs effects. Backstrom et al. (1999) reported LSD signaling at 2C receptors differs from that of endogenous 5-HT because of its inability to promote calcium release and dampened effects on phosphorylation. Interesting, calcium channel blockers have been shown to prevent biochemical changes resulting from LSD administration including 5-HT metabolism (Antkiewicz-Michaluk, Romanska, Vetulani, 1997).

LSD is a partial agonist for subtype 2 dopamine receptors (D_2) which are thought to be a primary target for anti-psychotic medications and may be related to LSD-induced psychosis (Seeman and Tallerico, 2005). Receptor binding assays have revealed LSD's interaction with D_2 receptor functioning is mediated by 5-HT_{2A} receptors (Borroto-Escuela et al., 2014). This effect is not seen with 5-HT agonists typically used in these studies. Moreover, drug discrimination research suggests LSD's interaction with D_2 receptors is temporal and may follow serotonergic agonism (Marona-Lewica, Thisted and Nichols, 2005). D_2 receptors are distributed widely throughout the nervous system including areas of the cortex and midbrain (Vincent, Khan, and Benes, 1993; Mansour et al., 1990). In the cortex, D_2 receptors play a role in the regulation of excitatory neurotransmission (Hsu et al., 1995) and may contribute to reward-related processing between cortical and subcortical structures. Interestingly, D_2 receptors in layer V of the neocortex are not uniformly distributed and produce novel after-depolarization signals that depend on L-type Calcium channels (Gee et al., 2012). L-type Calcium channels have been linked to

psychological disorders including schizophrenia, autism, and bipolar disorder (Bigos et al., 2010; Splawski et al., 2004; Sklar et al., 2011).

The neocortex is comprised of six neuronal layers containing two principal cell types, pyramidal or nonpyramidal. Pyramidal cells, named for their shape, are excitatory neurons that provide the only output from the neocortex and encompass the largest system of inputs (Nieuwenhuys, 1994). Research suggests the fifth layer may be important in the effects of atypical antipsychotic drugs as chronic treatment of olanzapine and clozapine in rats induces redistribution of the 5-HT_{2A} receptors in pyramidal neurons (Willins et al., 1999). Interestingly, the psychoactive properties of LSD may rely on neurons found in the same layer. Gonzalez-Maeso et al. (2007) found that a non-hallucinogenic alternative to LSD, lisuride, elicits different signaling patterns, indicating certain subcortical populations of 5-HT_{2A} are necessary for LSD's psychoactive effects.

Human neuroimaging studies have revealed functional connectivity is differentially affected following LSD (75-100 µg) administration (Muller and Borgwardt, 2019). Functional connectivity is a broad term to describe the communication between brain areas over time through statistical correlations obtained from functional magnetic resonance imaging (fMRI) (Tahedl et al, 2018). For LSD, decreases in connectivity are observed in visual and sensorimotor networks, while increases are demonstrated in thalamic areas during resting-state tests. The authors of the cited review (Muller and Borgwardt, 2019) note that thalamic connectivity is important for overall brain functioning and may provide the insights into LSD's psychoactive effects and therapeutic benefits. Surprisingly, at doses (13 µg) below the threshold of producing obvious subjective effects, LSD generates increases in amygdala connectivity that are weakly associated with increases in positive mood (Bershad et al., 2019). The antidepressant citalopram

produces a similar effect of normalizing amygdala responses and may be a target for its therapeutic action (Murphy et al., 2009). Taken together, these studies provide a substantial insight into the potential mechanisms by which LSD interacts with the mammalian nervous system to produce a profound effect on behavior.

LSD and BDNF. The profound, and often mystical, experiences offered by LSD are thought to be the result of perceptual alterations mediated, in part, by the serotonergic system (Fantegrossi, Murnane and Reissig, 2008). Additionally, Nichols and Sanders-Bush (2002) found that a single dose of LSD alters rodent gene expression related to synaptic plasticity, glutamate signaling, and cell structure in brain areas including the hippocampus, prefrontal cortex, and thalamus. The secretory protein Brain-Derived Neurotrophic Factor (BDNF) is a primary target involved in synaptic plasticity and glutamate signaling. BDNF binds to four receptors, Trk (A-C) and $p75^{NTR}$, located on neuronal and glial cells within the mammalian nervous system (Lewin and Carter, 2015). Interestingly, BDNF regulation has been shown to play a role in depression and anxiety phenotypes and is thought to underlie the latent therapeutic effects associated with selective serotonin reuptake inhibitor (SSRI) medication (Martinowich and Lu, 2008). Indirect evidence for BDNF upregulation following LSD administration has been demonstrated with a chronic exposure model in which rats received 45 injections of LSD (160 µg/kg) across 90 days (Martin et al., 2014). In this study, mRNA expression of BDNF remained upregulated four weeks after the discontinuation of the LSD treatment.

Only one study has been published providing direct evidence of LSD's effects on BDNF protein regulation. Ly et al. (2016) found that cortical neuron cultures treated with LSD (10 µM) for 24 hours produced two-fold increases in BDNF protein levels when compared to neuron cultures treated with vehicle. Unfortunately, generalization of these results is limited given the *in*

vitro experimental design. Taken together, these studies demonstrate a possible mechanism for the neuroadaptive nature of LSD. Future research will require *in vivo* experiments in order to correlate behavior with augmentations in neurotrophic factors.

Behavioral pharmacology. An important step in drug discovery is the use of animal models to determine a drug's behavioral profile. Although human behavior is significantly more complex, animal models of drug abuse, and a variety of behavioral pharmacology methods offer reliable prescreening tools capable of predicting human behavior. Unfortunately, preclinical work related to LSD's effect on behavior is minimal. Nonetheless, several important findings are outlined below.

Reward and reinforcement. Despite its schedule 1 status, LSD is not reliably self-administered in non-human primates (Goodwin, 2016). Self-administration procedures provide direct measures of drug reward and are considered the "gold-standard" for abuse-liability testing. In these procedures, organisms respond for intravenous infusions of a drug over a specified length of time. Drugs with high abuse potential produce higher rates of responding that those with lower abuse potential. Interestingly, some hallucinogens are self-administered at such a low rate that responding may not be readily maintained (Deneau et al., 1969; Griffiths, Bradley, and Bradford, 1979).

Indirect methods assessing the reinforcing value of LSD have revealed some evidence for abuse potential at high doses. In one study, 200 μg of LSD produced conditioned place preference in male rats after three pairings with a distinct contextual environment (Parker, 1996). Another study demonstrated similar results in male fawn-hooded rats after eight pairings, but not in female rats. Conditioned place preference is established when an organism spends significantly more time in an environment that was previously paired with a particular stimulus

compared to another contextually distinct environment. The lack of effect in female rats may be due to testing procedures overlapping with hormonal changes due to the estrus cycle (Meehan and Schechter, 1998).

Locomotor behavior. Ambulatory behavior is commonly measured following drug administration as a way to establish effective dose ranges. Primary measures for the detection of these behaviors include total movement, rearing, and preening. Rearing refers to standing on hind legs while preening consists of face scratches using the forearm. Several studies have been published documenting locomotor behaviors in rodents administered LSD. Dandiya et al. (1969) found LSD produces dose dependent increases in ambulatory frequency 15 minutes after the administration of a single dose. Interestingly, ambulatory frequency was significantly higher than placebo at doses as low as 2 µg/kg. Furthermore, rearing behaviors were significantly greater at moderate doses (2-32 µg/kg) and no different that placebo at higher doses (136 and 500 µgkg). Ouagazzal et al. (2001) reported similar increases in locomotion following administration of three escalating doses of LSD (30, 100, and 300 µg/kg). Peak elevations in ambulatory behavior was noted 15-20 minutes after the injection with behavior returning to baseline levels after 30 minutes.

Head twitch assay. LSD, and other serotonergic hallucinogens, elicit unique head twitch behaviors in rodents characterized by quick, rhythmic side-to-side head movements (Halberstadt and Geyer, 2011). Head twitch frequency has been demonstrated to increases as a function of dose (50, 100, 200, 400 µg) with peak responses occurring 5-10 minutes post injection (Halberstadt and Geyer, 2013; Yamamoto and Ueki, 1981). This dose-dependent relationship may reflect 5-HT_{2A} binding as several reports have demonstrated the role of these receptors in head-twitch behaviors. For example, mice bred to not express 5-HT_{2A} receptors do not display

head twitch responses after LSD administration (Gonzalez-Maeso et al., 2007). Furthermore, 5-HT_{2A} receptor antagonists prevent head-twitches from occurring after the administration of other hallucinogenic compounds (Fantegrossi et al., 2010; Fantegrossi et al., 2008). Correlations between preclinical head-twitch assays and human subjective reports have revealed incredible predictive validity of the assay in assessing hallucinogenic potency cross-species (Halberstadt, et al. 2020).

Operant behavior. Schedules of reinforcement demonstrate the powerful control of the environment on behavior and provide predictive tools for the analysis of operant responding. (Ferster and Skinner, 1957). Beginning with their initial conceptualization, reinforcement schedules have you been employed to describe the effects of drugs on behavior in preclinical screenings (Dews, 1956). Ratio schedules require a set number of responses before the presentation of a reward, while interval schedules require a specified passage of time before a response will produce reinforcement. LSD produces a reduction in response rates on ratio-based schedules and is thought to be due to increases in post-reinforcement pause (Freedman et al., 1964; Appel et al., 1968). Furthermore, Harris, Snell, and Loh (1977) demonstrated LSD (30, 100, 300 µg/kg) is up to 30 times more potent than other common hallucinogens with respect to its effects on response reduction on fixed-ratio 30 schedules. In the same study, LSD produced similar responding during fixed interval schedules of 2 minutes. However, a study a utilizing several concentrations of LSD (5, 10, 20, 40, 80, 160, 320 µg/kg) on a variable-interval schedule of 1 minute demonstrated a sigmoidal dose-response relationship indicating lower doses may have little effect of rate (Appel, 1971). Interestingly, the authors note a nonsignificant increase in response rate from the lowest dose tested, 5 µg/kg, to both 10 and 20 µg/kg before significant reductions in responding.

Differential reinforcement of low-rate (DRL) procedures reveal a disruption in timing behavior after high dose LSD administration that is similar to aforementioned findings regarding the effects on time perception in humans. At intervals of 10 and 25 seconds, 80-160 µg/kg LSD decreased responding without producing notable disruptions in motor functioning (Appel, 1971). Similar reductions have been demonstrated for 15 second intervals at doses of 100 and 200 µg/kg (Silva and Calil, 1975). The authors note that these reductions in responding may be due to post-reinforcement pauses but were less frequent at the highest dose. Nonetheless, decreases in response rate during DRL tasks result in less reinforcers earned over time and represents a detrimental outcome.

Choice and Impulsivity

Impulsivity is defined as a tendency to engage in maladaptive behaviors and is oftentimes associated with risk taking related to drug-use (de Wit, 2009). For example, individuals may continue to abuse drugs despite health issues stemming from their use. Additionally, drug-use may exacerbate other maladaptive behaviors including decisions based on monetary rewards. Recent research suggests impulsivity can be defined by three distinct categories including impulsive choice, impulsive action and personality traits (MacKillop et al., 2016). The latter is based on subjective, self-report measures and may be related to reward sensitivity and risk tolerance. Of the three domains, impulsive choice procedures are the most commonly utilized to assess impulsivity. For this reason, related procedures will be the focus of the remaining introduction.

Delay discounting. Delay discounting is of the most common behavioral measures of impulsive choice. In general, if an organism is given two response options producing differing magnitudes of the same reward, the organism will engage in the response that produces the larger

reward more frequently. Delay discounting represents a point at which an organism equally prefers the small immediate reward to the large but delayed alternative (Odum, 2011). However, if the larger reward is contingent upon a delay, the value of that response option diminishes as a function of the delay between the response and the expected reward.

Decisions such as these are typical of the human experience and often present themselves in economical contexts, such as decisions between choosing $100 dollars now or $150 dollars in a week. To most, the decision to choose $100 now might be obvious. However, as the monetary value for the delayed reward increases, the decision becomes more difficult. When 50% of total responding is allocated to either response option, an indifference point is met where the value of both rewards is said to be equivalent (Mazur, 1988). A similar phenomenon has been demonstrated in several other organisms including rats, pigeons, and non-human primates (Vanderveldt, Oliveira and Green, 2016), implicating an evolutionary benefit to this type of decision making.

A primary difference between humans and non-humans in delay discounting is the effectiveness of a given reward as a function of the delay in its presentation. For humans, complex verbal behavior mediates the expectancy of a reward for a given behavior that may present itself several months into the future. Given this, studies involving non-humans often use significantly shorter delays (seconds vs days or months) between the presentation of a delayed reward and the response (Vanderveldt, Oliveira and Green, 2016). Non-human variations of delay discounting procedures are carried out in traditional operant conditioning chambers with appetitive rewards contingent upon species specific responding (i.e. lever press, nose poke, or key peck). The first published study to measure systematically the influence of reinforcement delay on response rate used pigeons as a model organism (Ferster, 1953). Results from this work

revealed an almost complete reduction in responding (key pecks) when reinforcement was delayed 60 seconds from 0 on a response option with a variable-interval reinforcement schedule (~1 reinforcer/min). However, when the delay was gradually increased across several hours, normal rates of responding were maintained at the highest delays.

Rachlin and Baum (1969) extended Ferster's work in pigeons with the introduction of a second, concurrent response option with differing reinforcement schedules. In addition, the pigeons were required to position themselves next to the response choice as a form of time allocation to determine its predictive validity for response preference. Results from this experiment revealed that time allocation to a given response option is equal to the ratio of its values and that time allocation may provide a more general predictor of response preference. Moreover, the frequency of responding on a given response option is inversely proportional to the reinforcement delay. As mentioned previously, learning history has an impact on the relative rate of responding for a delayed reward (Ferster, 1953). When the delay to reinforcement for two response options is equal, but the magnitudes are different, gradually reducing the delay of the smaller magnitude response option will increase the likelihood for responses on the larger option (Mazur and Logue, 1978).

Discounting quantification. The reduction in value for a reinforcer as a function of time provides researchers with unique predictive modeling tools to interpret results of delay discounting experiments. These models employ exponential and hyperbolic decay functions to determine discounting behaviors and have demonstrated predictive validity across several different species (Mazur and Biondi, 2013; Woolverton, Myerson and Green, 2007). Hyperbolic function models are considered to be more robust than exponential functions because of their dynamic prediction (Myerson and Green, 1995). For example, exponential functions decay at a

constant rate while hyperbolic functions assume reward value declines rapidly at short delays but slows as the delay increases (Vanderveldt, Oliveira, and Green, 2016).

The formula for hyperbolic functions, as it relates to discounting, is defined as:

$$V = A/ (1+kD) \qquad (1)$$

where V represents subjective value of the large reward (A) and D represents the delay until the reward is received (Mazur, 1997). Additionally, the function contains one free parameter, *k*, which represents the degree of discounting for a specified delay (Odum, 2011). Given the equation's structure [1], it is not surprising that large integers of *k* indicate greater effects of delay on reinforcer value. Interestingly, impulsive individuals tend to display greater degrees of discounting, producing larger values of k, than those who are not (de Wit, 2009; Madden et al., 1997). For this reason, delay discounting is commonly utilized in clinical applications to assess impulsive behavior in vulnerable populations including those with substance abuse disorders.

Drug effects. As mentioned previously, delay discounting is utilized as a measure of impulsive choice in both humans and non-humans. Furthermore, drug-use has been cited as having an impact on impulsive behavior and other maladaptive behaviors related to consequence sensitivity (de Wit, 2009). Unfortunately, few human studies have directly assessed the acute effects of commonly abused substances on discounting behaviors and those that have generally consist of participants with specific drug histories. Animal models have been utilized extensively to aid in elucidating drug effects on complex discounting behaviors. In a comprehensive review, authors de Wit and Mitchell (2010) noted that most animal studies assessing the acute effects of commonly abused substances, across several drug classes, reveal increases in impulsivity in at least one dose. Interestingly, mixed results are reported for psychostimulants considered to be useful in treating impulsive behaviors in humans such as attention deficit disorders. Repeated

administration of psychostimulant cocaine increases impulsivity in rats, producing alterations in behavior for several days after a final dose has been administered (Dandy and Gatch, 2009). However, Slezak, Krebs, and Anderson (2012) reported no significant differences in discounting behavior for animals repeatedly administered amphetamine, another common psychostimulant. These discrepant results may indicate the procedure's sensitivity for elucidating impulsive behavior more readily after repeated administration of a drug and should be considered in future designs. Relevant to the present manuscript, LSD has never been tested in human or animals for its effects on discounting behaviors.

Current Research Objective

As mentioned previously, LSD administration in therapeutic environments produces significant reductions in alcohol abuse which may reflect changes in impulsive behavior. Additionally, low dose administration in humans and animals produces disruptions in temporal processing. Given that delay discounting procedures utilize temporally distinct events to quantify impulsive choices, assessing LSD's effect on these measures may be more advantageous than other measures of decision making or complex behavior. For this reason, the present study was designed to quantify potential augmentations in choice behavior following microdose LSD administration in a rodent model of delay discounting. To the author's knowledge, at the time of consideration, no study has been published with the intent of establishing optimal microdose concentrations of LSD in an animal model of complex behavior. Moreover, research quantifying the behavioral effects of LSD in animal models is limited in number and scope. A search of the preclinical behavioral literature revealed few published studies of LSD on complex operant behavior. One such study assessed a range of LSD doses on a time-based operant procedure involving a Differential Reinforcement of Low-Rate (DRL) schedule (Appel, 1971). Although

not originally intended to assess impulsivity, the results from Appel (1971) indicate LSD may produce alterations in responding related to impulsive action as rats received fewer reinforcers than controls. Receiving less reinforcers demonstrates maladaptive responding because DRL schedules require an interval of elapsed time before a response will produce a reinforcer.

The primary aim of the current study was to implement complex operant methods to evaluate the effects of LSD on delay discounting. Two separate experiments were conducted to determine whether LSD differentially affects steady state and transitional state responding as it pertains to discounting. Survey reports suggest LSD provides "cognitive enhancement" during daily activities (Johnstad, 2018) which may reflect differences during the presentation of novel stimuli in an organism's environment. For this reason, LSD was administered during the onset of a novel set of impactful delays in experiment I. Finally, a typical delay discounting procedure was carried out in experiment II to determine the effects of LSD on steady state behavior.

METHODS

Subjects

Thirty-two adult, male Sprague-Dawley rats (Charles River Laboratories, Wilmington, MA) weighing between 350-450 g were singly housed in polycarbonate cages containing corncob bedding within a temperature and humidity-controlled environment. Procedures were carried out during the rats' dark cycle (12/12h dark/light, 0600h-1800h) with measures to ensure minimal light exposure. Rats in the current study were approximately 24 weeks of age at the commencement of lever training. Previously, rats were exposed to single-lever operant training, as part of an instructional laboratory course, before beginning the formal lever training outlined herein. Rats were maintained between 85-90% of their free feeding weight during all experimental procedures with unrestricted access to water. All procedures were conducted in

accordance with the Guide for the Care and Use of Laboratory Animals (2011) and were approved by the Institutional Animal Care and Use Committee at Western Michigan University.

Apparatus

Behavioral testing was carried out in eight, standard rat operant conditioning chambers (ENV-001; Med Associates Inc. St. Albans, Vermont, USA) equipped with three retractable levers, a house light, stimulus lights mounted above each lever, and a food cup equidistant to the right and left levers. Chambers were housed in sound-attenuating cabinets with fans for ventilation and noise control. Food reinforcement was delivered via pellet dispensers containing 45mg Dustless Precision Pellets (F0021, BioServ, Flemington, NJ, USA). All equipment and experimental procedures were computer-operated through the MED-PC IV software package.

Drug

(+)-Lysergic acid diethylamide-tartrate (LSD) was provided by the National Institute on Drug Abuse Drug Control Supply Program (Bethesda, MD, USA). Bacteriostatic sodium chloride (0.9%) (saline) was used to dissolve the LSD salt. Solutions were administered via intraperitoneal (IP) injection at a 1 mg/kg volume and doses were expressed as weight of salt. Injections occurred 15 minutes prior to the start of testing in both experiments to ensure drug effects were present when testing began.

Magazine and Lever Training

A single session of exposure to the food delivery system was performed, utilizing a variable-time (60sec) schedule of reinforcement for 60 minutes. During this procedure, food pellets were delivered, on average, once every 60 seconds. Successful completion of this procedure required rats to consume all pellets delivered during the session. Immediately

following magazine training, rats were randomly assigned a lever (left or right) to begin fixed ratio 1 (FR1) operant training.

Single-lever training sessions lasted 30 minutes or until 100 reinforcers were delivered under an FR1 schedule of reinforcement. For the second session, rats were exposed to the lever not previously randomly assigned. During the third session, rats were exposed to both levers, simultaneously, for 60 minutes or until 200 reinforcers were delivered. Responses on each lever were considered to determine whether an individual rat would receive additional single-lever training on the non-preferred lever. Criteria for additional training required over 60% responding on a single lever during two-lever training sessions. Rats not meeting this criterion continued the two-lever procedure. After approximately two additional training sessions, the average among all rats for each lever was between 45-50% ($\mu_{left} = 95$, $\mu_{right} = 105$ responses on the final day of training). After three sessions of responding, delay discounting procedures began.

Delay Discounting Procedures

A varying delay rodent model of discounting was implemented based on general procedures outlined by several authors (Dandy and Gatch, 2009; Anderson and Woolverton, 2005; Evenden and Ryan, 1996). Before beginning the first delay discounting session, all rats were randomly assigned a delayed reinforcement and an immediate reinforcement lever for the entire duration of the experiments. Lever presses on one lever were followed by the immediate delivery of a single pellet while lever presses on the other lever produced three pellets after a specified period of time. Sessions were comprised of five blocks, each containing 12 trials, and were characterized by increases in delay for the large (delayed) reward lever. The first two trials of every block were considered "forced" choice trials in which only one lever was extended. Forced choice trials provide exposure to the increased delay before a "free" choice is presented.

During free choice trials (50 total), both levers were extended for 30 seconds and responses were recorded. If no choice was made within the 30 second interval, the levers retracted, and an error was recorded. During all trials, until a choice was made, stimulus lights were illuminated above extended levers and a house light was present. Intertrial intervals (ITI) were 30 seconds in length with no stimulus or house lights.

Four sets of five delays ([0, 1, 2, 4, 6]; [0, 2, 4, 8, 16]; [0, 5, 10, 20, 40]; [0, 10, 20, 40, 60] sec) were implemented over the course of training to provide gradual exposure to increases in reinforcement delay, culminating in a terminal set (0, 10, 20, 40, 60 sec) necessary for data collection. The first delay of every block, and every set, was 0 sec to allow for stability assessment across each session. In order to proceed to the next set of delays, rats were required to allocate 80% of their responses to the large reward lever during the first block (0 sec), for three consecutive sessions. No more than three additional sessions were required for stable responding across training sets. To assess the effects of LSD on acquisition of choice behavior related to an impactful, novel set of delays, two separate experiments were performed.

Experiment I. The purpose of the first experiment was to assess the effects of low dose LSD administration on the progression of choice behavior during exposure to the terminal set (0, 10, 20, 40, 60 sec) of delays. A between-group design was implemented with four conditions (n= 8/group), two experimental and two control conditions. The two experimental conditions encompassed two low doses of LSD, 20 and 40 µg/kg. A saline control group was used to assess direct differences between LSD and non-LSD induced choice behavior. Rats received a total six injections over 12 days, every other day, and were only tested on days in which an injection occurred. Finally, a training control group was utilized for comparison of typical choice responding in the absence of stimuli related to the injections.

LSD testing began on the first day of terminal baseline exposure and was always administered 15 minutes prior to testing in the rat's home-cage. Rats in the training control condition were handled briefly before being placed back into their home-cage. Following the injection interval, rats were placed into operant chambers with a programmed 30 seconds of habituation. After the final day of testing, rats receiving LSD or saline were sacrificed and brains were immediately frozen for future protein analysis. Training control rats were used experiment II, detailed below.

***Experiment II*.** A within-subjects design was carried out with the remaining rats (n = 8) from Experiment I to assess potential dose-dependent effects of LSD on choice-related behaviors. An escalating dosing pattern was utilized (20, 40, 80, 160 µg/kg), with a single LSD dose being testing each week. Four sessions occurred during week (Monday-Thursday) and consisted of two non-injection days (Monday and Tuesday) followed by a randomly determined drug or saline sessions (Wednesday and Thursday). Following the third week (80 µg/kg), two saline injections were administered, and no drug session was performed. For this reason, the final dose (160 µg/kg) was tested during the fifth week of Experiment II. No other differences in number of sessions, or time-off were noted, indicating consistency with the general experimental procedures.

Data Analysis

The primary dependent measure for the analysis of discounting behavior was the percentage of responding on the large reward lever across each delay (or block). Percentages were obtained by dividing the total number of responses for the large reward by the total number of free choice trials (10). Additionally, response latency (seconds) for every free choice trial was collected as a secondary dependent measure of interest. Generalized linear mixed-effect models

were computed to determine the presence of statistically significant interactions and main effects. Mixed-effect models provide more accurate estimates than repeated measure analyses when designs are unbalanced or include factors with several levels of repeated measurement. Furthermore, recent publications have demonstrated their powerful utility in delay discounting analyses because of the control provided over subject and temporal variability as a function of a given condition (Marshall and Kirkpatrick, 2016; Peterson and Kirkpatrick, 2016). This added precision is due to the addition of random effects which allow for individual intercepts to be calculated for specified repeatedly measured variables of the same value.

In the present experiment random effects were selected based on model convergence and Akaike information criterion (AIC). Model selection with AIC is a common procedure to determine the amount of information lost when variables are removed or supplemented (Wagenmakers and Farrell, 2004). Experiment I and II differed slightly in their model considerations due to differences in experimental design (i.e. between vs within). For example, day and delay were considered random effects for Experiment I because it was assumed a novel, impactful set of delays would require separate intercepts for each day. A similar consideration was given to repeatedly measuring the same set of delays within the same group. In Experiment II, rats had established baseline responding during the terminal set of delays before a single injection of LSD or saline was administered. Furthermore, rats were exposed to all doses of LSD and likely varied individually providing a rationale for individual intercept calculations. For these reasons, it was assumed utilizing rats as a random effect would be advantageous in model considerations.

All statistical analyses and figures were produced using the R programming language. For linear mixed-effect models, the package lme4 was used along with emmeans for multiple

comparisons purposes. Error bars on all figures represent standard variance computations and should not be used for visual inspection of statistical significance as no established method for their computation has been reported (Peterson and Kirkpatrick, 2016). Nonetheless, standard estimates provide relative estimates for reference purposes.

RESULTS

Experiment I

***Discounting*.** The goal of Experiment I was to determine the effects of low dose LSD (20 and 40 µg/kg) on a rodent model of delay discounting when a novel, impactful set of delays (0, 10, 20, 40, 60 sec) was first introduced. A total of six injections were administered over the course of 12 days, behavior was only assessed on injection days. Figure 1 represents choice (%) for the larger reward for each treatment averaged across all six days and Figure 2 displays choice data separated by day. A generalized linear mixed-effects model (fixed effect = treatment; random effects = day, delay) did not reveal any significant interactions among groups when compared across delay or days. Significant main effects of delay ($F_{(4,775)} = 21.04$; $p < 0.001$) and day ($F_{(5,775)} = 2.667$, $p = 0.0212$) were obtained and Tukey-corrected comparisons were performed. Significant decreases in large reward lever responding were noted between delay conditions of 60 vs 0 sec ($\mu_{Difference} = 26.25\%$; $p = 0.0154$), 60 vs 10 sec ($\mu_{Difference} = 30\%$; $p = 0.003$), and 40 vs 10 sec ($\mu_{Difference} = 23.75\%$; $p = 0.0384$) when compared across all days and treatments. Rats responded on the large reward lever less frequently on days 5 and 6 when compared to days 1 and 2 ($\mu_{5-1} = -11.73\%$, $p < 0.001$; $\mu_{5-2} = -12.93\%$, $p < 0.001$; $\mu_{6-1} = -10.18\%$, $p = 0.0046$; $\mu_{6-2} = -11.38\%$, $p < 0.001$). Additionally, significant decreases were noted between days 5 and 3 ($\mu_{5-3} = -9.417\%$, $p = 0.013$) with the comparison of days 6 and 3 approaching significance ($\mu_{6-3} = -7.870\%$, $p = 0.061$).

Figure 1

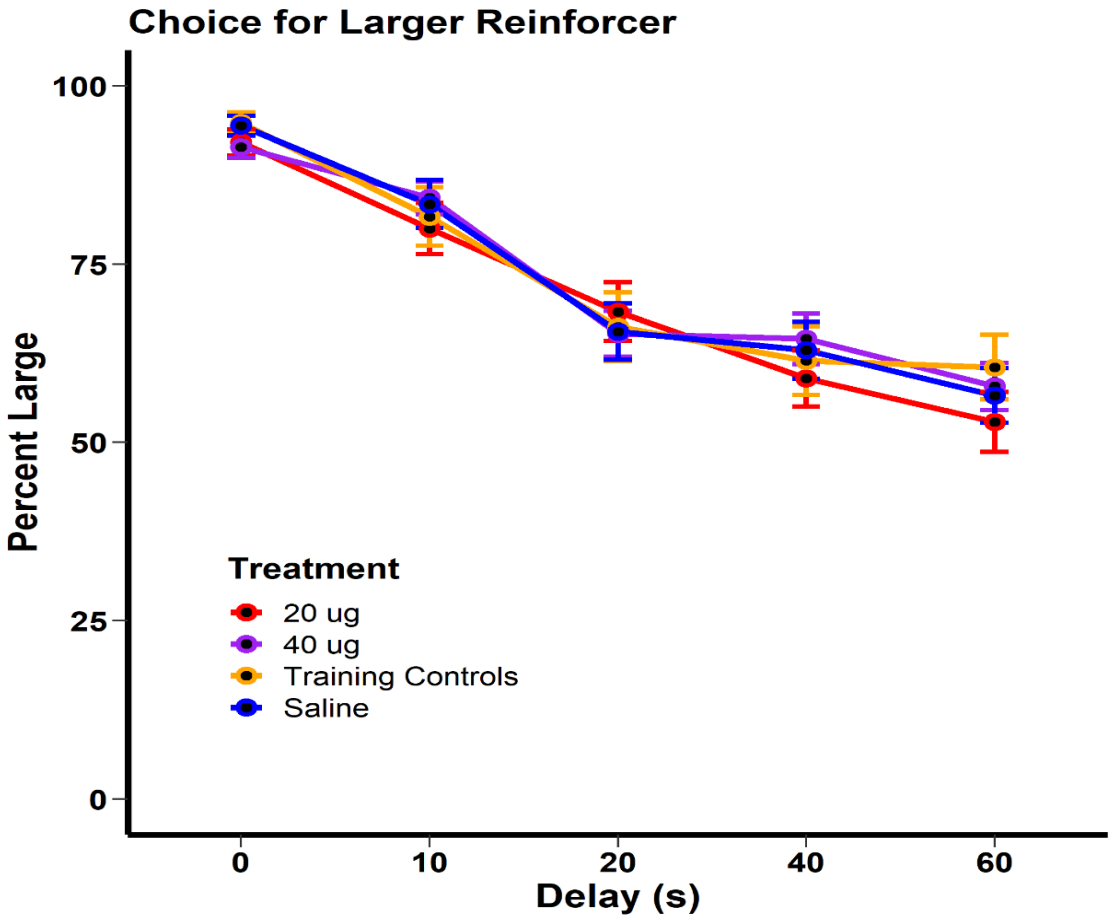


Figure 1. Percent Choice for Larger Reinforcer Between Treatment Conditions. Percent large data is displayed as an average across all days for each treatment in experiment I. Percent large represents the number of responses for the large reward over the total number of possible responses (10) in a given block (delay). Error bars represent standard variance calculations (S.E.M).

Figure 2

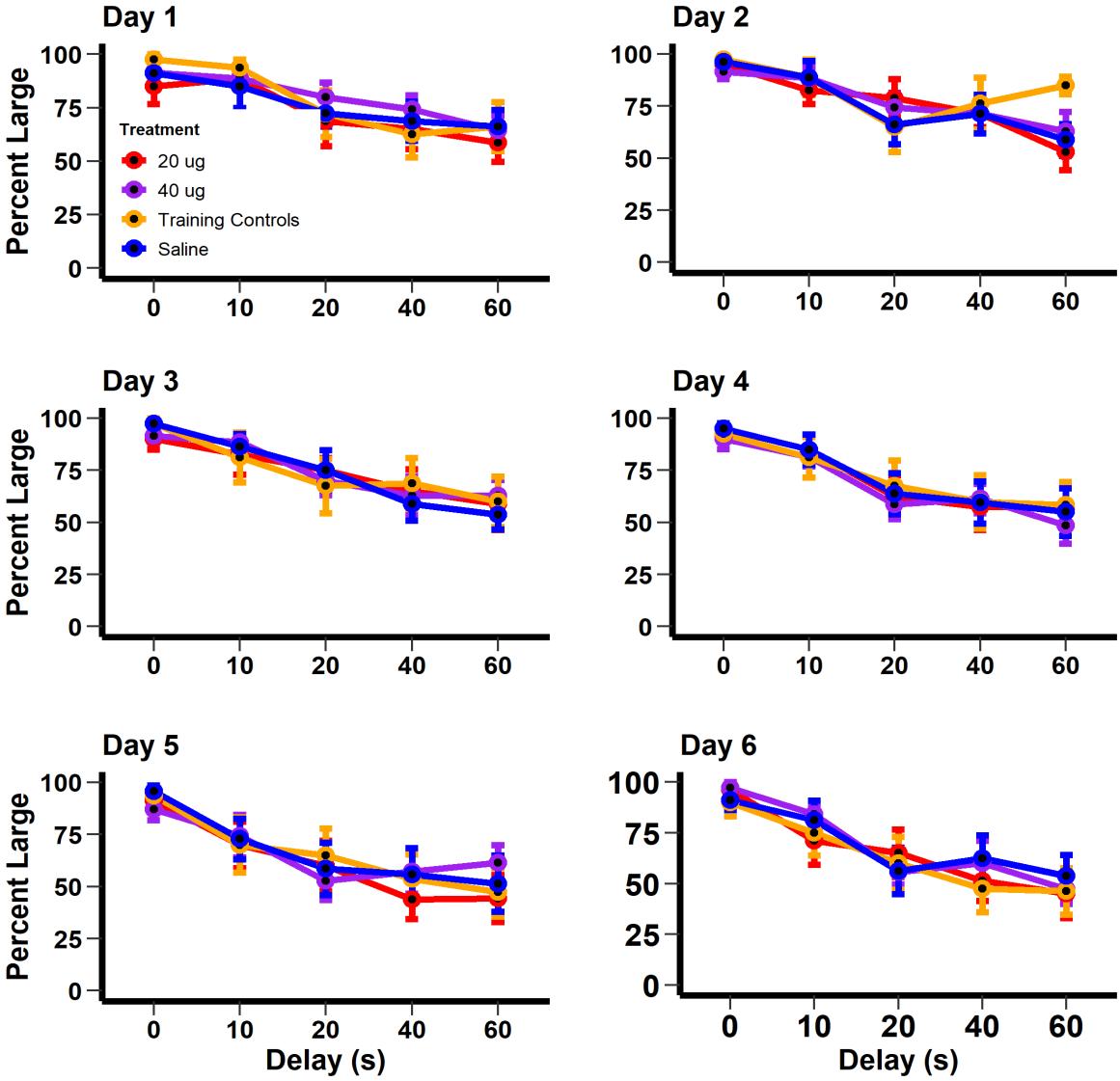


Figure 2. Percent Choice for Larger Reinforcer Between Treatment Conditions. Percent large data is displayed for each day of injections (6) and for each treatment in experiment I. Error bars represent standard variance calculations (S.E.M).

Table 1

Daily k Parameter Estimates for Experiment I

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Overall k
20 ug	0.1673	0.1642	0.1695	0.1855	0.2375	0.2196	0.1880
40 ug	0.1484	0.1561	0.1657	0.1982	0.2153	0.1987	0.1782
Training Controls	0.1556	0.1517	0.1762	0.1833	0.2185	0.2216	0.1829
Saline	0.1625	0.1658	0.1708	0.1838	0.2176	0.1977	0.1811

Note. Daily *k* parameter estimates are displayed for each treatment condition in experiment I. Nonlinear modeling was carried out to determine the best fits and Overall k values were calculated utilizing data averaged across all days.

Hyperbolic modeling was performed in order to determine degree of discounting and reinforcer value, denoted by parameter *k*, for each treatment. Table 1 displays all values of *k* for each day while Figure 3 illustrates hyperbolic curves for each treatment using an overall *k* parameter predicted from combining all days and groups. In general, overall *k* values were similar across treatments (20 µg/kg = 0.188; 40 = 0.178; Training Controls = 0.183; Saline = 0.181) and increased over the course of the sessions ($20_{Day1} = 0.167$, $20_{Day6} = 0.220$; $40_{Day1} = 0.148$, $40_{Day6} = 0.199$; $TrainingControls_{Day1} = 0.156$, $TrainingControls_{Day6} = 0.222$; $Saline_{Day1} = 0.162$, $Saline_{Day6} = 0.197$).

Figure 3

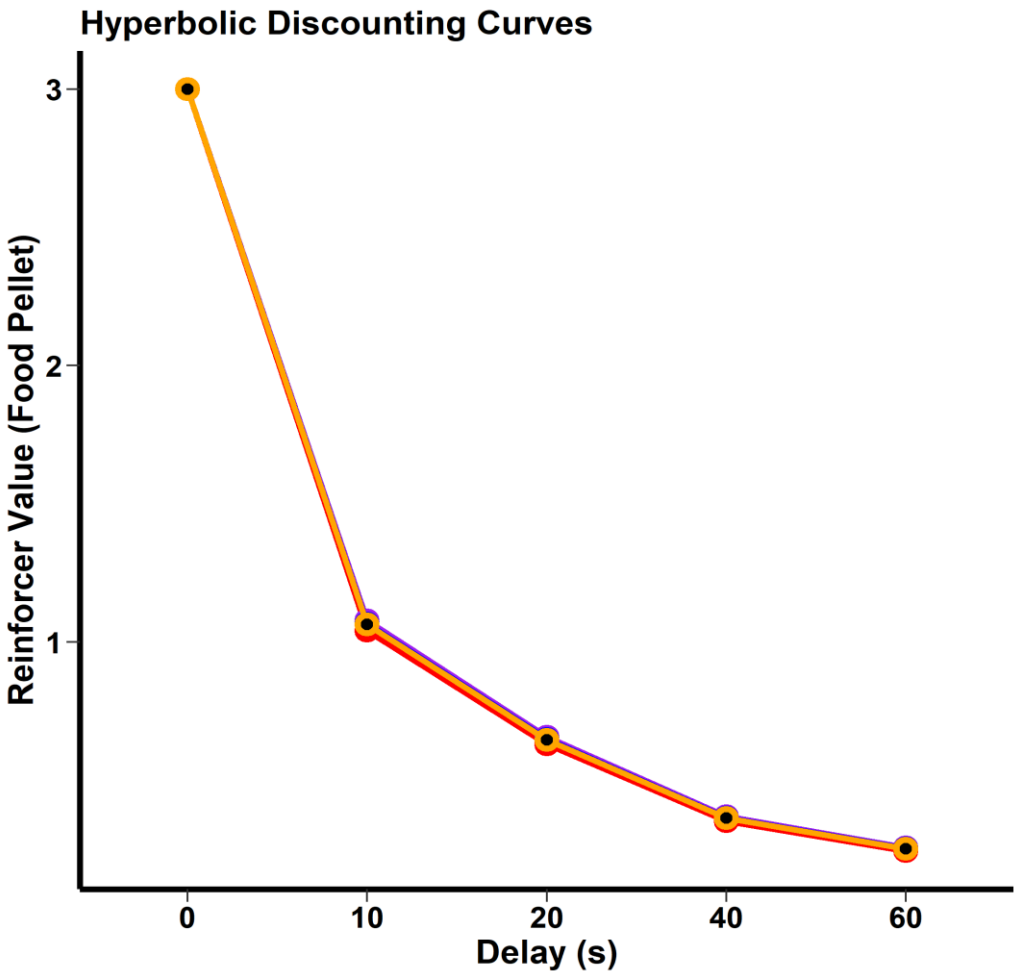


Figure 3. Hyperbolic Discounting Curves for all Treatment Conditions in Experiment I. Data for all days across each treatment in experiment I was modeled utilizing a hyperbolic function to determine k parameters representative of discounting value. Predicted values from the non-linear model are plotted and represent the value of the large reward (3 pellets) as a function of the delay in each block.

Latency. Latency to respond was recorded for every lever press during free choice trials and represents the time between lever extension and a response. Figure 4a represents between-treatment latency averages (seconds) for each block averaged across all days. Figure 4b displays overall choice trial latency data averaged across all days and blocks. Figure 5 represents data daily response latency for each treatment condition. A generalized linear mixed-effects model (fixed effect = treatment; random effect = day, delay) revealed a significant treatment x delay interaction ($F_{(12,9134)} = 2.357$, $p = 0.004$). Given the interaction, estimated least-squares means were calculated and pairwise comparisons were performed between treatment and delay using Tukey p-value correction. At the 0 second delay rats in the 20 µg/kg ($\mu_{20} = .765$ sec) condition were significantly slower in responding when compared to the 40 µg/kg ($\mu_{40} = 0.523$ sec, $p < 0.001$) and training control (TC) groups ($\mu_{TC} = 0.523$ sec, $p < 0.001$). Additional significant comparisons were obtained at the 20 second delay between the 20 and 40 µg/kg conditions ($\mu_{20} = 0.937$ vs. $\mu_{40} = 0.766$, $p = 0.048$) and between 40 µg/kg and TC ($\mu_{40} = 1.06$ vs $\mu_{TC} = 0.832$, $p = 0.044$).

Figure 4a

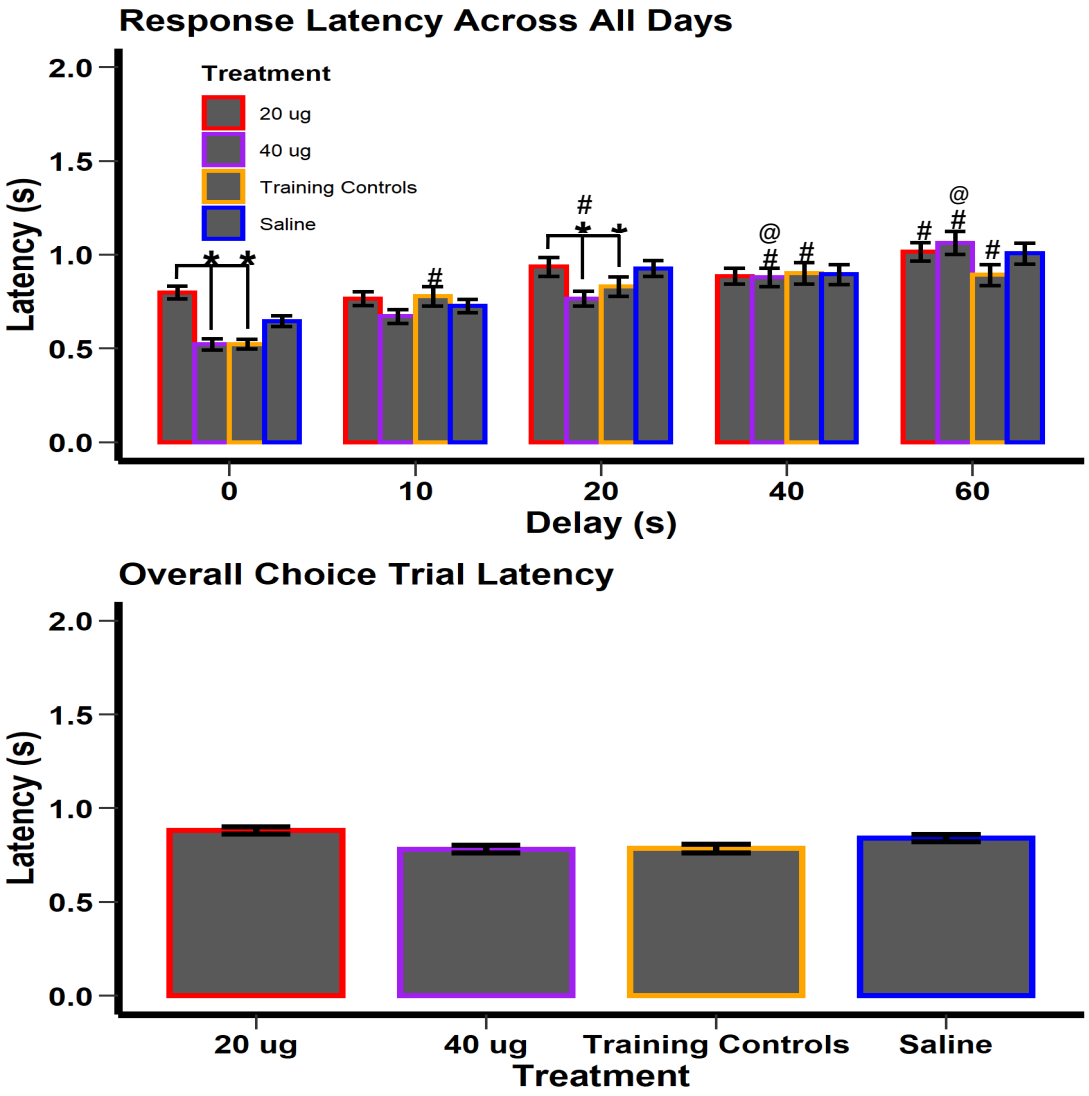


Figure 4. Response Latency by Delay for all Days and Overall Choice Trial Latency for all Treatment Conditions. (a) Response latency averaged across all days for each treatment is displayed for experiment I. (b) Response latency averaged across all delay conditions for each treatment is presented for experiment I. Error bars represent standard variance calculations (S.E.M). Statistical significance within a given delay condition is denoted by "*" whereas significance between delays are denoted by "#" and "@". "#" represents differences from the 0 second condition and "@" represents differences from the 10 second condition.

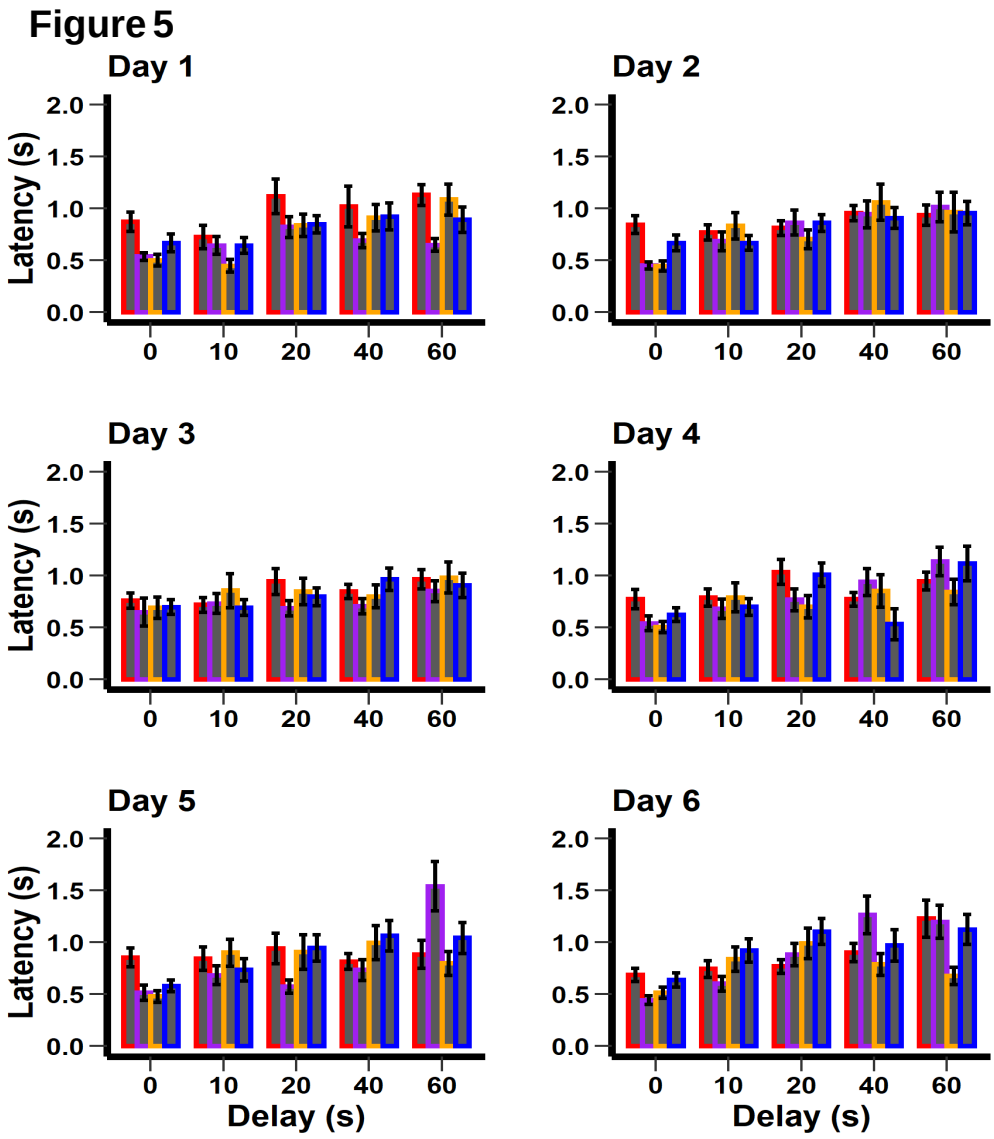


Figure 5. Response Latency by Day for all Treatment Conditions. Average response latency for each day and treatment is displayed for experiment I. Latency was recorded after every lever press during each session. The vertical axis represents each delay (s) or block each consisting of 10 total trials. Error bars represent standard variance calculations (S.E.M).

Due to the overall model complexity and small sample size, a two-factor mixed-effects model (fixed effect = treatment; random effect = day) was used to determine differences between delay for each treatment. A significant interaction of treatment and delay ($F_{(12,9134)} = 2.357$, $p = 0.004$) was obtained and multiple comparisons were performed based on estimated least-squares means with Tukey-corrected p-values. For the saline condition, significant increases in latency were observed between the 0 ($\mu = 0.65$) delay condition and the 20 ($\mu = 0.928$, $p = <0.001$), 40 ($\mu = 0.895$, $p = <0.001$) and 60 ($\mu = 1.007$, $p = <0.001$) second delay conditions. Furthermore, significant increases were observed between the 10 second delay condition ($\mu = 0.73$) and both the 20 ($\mu = 0.928$, $p = 0.149$) and 60 ($\mu = 1.007$, $p < 0.001$) second conditions. Comparisons between blocks in the TC group revealed significant increases in latency between the 0 sec delay ($\mu = 1.007$) and all other delays ($\mu_{10} = 0.778$, $\mu_{20} = 0.832$, $\mu_{40} = 0.902$, $\mu_{60} = 0.892$, $p < 0.001$ for all comparisons). The lowest LSD dose (20 µg/kg) produced a significant increase in latency when comparing the 0 ($\mu = 0.798$) and 10 ($\mu = 0.766$) second delay conditions to the 60 second delay ($\mu = 1.016$, $p = 0.005$ and < 0.001 respectively). LSD administered at 40 µg/kg produced significant increases in response latency when comparing the 0 second delay ($\mu = 0.523$) to the 20 ($\mu = 0.766$, $p = 0.003$), 40 ($\mu = 0.880$, $p < 0.001$), and 60 second delay ($\mu = 1.064$, $p < 0.001$). Finally, significant increases in latency were also identified for the 40 µg/kg LSD treatment at the 10 second delay ($\mu = 0.671$) when compared to the 40 ($\mu = 0.880$, $p = 0.019$) and 60 second delays ($\mu = 1.064$, $p < 0.001$).

Experiment II

Discounting. Training controls (TC) from Experiment I were used in Experiment II to determine the acute effects of LSD on choice behavior after reaching stable terminal baseline (0, 10, 20, 40, 60 sec) responding. A series of four escalating LSD concentrations (20, 40, 80, 160

µg/kg) were administered once per week. Figure 6 displays choice (% large reward) responding during sessions when LSD was administered for ease of visual comparison among doses. Figure 7 depicts choice (%) responding for the larger reward separated by dose (week) and includes respective saline and non-injection sessions for a given week. Choice data were averaged for the two non-injection tests each week. A generalized linear mixed-effects model (fixed effect = treatment, block; random effect = rat) revealed a significant interaction between treatment and delay ($F_{(20,524)} = 2.616$, $p < 0.001$). This interaction was due to a significant difference between the 80 and 160 µg/kg dose at a 10 second delay and several significant differences between LSD dose and saline or non-injection sessions. In the present model, both non-injection and saline conditions are analyzed across several sessions whereas each LSD dose was administered for a single session. For this reason, four separate mixed models (fixed effect = treatment, delay; random effect = rat) were computed for each week to assess differences within respective weeks. Significant treatment by delay interactions were obtained in models for the 40 (Week 1) and 160 (Week 4) µg/kg conditions and their respective saline or non-injection sessions. Administration of 40 µg/kg LSD produced decreases in choice responding at the 0 and 10 second delays when compared to both saline and non-injection sessions. For the 160 µg/kg condition, a significant decrease was found only at the 10 second delay compared to both saline and non-injection sessions.

Figure 6

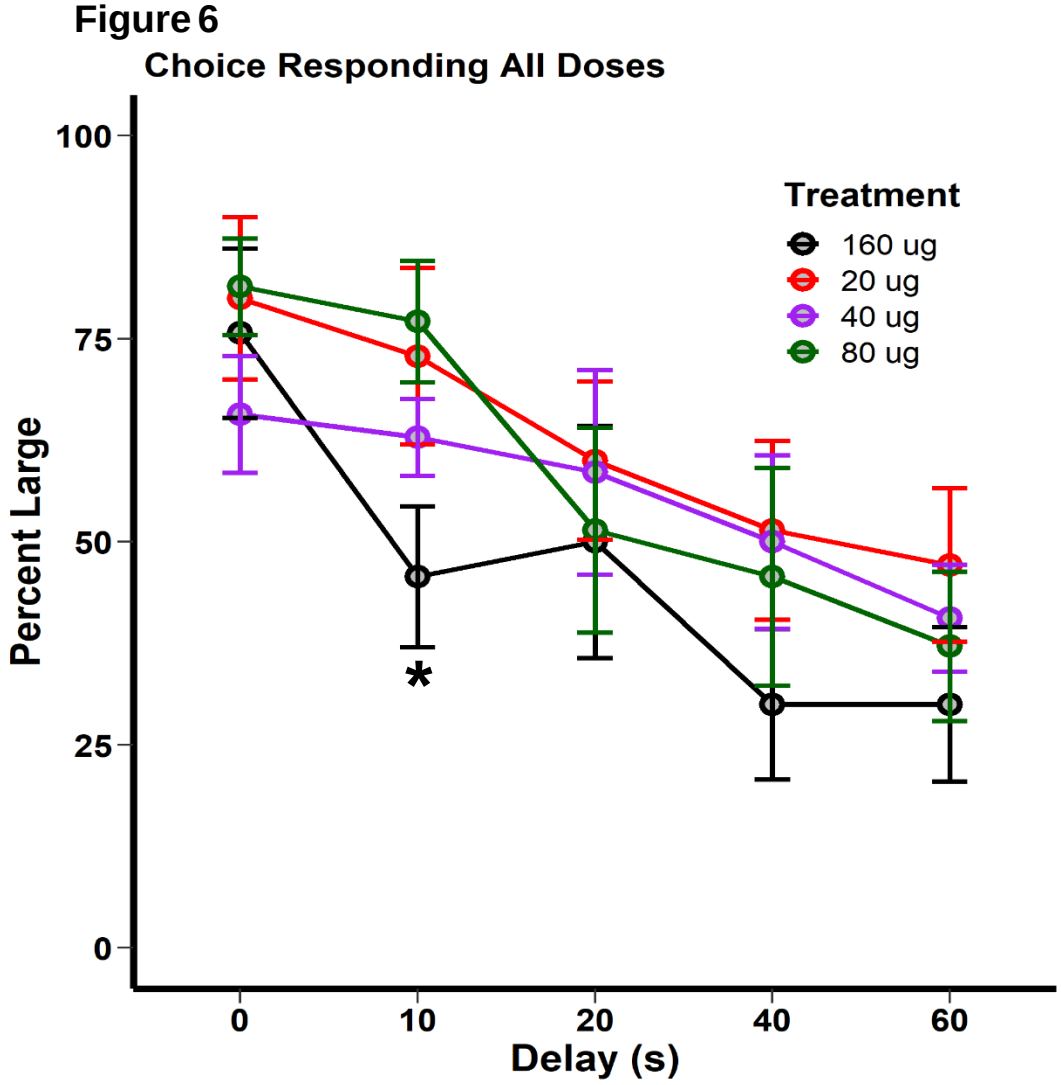


Figure 6. Percent Choice for Larger Reinforcer for all Doses of LSD in Experiment II. Percent large data for each week is presented for experiment II. Saline and No injection sessions are presented for the respective week. No injection sessions were averaged across two days. Percent large represents the number of responses for the large reward over the total number of possible responses (10) in a given block (delay). Error bars represent standard variance calculations (S.E.M). Significant differences within delay conditions are denoted by "*" ($p < 0.05$).

Figure 7

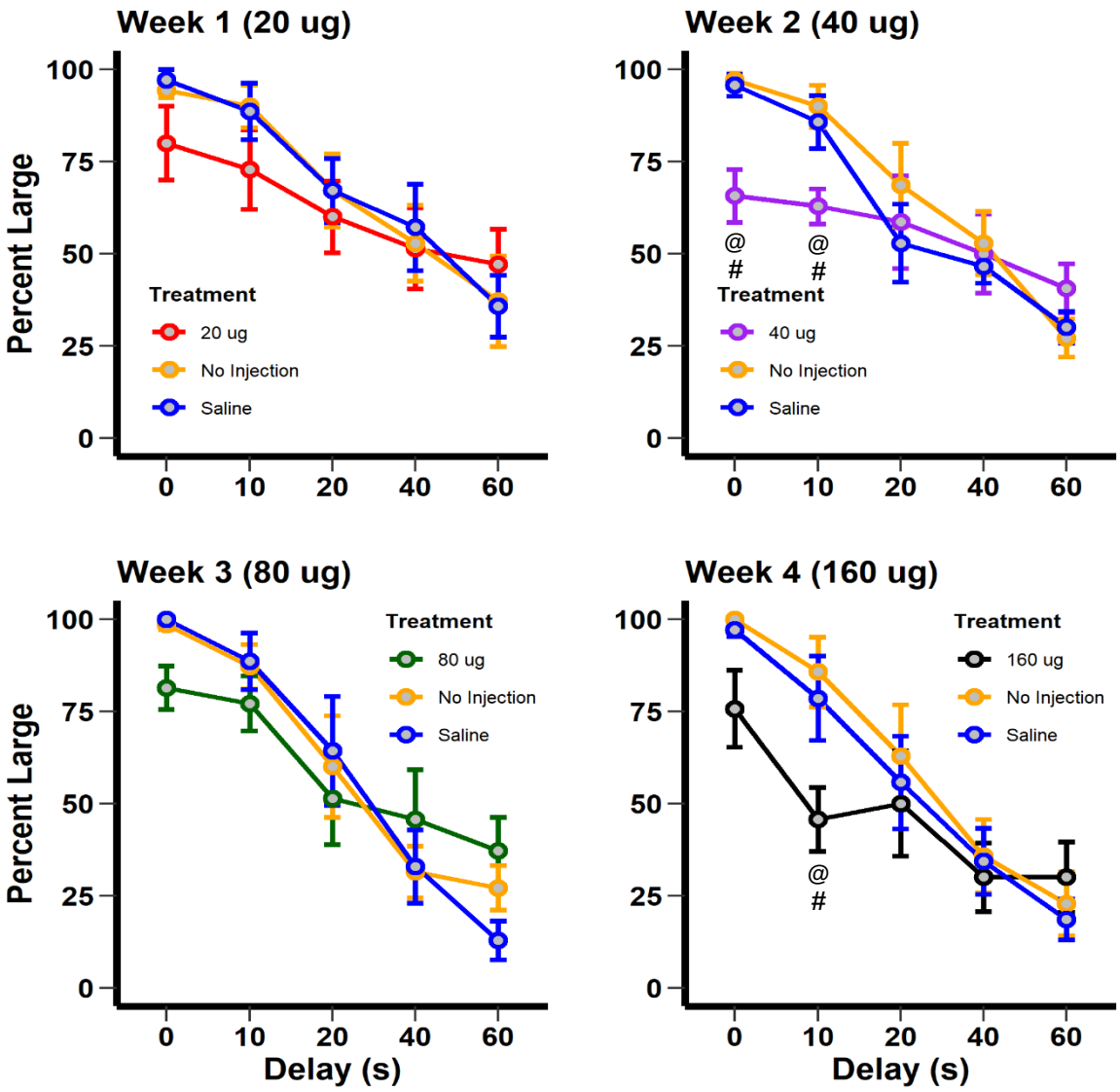


Figure 7. Percent Choice for Larger Reinforcer by Week (Dose). Percent large data for each dose of LSD from Experiment II is presented for purposes of visual comparison. Error bars represent standard variance calculations (S.E.M). Statistical significance ($p < 0.05$) from no injection sessions is represent by "@" whereas significance from saline is represented by "#".

Similar to the first experiment, *k* parameter values were calculated using hyperbolic modeling to determine the degree of discounting and relative reinforcer value. Table 2 displays *k* values for each LSD dose with saline and non-injection sessions represented by a model including all sessions. Discounting values increased each week for both saline (Week 1 = 0.186, Week 4 = 0.251, Overall = 0.218) and non-injection (Week 1 = 0.182, Week 4 = 0.242, Overall = 0.206) sessions. For LSD sessions, 20 µg/kg produced the lowest *k*-value (0.220) while the highest dose, 160 µg/kg, produced the greatest degree of discounting (0.375) for an LSD session. Intermediate doses of LSD (40 and 80 µg/kg) produced values similar to one another (0.254 and 0.237 respectively). Figure 8 illustrates hyperbolic modeling of the predicted reinforcer value given each dose's respective *k* parameter.

Table 2

Overall k Parameter Estimates for Experiment II

	20 ug	40 ug	80 ug	160 ug	Saline	No Injection
Overall *k*	0.2201	0.2540	0.2376	0.3753	0.2183	0.2062

Note. Overall k parameter estimates for each treatment condition in experiment I are displayed. Nonlinear modeling was carried out to determine the best fits. Estimates for LSD doses include one session while no-injection and saline are averaged across all sessions for each week.

Figure 8

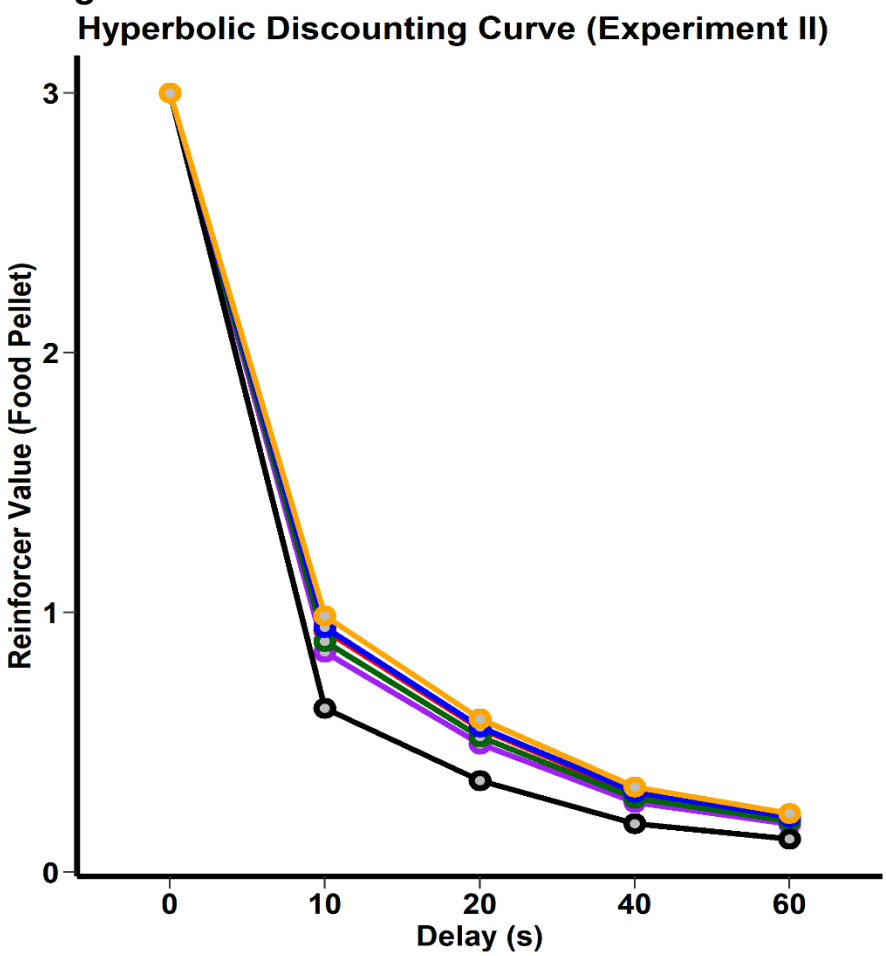


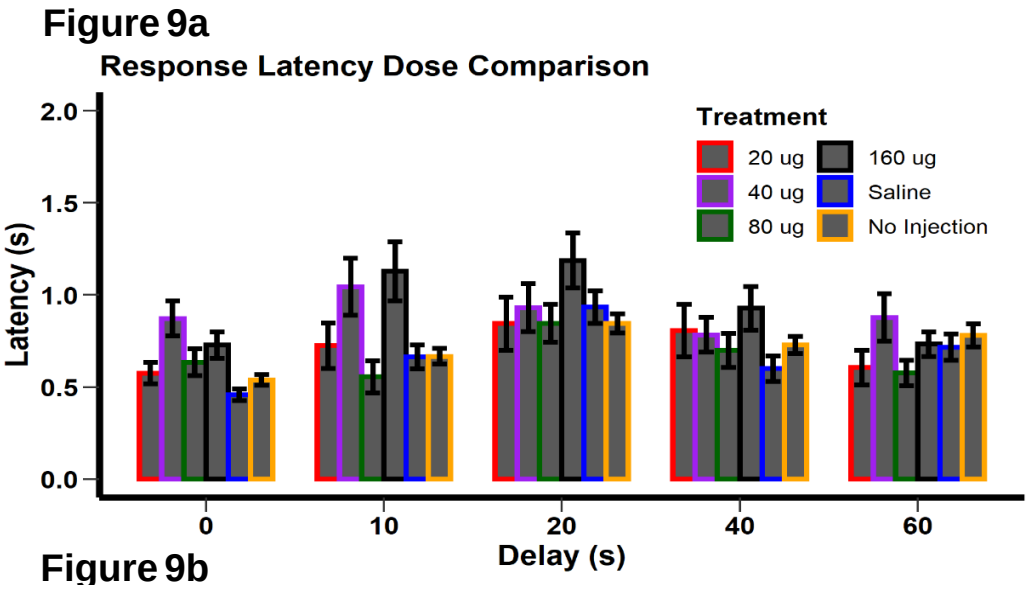
Figure 8. Hyperbolic Discounting Curves for all Treatment Conditions in Experiment II. Data for each treatment in experiment II was modeled utilizing a hyperbolic function to determine k parameters representative of discounting value. Predicted values from the non-linear model are plotted and represent the value of the large reward (3 pellets) as a function of the delay in each block.

Latency. Figure 9a graphically depicts response latency (s) for all treatment conditions for each block and 9b displays overall choice trial latency averaged across all blocks for each treatment condition. Figure 10 represents comparisons between each week of Experiment II. A

similar mixed-effects model to that used for choice comparisons in Experiment II was utilized to determine differences in response latency between LSD doses. Significant main effects were obtained for both dose ($F_{(5,5587)} = 6.55$, $p < 0.001$) and delay ($F_{(4,5587)}$ 7.15, $p < 0.001$) allowing for multiple comparisons. The 20 second delay condition produced the greatest overall response latency ($\mu = 0.934$) and was significantly higher than the 0 ($\mu = 0.634$, $p < 0.001$), 40 ($\mu = 0.758$, $p < 0.023$), and 60 ($\mu = 0.715$, $p = 0.001$) second delay conditions. Response latency during the 10 second delay was the next largest ($\mu = 0.803$) but was only significantly slower than the 0 second delay (($\mu = 0.634$, $p = 0.034$). Marginal mean differences for dose comparisons revealed an increase in overall latency from the 160 µg/kg ($\mu = 0.940$) condition compared to the 20 (, $p < 0.001$) and 80 µg/kg ($\mu = 0.670$, $p = 0.004$) conditions. Furthermore, 40 µg/kg LSD ($\mu = 0.950$) produced significantly greater response latency when compared to the 80 µg/kg ($\mu = 0.670$, $p < 0.001$) concentration.

Additional statistical significance was obtained for comparisons between saline and non-injection conditions but is not displayed for the present model. Similar to Experiment I, four separate models were computed to determine differences within each week. Main effects of delay are not addressed as they are adequately described by the previous model. For week 2 (40 µg/kg), a significant interaction between treatment and delay ($F_{(8,1397)} = 2.045$, $p = 0.038$) was obtained. Marginal mean comparisons revealed 40 µg/kg ($\mu = 0.872$) produced significantly greater response latency than saline ($\mu = 0.452$, $p = 0.023$) and non-injection sessions ($\mu = 0.419$, $p = 0.009$) at the 0 second condition. During the 10 second delay condition, 40 µg/kg ($\mu = 1.044$) produced significantly greater latency compared to only the non-injection control ($\mu = 0.533$, $p < 0.001$). Finally, a significant main effect of treatment was obtained during week 4 revealing significant increases in overall latency during the 160 µg/kg test session ($\mu = 0.940$) when

compared to the other conditions that week ($\mu_{saline} = 0.614$, $p < 0.001$; $\mu_{noninjection} = 0.713$, $p < 0.001$).



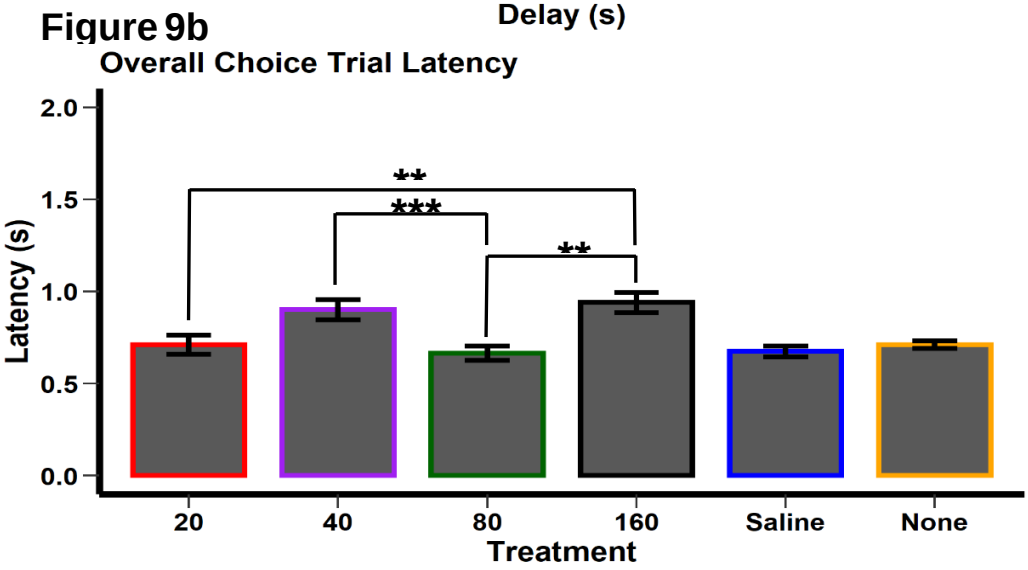


*Figure 9.*Response Latency by Delay for all Treatment Conditions and Overall Choice Trial Latency. (a) Response latency averaged across all weeks for each treatment is displayed for experiment II. (b) Response latency averaged across all delay conditions for each treatment is presented for experiment I. Error bars represent standard variance calculations (S.E.M). Between group statistical significance in 5b is represent by "*" ($p < 0.01$ = "**"; $p < 0.001$ = "***").

Figure 10

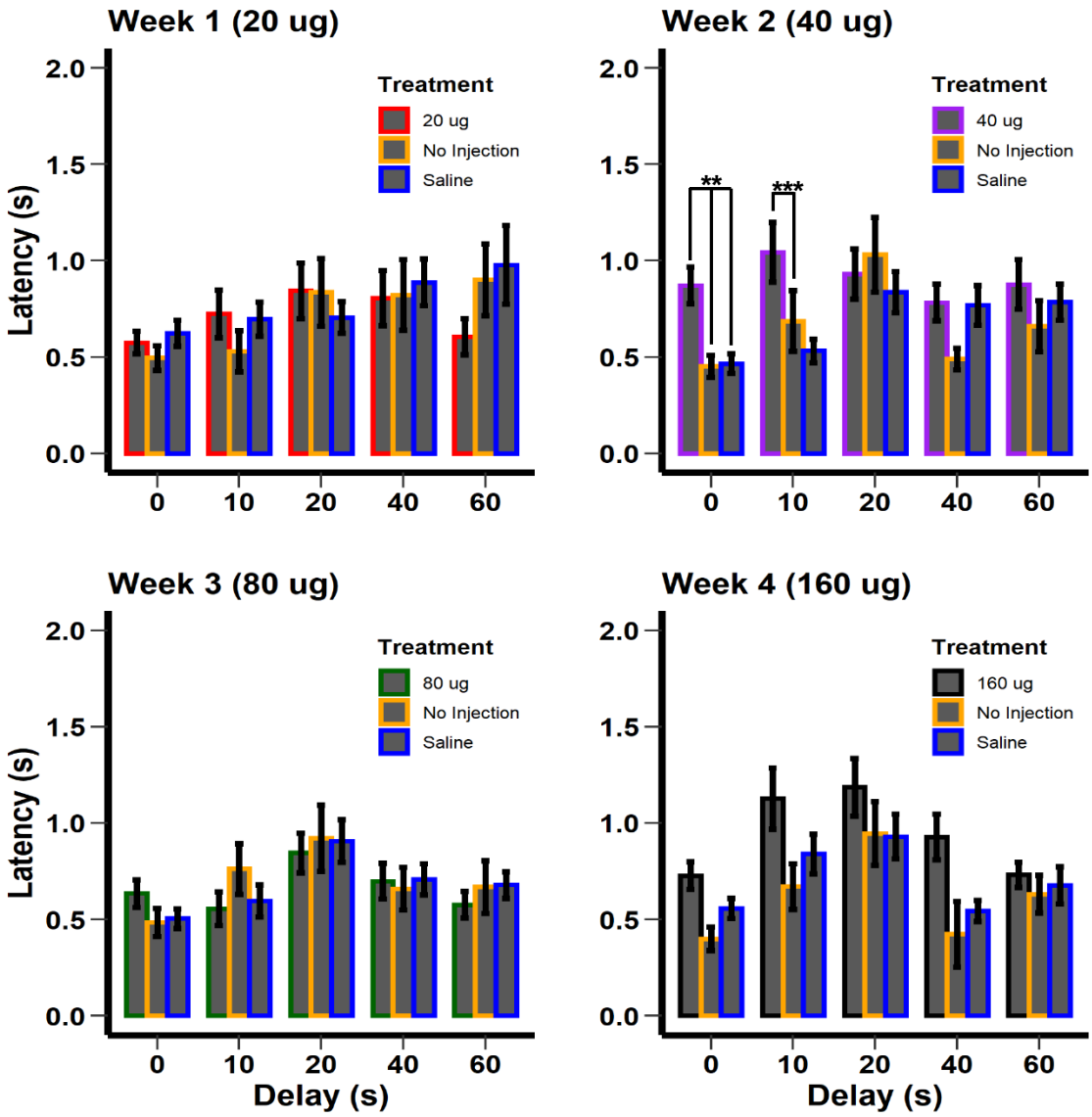


Figure 10. Response Latency by Week (Dose). Average response latency for each week is displayed for experiment II. No-injection sessions were averaged and represent two days of responding. Latency was recorded after every lever press during each session. The vertical axis represents each delay (s) or block each consisting of 10 total trials. Error bars represent standard variance calculations (S.E.M). Statistical significance within delay conditions is denoted by "*" ($p < 0.01$ = "**"; $p < 0.001$ = "***").

DISCUSSION

LSD is a popular hallucinogen characterized by intense perceptual alterations and increases in positive mood (Schmid, 2015; Dolder, 2016). Recent studies suggest LSD may provide therapeutic benefits for anxiety and substance abuse disorders (Krebs and Johansen, 2012; Gasser et al., 2014). Moreover, user reports suggest similar benefits at low dose, "microdose", LSD concentrations but experimental evidence is limited. Similarly, animal models addressing the potential therapeutic benefits of LSD are almost non-existent. For this reason, the primary aim of the present study was to assess the effects of LSD in a rodent model of delay discounting, a procedure predictive of impulsive behavior. Additionally, LSD's serotonergic agonism is of interest considering antidepressant drugs have been reported to increase responding for large-delayed rewards in rodent delay discounting procedures (Bizot et al., 1988). Commonly prescribed antidepressant drugs typically facilitate serotonin release through selective serotonin reuptake inhibition (SSRI). Moreover, rodent lesion studies suggest the serotonin system plays a role in impulsive choice behavior (Bizot et al., 1999). Literature detailing the effects of anxiolytics on rodent discounting behavior is conflicting. Acute treatment of anxiolytics has been shown to both increase (Thiebot et al., 1985) and decrease (Evenden and Ryan, 1996) discounting. Additionally, reductions in discounting have been demonstrated during chronic administration of anxiolytics (Evenden and Ryan, 1996; Huskinson and Anderson, 2012). The present study utilized two separate experiments to determine LSD's effects on rodent discounting behavior. For Experiment I, LSD's effects were assessed when a novel, impactful set of delays was first introduced. For the second experiment, rats from the training control group in the first experiment were used for a within-subject escalating dose assessment of LSD to

determine its effects on steady state behavior. Percentage of responses for the large reward lever and response latency were collected as dependent variables of interest in both experiments.

Results from choice data in Experiment I did not reveal any statistically significant differences among treatment conditions. Visual inspection of large reward lever responding across all days (Figure 1) suggests the lack of significance may not be due to inadequate sample size or inherent variability in the delay discounting task. Moreover, discounting curves for each treatment in Figure 3 are difficult to distinguish indicating comparable overall *k* values (Table 1). A similar trend is depicted when choice responding is plotted for each test day individually (Figure 2). Unsurprisingly, rats responded significantly less for the larger reward at longer delays (40 and 60 second) when compared to the shortest delays (0 and 10 seconds) irrespective of treatment group. This finding is important as it provides evidence that the terminal delays were impactful and the discounting procedure was successful. Differences between choice responding each day was expected given that it takes several sessions for an animal to achieve stable baseline performance (Mar and Robbins, 2007). In the present study, rats responded significantly less overall for the large reward on days 5 and 6 when compared to days 1, 2 and 3 ($\mu_{6\text{-}3}$ = -7.870%, p = 0.061) indicating an effect of exposure on choice.

Experiment II offered a comparison of LSD doses after rats had been exposed to a terminal set of delays for nine sessions. An overall treatment by block interaction revealed a significant decrease in percentage of responses for the large reward following treatment with 80 or 160 µg/kg at the 10 second delay. Although the variability (S.E.M) among groups was high (Figure 7), a distinct dose-response relationship is depicted by overall *k* values for each treatment (Table 2 and Figure 8). For example, non-injection sessions produced the lowest *k* value (0.2062) followed by saline (0.2183) and 20 µg/kg LSD (0.2201) whereas 160 µg/kg LSD produced the

largest (0.3753). Intermediate LSD doses (40 and 80 µg/kg) did not follow this relationship, but *k* values fell between both those obtained with 20 and 160 µg/kg LSD. Visual depictions of within-week comparisons (Figure 6) suggest the greatest differences between LSD and saline or non-injection sessions occurred during the first two delays (0 and 10 seconds). Interestingly, average estimates for large reinforcer choice are higher in all drug conditions when compared to their respective saline and non-injection sessions throughout the 60 second delay.

Statistically significant differences in choice for the larger reward were observed during administration of both 40 and 160 µg/kg LSD at the 10 second delay when compared to no injection and saline sessions. Additionally, 40 µg/kg LSD produced significant reductions when no delay was present (0 second condition) when compared to controls. However, for all LSD doses assessed in Experiment II, the 0 and 10 second delay conditions produced the largest discrepancy in choice behavior between treatments. These results are consistent with an earlier investigation of LSD in a different behavioral paradigm also presumed to model impulsivity. In rats trained to respond under a differential reinforcement of low rate (DRL) schedule, Appel (1971) found 80-160 ug/kg LSD decreased responding during 10 and 20 second intervals which resulted in fewer reinforcers obtained in a session. DRL schedules are commonly used as a measure of impulsive action. Considered together, the current results and previous findings by Appel (1971) suggest LSD's effects on temporal perception may be similar across different measurements of complex behavior. Interestingly, average estimates for large reinforcer choice are higher in all drug conditions when compared to their respective saline and non-injection sessions during the 60 second delay. The lack of significance among other doses may be a result of design complexity as there were several factors (block and treatment) containing many levels (5 and 3) and few rats (n = 7). Furthermore, due to the number of free choice trials per block

(10), a single response difference amounts to a 10% overall change in behavior and may inflate individual response variability.

Formal variance comparisons of the difference between 20 and 40 µg/kg LSD in Experiments I and II cannot be performed, but comparisons between *k* values are discussed. Given the similar increasing trend across treatment conditions in Experiment I, estimates from test day 6 provide the most accurate comparison between experiments. Interestingly, the discounting value on test day 6 for the 20 µg/kg LSD-treated group in experiment I is almost identical to the value obtained in Experiment II with this dose (0.2196 vs 0.220). This similarity may be due in part to the number of terminal delay exposures as 20 µg/kg was administered in Experiment II only three sessions after the final injection of 20 µg/kg in Experiment I. Moreover, exposure differences may explain the large discrepancy in *k* values obtained with 40 µg/kg LSD administration on day six versus week two of Experiment II (0.1987 vs. 0.2540). In general, drug administration commenced once responding was stable for at least five consecutive days during the 0 second condition of the terminal delay set (Dandy and Gatch, 2013). Statistical comparisons in the present experiment revealed no significant differences between days 4 and 5, suggesting behavior was stable for at least two days. Given this, the behavioral effects produced by low dose LSD may have been masked by the substantial augmentation of environmental conditions pertaining to increases in delay.

The absence of a significant overall effect of 40 µg/kg LSD on choice behavior in Experiment I was surprising given the significant acute effects of this dose on behavior in Experiment II. Literature describing tolerance to the behavioral effects of LSD in rats suggests tolerance is highly dependent on dose and behavior. For example, daily treatment with 130 µg/kg LSD produces tolerance to initial reductions in lever-pressing but not to escape behaviors related

to the onset of a shock (Freedman et al., 1964). In the present experiment, injections were administered every two days and should not have produced tolerance to the drug's behavioral effects. The difference in sensitivity between experiments is interesting considering the conflicting human literature regarding the subjective effects of low dose LSD. Bershad et al. (2019) utilized a within subject escalating dose procedure and found significant increases in drug "liking" and "feeling high" at 13 and 26 µg. Participants in this study were young and were included only if they had experience with hallucinogens. Yanakieva et al. (2019) utilized a between subject repeated dosing design and found no significant differences between low dose concentrations of LSD on similar measures. These participants were older and only included if they had no history of psychedelic use in the last five years. While the subjective measures reported have no relationship with impulsivity, the similarity in results given experimental design suggest a role of experience in the manifestation of LSD's psychoactive effects.

Response latency was collected as a secondary measure to determine whether LSD produced behavioral disruptions beyond discounting. As previously mentioned, temporal perception is significantly altered in both humans (Yanakieva et al, 2019; Aronson et al., 1959) and rats (Appel, 1971) following LSD administration. Furthermore, LSD produces intervals of limited responding under fixed ratio reinforcement schedules (Freedman et al., 1964) which may be elucidated through response latency measures. In Experiment I, latency tended to increase across each delay with no difference between days (Figure 4). Rats administered 20 µg/kg LSD were significantly slower in responding than those administered 40 µg/kg LSD throughout the 0 and 20 second delay conditions (Figure 5). By the final delay condition (60 seconds) in Experiment I, response latency of the rats administered 20 and 40 µg/kg doses was similar and significantly higher than the respective latency during the 0 second delay condition.

Results from Experiment II revealed a negative parabolic relationship between latencies across delays. For example, the 20 second delay condition produced the greatest overall response latency followed by the 10 second delay. Overall, 160 µg/kg LSD produced the slowest responding followed by 40 and 20 µg/kg LSD (Figure 10). Significant between treatment differences within each week for 160 and 40 µg/kg LSD were established because of increases at shorter delay conditions (i.e. 0, 10, 20 sec). These increases in response latency were not surprising given previous reports in the literature that LSD decreases response rates during operant tasks (Appel et al., 1971; Silva and Calil, 1975; Freedman et al., 1964). Although these measures are not identical because a single response is required per trial in the present study, rats may engage in similar disruptive behaviors (i.e. head-twitching) that influence locomotion or the ability to respond. For example, head twitch frequency increases as a function of dose and reaches the highest frequency around 10 minutes post injection (Halberstadr and Geyer, 2013; Yamamoto and Ueki. 1981).

Taken together, results from the present study indicate LSD may produce increases in discounting behavior at short delays when administered acutely. These results are consistent with the general notion that acute administration of commonly abused drugs produces impulsive responding in rodent models of delay discounting (de Wit and Mitchell, 2010). As mentioned, there are no direct comparisons to the present study, but cited therapeutic benefits in humans should be considered. For example, LSD, and other hallucinogens including psilocybin (Garcia-Romeu, Griffiths and Johnson, 2014; Johnson et al., 2014), have shown promise in the treatment of substance abuse disorders which are oftentimes associated with impulsive decision-making (de Wit, 2009). Given this, it is expected LSD may reduce impulsivity related to decision-making. Additionally, rodent models of delay discounting utilizing antidepressants with

serotonergic agonism similar to LSD have reported beneficial effects. However, this effect has been generalized to other antidepressants including those which influence the noradrenergic system (Bizot et al., 1988). This may indicate antidepressants produce decreases in discounting through additional non-serotonergic pathways. Future research should evaluate larger sample sizes across more doses to elucidate potential augmentations in discounting behavior following LSD administration.

A possible reason for reduced selection of the larger reward at the 0 sec delay may be due to a decrease in appetite as a result of serotonin agonism. Appetite is directly linked to fluctuations in the serotonergic system and drugs commonly utilized to suppress appetite do so by promoting serotonin release through the 1B and 2C subtypes (Blundell and Halford, 1998). Moreover, the serotonin 2C subtype is a primary target of LSD's action in the nervous system (Backstrom et al., 1999). In rats, LSD's effects on appetite suppression increase dose-dependently and tolerance does not develop to the suppressed effects after 10 days of repeated injections (Hamilton and Wilpizeski, 1961). Throughout saline and non-injection sessions, rats responded almost entirely for the large reward when no delay was present. This result was not observed when LSD was administered at any dose in Experiment II. Future research could evaluate LSD's effects on delay discounting at later post-injection intervals to overcome appetite suppression effects presumably mediated by serotonergic agonism. To evaluate whether increased discounting is only present at shorter delays, the presentation of delays could be arranged in a descending order in contrast to the ascending order conducted in the current study. As such, the first delay presented would be the largest and shorter delays would be presented at later post-injection intervals. Such investigations would be of interest in consideration of previous findings indicating differential involvement of serotonin and dopamine in the

discriminative stimulus effects maintained by LSD (Marona-Lewica, Thisted and Nichols, 2005; Marona-Lewicka and Nichols, 2007). In these studies, a pretreatment time of 90 minutes was necessary in order to elucidate the involvement of dopaminergic actions in the discriminative stimulus effects of LSD, whereas serotonergic actions predominate LSD discrimination at a 30 min post-injection interval. However, an *in vitro* analysis of dopamine release in freely moving rats following low dose LSD administration (2.5 and 25 µg/kg) did not find any significant alterations in dopamine or its metabolites in the striatum (Minuzzi et al., 2005). The absence of dopaminergic increases may be due low sample size (n = 5) and the pattern of injection in that study. Rats were administered two doses of LSD beginning with the lowest dose (2.5 µg/kg) followed by a higher dose (25 µg/kg) only two hours later. Nonetheless, evidence exists for dopamine involvement in LSD's behavioral and subjective effects and warrants further exploration.

Concluding Remarks

LSD is a remarkable drug with a therapeutic potential unlike many traditional drugs used to treat psychological illness. In recent years, published literature pertaining to all hallucinogens has increased in an attempt to prove their worth among alternatives. Unfortunately, research is still severely lacking in both human and non-human applications. This is exemplified in the present manuscript by the lack of a comparisons to existing literature pertaining to LSD's effects on complex behavior. Along with the necessity for additional direct measures of impulsivity, future research should evaluate the drug's effect on other rodent models of complex behavior including depression and anxiety phenotypes for translatable purposes. Moreover, studies with human participants should include objective report measures to evaluate discounting behaviors.

The inclusion of more dependent measures in both human and non-human work will allow a full characterization of the drug's effect on behavior.

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APPENDIX

WESTERN MICHIGAN UNIVERSITY

Institutional Animal Care and Use Committee

Date: May 8, 2019

To: Lisa Baker, Principal Investigator

From: Kathryn Ecker, Vice Chair

Re: IACUC Protocol Number 19-05-04

Your protocol entitled "Behavioral Assessment of Lsd Microdosing in Rats" has received approval from the Institutional Animal Care and Use Committee. The conditions and duration of this approval are specified in the Policies of Western Michigan University. You may now begin to implement the research as described in the application.

The Board wishes you success in the pursuit of your research goals.

Approval Termination: **May 7, 2020**

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