12-2023

Evaluating the Effects of Ketamine on Cognitive Flexibility in Rats Using a Probabilistic Reversal Learning Task

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EVALUATING THE EFFECTS OF KETAMINE ON COGNITIVE FLEXIBILITY IN RATS USING A PROBABILISTIC REVERSAL LEARNING TASK

by

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A dissertation proposal submitted in partial fulfillment
of the requirements for the degree
of

DOCTOR OF PHILOSOPHY

in

Psychology

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UTAH STATE UNIVERSITY
Logan, Utah

2023

ABSTRACT

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by

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Major depressive disorder (MDD) is a debilitating mental health condition that is among the leading causes of disability worldwide. However, traditional pharmacological treatments for MDD like selective serotonin reuptake inhibitors (SSRIs) are limited due to their delayed onsets of action, their chronic dosing requirements, and their unwanted and/or severe side effects that may cause discontinuation of use. One drug that seems capable of surmounting such shortcomings of traditional antidepressant treatments is the *N*-methyl-D-aspartate receptor antagonist ketamine. In clinical trials, ketamine significantly reduces depressive symptoms within just days, and the effects of a single infusion have been shown to last for weeks to months. Further, ketamine produces such therapeutic effects with limited side effects that persist for only a few hours post-treatment. Thus, it appears that ketamine may be both a viable and a superior alternative to traditional antidepressants such as SSRIs. However, clinical trials are still ongoing, and more research is needed to fully understand ketamine's beneficial effects. To this end, laboratory animal models using a paradigm known as reinforcement learning have been

shown to be particularly useful. Using reinforcement learning procedures allows for the examination and quantification of decision-making and reward-processing, two deficits of those diagnosed with MDD. The experiment in Chapter 2 was designed to test the potential dose-dependent effects of ketamine on cognitive flexibility, the ability to adapt to changing environmental circumstances, using a reinforcement learning procedure known as the probabilistic reversal learning task (PRL). Regardless of dose, ketamine did not enhance cognitive flexibility, and instead caused acute impairments in healthy rats. The experiment in Chapter 3 was designed to assess the effects of ketamine on behavior in the PRL when mild electric footshock was either combined with timeout periods or not, as timeouts alone may not always function as effective punishers. Although ketamine did not have any systematic effects beyond 1-hour post-injection, probabilistic shock punishment increased rats' performance on the task significantly. Implications for how these data might be related to underlying processes in MDD are discussed, as well as limitations that may be addressed in future research.

(129 pages)

PUBLIC ABSTRACT

Evaluating the Effects of Ketamine on Cognitive Flexibility in Rats Using a Probabilistic Reversal Learning Task

Anthony N. Nist

Depression is one of the most debilitating and widespread mental health conditions in the world today. Drugs that are traditionally prescribed to combat depression are flawed in several ways, and because of this, new treatments are needed. One drug that seems capable of overcoming the limitations of traditional antidepressants is ketamine. In clinical research, a single dose of ketamine can significantly reduce symptoms of depression quickly, its effects may last for weeks to months, and its side effects appear to be limited and relatively harmless. However, clinical research is ongoing, and more research is needed to fully understand ketamine's beneficial effects. One way that research can help understand how ketamine works is by using animal models of behavior. These models are beneficial because they allow researchers to isolate very specific variables that sometimes are not possible with human research. The specific approach used here is called reinforcement learning, which is well-suited to studying basic decision-making processes and how behavior changes based on receiving rewards and punishments. The experiment in Chapter 2 was designed to test the effects of different ketamine doses on behavioral adaptation, something that depressed individuals struggle with. Regardless of dose, ketamine did not enhance this ability, and instead appeared to cause short-lived impairments in healthy rats. The experiment in Chapter 3 was designed to assess the effects of ketamine on behavior when two different forms of

negative outcomes were either combined or not. Ketamine again did not have any long-lasting effects, but rats showed enhanced behavioral adaptation and persistence when they experienced a combination of two negative outcomes. Together, these studies aimed to improve our understanding of what aspects of depression ketamine might be useful for, and how to improve upon future research using reinforcement learning procedures with non-human animals.

ACKNOWLEDGMENTS

First and foremost, I'd like to thank my advisor, Tim Shahan. I will be forever grateful to you for shaping me as a scientist. You always allowed me the freedom to pursue my own interests and to make and learn from my own mistakes. But at the same time, you were always there when I really needed it. Looking back on it now, I could not imagine having had a better scientific mentor than you. I'd like to thank my dissertation committee, Drs. Greg Madden, Mona Buhusi, Katie Brown, and especially Dr. Steve Walsh. Thank you to Steve for allowing me, someone who had not taken a math class in almost 10 years, to take his graduate level statistics course, and afterward for spending so much of his precious time with me to foster my academic growth when he did not have to. I am thankful for all of the labmates I have been so fortunate to work with over the years. Especially the group that was there when I first started at USU, Drs. Rusty Nall, Paul Cunningham, Kaitlyn Browning (lab mom!), and Rafaela Fontes. Your unconditional guidance, support, and friendship were crucial for me in my first year and beyond. I am also grateful for my undergraduate mentor Dr. Matt Locey. Thank you for giving me the opportunity to learn about behavioral science in your lab, and for pushing me to seek out my graduate training elsewhere. I'd like to thank my family, especially my parents and grandparents for their unwavering support. I'd like to thank Jolyn for being my number one supporter throughout the entire process of this dissertation, from formulation to data collection, to writing in her basement during the summer. Without your love, patience, generosity, thoughtfulness, and understanding I wouldn't have been able to do it. Finally, thank you to the USU psychology department and Tim Shahan for

providing the funds necessary to complete this dissertation.

Tony Nist

CONTENTS

Page

LIST OF FIGURES

CHAPTER I

INTRODUCTION

Major depressive disorder (MDD) is a debilitating mental health condition that may be broadly characterized by persistent and lasting psychological symptoms including changes in mood, a loss of interest in or lack of pleasure for previously rewarding activities, and changes in cognition, among others (e.g., Otte et al., 2016). According to the World Health Organization, depression is one of the leading causes of disability worldwide, affecting an estimated 5% of the world's adult population (World Health Organization, 2021). MDD has also been associated with an increased risk of developing other health conditions such as diabetes, heart disease, and stroke (e.g., Whooley & Wong, 2013), therefore amplifying its burden to the public. As such, research aimed at the development of effective treatments for depression represents a critical need.

Specifically, more effective pharmacological treatments are needed to combat depressive symptoms. This is because as many as 50% of patients being treated with traditional antidepressant drugs such as selective serotonin reuptake inhibitors (SSRIs) have no response to such treatments the first time around (e.g., Rush et al., 2006), and an additional 20–30% still fail to respond following two or more medication trials (e.g., Rizvi et al., 2014; Rush et al., 2006). This issue notwithstanding, even those patients who do experience positive effects of such antidepressant treatments experience delays as long as several weeks or months until the onset of therapeutic effects occurs (e.g., Frazer & Benmansour, 2002; Machado-Viera et al., 2008; Price et al., 2009). This delayed onset of action for traditional antidepressant drugs is critical for two main reasons. First,

improvements in depressive symptoms that occur within the first several weeks of antidepressant treatments may be crucial for clinical stability in the long-term (e.g., Lingam & Scott, 2002). Second, suicidal ideation in depressed patients may be enhanced during the first month of treatment with antidepressants (e.g., Goforth & Holsinger, 2007; Jick et al., 2004; Rucci et al., 2009). Other notable limitations of traditional antidepressant drugs include that they require chronic dosing regimens that may last for years (e.g., see Everleigh et al., 2019; Maund et al., 2019), and that they may cause unpleasant side effects including but not limited to sexual dysfunction, gastrointestinal distress, headaches, dizziness, and in more extreme cases weight gain, sedation, seizures, and the induction of mania or suicidal ideation (see Stahl, 2020). Such negative side effects have been shown to cause up to a 50% discontinuation of use rate (e.g., Hawley et al., 1998). Therefore, it seems quite clear that more research should be aimed at testing the efficacy of other drugs that have faster therapeutic onsets, require less frequent dosing regimens, and have fewer intolerable side effects.

To this end, one such drug that has shown promise in these areas is the *N*-methyl-D-aspartate receptor antagonist ketamine. In numerous clinical trials, single infusions of ketamine have been shown to robustly decrease depressive symptoms within hours to days (e.g., Berman et al., 2000; McGirr et al., 2015; Zarate et al., 2006). Importantly, this antidepressant effect has also been shown to be somewhat persistent, with studies showing benefits of a single infusion lasting for two weeks and as long as two months (e.g., Correll & Futter, 2006; Ibrahim et al., 2012; Murrough et al., 2013). In contrast to findings with antidepressants such as SSRIs, single infusions of ketamine also appear to significantly reduce suicidal ideation in depressed patients after just 24 hours (e.g.,

Grunebaum et al., 2018; Price et al., 2009, 2014). Ketamine has also been shown to have very limited and relatively harmless side effects such as drowsiness, dizziness, poor coordination, blurred vision, and feelings of strangeness that generally only occur for about four hours post-treatment before subsiding (e.g., Andrade, 2017). Further, a meta-analysis of nine ketamine trials found that there was no significant difference in treatment discontinuation rates across ketamine and control groups, but found that dissociative and psychedelic-like effects, although short-lasting and not clinically significant, were more common with ketamine (Kishimoto et al., 2016). However, there is some limited evidence to support that such effects may actually be related to ketamine's overall effectiveness as an antidepressant (Luckenbaugh et al., 2014). In sum, therefore, it appears that ketamine is a viable and, in many ways, a superior alternative drug to traditional antidepressants such as SSRIs. However, clinical trials are still ongoing, and more research is needed to understand the full spectrum of effects that ketamine has on depressed patients as well as on cognition, learning, and behavior more generally.

In this vein, animal models of behavior can be extremely useful to fill existing gaps in knowledge about disease states and treatment options (e.g., Fernando & Robbins, 2011; Nestler & Hyman, 2010), behavioral and/or cognitive deficits present in mental health conditions (e.g., see Luc et al., 2021; Nogo et al., 2022), and underlying neurological mechanisms (e.g., Pulcu et al., 2022). A problem with randomized controlled clinical trials (RCTs) in the context of MDD in particular is that many may suffer from experimental confounds. For example, MDD is a multifaceted condition that is diagnosed based on a patient presenting at least five of the nine diagnostic symptoms which vary across individuals (e.g., Kennedy, 2008). In addition, it has been found that

MDD is highly comorbid with other neuropsychiatric conditions, with as many as 35% of patients having one or more comorbidities including but not limited to anxiety, personality, and panic disorders, post-traumatic stress, phobias, and drug-dependence (e.g., Thaipisuttikul et al., 2014). Further, such RCTs may be impacted by past experience or interactions with other medications and/or ongoing regimens of therapy (e.g., Goforth & Holsinger, 2007), and subject-expectancy effects (e.g., Berman et al., 2000; Zarate et al., 2006). Thus, in contrast to clinical research with human subjects, animal models allow for the isolation of very specific variables which significantly enhance experimental control and the precision of results. Specifically, laboratory animal models using a paradigm known as reinforcement learning have been shown to be particularly useful in the context of MDD, as they provide a means by which decision-making and reward and punishment learning can be examined and quantified (e.g., Dayan & Niv, 2008). In general, experimental reinforcement learning approaches may employ asymmetrical reward probabilities across two or more response options or stimuli that the subject must discriminate across trials in order to maximize their reward earnings. These differing probabilistic contingencies allow for the effective examination and quantification of decision-making and reward-learning (e.g., Kangas, 2022). Such models are especially relevant when it comes to depression, as it has been found that MDD patients commonly display deficits in decision-making (e.g., Meiran et al., 2011; Mukherjee et al., 2020), show hypersensitivity to negative outcomes (e.g., Eshel & Roiser, 2010; Murphy et al., 2003; Taylor Tavares et al., 2008), and in general fail to effectively integrate past reward values when planning future actions (e.g., Treadway & Zald, 2011). Importantly, research employing reinforcement learning models has

produced similar findings across human and non-human subjects in the context of depression (e.g., Der-Avakian et al., 2017; Kangas et al., 2022; Pizzagalli et al., 2005, 2008). Further, such models have been shown to be sensitive to pharmacological manipulations in both humans (e.g., Kandroodi et al., 2021; Kanen et al., 2022) and rats (e.g., Bari et al., 2010; Kangas et al., 2020; Rychlik et al., 2017) and are amenable to computational modeling strategies (e.g., Huys et al., 2013; Kanen et al., 2019; Wilkinson et al., 2020), which make them important tools that can help in the search for more effective drug treatments for depression.

Therefore, the overarching goal of this dissertation was to examine the potential therapeutic effects of ketamine, a promising and potentially powerful alternative drug treatment for MDD, in the context of a model of reinforcement learning, the probabilistic reversal learning task (PRL) using rat subjects. The experiment described in Chapter 2 was aimed at examining the potential dose-dependent effects of ketamine on cognitive flexibility using the PRL. Cognitive flexibility is the ability for organisms to make adaptive decisions in response to changing environmental circumstances (e.g., Armbruster et al., 2012; Dajani & Uddin, 2015; Hamilton & Brigman, 2015). Such an ability has relevance to depression as those with MDD have been shown to have deficits in cognitive flexibility (e.g., Meiran et al., 2011; Mukherjee et al., 2020). Further, lysergic acid diethylamide (i.e., LSD), a drug with subjective and/or behavioral effects similar to those of ketamine (e.g., Ly et al., 2018, 2021), has recently been shown to enhance this ability in healthy human subjects engaging in the PRL (Kanen et al., 2022). The proposed experiment described in Chapter 3 is aimed at examining the effects of ketamine on cognitive flexibility and feedback sensitivity when electric foot shock was

combined with timeout periods or not. Almost all non-human versions of the PRL only employ brief timeout periods as the negative outcome or punishing stimulus (e.g., see Bari et al., 2010; Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020; but see Rygula & Popik, 2016), however, it has long been known that timeout periods do not always function as punishers (e.g., Solnick et al., 1977; see Hackenberg & DeFulio, 2007; Fontes & Shahan, 2021; Leitenberg, 1965 for review). As such, the goal of this experiment was to measure the effects of what would likely be a more potent punisher on behavior in the PRL, and to further examine the effects of ketamine in this context.

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CHAPTER II

EVALUATING THE DOSE-DEPENDENT EFFECTS OF KETAMINE ON COGNITIVE FLEXIBILITY IN A PROBABILISTIC REVERSAL LEARNING TASK WITH RATS

Abstract

Patients diagnosed with major depressive disorder (MDD) often experience abnormalities in behavioral adaptation following environmental changes (i.e., cognitive flexibility), and tend to undervalue positive outcomes and simultaneously overvalue negative ones. The probabilistic reversal learning task (PRL) has been used across species to study these deficits, and drugs that may have therapeutic value. Selective serotonin-reuptake inhibitors (SSRIs) are limited in their effectiveness as an MDD treatment and have shown inconsistent effects in non-human versions of the PRL. As such, ketamine, a drug that appears to overcome some limitations of SSRI-treatment of MDD, has begun to be examined in the context of the PRL. However, in this context, the only two studies to date have shown conflicting results, while using experimental designs and/or analytical strategies that may not be well-suited to fully capture ketamine's effects. Thus, the present experiment sought to add clarity with respect to these mixed findings using 40 rat subjects. After 5 sessions of PRL training, groups of rats received a single ketamine injection of either 0, 10, 20 or 30 mg/kg body weight. 1-hour post-injection, rats engaged in the PRL, and subsequently, sessions continued daily for 2 weeks. Results showed that ketamine had acute effects at the 1-hr post-injection timepoint, which were likely due to impairment, but no other effects of ketamine were

detected, regardless of dose. Overall, the present results suggest that the range of ketamine doses examined do not affect reward-processing in healthy rats as measured by the PRL.

Introduction

Cognitive flexibility refers to the ability of organisms to make adaptive decisions in response to changing environmental circumstances (e.g., Armbruster et al., 2012; Dajani & Uddin, 2015; Hamilton & Brigman, 2015). This ability is crucial for survival, as it allows for the functional processing of both positive and aversive environmental stimuli. As such, anomalies in the ability to adapt behavior flexibly are characteristic of a variety of mental health conditions and/or neurological disorders (e.g., Geurts et al., 2009; Meiran et al., 2011; Peterson et al., 2009). In major depressive disorder (MDD), for example, patients tend to undervalue positive outcomes and show a disproportionate sensitivity to negative ones (e.g., Eshel & Roiser, 2010; Murphy et al., 2003; Taylor Tavares et al., 2008). This impairment presents a challenge to normal functioning, and as a result, a considerable amount of research has been devoted to understanding the underlying processes involved as well as potential treatment approaches.

One of the most frequently used methods to study cognitive flexibility in laboratory settings is a paradigm known as the probabilistic reversal learning task (PRL). The PRL usually involves the choice between two concurrent stimuli or response options that differ in their probability of reward delivery (e.g., 80% vs. 20%). After either a pre-determined number of trials or an exclusive preference for the richer option is shown, the probabilities are reversed such that the previously rich option becomes the previously lean option and vice-versa. Importantly, this unsignaled reversal in reward contingencies allows for the effective examination of flexible behavior as well as how individuals might differentially process positive and negative outcomes (e.g., Kangas, 2022; Kehagia et al., 2010). Enhanced flexibility in the PRL is measured by increases in the rate at which a

subject shows a switch in preference for the previously lean (now the richer) option (e.g., Kanen et al., 2022), or in the case of non-human studies, the number of reversals that occur in a single session (e.g., Bari et al., 2010). The effects of positive and negative feedback on choice are usually measured in terms of win-stay and lose-shift proportions, where the former refers to the repetition of a choice for the rich option following the receipt of a reward, and the latter refers to changing options following a non-rewarded trial. The PRL has been widely used in clinical research with human subjects (e.g., den Ouden et al., 2013; Lawrence et al., 1999; Mukherjee et al., 2020), and with different strains of rats (e.g., Bari et al., 2010; Rychlik et al., 2017; Wilkinson et al., 2020), and monkeys (Bari et al., 2022). Although procedurally the task may vary slightly depending on the species being examined, studies with non-humans have revealed similar findings to those with humans concerning the processing of positive and negative outcomes (e.g., Costa et al., 2015; Kanen et al., 2019), making the PRL a valid procedure for translational examinations of cognitive flexibility.

Many of the mental health disorders for which the inability to behave flexibly is a hallmark may stem from low levels of the neurotransmitter serotonin (e.g., Bari et al., 2010; Kandroodi et al., 2021; Kanen, 2021, 2022). For example, Kanen et al. (2021) and Bari et al. (2010) found that serotonin depletion caused impairments in reversal learning in humans and rats, respectively. Conversely, Bari et al. (2010) also found that both acute and sub-chronic doses of the selective serotonin reuptake inhibitor (SSRI) citalopram enhanced reversal learning. However, SSRIs have been found to have inconsistent effects when used in the PRL. For example, Bari et al. (2010) found that a single dose of citalopram (1 mg/kg) caused fewer reversals per session and increased lose-shift behavior

but found the opposite when the dose was increased to 10 mg/kg or when the doses were repeated daily at 5 or 10 mg/kg. In contrast, Wilkinson et al. (2020) found that an acute citalopram dose of 10 mg/kg increased rats' proportion of win-stay behavior, but a range of doses (1, 3, and 10 mg/kg) did not affect lose-shift behavior nor the number of reversals per session. Similarly, Drozd et al. (2019) administered doses of the SSRI escitalopram (0.5, 1, and 3 mg/kg) to rats and found no effect on reversals per session nor on win-stay/lose-shift behavior.

In addition to these experimental inconsistencies in the PRL, SSRI-based treatments for depression are known for being limited in their effectiveness. For example, most if not all patients experience long latencies to the onset of therapeutic effects, with the peak of effectiveness often not being reached for weeks or months (e.g., Frazer & Benmansour, 2002; Price et al., 2009). This problem is exacerbated by the fact that suicidality in depressed patients often is enhanced in the first month of treatment with SSRIs (e.g., Goforth & Holsinger, 2007; Jick et al., 2004; Rucci et al., 2009). Additionally, despite the fact that most patients undergo long-term treatment with SSRIs, a considerable proportion experience no satisfactory clinical improvement (e.g., Machado-Viera et al., 2008; Rush et al., 2006). Further, even if SSRIs are effective in reducing depressive symptoms, many patients experience unpleasant side effects (e.g., Alli & Hendawy, 2018) that may lead to discontinuation of use. Consequently, recent research has been devoted to exploring the efficacy of other drugs that may have faster onsets, greater tolerability, and require less frequent dosing regimens than SSRIs. One such drug that has shown promise in these areas is the *N*-methyl-D-aspartate receptor antagonist ketamine. In clinical trials, a single infusion of ketamine has been shown to

significantly reduce depressive symptoms within days, with therapeutic effects lasting for weeks and sometimes up to or longer than one month (e.g., Berman et al., 2000; Correll & Futter, 2006; McGirr et al., 2015; Zarate et al., 2006). Thus, it seems that ketamine can overcome at least two primary concerns of SSRI therapy for depression.

Like SSRIs, ketamine's capacity to modulate cognitive flexibility has begun to be explored with non-humans in the PRL, albeit with mixed results. For example, Wilkinson et al. (2020) administered a range of acute ketamine doses (1, 3, and 10 mg/kg) to rats and found that a 10 mg/kg dose decreased reversals per session, the proportion of win-stay behavior, and motivation, as measured by the latency to initiate a trial. The lower doses had no significant effects on any behavioral measures. In contrast, Rychlik et al. (2017) administered acute doses of ketamine (5, 10, and 20 mg/kg) to rats and found that a 20 mg/kg dose reduced lose-shift behavior following misleading negative feedback (i.e., a non-rewarded trial after a response to the rich option) but the effect of lose-shift behavior following true negative feedback (i.e., a non-rewarded trial after a response to the lean option) failed to reach statistical significance compared to placebo. No other significant behavioral effects were found for the high dose, nor either of the two lower doses. Interestingly, unlike Wilkinson et al., no negative motivational effects were found with the 10 nor with the higher 20 mg/kg dose. Therefore, despite the growing body of clinical and experimental evidence showing the efficacy of ketamine as an antidepressant, the results of ketamine's effectiveness in the PRL seem inconclusive, and at best inconsistent.

In addition, there are limitations of each of these studies that, if remedied, might both clarify some inconsistencies in results as well as add additional important data to the

literature. One such limitation of both studies is that the post-ketamine PRL testing was conducted only once one hour following administration in the Wilkinson et al. study and at a maximum of 48-hrs following administration in the Rychlik et al. study. Although each found that ketamine had some detectable effect (whether positive or negative) at the 1-hr post-injection PRL test, a study by Gastambide et al. (2013) showed that a single 10 mg/kg dose of ketamine given to rats caused response suppression, impairments in reaction time, and motivational deficits that lasted for at least 1-hr. Other non-PRL studies with non-humans have shown that longer post-drug intervals may be necessary to observe the peak of ketamine's therapeutic effects. For example, McGowan et al. (2017) trained mice in a contextual fear conditioning procedure and found that a single 30 mg/kg injection of ketamine did not affect freezing behavior after 1-hr but did significantly reduce freezing 1-week later. Further, other studies have shown therapeutic effects of ketamine that persisted for weeks (e.g., Autry et al., 2011; Yilmaz et al., 2002). Thus, these results seem to coincide with the finding that in humans a single dose of ketamine might have effects that occur quickly and persist for weeks or longer, however, it may be that 1-hr post-injection is too short of an interval to see such effects. Therefore, it appears that extending the interval of post-drug PRL testing would prove valuable for understanding the time course of ketamine's effects on cognitive flexibility, as this has yet to be examined in animal models of the PRL.

Another noteworthy limitation of the study conducted by Rychlik et al. (2017) is that only conventional behavioral measures (e.g., win-stay/lose-shift proportions, reversals completed, proportion of "correct" lever presses) were included as part of their analysis. Several recent papers using the PRL have demonstrated that computational

modeling strategies are able to detect more nuanced and subtle differences in cognitive flexibility more robustly than conventional behavioral measurements alone[1] (Kanen et al., 2019, 2022). For example, in a human version of the PRL, Kanen et al. (2022) gave participants a dose of the psychedelic drug lysergic acid diethylamide (i.e., LSD) to examine its effects on cognitive flexibility and found no effect on win-stay/lose-shift behavior, but did find that LSD caused an increase in reward and punishment learning rates, and a decrease in "stimulus stickiness" (i.e., a measure of perseverative behavior) as evidenced by statistically significant changes in their model parameters across conditions (i.e., drug vs. placebo). Therefore, a similar computational analysis in the context of the PRL with ketamine could be informative and provide additional clarity on how the drug might be affecting cognitive flexibility as a function of different doses and time points post-injection.

As such, the present experiment had three main goals. First, the effects of a range of ketamine doses were examined in the PRL with rats to provide clarity with respect to the mixed findings in the current literature. Second, the time course of the examination of ketamine's effects on cognitive flexibility in the PRL were extended beyond previous examinations (Rychlik et al., 2017; Wilkinson et al., 2020) to assess the correspondence between the human clinical data and findings with non-humans outside of the PRL. Finally, a computational modeling approach similar to Kanen et al. (2022) was applied to

[1] Wilkinson et al. (2020) did include one such model but found only that a 10 mg/kg ketamine dose reduced the value of their learning-rate parameter, corresponding with behavioral data showing motivational deficits.

the behavioral data obtained in the PRL to provide additional depth of analysis with respect to the effects of ketamine on cognitive flexibility.

Methods

Subjects

40 experimentally naïve male Long-Evans rats (Charles River, Portage, MI) were used in the present study. Rats were 71-90 days old upon arrival and were maintained at 80% of their free-feeding weights. Rats were individually housed with free access to water in a temperature-controlled colony room with a 12:12 hour light/dark cycle (lights on at 7:00 AM). Care of animals and all procedures below were approved by Utah State University's Institutional Animal Care and Use Committee.

Apparatus

Ten modular Med Associates (St. Albans, VT) operant chambers were in the experiment. These chambers measured 30 cm X 24 cm X 21 cm and were housed in sound and light attenuating cubicles. Each chamber had aluminum panels on the front and back walls, as well as Plexiglas walls on each side. In the center of the front panels, there was a food pellet receptacle which was illuminated when delivering 45-mg food pellets (Bio Serv, Flemington, NJ). On the back wall of each chamber opposite the food receptacle were five small, evenly spaced nose-poke (NP) ports equipped with LED lights and photo beams that could detect head entries. Only the furthest left and furthest right NP ports were ever active. Thus, the other three NP ports were covered with metal stoppers throughout the duration of the experiment. Each chamber was also equipped with a house light centered on the ceiling of the chamber above the nose-poke ports. The

timing of experimental events and data collection were controlled by Med-PC IV (Med Associates) software run on a computer in an adjacent control room.

Drug

Ketamine hydrochloride (VetOne, Boise, ID) was diluted from its original concentration of 100 mg/ml using 0.9% sterile saline solution to concentrations of 10, 20, and 30 mg/ml. Rats received a single injection of either 0 (i.e., saline), 10, 20, or 30 mg/kg body weight. Injections were given via the intraperitoneal route at a volume of 1 ml/kg body weight. Injections took place the day following the final session of PRL training (described below).

Procedure

Magazine Training. Rats were first trained to collect and consume food pellets from the food magazine for one 30-min session. During this session, there were no stimuli present, and food pellets were delivered response-independently according to a variable-time 60-s schedule (Fleshler & Hoffman, 1962). Each food delivery was accompanied by an audible click and illumination of the magazine for 3-s.

Response Training. Sessions during this phase began with the illumination of the house light and one NP port (i.e., 50% chance of left or right). A response made into the illuminated location resulted in the delivery of a single food pellet. Once the pellet was collected, the next trial was initiated immediately. During trials, a response made into any of the non-illuminated locations resulted in a 5-s timeout period during which all chamber stimuli were turned off. The number of illuminations per location was arranged such that both locations were rewarded an equal number of times per training session. Thus, rats earned a total of 90 food pellets (i.e., 45 from each location) during each

training session. Rats remained in this phase until their performance reached a criterion of at least 80% accuracy (i.e., correct choices/total choices). Thus, this phase lasted a total of 7 sessions.

Phase 1 – PRL Training. Sessions during this phase began as in the prior phase, with the exception that now both NP locations were illuminated simultaneously. The first NP port to which a response was made resulted in the delivery of a single food pellet. Subsequently, this location initially became the high probability reward (i.e., rich) location and triggered the delivery of food pellets on 80% of trials. By default, the remaining NP location initially became the low probability reward (i.e., lean) location and triggered the delivery of food pellets on 20% of trials. These light stimuli were presented for 30-s and, if no choice was made within this time, the trial was considered an omission which initiated a 5-s timeout period during which all stimuli were turned off. On trials in which a food reward was not presented, there was a 2.5-s timeout period before the initiation of the next trial. If a rat chose the rich location on eight consecutive trials (whether each trial was rewarded or not), the reward contingencies were reversed such that the rich and lean locations switched (i.e., the previously 80% rewarded location became 20% rewarded, and vice-versa). Each session during this phase consisted of 200 trials or a maximum of 40 minutes. This phase lasted a total of 5 sessions.

Phase 2 – Drug Administration and PRL test. Following the conclusion of PRL training, rats were placed into 1 of 4 groups based on ketamine dose: 0 (i.e., vehicle), 10, 20, or 30 mg/kg. One rat died shortly after the injections took place, leaving the final group sizes as: 10, 9, 10, and 10 for the 0, 10, 20, and 30 mg/kg groups, respectively. This rat was removed from all data analyses. Ketamine was administered to each subject based on

their grouping, and then subjects were placed back into their home cages. One hour following injections, experimental sessions began. Subsequently, sessions continued daily for 2 weeks post-injection. All parameters of the PRL task remained the same as described in the previous phase.

Data Analysis and Measures

Feedback sensitivity was assessed as in previous studies (e.g., Bari et al., 2010; Rychlik et al., 2017; Wilkinson et al., 2020) via win-stay/lose-shift probabilities. Win-stay probability was computed as the number of times a rewarded trial was followed by the repetition of the same response option in the subsequent trial, divided by the total number of rewarded trials. Similarly, lose-shift probability was calculated as the number of times a non-rewarded trial was followed by a trial in which the other response option was chosen, divided by the total number non-rewarded trials. Following previous studies (e.g., Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020) these measures were further broken down to either true or misleading feedback, where, for example, true positive feedback was the delivery of a reward following a trial in which the rich option was chosen, and misleading positive feedback was the delivery of a reward following a trial in which the lean option was chosen. Conversely, true negative feedback was the initiation of a timeout period following a trial in which the lean option was chosen, and misleading negative feedback was the initiation of a timeout period following a trial in which the rich option was chosen. Other behavioral measurements of interest included: the total number of reversals that occurred per session (i.e., a measure of cognitive flexibility), rich response percentage (i.e., number of responses made to the “correct” response / total responses), the latencies to collect pellets and respond to the trial stimuli,

and omission percentage (number of omissions / total number of trials) per session. As in previous studies with similar designs (e.g., Rychlik et al., 2017; Wilkinson et al., 2020), each of these measures was assessed via a (Dose x Session) repeated-measures (RM) analysis of variance test (ANOVA) where drug dose (i.e., 0, 10, 20, and 30 mg/kg) was treated as a continuous between-subjects variable and session (i.e., 1 hour, 24 hours, 1 week, and 2 weeks post-injection) was the within-subjects factor. All significance testing was conducted at $\alpha = 0.05$. Mauchly's test of sphericity was applied and when necessary, degrees of freedom and *p*-values were corrected using the Greenhouse-Geisser method. The alpha-correction method described by Benjamini and Hochberg (1995) was applied to all simple-effects and pairwise post-hoc analyses.

Computational Modeling

To add additional depth to the present analysis, a reinforcement learning model was fit to the behavioral data using maximum likelihood estimation (MLE). This model was based on the best fitting model from Kanen et al. (2022), and is an adaptation based on the model of associative learning developed by Rescorla and Wagner (1972). All modeling was conducted using R version 4.3.0 (R Core Team, 2023). This model incorporated four separate parameters. The parameters α^{rew} and α^{pun} are the reward and punishment learning rate parameters, respectively. These parameters dictate the speed at which the value of a specific nose-poke location was updated across trials, with higher rates being indicative of faster updating. A reward earned on a given trial t, lead to an increase in the value V_i of the location i that was chosen, via the equation $V_{i,t+1} = V_{i,t} + \alpha^{rew}(R_t - V_{i,t})$, where R_t represents the outcome on trial t, which was defined as $R_t = 1$ for trials in which a reward was earned. Conversely, $R_t = 0$ for trials in which no

reward was earned, which lead to a decrease in location value according to $V_{i,t+1} = V_{i,t} + \alpha^{pun}(R_t - V_{i,t})$. Overall location value was then incorporated into a final quantity according to $Q_t^{reinf} = \tau^{reinf} V_t$, where the additional parameter τ^{reinf} is called the reinforcement sensitivity parameter, which governs the impact of reinforcement history on current behavior. A greater value for this parameter would be indicative of a greater weight being placed upon past reinforcements when a choice was made. The fourth and final model parameter, τ^{stim}, governs the tendency to repeat a response, regardless of the outcome that followed (i.e., perseverative behavior), and is called the "stimulus stickiness" parameter. Higher values of this parameter would be indicative of less exploratory behavior (i.e., switching to a new stimulus). This effect was modeled according to $Q_t^{stim} = \tau^{stim} S_{t-1}$, where S_{t-1} is 1 for the location that was chosen on the previous trial, and 0 for the other location. With these four parameters, the final quantity controlling choice was determined by $Q_t = Q_t^{reinf} + Q_t^{stim}$. The quantities Q, associated with the two locations, for a given trial, was then input into a SoftMax choice function that computed the probability of each choice according to $P(action_a) = softmax^a(Q_1 \dots Q_n) = \frac{e^{Qa}}{\sum_{k=1}^{n} e^{Q_k}}$ for $n = 2$ location choices. This model allowed for the assessment of whether ketamine was differentially affecting the impact of positive versus negative feedback (i.e., reward vs. timeout), how it modulated the impact of prior choices on current ones (i.e., reward history), and whether it influenced perseverative behavior.

The explanation of the MLE process for these model parameters is as follows. In a Markov Decision Process (MDP), a particular environment is modeled as a set of states, where actions (i.e., behaviors) can be performed by an agent to control the system's state

(e.g., see Sutton & Barto, 1998; van Otterlo & Wiering, 2012). The MDP/agent interaction gives rise to a trajectory:

$$S_0, A_0 \Rightarrow R_{1,} S_1, A_1 \Rightarrow R_{2,} S_2, A_2 \Rightarrow \cdots \Rightarrow R_{t,} S_t, A_t$$

where S is the state of the environment, A is the choice or action of the agent (i.e., written above as $action_a$), and R is the reward state from the environment given action, all at time t. The general probability model for the system (i.e., the agent-environment interaction) is a dynamic Markov process which puts a joint probability distribution on the trajectory and breaks it up via a set of conditional independence assumptions:

$p\big(S_0, A_0, R_{1,} S_1, A_1, R_{2,} S_2, A_2, \ldots, R_{t,} S_t, A_t\big) =$

$$= \quad p(S_0, A_0) * p\big(R_{1,} S_1 | S_0, A_0\big) * p(A_1 | S_1, R_1) * p\big(R_{2,} S_2 | S_1, A_1\big) * p(A_2 | S_2, R_2) * \cdots * p\big(R_{t,} S_t | S_{t-1}, A_{t-1}\big) * p(A_t | S_{t-1}, R_{t-1})$$

where probability terms $p\big(R_{t,} S_t | S_{t-1}, A_{t-1}\big)$ represent the parameterization of the environment (i.e., the reward structure) via the set of rules established by the experimenter to govern the reward structure given previous selections and actions taken by the agent. The probability terms $p(A_t | S_{t-1}, R_{t-1})$ represent the behavior of the agent based on previous selections and rewards, and as currently written, is an implicit function of the reward learning parameters. Based on the above definition of probability of action (i.e., via SoftMax choice function and Q quantities) the probability of action is best written:

$$p\big(A_t = a | S_{t-1}, R_{t-1}, \alpha^{rew}, \alpha^{pun}, \tau^{reinf}, \tau^{stim}\big) = \frac{\exp\{Q_{a,t}\}}{\sum_{k=1}^{n} \exp\{Q_{k,t}\}} \quad (1),$$

where $Q_{k,t}$ is a function of actions, rewards, and the reinforcement learning parameters as defined above. Given a trial run and corresponding agent action data $\mathcal{D} = \{A_1, A_2, \ldots, A_T\}$, where T is the total number of trials in a run by the agent, the likelihood function is defined as:

$$L\left(\alpha^{rew}, \alpha^{pun}, \tau^{reinf}, \tau^{stim} | \mathcal{D}\right) = \prod_{t=1}^{T} p\left(A_t | S_{t-1}, R_{t-1}, \alpha^{rew}, \alpha^{pun}, \tau^{reinf}, \tau^{stim}\right) \quad (2),$$

and maximum likelihood estimates of the reinforcement learning parameters are then defined as:

$$\left\{\widehat{\alpha^{rew}}, \widehat{\alpha^{pun}}, \widehat{\tau^{reinf}}, \widehat{\tau^{stim}}\right\} \coloneqq \arg\min\left(-\log L\left(\alpha^{rew}, \alpha^{pun}, \tau^{reinf}, \tau^{stim} | \mathcal{D}\right)\right) \quad (3),$$

and Equation 3 is solved by numerical optimization using R software.

Maximum likelihood estimates for each of the four reinforcement learning parameters were obtained for individual rats across all sessions of the PRL task. These estimates were then analyzed in the same manner as described above for the behavioral measurements. Parameter estimates were considered outliers if they fell outside the interquartile range as defined by: $x < Q_1 - 1.5 * IQR \; or \; x > \; Q_3 + 1.5 * IQR$, where Q_1 and Q_3 were the 25th , and 75th percentiles, respectively, and $IQR = \; Q_3 - \; Q_1$. If a given parameter estimate was deemed an outlier it was removed from subsequent analyses. Degrees of freedom in statistical analyses conducted on parameter estimates detailed below reflect the removal of outliers.

Results

Behavioral Measurements

After establishing a baseline of performance in the PRL across five sessions, rats were divided into four groups based on number of reversals completed, rich response percentage, and win-stay/lose-shift probabilities. Separate one-way ANOVAs confirmed that there were no group differences in any of these measures, reversals, $F(3, 191) = 0.04$, $p = 0.98$, $\eta_p^2 = 0$, rich response percentage, $F(3, 191) = 0.11$, $p = 0.96$, $\eta_p^2 = .002$, win-stay, $F(3, 191) = 1.13$, $p = 0.34$, $\eta_p^2 = .02$, and lose-shift, $F(3, 191) = 0.85$, $p = 0.47$, $\eta_p^2 = .01$.

Figure 2-1 shows the 5-session average of these measures for each group during Phase 1, as well as the effect of ketamine dose on each of these measures at four timepoints post-injection: 1 hr, 24 hr, 1 week, and 2 weeks. Each group is represented by a different bar color. Figure 2-1A shows that, overall, reversals increased for all groups as a function of experience with the task across sessions. The RM ANOVA revealed a non-significant Dose x Session interaction, $F(9, 105) = 0.58$, $p = .89$, $\eta_p^2 = .05$ and no main effect of Dose, $F(3, 35) = 0.30$, $p = .82$, $\eta_p^2 = .03$ but a significant main effect of Session, $F(3, 105) = 22.02$, $p < .001$, $\eta_p^2 = .39$. Therefore, despite that the number of reversals increased over time, ketamine dose had no significant impact. Figure 2-1B similarly shows that rich response percentage appeared to increase slightly over time for all groups. The RM ANOVA revealed a significant Dose x Session interaction, $F(9, 105) = 2.37$, $p = .02$, $\eta_p^2 = .17$, a significant main effect of Session, $F(3, 105) = 19.17$, $p < .001$, $\eta_p^2 = .35$, but no significant main effect of Dose, $F(3, 35) = 1.47$, $p = .23$, $\eta_p^2 = .11$. Simple effects tests identified the 1-hr post-injection timepoint as the source of the interaction, with the two higher doses of ketamine (i.e., 20 and 30 mg/kg), but not the 10 mg/kg dose, producing a significant decrease in rich response percentage relative to the vehicle

control group. However, aside from this first session post-ketamine, no other group differences occurred, and rich response percentage increased as rats were given more exposure to the task. Figure 2-1C and Figure 2-1D show win-stay and lose-shift percentages, respectively. As with reversals and rich response percentage, win-stay percentage appeared to increase across sessions regardless of group. This was confirmed by the RM ANOVA which revealed a significant main effect of Session, $F(2.5, 86.6) = 38.87$, $p < .001$, $\eta_p^2 = .53$, but no significant main effect of Dose, $F(3, 35) = 0.62$, $p = .61$, $\eta_p^2 = .05$, nor a significant Dose x Session interaction, $F(7.4, 86.6) = 0.48$, $p = .88$, $\eta_p^2 = .04$. Thus, like reversals per session, win-stay percentage did increase with exposure to the task, but the rate of increase did not differ significantly as a function of ketamine dose. There was an apparent effect of ketamine dose on lose-shift percentage at the 1-hr post-injection timepoint, after which there appeared to be no group differences. This was confirmed by the RM ANOVA which revealed a significant Dose x Session interaction, $F(9, 105) = 4.06$, $p < .001$, $\eta_p^2 = .26$, and significant main effects of Dose, $F(3, 35) = 3.13$, $p = .04$, $\eta_p^2 = .21$, and Session, $F(3, 105) = 27.86$, $p < .001$, $\eta_p^2 = .44$. Simple effects tests again identified the 1-hr timepoint as the source of the interaction, with the 20 and 30 mg/kg groups showing statistically significant decreases in lose-shift percentages compared to the 10 mg/kg or vehicle control groups. Following the 1-hr post-injection timepoint however, there were no longer any group differences or effects of time on lose-shift percentage.

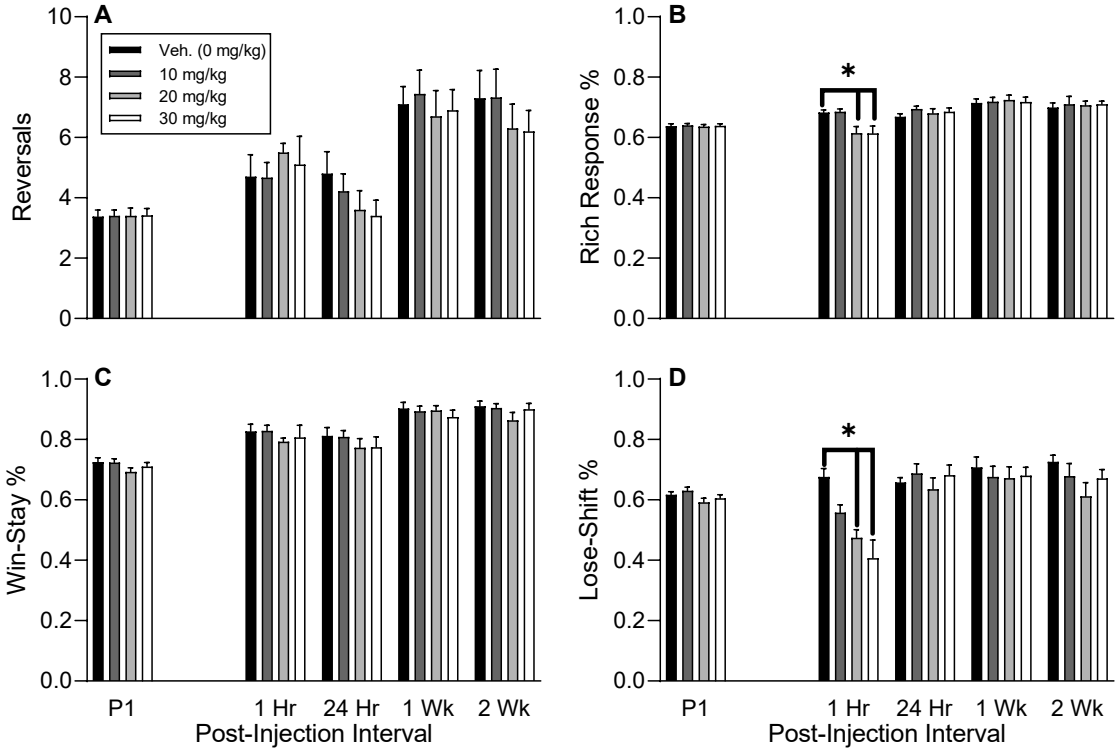

Figure 2-1. Reversals (A), rich response % (B), Win-Stay % (C), and Lose-Shift % (D) across the five sessions of Phase 1 – PRL Training (average), and at selected intervals (i.e., 1 hour, 24 hours, 1 week, and 2 weeks) following administration of ketamine. Each group is represented by a different bar color as denoted in the figure legend. Error bars represent standard error of the mean.

In the same style as in Figure 2-1, Figure 2-2A and 2-2B show win-stay percentages after true positive and misleading positive feedback, respectively. As with total win-stay percentage, there was no significant main effect of ketamine dose nor a Dose x Session interaction for either of these measures: true positive Dose, $F(3, 35) = 0.64$, $p = .59$, $\eta_p^2 = .05$, interaction, $F(7.4, 85.9) = 0.42$, $p = .89$, $\eta_p^2 = .03$, and misleading positive Dose, $F(3, 35) = 1.44$, $p = .25$, $\eta_p^2 = .11$, interaction, $F(7.3, 85.2) = 1.44$, $p = .20$, $\eta_p^2 = .11$. However, like total win-stay percentage, each of these measures showed a

significant main effect of Session, true positive, $F(2.5, 85.9) = 36.52$, $p < .001$, $\eta_p^2 = .52$, misleading positive, $F(2.4, 85.2) = 8.67$, $p < .001$, $\eta_p^2 = .20$. Thus, regardless of how it was analyzed, ketamine had no significant effects on win-stay behavior in the task, and it appeared that this measure only increased as a function of exposure to the task conditions. Figure 2-2C and 2-2D show lose-shift percentages after true negative and misleading negative feedback, respectively. Similar to total lose-shift percentage, there was a significant Dose x Session interaction for lose-shift percentage following true negative feedback, $F(9, 105) = 3.86$, $p < .001$, $\eta_p^2 = .25$, and a significant main effect of Session, $F(3, 105) = 19.75$, $p < .001$, $\eta_p^2 = .36$, but no significant main effect of Dose, $F(3, 35) = 1.92$, $p = .14$, $\eta_p^2 = .14$. Simple effects tests again identified the 1-hr post-injection timepoint as the source of the interaction, with the 20 and 30 mg/kg groups showing significantly lower levels of lose-shift behavior following true negative feedback compared to the 10 mg/kg or vehicle control groups. Beyond this session, no other significant effects of ketamine dose were found. Likewise, there was a significant Dose x Session interaction for lose-shift percentage following misleading negative feedback, $F(9, 105) = 2.23$, $p < .001$, $\eta_p^2 = .16$, as well as significant main effects of Dose, $F(3, 35) = 3.65$, $p = .02$, $\eta_p^2 = .24$, and Session, $F(3, 105) = 21.14$, $p < .001$, $\eta_p^2 = .38$. This time, simple effects tests identified that the 20 and 30 mg/kg groups were statistically different than only the vehicle control group at the 1-hr post-injection timepoint. No other significant effects of ketamine dose were found. Thus, as with the total lose-shift measure, the only significant effects of ketamine dose occurred at the 1-hr post-injection timepoint, and otherwise no group differences occurred.

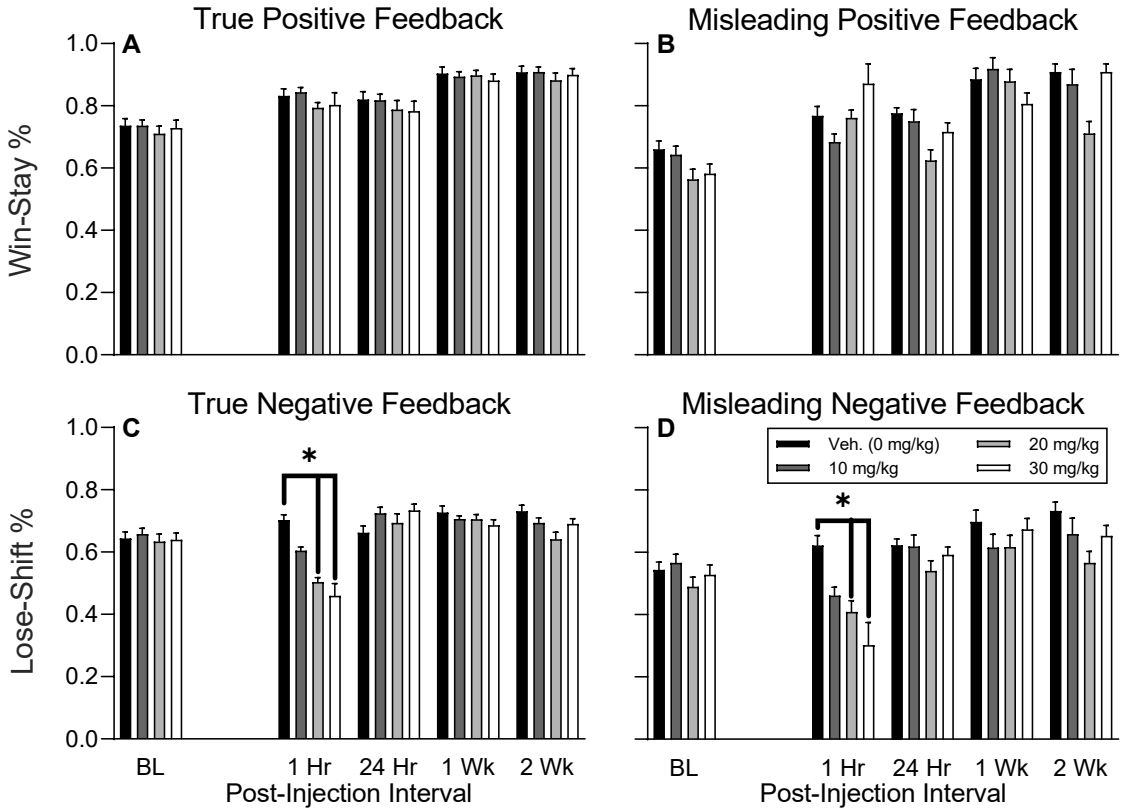

Figure 2-2. Win-stay % after true positive feedback (A) and misleading positive feedback (B). Lose-shift % after true negative feedback (C) and misleading negative feedback (D). All other details are as in Figure 2-1.

Figure 2-3A shows the effects of ketamine dose on the average latency (s) per session to initiate a trial via a nose-poke into one of the two response options. As shown in the figure, it appeared that ketamine increased trial response latency as a function of dose in the 1-hr post-injection session. The RM ANOVA revealed a significant Dose x Session interaction, $F(3.9, 45.6) = 7.37$, $p < .001$, $\eta_p^2 = .39$, and significant main effects of Dose, $F(3, 35) = 3.26$, $p = .03$, $\eta_p^2 = .22$, and Session, $F(1.3, 45.6) = 55.39$, $p < .001$, $\eta_p^2 = .61$. Simple effects tests confirmed that the 1-hr timepoint was the source of the

interaction, with each dose of ketamine showing statistically significant increases in trial response latency compared to the vehicle control group. Beyond this session, no other significant effects of ketamine were detected. Similarly, Figure 2-3B shows the effects of ketamine dose on the average latency (s) per session to collect reward pellets after they were earned. Although there was a visibly large increase in food collection latency for the 30 mg/kg group during the 1-hr post-injection session, following correcting for violations of sphericity, the Dose x Session interaction failed to reach statistical significance, $F(3, 35.4) = 1.78$, $p = .08$, $\eta_p^2 = .13$. No significant main effects of Dose, $F(3, 35) = 1.41$, $p = .26$, $\eta_p^2 = .11$, nor Session, $F(1, 35.4) = 1.93$, $p = .13$, $\eta_p^2 = .05$, were found either. Finally, Figure 2-3C shows the effects of ketamine dose on omission percentage. As with trial and food collection latencies, there was a large, visually apparent, increase in omission percentage at the 1-hr post-injection timepoint for all ketamine doses. The RM ANOVA revealed a significant Dose x Session interaction, $F(3.3, 37.1) = 2.68$, $p = .05$, $\eta_p^2 = .19$, and Session, $F(1.1, 37.1) = 4.76$, $p = .03$, $\eta_p^2 = .12$, but no significant main effect of Dose, $F(3, 35) = 2.67$, $p = .06$, $\eta_p^2 = .19$. Simple effects tests indicated that the 1-hr post-injection timepoint was the source of the interaction, with the 30 mg/kg group showing a statistically significant increase in omission percentage compared to all other groups, but no other significant effects of ketamine dose were detected. Thus, as with the other measures detailed above, the only significant effects of ketamine dose appeared at the 1-hr post-injection timepoint, with the majority of the significant effects being found with the 20 and 30 mg/kg doses.

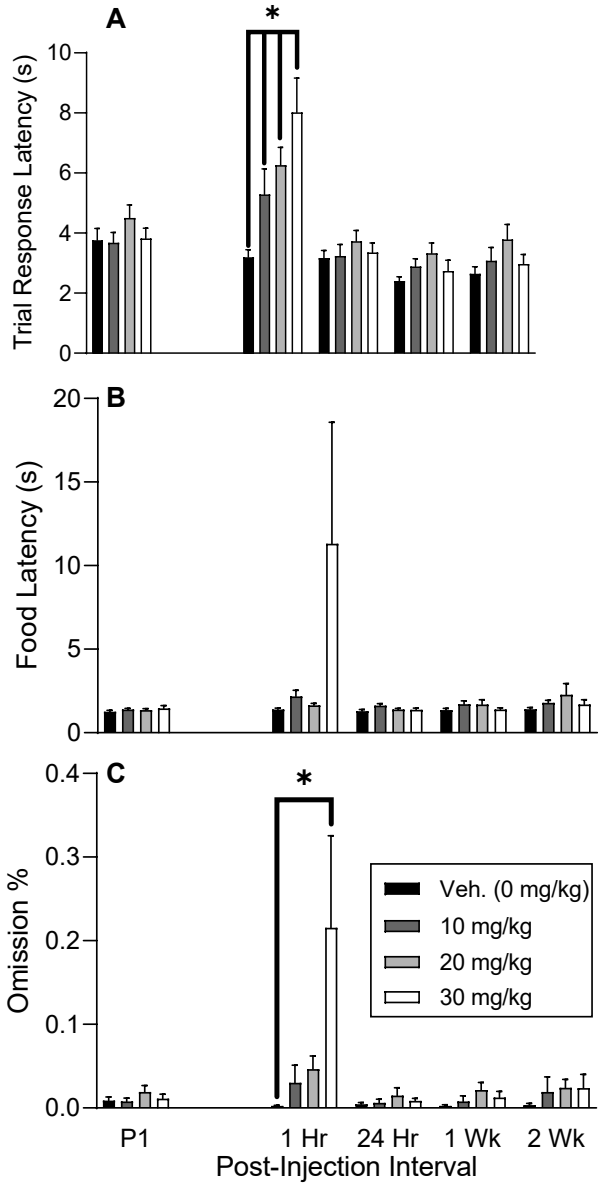

Figure 2-3. Average latency (s) to initiate a trial (A), average latency (s) to collect food pellets (B), and omission % (C). All other details are as in Figure 2-1.

Computational Modeling Results

As with the behavioral measurements detailed above, there were no statistically significant differences detected across groups during Phase 1 for any of the four

reinforcement learning model parameter estimates when one-way ANOVAs were utilized: α^{rew}, $F(3, 191) = 0.25$, $p = .49$, $\eta_p^2 = .003$, α^{pun}, $F(3, 191) = 1.19$, $p = .31$, $\eta_p^2 = .02$, τ^{reinf}, $F(3, 152) = 1.09$, $p = .35$, $\eta_p^2 = .02$, and τ^{stim}, $F(3, 182) = 0.73$, $p = .54$, $\eta_p^2 = .01$.

In the same style as the previous figures, Figure 2-4 shows the grouped maximum likelihood estimates for each of the four reinforcement learning model parameters across Phase 1, and the same post-injection intervals as above. Figure 2-4A and 2-4B show the reward and punishment learning rate parameters (i.e., α^{rew} and α^{pun}), respectively. In both figures, it appears visually that there were acute effects of ketamine dose at the 1-hr post-injection timepoint, but otherwise no systematic effects of ketamine dose appear, and the values of the learning rate parameters appear to increase with time. The RM ANOVA conducted for the reward learning rate parameter revealed a significant main effect of Session, $F(3, 75) = 7.47$, $p < .001$, $\eta_p^2 = .23$, but no significant main effect of Dose, $F(3, 25) = 2.03$, $p = .14$, $\eta_p^2 = .20$, nor a significant Dose x Session interaction, $F(9, 75) = 1.30$, $p = .25$, $\eta_p^2 = .13$. In contrast, the RM ANOVA conducted for the punishment learning rate parameter revealed a significant Dose x Session interaction, $F(7, 58.3) = 2.24$, $p = .04$, $\eta_p^2 = .21$, and a significant main effect of Session, $F(2.3, 58.3) = 21.34$, $p < .001$, $\eta_p^2 = .46$, but no significant main effect of Dose, $F(3, 25) = 0.50$, $p = .68$, $\eta_p^2 = .06$. Simple effects tests revealed the 1-hr timepoint as the source of the interaction, with the 30 mg/kg dose significantly decreasing punishment learning rate compared to the vehicle control. However, no other comparisons revealed any significant differences. As such, these findings closely mirrored those mentioned above with respect to win-stay and lose-shift behavior. Figure 2-4C and 2-4D show the remaining two

reinforcement learning model parameters, reinforcement sensitivity (i.e., , τ^{reinf}) and stimulus stickiness (i.e., τ^{stim}), respectively. Visually, it appeared that the reinforcement sensitivity parameter increased with time; however, the RM ANOVA conducted for this parameter revealed interaction and main effects that were close but did not reach the significance threshold, Dose x Session interaction, $F(9, 54) = 1.83$, $p = .08$, $\eta_p^2 = .23$, Dose, $F(3, 18) = 2.44$, $p = .10$, $\eta_p^2 = .29$, and Session, $F(3, 54) = 2.66$, $p = .06$, $\eta_p^2 = .13$. Finally, there was an obvious effect of ketamine dose on the stimulus stickiness parameter at the 1-hr post-injection timepoint, but beyond this, no other systematic effects are visually apparent. The RM ANOVA revealed a significant main effect of Dose, $F(3, 16) = 3.98$, $p = .03$, $\eta_p^2 = .43$, but no significant effects of Session, $F(2.1, 33.1) = 1.14$, $p = .34$, $\eta_p^2 = .07$, nor a significant Dose x Session interaction, $F(6.2, 33.1) = 1.16$, $p = .34$, $\eta_p^2 = .18$. Pairwise comparisons revealed no significant group differences following corrections for familywise error rate.

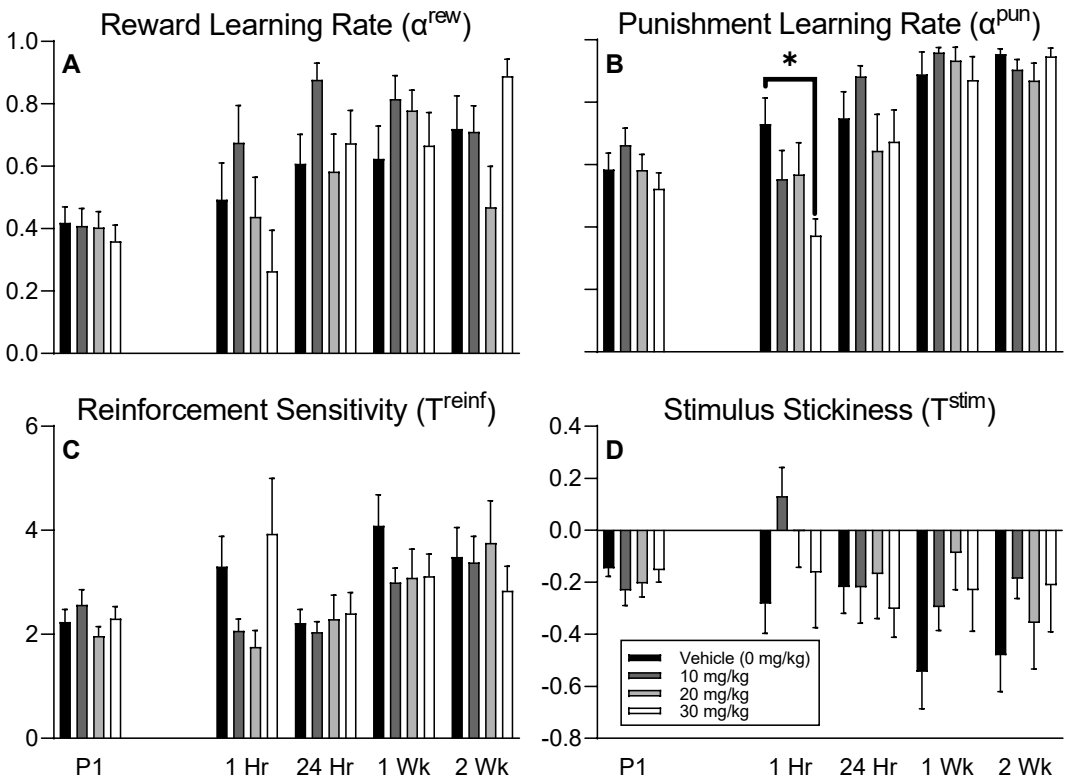

Figure 2-4. Maximum likelihood estimates for each of the reinforcement learning model parameters: reward learning rate (A), punishment learning rate (B), reinforcement sensitivity (C), and stimulus stickiness (D). All other details are as in Figure 2-1.

Discussion

The purpose of the present experiment was threefold. First, given that past examinations of ketamine within the context of rodent versions of the PRL have produced conflicting results (e.g., Rychlik et al., 2017; Wilkinson et al., 2020), the primary objective of this experiment was to provide further evidence that ketamine either would or would not enhance performance in this context to bring clarity to the mixed literature. For example, Wilkinson et al. (2020) found that a 10 mg/kg dose of ketamine

decreased reversals, win-stay percentage, and motivation (as measured by an increased trial response latency) 1-hr post administration. In contrast, Rychlik et al. (2017) found no significant effects of a 10 mg/kg dose but found that 20 mg/kg of ketamine significantly reduced lose-shift behavior following misleading negative feedback 1 -, 24-, and 48-hrs post administration. Curiously, however, Rychlik et al. reported no motivational impairments, even with a significantly higher dose and at the same timepoint as in the Wilkinson et al. study. As such, in the present experiment, following the establishment of a behavioral baseline in the PRL, rats were assigned to one of four groups that received a single injection of either 0 (i.e., saline) 10, 20, or 30 mg/kg ketamine.

Similar to the findings of Wilkinson et al. (2020), the present study found that ketamine caused acute impairments that appeared to be motivational in nature. For example, like the experiment conducted by Wilkinson et al. (2020), trial response latency was significantly increased by ketamine 1-hr after administration. In the present study, this effect was found to increase as a function of ketamine dose. Other evidence for motivational impairment caused by ketamine included that, 1-hr post-injection, rats given the highest dose of ketamine (i.e., 30 mg/kg) showed a significantly increased omission percentage. In other words, for these rats in this first session post-ketamine, the number of trials in which no choice was made increased relative to rats who received vehicle injections. In addition, although the effect did not reach statistical significance, the latency to collect food pellets after they were earned for rats in the 30 mg/kg group 1-hr post-injection was increased both relative to the other groups as well as prior to ketamine administration. Given that all rats included in this experiment were held under food-

restriction conditions to maintain motivation for food reinforcement, this effect seems clearly related to a motivational impairment. Finally, although unlike the findings of Wilkinson et al. (2020), the present experiment found no impact of ketamine at the 1-hr post-injection timepoint on either reversals nor win-stay percentage. There was, however, a significant decrease in rich response percentage (i.e., the proportion of choices for the high probability option) for the 20 and 30 mg/kg groups compared to the control group. Rychlik et al. (2017) asserted that this measure indexes the ability to discriminate between the rich and lean options. Thus, in addition to causing motivational impairments at the 1-hour post-injection timepoint, it also appears that the two higher doses of ketamine may have caused the response options to become less discriminable.

All of these findings with respect to motivation and discrimination are in contrast to the findings of Rychlik et al. (2017), however, who reported no impact of a 20 mg/kg ketamine dose on trial response latency, omissions, or rich response percentage 1-hour post-injection. Like the present findings, Rychlik et al. also found no significant effect of ketamine on reversals, nor on win-stay percentage, regardless of whether it was decomposed further into true positive or misleading positive feedback. The only statistically significant finding reported from the Rychlik et al. (2017) experiment was that 20 mg/kg of ketamine reduced lose-shift following misleading negative feedback compared to the vehicle control group. This effect was detected at 1-, 24-, and 48-hrs post ketamine administration, but no effects were found at the smaller 5 and 10 mg/kg doses. In the present study, we found a similar effect on lose-shift behavior, in that the 20 and 30 mg/kg doses significantly reduced all lose-shift behavior metrics including total lose-shift (Figure 2-1D), lose-shift following true negative feedback (Figure 2-2C), lose-shift

following misleading negative feedback (Figure 2-2D). However, in contrast to the findings of Rychlik et al., this decrease in lose-shift behavior only occurred in the present study for the 1-hr post-injection timepoint, and by 24-hrs post-injection, no differences as function of ketamine dose were detected. Similarly, although the effect did not reach statistical significance, Wilkinson et al. (2020) reported a trend toward decreased lose-shift behavior 1-hr following the administration of 10 mg/kg ketamine. Therefore, as mentioned by Wilkinson et al., it seems difficult to separate what appear to be motivational and/or discriminative impairments from the decrease on lose-shift behavior caused by ketamine. This seems especially valid given that in the present study, once the impairments were no longer present 24-hrs later, so too were the effects on lose-shift behavior.

Thus, although it appears as though the effect of ketamine on lose-shift behavior was due more to impairment than a therapeutic effect in the present study, additional interpretative difficulty arises due to procedural differences between the present study and the studies conducted by Wilkinson et al. (2020) and Rychlik et al. (2017). For example, Wilkinson et al. did not include any further examinations of the effects of ketamine beyond 1-hr. Therefore, it is impossible to know whether the rats in their study would have shown a similar recovery from the acute impairments as did the rats in the present study. Had this been a feature in their experimental design, perhaps it would have been easier to draw more firm conclusions about the differences between the findings from Rychlik et al. and the present study. Relatedly, because Rychlik et al. only included PRL testing a maximum of 48-hrs post ketamine administration, it is equally impossible to know if the effect of ketamine on lose-shift would have persisted beyond this point, or,

if it would have reached statistical significance at all with additional timepoints included in the analysis. Further, Rychlik et al. reported that data from 1 rat in their 20 mg/kg group was discarded due to a "…high level of omissions in the 1 h post-injection test…" (Rychlik et al., 2017, p. 616). This is potentially problematic given that they also reported no impairing effects of ketamine but show no graphic depictions of these data as in the present study. Also, unlike the present study, Rychlik et al. found a trend toward significance in terms of the effect of 20 mg/kg ketamine on lose-shift following true negative feedback, however, they did not report statistics nor show any figures of what lose-shift behavior looked like as a whole, irrespective of the feedback being true or misleading. Given that the present study found effects of ketamine on every lose-shift metric at the 1-hr timepoint, this could be another indication of problems with the data analysis conducted by Rychlik et al. (2017). Other, more minor procedural differences between the Rychlik et al. study and the present study may have further contributed to the differences in findings. Such procedural differences include that Rychlik et al. used group-housed rats (i.e., four per cage), levers as the response operanda, and 0.1 mL of 20% sucrose solution as reinforcers, compared to the individually housed rats, nose-poke operanda, and 45mg food pellets used in the present study. Although these differences are slight, it is unclear whether they would have been enough to contribute to the discrepancy in results. In any case, what seems clear is that future experiments should continue to explore this area of research, as rigorous testing of ketamine in this context may be necessary to establish definitive conclusions regarding its utility and the consistency of its effects. Further, it seems that better consistency in experimental design, where possible, would be beneficial.

A secondary but related purpose of this experiment was to expand the time window following ketamine administration beyond that of the two previously conducted studies (Rychlik et al., 2017; Wilkinson et al., 2020). As mentioned above, previous research has shown that ketamine can have therapeutic effects in both humans and non-humans that may last much longer than 48 hours (e.g., Autry et al., 2011; Berman et al., 2000; Yilmaz et al., 2002; Zarate et al., 2006). But further, ketamine has been shown to cause acute impairments in rats that remain present 1-hr later (e.g., Gastambide et al., 2013; Wilkinson et al., 2020). Yet, ketamine can also have therapeutic benefits that may not appear initially, but eventually do with the passage of time (e.g., McGowan et al., 2017). As such, the present experiment began post-ketamine testing on the PRL 1-hr following ketamine injections like the experiments conducted within the context of the PRL (Rychlik et al., 2017; Wilkinson et al., 2020). But the present experiment also extended examinations of behavior in the PRL daily for 2 weeks post-ketamine to create an experiment with more continuity relative to the broader literature outside of the context of the PRL. Despite that additional time was included in the present analysis, as already noted, there were no statistically significant effects of ketamine found beyond the 1-hr post-injection timepoint. Rats did improve their performance overall as experience with the task increased. This was evidenced by significant increases in reversals and win-stay percentage in all groups over time. Thus, even though the extended time-window did not seem to matter for examining the effects of ketamine, the fact that rats were able to increase their performance over time suggests that the PRL may be a useful tool for more longitudinal examinations of cognitive flexibility and feedback sensitivity.

The third and final purpose of the present experiment was to apply a computational modeling strategy to PRL data, as has been done before with data from human (Kanen et al., 2019, 2022) and non-human (Wilkinson et al., 2020) versions of the PRL. The present model was adapted from that described by Kanen et al. (2019, 2022) and altered slightly to better fit the structure of the PRL data that is obtained from non-human versions of the task. The only previous experiment to computationally model both the effects of ketamine and behavior in the PRL was the above-mentioned study conducted by Wilkinson et al. (2020), which found only that 10 mg/kg ketamine significantly reduced their learning rate parameter 1-hr post-injection. Although in the Wilkinson et al. study, there was only a single learning rate compared to separate learning rates for reward and punishment learning in the present study, it was similarly found that the highest dose of ketamine (i.e., 30 mg/kg) significantly reduced the punishment learning rate 1-hr following administration. This finding appears to mirror the finding that ketamine significantly reduced lose-shift behavior 1-hr following ketamine administration. Yet, like with the behavioral findings previously mentioned, the present modeling approach was not able to detect any other significant effects of ketamine past the 1-hr post-injection timepoint. However, seemingly in correspondence with the behavioral data showing increases in win-stay and reversals over time, it was found that the reward learning rate parameter also significantly increased with time. Thus, despite that no underlying effects of ketamine were detected, the fact that the present modeling approach was able to capture such an effect seems indicative of an overall appropriate fit to the data.

Overall, the primary finding of the present study was that, regardless of dose, ketamine did not enhance cognitive flexibility, and, while it did cause an initial significant decrease in negative feedback sensitivity (i.e., lose-shift behavior), it appeared that this was more of a function of impairment than a therapeutic effect. However, as mentioned above, given some of the novel aspects of this study, as well as some of the differences in procedural details from past related studies (e.g., Rychlik et al., 2017; Wilkinson et al., 2020), it seems that more research should be devoted to understanding the effects of ketamine within the context of the PRL. One notable limitation of the present study that may have contributed to the present was that the subjects were healthy rats. Thus, there may have been a ceiling effect with cognitive flexibility and feedback sensitivity in the task (Rychlik et al., 2017). Using the PRL, future research may usefully examine the capacity of ketamine to reverse depression-like behaviors and/or deficits in reward-processing induced in some way by the experimenter as such experiments would likely aid in our understanding of how ketamine may be beneficial for MDD.

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CHAPTER III

THE EFFECTS OF KETAMINE ON PROBABILISTIC REVERSAL LEARNING WITH A COMBINATION OF NEGATIVE OUTCOMES

Abstract

The probabilistic reversal learning task (PRL) is a paradigm that is used in the context of major depressive disorder (MDD) to assess impairments in cognitive flexibility and feedback sensitivity. The PRL is widely utilized for several reasons, but namely because behavior in the task is sensitive to pharmacological interventions. This is crucial, as traditional antidepressants are limited in their effectiveness, and new drugs are needed to combat MDD. As such, the drug ketamine, which may be able to overcome some of the limitations associated with traditional antidepressants, has begun to be explored in the context of the PRL, but the results that speak to its therapeutic efficacy thus far are mixed. One reason for this could be that almost all non-human versions of the PRL use timeout periods as the punishing stimulus, but, it has long been known that timeout periods do not always function as punishers. Including a truly punishing stimulus is critical to ensure the task has sufficient translational value. Thus, the present experiment sought to examine the effects of ketamine in the PRL when electric footshocks were either combined with timeout periods or not. 40 rats first established a baseline of PRL performance where timeouts were used as punishers, and in Phase 2, 20 rats probabilistically received footshock punishment at the onset of non-rewarded trials, while the other 20 continued under the same conditions as before. Finally, a single dose of ketamine was administered to half of the rats (n = 10) in each condition (i.e., shock and

no shock), giving four different groups total. Ketamine had no effect on behavior in the task beyond causing acute impairments, but the addition of shock punishment appeared to increase behavioral persistence (i.e., less lose-shift behavior) and cognitive flexibility (i.e., more reversals) in the task. Overall, this suggests further that ketamine may not impact reward processing in healthy rats, but also that changes in punishment conditions within the PRL will impact behavior.

Introduction

The probabilistic reversal learning task (i.e., PRL) is among the primary paradigms employed in both clinical and laboratory settings to detect cognitive deficits across a range of mental health conditions (e.g., Cools et al., 2001; Lawrence et al., 1999; Roiser et al., 2009; Waltz & Gold, 2007). Notably, this task is frequently used in the context of major depressive disorder (MDD) as a method to assess cognitive flexibility, the ability of organisms to make adaptive decisions in response to changing environmental circumstances (e.g., Armbruster et al., 2012; Dajani & Uddin, 2015; Hamilton & Brigman, 2015). The PRL generally involves the choice between two stimuli with asymmetric reward contingencies (e.g., 80% vs. 20%) in a trial-based procedure. Once either an exclusive preference for the rich option is shown or a pre-determined number of trials are completed, the reward probabilities undergo an unsignaled reversal such that the previously rich option becomes the previously lean option and vice-versa. This reversal in reward contingencies allows for the examination of cognitive flexibility as well as individual differences in the processing of positive and negative outcomes (e.g., Kangas, 2022; Kehagia et al., 2010). Greater levels of cognitive flexibility are measured by increases in the rate at which a subject shows a switch in preference for the previously lean (now the richer) option (e.g., Kanen et al., 2022), or in the case of non-human studies, the number of reversals that occur in a single session (e.g., Bari et al., 2010). The effects of positive and negative feedback on choice are usually measured in terms of win-stay and lose-shift proportions, where the former refers to the repetition of a choice for the rich option following the receipt of a reward, and the latter refers to changing options following a non-rewarded trial. Each of these measures are relevant to

MDD as it has been found that, relative to healthy controls, those with MDD display deficits in cognitive flexibility (e.g., Meiran et al., 2011; Mukherjee et al., 2020), show hypersensitivity to negative outcomes (e.g., Eshel & Roiser, 2010; Murphy et al., 2003; Taylor Tavares et al., 2008), and in general, suffer from abnormalities in reward-processing (e.g., Whitton et al., 2015).

There are at least three other important factors that contribute to the widespread use of the PRL to study such reward-processing deficits. First, findings from experiments using the PRL have shown significant continuity across human and non-human subjects (e.g., see Bari et al., 2010; Costa et al., 2015; Kanen et al., 2019, 2022), making it a useful tool for studying the cognitive processes underlying MDD and related disorders in laboratory animals. Second, computational models of reinforcement learning have been successfully applied to PRL data (e.g., Kanen et al., 2019, 2022; Mukherjee et al., 2020; Wilkinson et al., 2020). Such modeling strategies have proven to be informative, as they allow researchers to make inferences regarding the underlying mechanisms of mental illnesses that may contribute to observable dysfunctional behavior (e.g., Huys et al., 2016; Stephan & Mathys, 2014). Finally, behavior in the PRL has been shown to be sensitive to pharmacological interventions in both humans (e.g., Kandroodi et al., 2021; Kanen et al., 2022) and rats (e.g., Bari et al., 2010; Rychlik et al., 2017), making it a useful procedure by which to test the effects of novel pharmacological treatments.

Such treatments are sorely needed, as first-line drugs like selective serotonin reuptake inhibitors (SSRIs) are limited in their effectiveness (e.g., see Machado-Viera et al., 2008; Rizvi et al., 2014; Rush et al., 2006), and have little to no impact on the reward-processing deficits commonly present in MDD (e.g., Craske et al., 2016; Kieslich et al.,

2022). As such, much recent research has been directed toward examinations of alternative drugs that may be beneficial in the treatment of MDD. In this vein, the *N*-methyl-D-aspartate receptor antagonist ketamine has shown promise as a drug that can overcome some of the limitations of SSRI-based treatments for MDD including their long latency to therapeutic onset (e.g., Machado-Viera et al., 2008; Price et al., 2009), their chronic dosing requirements (e.g., Everleigh et al., 2019; Maund et al., 2019), and their sometimes unpleasant and/or severe side effects (e.g., Hawley et al., 1998; Stahl, 2020). In clinical trials, single infusions of ketamine have been shown to reduce depressive symptoms within hours to days (e.g., Berman et al., 2000; Zarate et al., 2006), while producing only limited side-effects that appear to be short-lasting and relatively harmless (e.g., Andrade et al., 2017; Kishimoto et al., 2016).

Despite that ketamine has thus far shown promise as an antidepressant in human clinical trials (see Brendle et al. 2023; Jelen & Stone, 2021 for recent reviews), its therapeutic effects in non-human animal research, although mostly positive, have at times been mixed (e.g., see Bartsch et al., 2023; Polis et al., 2019). In studies where ketamine has been administered to rats engaging in the PRL, for example, this has been the case to date. Wilkinson et al. (2020) administered a range of ketamine doses (1, 3, and 10 mg/kg) to rats and found that a 10 mg/kg dose decreased reversals per session, the proportion of win-stay behavior, and motivation, as measured by the latency to initiate a trial. The lower doses had no significant effects on any behavioral measures. In contrast, Rychlik et al. (2017) administered acute doses of ketamine (5, 10, and 20 mg/kg) to rats and found that a 20 mg/kg dose reduced lose-shift behavior following misleading negative feedback (i.e., a non-rewarded trial after a response to the rich option) but the effect of lose-shift

behavior following true negative feedback (i.e., a non-rewarded trial after a response to the lean option) failed to reach statistical significance compared to placebo. No other significant behavioral effects were found for the high dose, nor either of the two lower doses. Interestingly, unlike Wilkinson et al., no negative motivational effects were found with the 10 nor with the higher 20 mg/kg dose. Similar to the findings of Wilkinson et al., the experiment described in Chapter 2 of this dissertation also found that ketamine initially caused increases in trial response latency and omissions (i.e., trials in which no response is made), and did not produce any significant effects beyond 1-hour post-injection. Thus, overall, the effects of ketamine in the context of the PRL seem to be more toward the side of null or even negative effects on cognitive flexibility and feedback sensitivity. One possibility for this could be due to the nature of the consequences arranged by the PRL.

As mentioned above, the PRL arranges asymmetric reward probabilities such that selection of the rich response option will result in a positive outcome (i.e., reward) on 80% of trials, whereas selection of the lean response option will result in a positive outcome on only 20% of trials. Conversely, selection of the rich response option will result in a negative outcome (i.e., punishment) on only 20% of trials, whereas selection of the lean response option will result in a negative outcome on 80% of trials. In human studies, such positive and negative outcomes most commonly come in the form of arbitrary stimuli like shapes for which specific meanings must be inferred over time (e.g., Cools et al., 2001; Waltz & Gold, 2007) or in the form of more meaningful stimuli such as colors like green and red (e.g., Lawrence et al., 1999; Roiser et al., 2009), smiling and frowning faces (e.g., Brolsma et al., 2022; Kanen et al., 2019, 2022), plus signs and

minus signs (e.g., Pilhatsch et al., 2020), words like “CORRECT” and “WRONG” (e.g., Murphy et al., 2003; Taylor Tavares et al., 2008), or the gain and loss of money (e.g., Mukherjee et al., 2020). In contrast, the positive outcome on rewarded trials in studies with rat subjects are food pellets (i.e., Bari et al., 2010; Wilkinson et al., 2020) or sucrose solution (e.g., Drozd et al., 2019; Rychlik et al., 2017; Rygula & Popik, 2016), whereas the negative outcome for punished trials is almost always a brief timeout period (i.e., reward omission; e.g., Bari et al., 2010; Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020).

The use of timeout periods as punishers in non-human versions of the PRL, although common, may not be optimal to study the processing of negative outcomes. This is because timeout periods may not always function as punishers, or stimuli that decrease the probability of a future behavior (e.g., Solnick et al., 1977; see Hackenberg & DeFulio, 2007; Fontes & Shahan, 2021; Leitenberg, 1965 for review). For example, Solnick et al. (1977) used timeouts in an attempt to punish problem behavior in two children with neurological conditions and found that when timeout periods followed instances of problem behavior, the problem behavior increased in frequency. In a follow-up study, the experimenters manipulated reinforcer quality during “time-in” periods and found that timeout periods became more effective punishers when reinforcement conditions were qualitatively richer. Thus, in addition to the reinforcing efficacy of the time-in period directly impacting the punishing effects of timeout, other contextual variables such as motivation and the availability of non-punished alternatives may play also play a role (Hackenberg & DeFulio, 2007). In addition, timeouts are sometimes considered to be a distinct and separate outcome from “real” aversive events like electric

shock (e.g., Orsini et al., 2015; Rygula & Popik, 2016). Though research has shown many similarities and differences between timeout and shock (e.g., see Fontes & Shahan, 2021), these differences may be of relatively greater importance when the effects of drugs are examined in tandem with punishment (see Poling, 2000). For example, the drug amphetamine has been shown to increase preference for large, uncertain rewards when timeout periods were used as punishers (St. Onge & Floresco, 2009), but decrease such preference when electric shocks were used as punishers (Simon et al., 2011). Therefore, it seems logical that incorporating electric shock punishment into the PRL could likely change the effects of ketamine within the task. Further, if there are scenarios in which timeouts are not functioning as punishers, using a stimulus like electric shock may enhance the translational value of the PRL in terms of its utility in assessing negative feedback sensitivity (Rygula & Popik, 2016).

As such, the present study had two main goals. First, the behavioral effects of ketamine were further examined in the context of the PRL, but when electric shock, a likely more effective punisher, was either combined with timeout periods or not. Given some of the past research noted above, there was good reason to believe that ketamine may have different effects as a function of the type of negative consequence experienced. Second, computational modeling was applied to the PRL data to quantify both the effects of ketamine, as well as the effects of incorporating shock into the PRL procedure. This modeling approach adapted from Kanen et al. (2019, 2022) used dual learning rates, with one rate to capture the effects of rewards and punishments on learning separately. Thus, this model would allow for the quantification of how adding shocks into the PRL

procedure might be affecting the punishment learning rate similarly or differently than timeouts alone, which has yet to be explored in this context.

Methods

Subjects

40 experimentally naïve male Long-Evans rats (Charles River, Portage, MI) were used in the present study. Rats were 71-90 days old upon arrival and were maintained at 80% of their free-feeding weights. Rats were individually housed with free access to water in a temperature-controlled colony room with a 12:12 hour light/dark cycle (lights on at 7:00 AM). Care of animals and all procedures below were approved by Utah State University's Institutional Animal Care and Use Committee.

Apparatus

Ten modular Med Associates (St. Albans, VT) operant chambers were in the experiment. These chambers measured 30 cm X 24 cm X 21 cm and were housed in sound and light attenuating cubicles. Each chamber had aluminum panels on the front and back walls, as well as Plexiglas walls on each side. In the center of the front panels, there was a food pellet receptacle which was illuminated when delivering 45-mg food pellets (Bio Serv, Flemington, NJ). On the back wall of each chamber opposite the food receptacle were five small, evenly spaced nose-poke (NP) ports equipped with LED lights and photo beams that could detect head entries. Only the furthest left and furthest right NP ports were ever active. Thus, the other three NP ports were covered with metal stoppers throughout the duration of the experiment. Each chamber was also equipped with a house light centered on the ceiling of the chamber above the nose-poke ports. Finally, each chamber was also equipped to deliver 50 ms of scrambled foot shock

through the metal grid floor. The timing of experimental events and data collection were controlled by Med-PC IV (Med Associates) software run on a computer in an adjacent control room.

Drug

Ketamine hydrochloride (VetOne, Boise, ID) was diluted from its original concentration of 100 mg/ml using 0.9% sterile saline solution to a concentration of 20 mg/ml. Rats received a single injection of either 0 (i.e., saline) or 20 mg/kg body weight. Injections were given via the intraperitoneal route at a volume of 1 ml/kg body weight. Injections took place the day following the final session of Phase 2 (described below).

Procedure

Magazine Training. Rats were first trained to collect and consume food pellets from the food magazine for one 30-min session. During this session, there were no stimuli present, and food pellets were delivered response-independently according to a variable-time 60-s schedule (Fleshler & Hoffman, 1962). Each food delivery was accompanied by an audible click and illumination of the magazine for 3-s.

Response Training. Sessions during this phase began with the illumination of the house light and one NP port (i.e., 50% chance of left or right). A response made into the illuminated location resulted in the delivery of a single food pellet. Once the pellet was collected, the next trial was initiated immediately. During trials, a response made into any of the non-illuminated locations resulted in a 5-s timeout period during which all chamber stimuli were turned off. The number of illuminations per location was arranged such that both locations were rewarded an equal number of times per training session. Thus, rats earned a total of 90 food pellets (i.e., 45 from each location) during each

training session. Rats remained in this phase until their performance reached a criterion of at least 80% accuracy (i.e., correct choices/total choices). Thus, this phase lasted a total of 7 sessions.

Phase 1 – PRL Training. Sessions during this phase began as in prior phase, with the exception that now both NP locations were illuminated simultaneously. The first NP port to which a response was made resulted in the delivery of a single food pellet. Subsequently, this location initially became the "correct" location and triggered the delivery of food pellets on 80% of trials. By default, the remaining NP location initially became the "incorrect" location and triggered the delivery of food pellets on 20% of trials. These light stimuli were presented for 30-s and, if no choice was made within this time, the trial was considered an omission which initiated a 5-s timeout period during which all stimuli were turned off. On trials in which a food reward was not presented, there was a 2.5-s timeout period before the initiation of the next trial. If a rat chose the "correct" location on eight consecutive trials (whether each trial was rewarded or not), the reward contingencies were reversed such that the "correct" and "incorrect" locations switched (i.e., the previously 80% rewarded location became 20% rewarded, and vice-versa. Each session during this phase consisted of 200 trials or a maximum of 40 minutes. This phase lasted a total of 5 sessions.

Phase 2 – PRL with or without Shock. Following the final session of Phase 1, rats were assigned to either Shock (n = 20) or No Shock (n = 20). For Group No Shock, all experimental parameters of the PRL task remained the same as described in the previous phase. For Group Shock, all PRL parameters remained the same except that now non-rewarded trials had a p = .5 probability of delivering a single foot shock at the onset of

the timeout period. On trials in which a shock occurred, it began immediately at the onset of the timeout period and then terminated after 50 ms. The remainder of the timeout period following the termination of the foot shock was the same as previously described. Shock intensity began at 0.2 mA for the first session of this phase and was subsequently titrated up or down in increments of 0.1 mA per day to maintain overall trial response rates (i.e., correct + incorrect responses / session time) between 40% to 60% of each individual rat's maximum response rate across all prior PRL sessions. This procedure was used as in a prior study to ensure that rats would not habituate over time to shock conditions or, conversely, that the shock would not suppress responding too much (Shahan et al., 2023). As described previously by Shahan et al. (2023), if a rat's trial response rate for a given session was more than 60% of its respective maximum response rate, the shock intensity was increased by 0.1 mA in the subsequent session. If a rat's trial response rate fell below 40%, the shock intensity for the subsequent session was decreased by 0.1 mA. Otherwise, shock intensity remained unchanged. Throughout these sessions and all subsequent sessions, a rat's maximum response rate was adjusted if its previous maximum was exceeded. This phase lasted a total of 5 sessions.

Phase 3 – Drug Administration and PRL test. Following the conclusion of Phase 2, rats were further divided into sub-groups based on whether they would receive ketamine or not (i.e., saline) such that there were now 4 groups total: No Shock + Ketamine, No Shock Control, Shock + Ketamine, and Shock Control. Ketamine or saline was administered to each subject based on their grouping, and then subjects were placed back into their home cages. One hour following injections, experimental sessions began. Subsequently, sessions continued daily for 2 weeks post-injection. All parameters of the

PRL task remained the same as described in the previous phase, dependent on group assignment.

Data Analysis

Feedback sensitivity was assessed via win-stay/lose-shift probabilities. Win-stay probability was computed as the number of times a rewarded trial was followed by the repetition of the same response option (regardless of if the trial was rewarded or not) in the subsequent trial, divided by the total number of rewarded trials. Similarly, lose-shift probability was calculated as the number of times a non-rewarded trial was followed by a trial in which the other response option was chosen, divided by the total number non-rewarded trials. Following previous studies (e.g., Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020) these measures were further broken down to either true or misleading feedback, where, for example, true positive feedback was the delivery of a reward following a trial in which the rich option was chosen, and misleading positive feedback was the delivery of a reward following a trial in which the lean option was chosen. Conversely, true negative feedback was the initiation of a timeout period following a trial in which the lean option was chosen, and misleading negative feedback was the initiation of a timeout period following a trial in which the rich option was chosen. Other primary behavioral measurements of interest included: the total number of reversals that occurred per session and rich response percentage (i.e., number of responses made to the "correct" response / total responses). Secondary behavioral measurements included the latencies to collect pellets and respond to the trial stimuli, omission percentage (number of omissions / total number of trials) per session, and the overall trial response rate (i.e., computed as trials completed / session time). Each of the

primary behavioral measures during Phase 1 were assessed via a one-way analysis of variance (ANOVA) where group was the between-subjects factor to ensure lack of statistical differences across the four groups. Phase 2 data were analyzed via one-way ANOVAs where shock was the sole between-subjects factor. Finally, Phase 3 data were analyzed via two-way (Shock x Ketamine) ANOVAs. Significant two-way interactions were assessed post-hoc via Tukey's honestly significant difference (HSD) test. All significance testing was conducted at $\alpha = 0.05$.

Computational Modeling

All details of the computational model in this chapter are as described for Chapter 2 of this dissertation.

Results

Behavioral Measurements

Following the completion of the five sessions of PRL training, rats were initially placed into one of two groups: Shock or No Shock. Group assignments were made based on the primary behavioral measurements detailed above: reversals, rich response percentage, and win-stay/lose-shift percentages. However, because rats were ultimately placed into one of four different groups by the end of the experiment, all data from Phase 1 were analyzed as a comparison between these four groups (i.e., No Shock + Ketamine, No Shock Control, Shock + Ketamine, and Shock Control). Separate one-way ANOVAs conducted across all Phase 1 sessions revealed no significant group differences in reversals, $F(3, 196) = 0.39$, $p = .76$, $\eta_p^2 = .01$, rich response percentage, $F(3, 196) = 0.42$, $p = .74$, $\eta_p^2 = .01$, win-stay percentage, $F(3, 196) = 1.05$, $p = .37$, $\eta_p^2 = .02$, or lose-shift percentage, $F(3, 196) = .77$, $p = .51$, $\eta_p^2 = .01$. Because no group differences were found

during Phase 1 for these measurements, all Phase 2 analyses were conducted using shock as the sole between-subjects factor in separate one-way ANOVAs. All analyses beyond Phase 2, however, were again analyzed to illustrate potential differences or lack thereof among the four groups. As such, two-way ANOVAs were conducted for Phase 3 data (i.e., at timepoints of 1-hr, 1-day, 1-week, and 2-weeks post-injection) where shock and ketamine were the two between-subjects factors.

Figure 3-1 shows the 5-session average of these measures for each group during Phase 1 and Phase 2, as well as the effect of ketamine on each of these measures at four timepoints post-injection: 1 hr, 24 hr, 1 week, and 2 weeks. Each of the four groups are represented by a different bar color. Beginning with the data from Phase 2, Figure 3-1A shows that the rats probabilistically receiving shock punishment during non-rewarded trials had an increased number of reversals relative to those receiving timeout only during non-rewarded trials. The introduction of shock was confirmed to cause a statistically significant increase in reversals by a one-way ANOVA, $F(1, 198) = 13.33$, $p < .001$, $\eta_p^2 = .06$. Figures 3-1B and 3-1C show that, in contrast, shock had no significant effect on rich response percentage, $F(1, 198) = 0.96$, $p = .33$, $\eta_p^2 = .004$, nor win-stay percentage, $F(1, 198) = 1.71$, $p = .19$, $\eta_p^2 = .01$. Finally, as with reversals, Figure 3-1D shows that shock had a different effect on lose-shift percentage than timeout, with the rats receiving shock showing lower percentages of lose-shift behavior. This decrease in lose-shift was confirmed to be statistically significant, $F(1, 198) = 44.98$, $p < .001$, $\eta_p^2 = .19$. Therefore, overall, the introduction of probabilistic shock punishment during non-rewarded trials both significantly increased reversals and decreased lose-shift percentage compared to timeout during non-rewarded trials alone.

During Phase 3, it appeared that reversals may have also been affected by ketamine. A two-way (Shock x Ketamine) ANOVA revealed a non-significant interaction between shock and ketamine, $F(1, 156) = 1.06$, $p = .31$, $\eta_p^2 = .01$. As such, the ANOVA model was re-fit without the interaction term. This model revealed a significant main effect of shock, $F(1, 157) = 7.47$, $p = .007$, $\eta_p^2 = .05$, but no significant effect of ketamine, $F(1, 157) = 1.45$, $p = .23$, $\eta_p^2 = .01$. Post-hoc pairwise comparisons confirmed that both groups receiving shock had significantly more reversals than the no-shock groups but did not differ significantly from one another. Like during Phase 2, rich response percentage was not significantly affected by shock, nor by ketamine during Phase 3. There was no significant interaction, $F(1, 156) = 1.89$, $p = .17$, $\eta_p^2 = .01$, nor significant main effects of shock, $F(1, 157) = 0.25$, $p = .62$, $\eta_p^2 = .001$, or ketamine, $F(1, 157) = 0.58$, $p = .45$, $\eta_p^2 = .003$. Also like Phase 2, it did not appear that ketamine had a large effect on win-stay percentage during Phase 3. A two-way ANOVA (Shock x Ketamine) revealed a significant interaction between shock and ketamine, $F(1, 156) = 3.96$, $p = .05$, $\eta_p^2 = .02$. Tukey's HSD was used to determine the source of the interaction, but this test revealed no significant group differences after correcting for familywise error rate. Thus, neither shock nor ketamine produced any significant effects on win-stay percentage. Finally, the effect of ketamine on lose-shift percentage was tested via the same two-way (Shock x Ketamine) ANOVA which revealed a significant interaction between shock and ketamine, $F(1, 156) = 4.51$, $p = .04$, $\eta_p^2 = .03$. Tukey's HSD revealed that the shock control group was significantly less likely to shift following a loss than both no shock groups. Similarly, the Shock + Ketamine Group was less likely to shift following a loss compared to the no shock control group, but not compared to the No

Shock + Ketamine Group. All other group comparisons were not significantly different. Thus, although the effects of shock on lose-shift percentage persisted throughout Phase 3, there were no statistically significant effects of ketamine. Overall, there were no significant nor systematic effects of ketamine on any of these measurements.

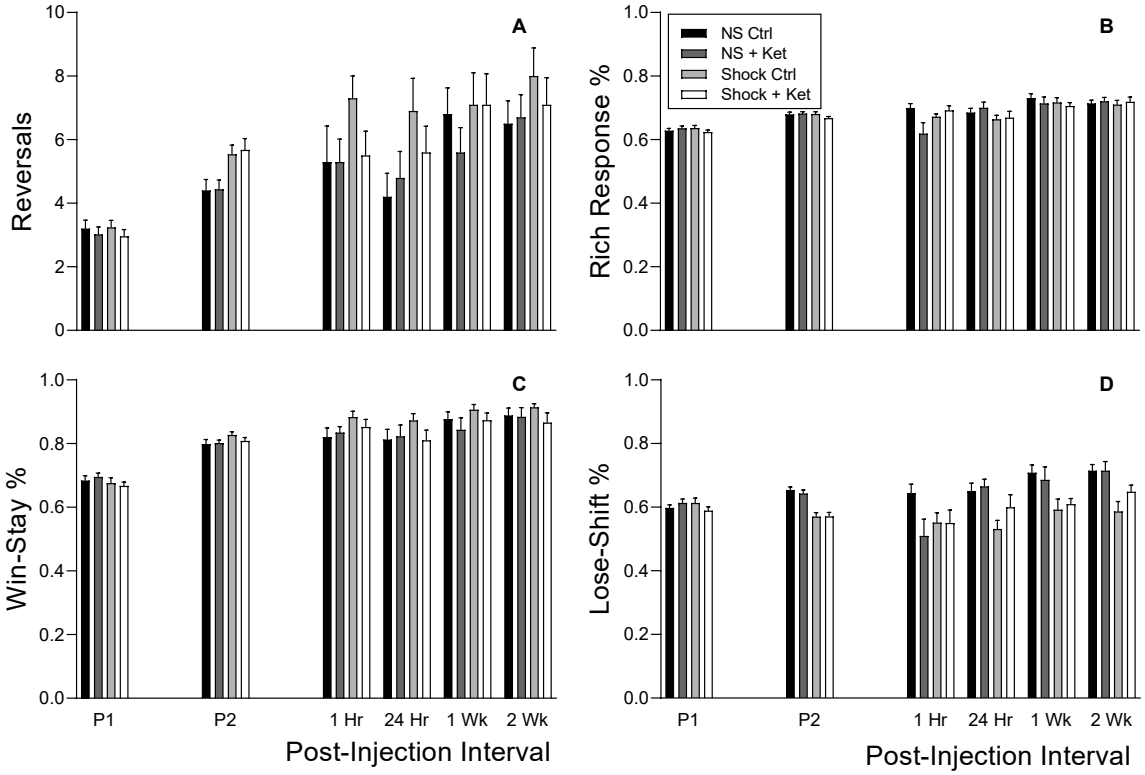

Figure 3-1. Reversals (A), rich response % (B), Win-Stay % (C), and Lose-Shift % (D) across the five sessions of Phase 1 – PRL Training (average), Phase 2 – PRL with or without Shock, and at selected intervals (i.e., 1 hour, 24 hours, 1 week, and 2 weeks) following administration of ketamine. Each group is represented by a different bar color as denoted in the figure legend. Error bars represent standard error of the mean.

In the same style as Figure 3-1, Figure 3-2 shows trial response latency, food collection latency, omission percentage, and trial response rate in Phase 1, Phase 2, and at

the same Phase 3 intervals as in the previous figure. Beginning with Phase 2 data, Figure 3-2A shows that the groups receiving shock appeared to show increased trial response latencies compared to those receiving timeout during non-rewarded trials. A one-way ANOVA confirmed that this effect was significant, $F(1, 198) = 69.02$, $p < .001$, $\eta_p^2 = .26$. In contrast, as can be seen in Figure 3-2B, there was not a visually apparent effect of shock on food collection latency during Phase 2. A one-way ANOVA confirmed that shock had no significant effect on this measure, $F(1, 198) = 2.46$, $p = .12$, $\eta_p^2 = .01$. Figures 3-2C and 3-2D show that shock did have an effect on both omission percentage and trial response rate. Both of these effects were confirmed to be statistically significant via separate one-way ANOVAs, omission percentage, $F(1, 198) = 27.6$, $p < .001$, $\eta_p^2 = .12$, and trial response rate, $F(1, 198) = 77.5$, $p < .001$, $\eta_p^2 = .28$. Thus, the introduction of probabilistic shock punishment during Phase 2 significantly increased trial response latency and omissions, and significantly decreased overall response rate.

During Phase 3 it also appeared that some of these measures were affected by ketamine administration. A two-way (Shock x Ketamine) ANOVA revealed a non-significant interaction of shock and ketamine on trial response rate, $F(1, 156) = 2.93$, $p = .09$, $\eta_p^2 = .02$. Refitting this model without the interaction term revealed significant main effects of both shock, $F(1, 157) = 62.14$, $p < .001$, $\eta_p^2 = .28$, and ketamine, $F(1, 157) = 14.82$, $p < .001$, $\eta_p^2 = .09$, on trial response latency. Tukey's HSD revealed that groups receiving shock or ketamine showed a significant increase in trial latency compared to the no shock control, but the Shock + Ketamine Group did not differ from the Shock Control Group. Like shock during Phase 2, ketamine appeared to have no systematic effects on food collection latency during Phase 3. This was confirmed by a two-way

(Shock x Ketamine) ANOVA which revealed a non-significant interaction between shock and ketamine, $F(1, 156) = 0.83$, $p = .36$, $\eta_p^2 = .01$, and non-significant main effects of shock, $F(1, 157) = 1.93$, $p = .17$, $\eta_p^2 = .01$, and ketamine, $F(1, 157) = 0.21$, $p = .64$, $\eta_p^2 = .001$. As with shock in Phase 2, it appeared that ketamine may have had an effect on omission percentage in Phase 3. However, a two-way (Shock x Ketamine) ANOVA revealed a non-significant interaction between shock and ketamine, $F(1, 156) = 0.73$, $p = .40$, $\eta_p^2 = .004$. Refitting the model without the interaction term revealed a significant main effect of shock, $F(1, 157) = 24.29$, $p < .001$, $\eta_p^2 = .13$, but the main effect of ketamine failed to reach statistical significance, $F(1, 157) = 3.41$, $p = .07$, $\eta_p^2 = .02$. Post-hoc pairwise comparisons confirmed that both groups receiving shock had significantly higher omission percentages than the no-shock groups but did not differ significantly from one another. Finally, to determine the how shock and ketamine may have affected overall response rate, a two-way (Shock x Ketamine) ANOVA was conducted which revealed a significant interaction between shock and ketamine, $F(1, 156) = 4.73$, $p = .03$, $\eta_p^2 = .03$. Tukey's HSD revealed that groups receiving either shock or ketamine showed a significant decrease in response rate compared to the no shock control group, but the two shock groups did not differ significantly from one another. Thus, overall, the only significant effects of ketamine found during Phase 3 were that it increased trial response latency and decreased response rate compared to the No Shock Control group.

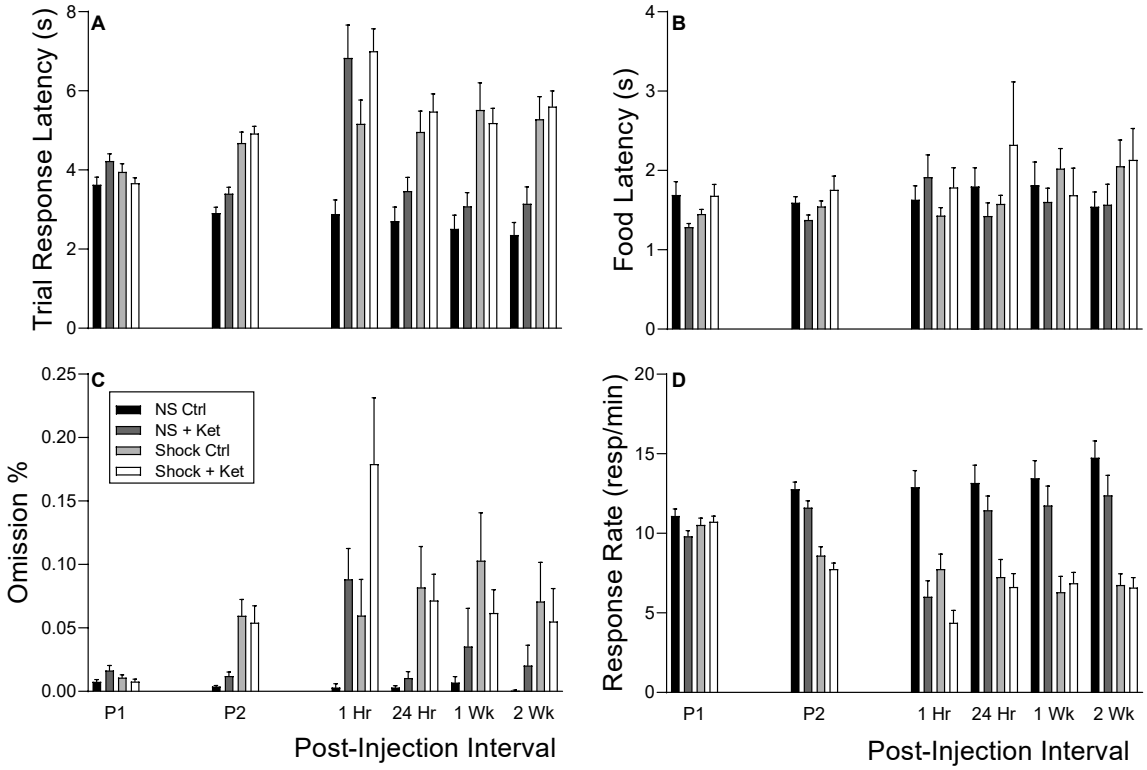

Figure 3-2. Average latency (s) to initiate a trial (A), average latency (s) to collect food pellets (B), omission % (C), and trial response rate (D). All other details are as in Figure 3-1.

To further assess the effects of shock versus no shock and ketamine on feedback sensitivity, Figure 3-3A and 3-3B show win-stay percentages following either true positive feedback or misleading positive feedback, respectively. Beginning with Phase 2, the introduction of shock punishment did not significantly impact either of these measures, true positive, $F(1, 198) = 2.66$, $p = .10$, $\eta_p^2 = .01$, misleading positive, $F(1, 198) = 0.20$, $p = .66$, $\eta_p^2 = .001$. Similarly, during Phase 3 neither shock nor ketamine had a significant effect on win-stay behavior following true positive feedback. This was confirmed by a two-way (Shock x Ketamine) ANOVA which revealed a nonsignificant interaction, $F(1, 156) = 2.78$, $p = .10$, $\eta_p^2 = .02$. Refitting this model without the interaction term revealed a nonsignificant main effect of ketamine, $F(1, 157) = 1.38$, p =

.24, η_p^2 = .01, and a main effect of shock that approached, but did not reach statistical significance, $F(1, 157) = 3.44$, $p = .07$, η_p^2 = .02. In contrast, win-stay behavior following misleading positive feedback did appear to be impacted by ketamine. This was confirmed by a two-way (Shock x Ketamine) ANOVA which revealed a significant interaction, $F(1, 156) = 4.57$, $p = .03$, η_p^2 = .03. Tukey's HSD revealed that the Shock + Ketamine Group was significantly less likely to stay following misleading positive feedback than the Shock Control Group, but no other significant group differences were found. Therefore, neither shock nor ketamine alone significantly impacted win-stay following true positive feedback, but when shock and ketamine were combined, this reduced the impact of misleading positive feedback compared to shock alone. Figures 3-3C and 3-3D show the effects of shock and ketamine on lose-shift following true negative feedback and misleading negative feedback, respectively. During Phase 2, the introduction of shock punishment significantly decreased both measures compared to timeout alone, true negative, $F(1, 198) = 36.14$, $p < .001$, η_p^2 = .15, and misleading negative, $F(1, 198) = 21.74$, $p < .001$, η_p^2 = .10. During Phase 3, it appeared that ketamine may have also had an effect on lose-shift following true negative feedback. A two-way (Shock x Ketamine) ANOVA revealed a significant interaction, $F(1, 156) = 5.58$, $p = .02$, η_p^2 = .03. Tukey's HSD revealed however, that the Shock Control and Shock + Ketamine Groups did not differ significantly, nor did the No Shock Control and No Shock + Ketamine Groups. However, the Shock Control Group was significantly less likely to shift following true negative feedback than either of the No Shock Groups. Thus, shock had significant effects on this measure, but not ketamine. There were also no obvious effects of ketamine on lose-shift following misleading negative feedback. A two-

way (Shock x Ketamine) ANOVA confirmed this by revealing a nonsignificant interaction, $F(1, 156) = 0.90$, $p = .35$, $\eta_p^2 = .01$. The main effects only model also revealed a nonsignificant main effect of ketamine, $F(1, 157) = 0.05$, $p = .83$, $\eta_p^2 = 0$, but a significant main effect of shock, $F(1, 157) = 9.63$, $p = .002$, $\eta_p^2 = .06$. Thus, although there was no significant statistical effect of ketamine, both groups receiving shock were significantly less likely to shift following misleading negative feedback than groups receiving timeout only.

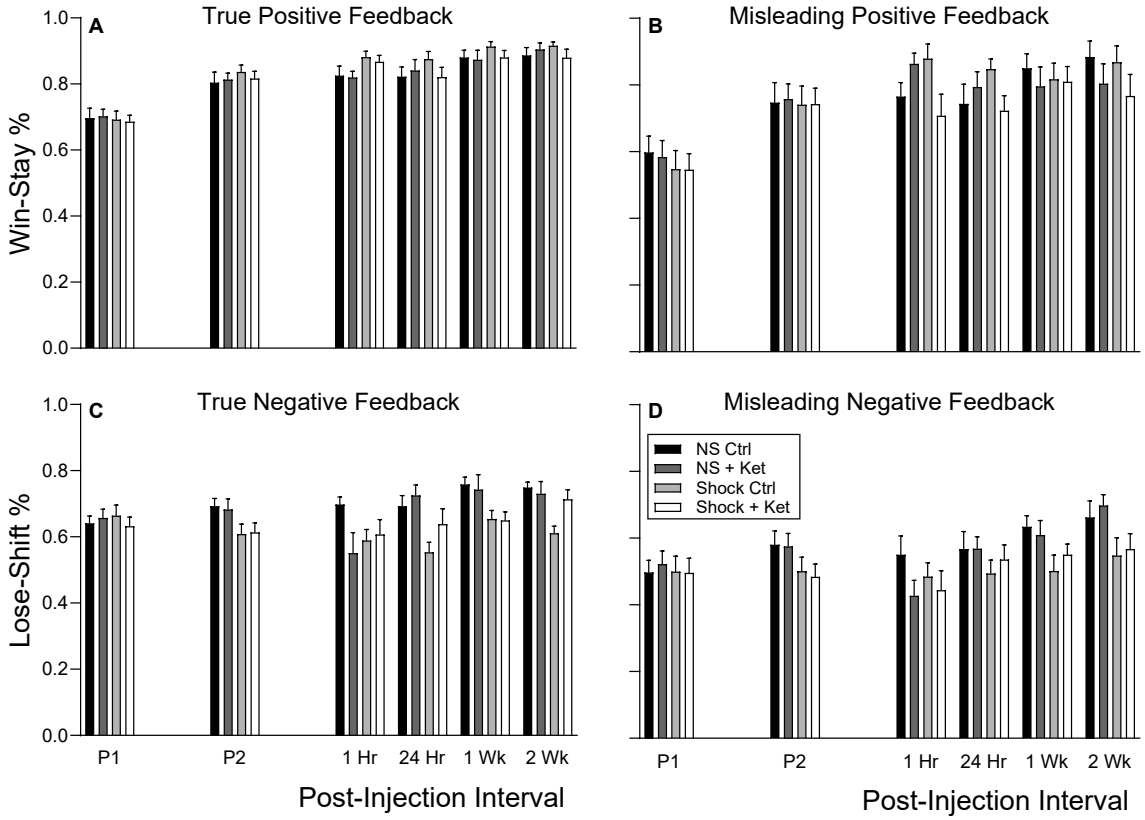

Figure 3-3. Win-stay % after true positive feedback (A) and misleading positive feedback (B). Lose-shift % after true negative feedback (C) and misleading negative feedback (D). All other details are as in Figure 3-1.

Because shock was found to significantly impact all lose-shift metrics reported above but ketamine was not, Figure 3-4 shows within-group comparisons of the effects of shock versus no shock on lose-shift behavior for the two groups that experienced probabilistic shock punishment during Phases 2 and 3. Figures 3-4A and 3-4B show total lose-shift percentage (i.e., shocks and timeouts combined) for the Shock Control Group and Shock + Ketamine Group, respectively. Figures 3-4C and 3-4D show lose-shift percentage following true negative feedback for the respective groups, and similarly, Figures 3-4E and 3-4F show lose-shift percentage following misleading negative feedback. Non-rewarded trials resulting in shock + TO and TO alone are denoted by the grey and white bars, respectively. Paired-samples *t*-tests were conducted for each of these within-group comparisons (i.e., the effect of shock vs. timeout alone on lose-shift), and all tests returned as nonsignificant (all *p*s > .10). Thus, within the groups receiving probabilistic shock punishment for non-rewarded trials, the effects of receiving shock or timeout on lose-shift behavior did not differ statistically. Therefore, despite that the groups receiving shock were statistically different in all lose-shift measures compared to the groups receiving only timeouts, within the Shock Control and Shock + Ketamine Groups, shock + TO and timeout alone were treated similarly to one another in terms of lose-shift behavior.

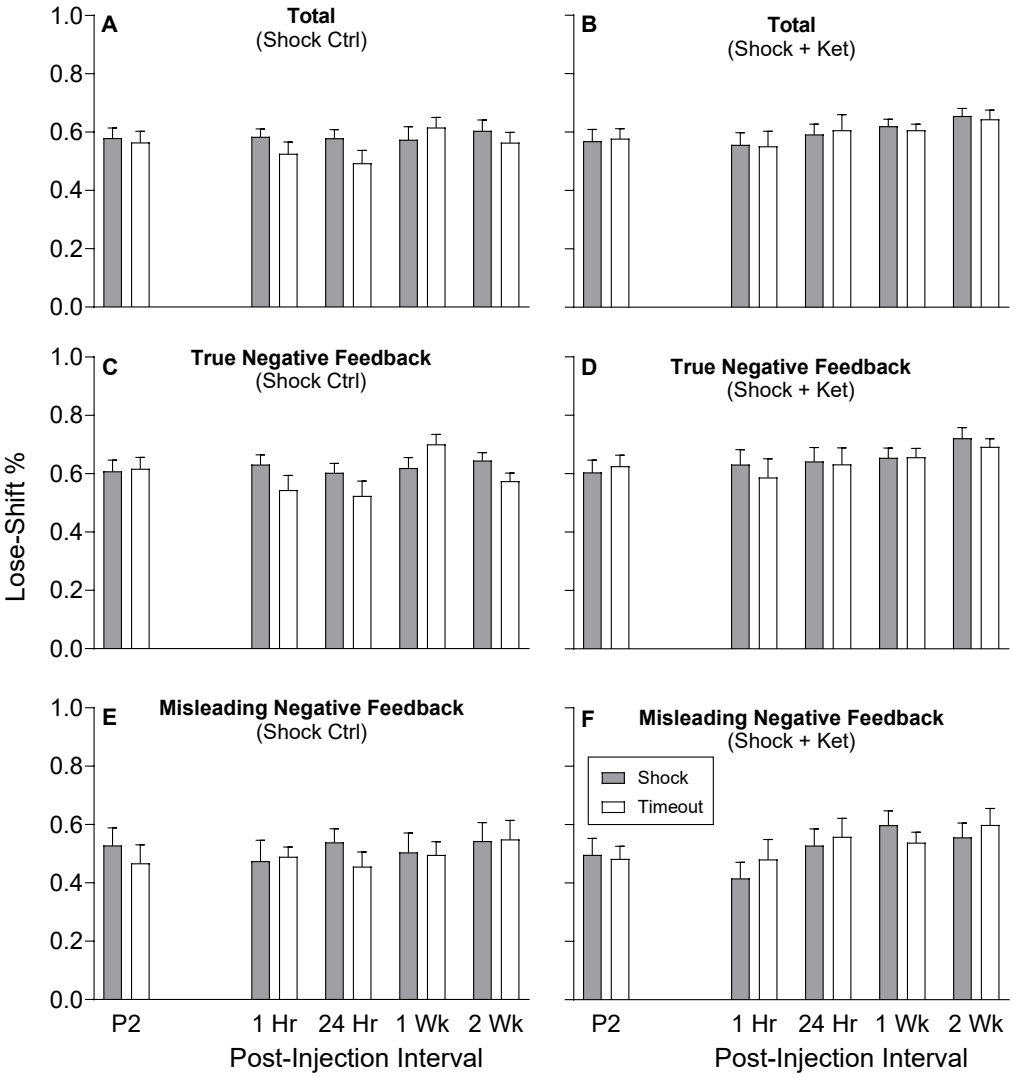

Figure 3-4. Lose-Shift % by feedback type, group, and punishment type across Phase 2 (average), and at selected intervals following administration of ketamine. Total % for Shock Control Group (A) and Shock + Ketamine Group (B), after true negative feedback for Shock Control (C) and Shock + Ketamine Group (D), and after misleading negative feedback for Shock Control (E) and Shock + Ketamine Group (F). Shock and timeout are represented by grey and white bars respectively. Error bars are standard error of the mean.

Computational Modeling Results

As with the primary behavioral measurements detailed in the above section, separate one-way ANOVAs revealed there were no statistically significant differences across groups during Phase 1 for the reinforcement learning model parameter estimates: α^{rew}, $F(3, 196) = 1.85$, $p = .14$, $\eta_p^2 = .03$, α^{pun}, $F(3, 196) = 1.14$, $p = .34$, $\eta_p^2 = .02$, τ^{reinf}, $F(3, 152) = 0.34$, $p = .80$, $\eta_p^2 = .01$, and τ^{stim}, $F(3, 193) = 0.97$, $p = .41$, $\eta_p^2 = .01$.

Figure 3-5 shows the grouped maximum likelihood estimates for each of the four model parameters across Phase 1, Phase 2, and at the same post-injection intervals as in the above figures. Figure 3-5A and 3-5B show the reward and punishment learning rate parameters (i.e., α^{rew} and α^{pun}), respectively. As with the analyses above, because no group differences were detected across Phase 1, data from Phase 2 were analyzed with shock as the sole between-subjects factor. Separate one-way ANOVAs found that shock significantly reduced both reward learning rate, $F(1, 190) = 29.55$, $p < .001$, $\eta_p^2 = .13$, and punishment learning rate, $F(1, 190) = 7.23$, $p = .008$, $\eta_p^2 = .04$, during Phase 2 compared to the No Shock Groups. Figure 3-5C and 3-5D show the remaining two reinforcement learning model parameters, reinforcement sensitivity (i.e., , τ^{reinf}) and stimulus stickiness (i.e., τ^{stim}), respectively. During Phase 2, the effect of shock on reinforcement sensitivity approached, but did not reach statistical significance compared to timeout alone, $F(1, 172) = 3.65$, $p = .06$, $\eta_p^2 = .02$. Conversely, shock significantly increased the stimulus stickiness parameter compared to timeout alone, $F(1, 188) = 23.73$, $p < .001$, $\eta_p^2 = .11$.

During Phase 3, it appeared that the effect of shock on the reward learning rate parameter persisted, regardless of ketamine treatment. A two-way (Shock x Ketamine) ANOVA found a nonsignificant interaction, $F(1, 140) = 1.03$, $p = .31$, $\eta_p^2 = .01$. As such,

the model was refit without the interaction term and found a significant main effect of shock, $F(1, 141) = 13.66$, $p < .001$, $\eta_p^2 = .01$, but no significant main effect of ketamine, $F(1, 141) = 0.53$, $p = .47$, $\eta_p^2 = .01$. Thus, shock significantly and persistently reduced the value of reward learning rate parameter compared to timeout alone, but ketamine did not have a significant impact. In contrast, there appeared to be no systematic effect of shock nor ketamine on the punishment learning rate parameter during Phase 3. This was confirmed by a two-way (Shock x Ketamine) ANOVA that revealed a nonsignificant interaction, $F(1, 140) = 0.002$, $p = .96$, $\eta_p^2 = .01$. The main effects only model further showed nonsignificant main effects of both shock, $F(1, 141) = 0.03$, $p = .85$, $\eta_p^2 = .01$, and ketamine, $F(1, 141) = 1.06$, $p = .31$, $\eta_p^2 = .01$. Therefore, although shock did have a significant effect on the punishment learning rate parameter initially (i.e., during Phase 2), it appears that this effect was only transient in nature. Further, as with reward learning rate, punishment learning rate was not systematically affected by ketamine. In contrast, it appeared that the reinforcement sensitivity parameter may have been affected by both shock and ketamine during Phase 3. However, a two-way (Shock x Ketamine) ANOVA revealed a nonsignificant interaction, $F(1, 131) < .001$, $p = .99$, $\eta_p^2 = .01$. Refitting the model without the interaction term gave a significant main effect of shock, $F(1, 132) = 3.84$, $p = .05$, $\eta_p^2 = .01$, but a nonsignificant main effect of ketamine, $F(1, 132) = 0.06$, $p = .80$, $\eta_p^2 = .01$. Post-hoc pairwise comparisons revealed that both groups receiving probabilistic shock punishment had significantly greater values of the reinforcement sensitivity parameter compared to the groups receiving only timeout as punishment, but the Shock Control and Shock + Ketamine Groups did not differ significantly from one another. Thus, once again, shock, but not ketamine, had a significant effect. Finally,

although there appeared to be clear differences for the stimulus stickiness parameter, these differences did not appear to be systematic across groups. A two-way (Shock x Ketamine) ANOVA revealed a significant interaction, $F(1, 127) = 8.01$, $p = .005$, $\eta_p^2 = .01$. Tukey's HSD found that the only significant group differences were between the Shock Control and No Shock Control Groups, with the Shock Control Group showing a significantly greater stimulus stickiness value. Therefore, it appears that shock increased stimulus stickiness, but only when ketamine was not also administered.

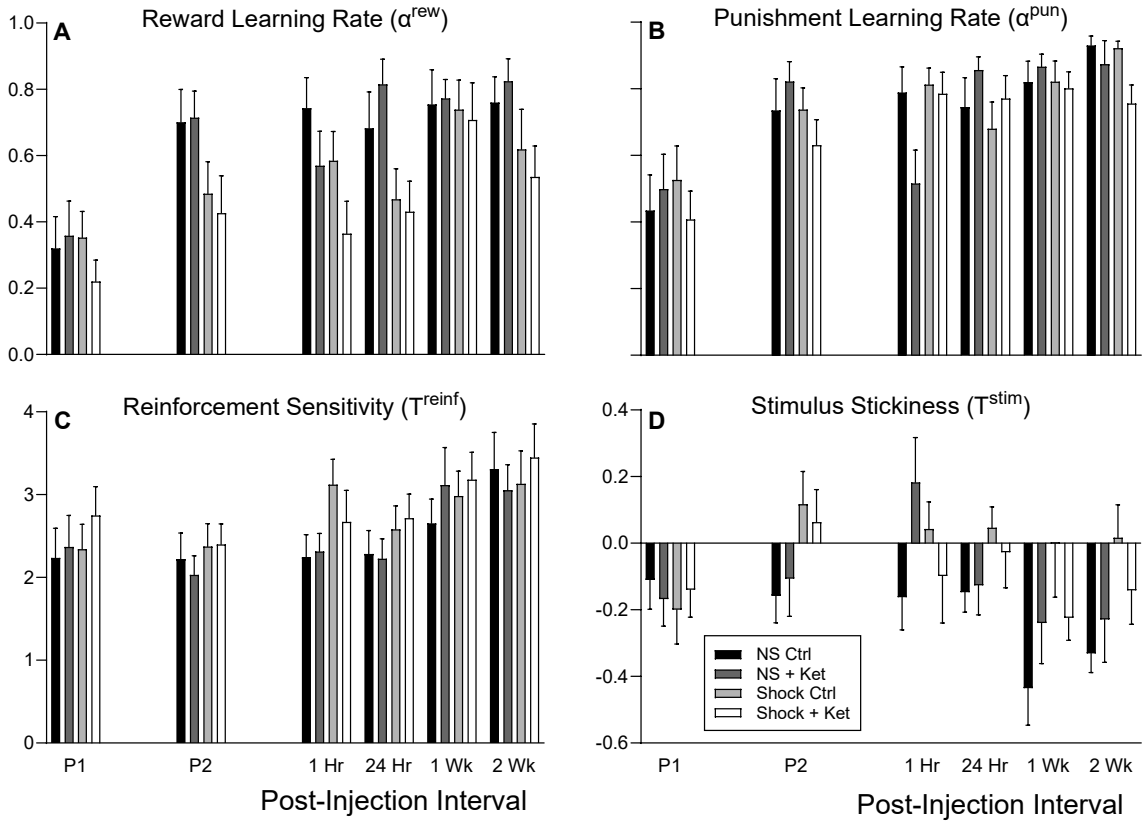

Figure 3-5. Maximum likelihood estimates for each of the reinforcement learning model parameters: reward learning rate (A), punishment learning rate (B), reinforcement sensitivity (C), and stimulus stickiness (D). All other details are as in Figure 3-1.

Discussion

The primary purpose of the present experiment was to examine if ketamine would have different effects on performance in the PRL when electric shock was either combined or not, with timeouts periods. This is because the overweighting of negative outcomes (i.e., punishments) in decision-making processes is a hallmark of MDD (e.g., Eshel & Roiser, 2010). The PRL is often used as a means by which to study the effects of drugs on such processes, but most commonly timeout periods are employed as the negative outcome following a non-rewarded trial (e.g., Bari et al., 2010; Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020). The problem with this is that past research has demonstrated that timeout periods may not always function as punishers (e.g., Solnick et al., 1977; see Fontes & Shahan, 2021; Hackenberg & DeFulio, 2007). Further, the behavioral effects of drugs may be modulated dependent on the type of punishment in place (e.g., see Orsini et al., 2015; Poling, 2000). Therefore, following the establishment of a behavioral baseline on the PRL task in which 2.5 s timeout periods were used as the negative outcome, rats were split into two even groups. The first group continued to experience the PRL as before, but the second group now had a 50% probability that non-rewarded trials would begin with a 50 ms footshock. Finally, rats were given a single injection of either 20 mg/kg ketamine or saline based on their final grouping, and then continued daily PRL sessions for two weeks. To supplement the analysis of these data, a second aim of this experiment was to employ a computational modeling strategy. This model allowed for the quantification of the effects of ketamine with or without shocks, as well as if the effect of adding shocks differed from timeout alone.

Overall, the present experiment found that ketamine had few systematic effects on behavior in the PRL, regardless of whether probabilistic shocks were included as part of a negative outcome or not. One notable exception to this was that the combination of shock and ketamine (i.e., the Shock + Ketamine Group) was found to significantly decrease win-stay behavior following misleading positive feedback compared to shock alone (i.e., the Shock Control Group). No significant effect of shock on win-stay behavior was found during Phase 2, nor was there a similar effect found in Phase 3 for the groups receiving shock alone (i.e., Shock Control) or ketamine alone (i.e., No Shock + Ketamine). Previous examinations of the effects of ketamine in the PRL have found mixed results in terms of ketamine's effects on win-stay behavior. For example, Wilkinson et al. (2020) found that 10 mg/kg ketamine significantly reduced total win-stay behavior (i.e., regardless of whether feedback was true or misleading) 1-hr post-injection. In contrast, Rychlik et al. (2017) found no effects of 5, 10, or 20 mg/kg ketamine on total win-stay behavior. The former study argued that motivational impairment was responsible for this effect, while the latter study argued that the lack of effect of ketamine on win-stay behavior was due to a "ceiling effect" from using healthy rat subjects. Either of these explanations seems plausible in the context of their own data, but neither are totally applicable to the findings of the present study. Interpretation is further complicated by the fact that both of these studies used only timeouts (as is traditional) as the negative outcome for non-rewarded trials. But, as mentioned above, it is not without precedent to find that the behavioral effects of drugs may vary with different forms of punishment (e.g., Orsini et al., 2015; Poling, 2000). Thus, it is unclear whether the reduction win-stay behavior following misleading positive feedback caused by the combination of shock and

ketamine found in the present experiment is an anomaly or should be anticipated. Beyond this effect, however, the only other significant effects of ketamine found were that it caused an initial increase in trial response latency and a corresponding decrease in response rate. In terms of the previous research in this context, once again the findings are mixed as Wilkinson et al. (2020) found that 10 mg/kg of ketamine increased trial response time 1-hr later, but Rychlik et al. (2017) found no impact of ketamine on this measure with a 10 mg/kg dose, nor with a higher, 20 mg/kg dose. Given that these effects in the present study only appeared to be large 1-hr post-injection of ketamine, and after they returned to pre-ketamine levels, it seems most logical that they were from an acute impairment effect as was concluded by Wilkinson et al. (2020). Further, other non-PRL studies have shown that a single 10 mg/kg dose of ketamine is enough to cause acute motor and motivational deficits in rats (e.g., Dix et al., 2010; Gastambide et al., 2013; Smith et al., 2011). As such, this appears to be the case for the present study as well.

Similar to the limited effects of ketamine found on behavioral measurements, there was also no effect of ketamine detected with the computational modeling approach employed here. Specifically, there was no effect of ketamine found on either the reward or punishment learning rate, nor on the reinforcement sensitivity parameters. The only noteworthy effect of ketamine found through the present modeling approach was that ketamine seemed to reduce the value of the stimulus stickiness parameter, but only for rats who also experienced probabilistic shock following non-rewarded trials. As can be seen in Figure 3-5D, this effect was persistent throughout Phase 3. Stimulus stickiness is a measure of perseveration, or the tendency to choose the same stimulus again on the following trial regardless of the outcome that followed, where higher values are

indicative of less exploratory behavior (e.g., Kanen et al., 2019, 2022). Thus, it seems that ketamine produced more exploratory behavior in the Shock + Ketamine Group relative to the Shock Control Group, but it had no significant effect when timeouts were the only form of negative outcome experienced.

In contrast to the effects of ketamine, the introduction of probabilistic shock punishment during Phase 2 of the experiment had an immediate effect on behavior in several notable ways. First, relative to the timeout only condition, rats receiving shocks completed more reversals per session, which is indicative of enhanced cognitive flexibility (e.g., Kangas, 2022). Further, these rats were also less likely to shift to the other option following non-rewarded (or punished) trials, regardless of whether the feedback was true or misleading. All rats, regardless of whether they were receiving shocks or not, were less likely to shift away from an option following misleading negative feedback, but the rats receiving shock were much less likely to do so compared to the rats receiving only timeouts. Second, shock increased both average trial response latency and omission percentage, while also causing a significant decrease in overall response rate. Therefore, despite that, on average, rats receiving shocks took longer to complete the task and were less likely to complete all of their trials, they still outperformed the rats receiving only timeout periods following non-rewarded trials. Though at first it may seem counterintuitive that rats receiving electric shock would be less likely to shift away from the response option that was sometimes delivering electric shock, this is actually the most adaptive strategy. For example, it has been shown that compared to healthy controls, MDD patients are more likely to shift to another option following misleading negative feedback, even when they are told beforehand that they

should ignore it (e.g., Murphy et al., 2003, Taylor Tavares et al., 2008). Given the probabilistic nature of the response options in the PRL, ignoring this misleading negative feedback, and persisting with choices on that same option (i.e., the rich option) despite being punished for it will eventually lead to a reward. Although, as mentioned above, all rats in the present experiment were less likely to shift away from the rich option following a non-rewarded trial, the rats receiving shock appeared to understand this probabilistic relationship more so than the rats receiving only timeouts. Another interesting finding speaking to this was that, within the two groups of rats receiving probabilistic shock punishment, shocks and timeouts were treated similarly. In other words, the rats experiencing probabilistic shocks were equally likely on average to shift to the other response option regardless of whether they had just experienced a shock or not. If a shift to the other response option occurred more often following the receipt of shock than following a timeout alone, it is likely that these rats would not have completed more reversals per session than the rats receiving only timeouts due to the probabilistic nature of the task.

Similar to the effects of shock on behavior, the present computational modeling approach found that the model parameters also differed between the shock and no shock groups. For example, during Phase 2, compared to timeout alone, shock significantly decreased both the reward and punishment learning rates. These parameters index the rate at which rewards and punishments update the value of the two nose-poke locations across trials. The interpretation of this reduced reward learning rate, therefore, would be that the receipt of rewards had less of an impact on subsequent choices for the groups receiving shock than they did for the groups receiving only timeouts. This appears to make intuitive

sense if the rats receiving shocks were more focused on how the receipt of punishments impacted the value of each response (i.e., an increased sensitivity to negative feedback). Although the value of the punishment learning rate was also reduced by shock during Phase 2, indicative of less learning from negative outcomes, this effect appeared to be transient in nature, as there was no longer any detectable group differences in this parameter during Phase 3.The rats receiving probabilistic shock punishment always had a greater value of their punishment learning rate parameter relative to their reward learning rate parameter, which ultimately is indicative that punishments were more important to their overall decision-making processes than rewards. This is in contrast to the expectation that shock would increase the punishment learning rate relative to timeout alone, however, higher punishment learning rates are generally considered to be indicative of a learning deficit (e.g., Eshel & Roiser, 2010, Murphy et al., 2003; Taylor Tavares et al., 2008). In addition, although this effect did not appear initially, during Phase 3, shock significantly increased the value of the reinforcement sensitivity parameter relative to the groups receiving timeout only. Lower values of this reinforcement sensitivity parameter have been found in MDD patients (e.g., Huys et al., 2013), and thus, a higher value of this parameter appears to be more adaptive. This appears to be further evidence that shock increased behavioral adaptation within the PRL. Finally, the introduction of shock also significantly increased the value of the stimulus stickiness parameter, indicating a decrease in exploratory behavior. In other words, shock made rats significantly less likely to switch to the other option, regardless of whether they received a positive or a negative outcome. This corresponds with the lower percentage of lose-shift behavior found in this group. Generally, greater stimulus stickiness values have

been associated with maladaptive behaviors such as in substance use disorder (Kanen et al., 2019). However, as mentioned above, given the probabilistic nature of the task, "sticking" to an option even after receiving a punishment appears to be the most adaptive strategy.

Thus, in conclusion, the primary and novel finding of the present study was that the receipt of probabilistic shocks increased performance in the PRL relative to the receipt of timeouts alone. This may suggest that, in this context, the addition of electric shocks to timeout periods functioned as a more potent stimulus than did timeout periods alone. This was evidenced by multiple behavioral measurements, as well as by the computational modelling approach used herein. The present study also found additional evidence that ketamine did not significantly impact cognitive flexibility or feedback sensitivity in the PRL, regardless of the type of negative outcome in place. However, as mentioned by previous studies, this lack of effect may be due to the fact that healthy rats were used as subjects (Rychlik et al., 2017). Future studies may need to continue to explore the effects of ketamine in the context of rodent models of depression using the PRL to more fully understand how it might have beneficial therapeutic effects.

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CHAPTER IV

GENERAL DISCUSSION

The *N*-methyl-D-aspartate receptor antagonist ketamine has shown promise as a potential alternative pharmacological treatment for major depressive disorder (MDD) across numerous clinical trials (e.g., Grunebaum et al., 2018; McGirr et al., 2015; Murrough et al., 2013). However, studying the effects of ketamine in the context of randomized controlled clinical trials with MDD patients can be complicated due to a number of potentially confounding factors including but not limited to diagnostic criteria (e.g., Kennedy, 2008), other concurrent psychiatric conditions (e.g., Thaipisuttikul et al., 2014), interactions other medications and/or ongoing regimens of therapy (e.g., Goforth & Holsinger, 2007), and subject-expectancy effects (e.g., Berman et al., 2000; Zarate et al., 2006). Therefore, using animal models of behavior can be extremely useful in isolating very specific variables which may not be possible in human clinical research. As such the purpose of this dissertation was to further examine the effects of ketamine on cognitive flexibility and feedback sensitivity, two aspects of cognition that appear to be impaired in MDD (e.g., Eshel & Roiser, 2010; Meiran et al., 2011), using a paradigm known as the probabilistic reversal learning task (PRL).

The experiment in Chapter 2 was designed to provide clarity with respect to the mixed findings of ketamine's effects on behavior in the PRL. This was done by attempting to replicate certain methodological aspects of the prior experiments in this context (Rychlik et al., 2017; Wilkinson et al., 2020) like dose, and post-injection intervals. In addition, this experiment sought to go beyond and remedy some of the

limitations of these prior studies. For example, this was the first study to extend examinations of ketamine's potential effects in the PRL beyond 48 hours post-administration. The goal of this change was to create more continuity with human clinical research showing that ketamine's antidepressant effects may last for weeks or longer (e.g., Correll & Futter, 2006; Ibrahim et al., 2012). The experiment in Chapter 2 also applied a computational modeling approach with the goal of better characterizing the effects of ketamine. For the most part, the results of the experiment in Chapter 2 appeared to coincide with a prior study showing that ketamine can cause acute impairment effects (Wilkinson et al., 2020). These effects did seem to dissipate quickly, yet still no significant nor systematic effects of ketamine on behavior or the model parameters were found when compared to a control condition in which rats were injected with saline. Overall, Chapter 2 suggests that ketamine may not enhance cognitive flexibility nor change the function of positive or negative feedback with respect to decision-making in healthy rats.

The experiment in Chapter 3 was designed to assess whether ketamine might differentially affect behavior in the PRL when electric shocks were either combined with timeout periods or not. In nearly all non-human animal versions of the PRL, timeouts are used as the negative outcome (e.g., Bari et al., 2010; Drozd et al., 2019; Rychlik et al., 2017; Wilkinson et al., 2020), but it has previously been shown that timeout periods may not always function as punishers (e.g., Solnick et al., 1977; see Fontes & Shahan, 2021; Hackenberg & DeFulio, 2007). This may be a problem for the task's overall translational value, as it has been shown that those with MDD may be disproportionately sensitive to negative outcomes (e.g., Murphy et al., 2003; Taylor Tavares et al., 2008). Further, the

behavioral effects of drugs may change with different forms of punishment in place (e.g., Poling, 2000), and have been shown to do so specifically when shock is used instead of timeout (e.g., Orsini et al., 2015). A secondary goal of this experiment was to again use a computational modeling approach to quantify the effects of adding shocks within the PRL task, as no previous study had done so to date. In Chapter 3, like Chapter 2, there were few notable effects of ketamine beyond that it appeared to cause acute impairments that seemed to wear off quickly. In contrast, the addition of shock punishment increased performance on the task relative to timeout alone in terms of the number of reversals completed per session, an index of cognitive flexibility (e.g., Kangas, 2022). Shock also decreased lose-shift percentage, and increased the value of the stimulus stickiness parameter both of which are indicative of increased behavioral persistence. Finally, compared to timeout alone, the addition of shock both decreased the reward learning rate parameter, and increased the reinforcement sensitivity parameter, while having only a transient effect on the punishment learning rate parameter. All of this, combined with the behavioral measures, seemed to indicate that the addition of probabilistic shock made rats behave more adaptively in the PRL. Thus, overall Chapter 3 suggests that the addition of shock to timeout periods may have been a more potent punisher than timeout alone, but despite this, ketamine did not have an impact on behavior in healthy rats.

One of the more notable similarities between the findings in both of these Chapters was that irrespective of dose, ketamine did not seem to enhance cognitive flexibility nor impact feedback sensitivity beyond the acute, 1-hr post-injection timepoint. In fact, the majority of the effects of ketamine found appeared to be negative in that they temporarily decreased motivation and/or the discriminability of stimuli. Overall, this

appears to be in contrast to the now large empirical literature demonstrating the effectiveness of ketamine for treating MDD (see Brendle et al., 2023; Jelen & Stone, 2021 for recent reviews). There are a few different possibilities that may explain this discrepancy between the human clinical research and the present data. First, the mechanism(s) responsible for the antidepressant effect of ketamine in rodents may be different than those that enhance cognitive flexibility. For example, in contrast to the present findings, single doses of ketamine have been found to have antidepressant effects in both the forced swim test and the learned helplessness models of depression (e.g., Autry et al., 2011; Dwyer et al., 2015; Shirayama & Hashimoto, 2016). Further, beyond the task being employed, other contextual variables such as age, sex, the timing and location of administration, and whether the racemic *(R,S)* or the enantiomer (*S*) form of ketamine is used may determine its observed behavioral effects (e.g., Bartsch et al., 2023; Polis et al., 2019). Perhaps most importantly for the present experiments, whether the animals are subjected to psychosocial stressors or not prior to ketamine administration may make the biggest impact of all. For example, Polis et al. (2019) conducted a meta-analysis of antidepressant effects of ketamine and found that negative or null results were far more common in unstressed (i.e., healthy) animals compared to those that had been subjected to some form of psychosocial stress. This suggests that ketamine may be able to reverse symptoms of depression after they are induced by the experimenter by some means, but perhaps that there is some kind of ceiling effect in healthy animals. Finally, outside of these considerations, it may be the case that differences in the verbal capabilities of humans and non-humans may play a role in the effects of drugs like ketamine. For example, the therapeutic effects of ketamine have been shown to be

enhanced by psychotherapy (e.g., Joneborg et al., 2022). Due to the nature of ketamine's subjective effects, which can be similar in nature to psychedelic drugs like LSD or psilocybin (e.g., Ly et al., 2018, 2021), it may be impossible to have a true double-blinded placebo-controlled trial, creating the possibility of subject-expectancy effects (e.g., Engin et al., 2009; Griffiths et al., 2006). Similarly, it has been speculated that the therapeutic efficacy of ketamine in humans may be confounded by interactions with ongoing regimens of therapy (e.g., Goforth & Holsinger, 2007).

However, regardless of the reason, it appears that a major limitation of the present experiments was the use of healthy rat subjects. As such, although this dissertation did not find any beneficial therapeutic effects of ketamine, the procedures used in both Chapter 2 and Chapter 3 may be usefully built upon and further utilized in animal models of depression induced by stress. One such procedure, known as the social defeat stress protocol (SDS) has been shown to induce depression-like symptoms in rats (e.g., Miczek et al., 2008). Importantly, such deficits have been found to be detectable using a reinforcement learning approach similar to the PRL known as the probabilistic reward task (Der-Avakian et al., 2017). As such, using the PRL procedures developed herein combined with the SDS could be extremely informative in a number of ways. For one, the effects of the SDS protocol could be captured using the reinforcement learning model used in the present experiments which would add further evidence that may suggest what underlying processes are affected by depression. In addition, utilizing the structure of the PRL task with different qualitative negative outcomes as performed in Chapter 3 could allow for examinations of how animals with depression-like symptoms respond to different forms or intensities of negative feedback. As stated in Chapter 3, using these

different negative outcomes would seem to enhance the overall translational value of the task.

Overall, despite that no therapeutic benefits of ketamine were found in the context of the PRL, these two Chapters have still contributed some important findings, and have hopefully provided a solid basis for future experiments. Due to the complicated and sometimes contextually dependent effects of ketamine, it appears that much more rigorous research may be needed in order to fully understand when, and why it has beneficial effects.

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Bachelor of Arts, Psychology
University of Nevada, Reno, NV, December 2016
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PROFESSIONAL POSITIONS

Aug. 2022 – Aug. 2023 **Graduate Instructor – Analysis of Behavior: Basic Principles,** Utah State University, Logan, UT
Supervisor: Gregory J. Madden, Ph.D.

Aug. 2021 – May 2022 **Graduate Teaching Assistant – Research Methods in Psychology,** Utah State University, Logan, UT
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Aug. 2018 – May 2023 **Graduate Research Assistant,** Utah State University, Logan, UT
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May 2017 – July 2018 **Registered Behavior Technician**, Alliance Family Services, Reno, NV
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Jan. 2017 – May 2018 **Teaching Assistant**, **Psychology 101,** University of Nevada, Reno, NV
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Aug. 2016 – Dec. 2016 **Undergraduate Proctor**, **Psychology 101,** University of Nevada, Reno, NV
Supervisor: Ramona A. Houmanfar, Ph.D.

Aug. 2015 – May 2018 **Undergraduate Research Assistant**, University of Nevada, Reno, NV
Supervisor: Matthew L. Locey, Ph.D.

AWARDS & GRANTS

March 2021 **Anthony LaPray Scholarship,** Department of Psychology, Utah State University, Logan, UT

Dec. 2020 **Society for the Advancement of Behavior Analysis Senior Student Presenter Grant,** Association for Behavior Analysis International

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Aug. 2018 – May 2022 **Presidential Doctoral Research Fellowship,** Utah State University, Logan, UT

CERTIFICATIONS

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PROFESSIONAL ACTIVITIES

Publications

Nist, A. N., Walsh, S. J., & Shahan, T. A. (under review). Evaluating the Dose-Dependent Effects of Ketamine on Cognitive Flexibility in a Probabilistic Reversal Learning Task with Rats. *Psychopharmacology*

Nist, A. N., & Shahan, T. A. (2023). Examining resurgence in rats following expanded-operant treatments. *Journal of the Experimental Analysis of Behavior, 120*(2), 186-203. https://doi.org/10.1002/jeab.870

Shahan, T. A., Sutton, G. M., **Nist, A. N.**, & Davison, M. (2023). Aversive control versus stimulus control by punishment. *Journal of the Experimental Analysis of Behavior, 119*(1), 104-116. https://doi.org/10.1002/jeab.805

Browning, K. O., Sutton, G. M., **Nist, A. N.,** & Shahan, T. A. (2022). The Effects of Large, Small, and Thinning Magnitudes of Alternative Reinforcement on Resurgence. *Behavioural Processes, 195,* 104586. https://doi.org/10.1016/j.beproc.2022.104586

Nist, A. N., & Shahan, T. A. (2021). The Extinction Burst: Impact of reinforcement time and level of analysis on measured prevalence. *Journal of the Experimental Analysis of Behavior, 116*(2), 131-148. https://doi.org/10.1002/jeab.714

Sutton, G. M., **Nist, A. N.**, Nall, R. W., Browning, K. O., & Shahan, T. A. (2021). Resurgence of alcohol seeking following abstinence induced by punishment in male and female rats. *Behavioral Brain Research, 410*(23), 113345. https://doi.org/10.1016/j.bbr.2021.113345

Nist, A. N., & Shahan, T. A. (2021). Resurgence and Repeated Within-Session Progressive-Interval Thinning of Alternative Reinforcement. *Journal of the Experimental Analysis of Behavior*, *115*(2), 442-459. https://doi.org/10.1002/jeab.672

Shahan, T. A., Browning, K. O., **Nist, A. N.,** & Sutton, G.M. (2020). Resurgence and Downshifts in Alternative Reinforcement Rate. *Journal of the Experimental Analysis of Behavior, 114*(2), 163-178. https://doi.org/10.1002/jeab.625

Presentations

Nist, A.N., & Shahan, T.A. Examining resurgence in rats following expanded-operant treatments. Paper presented at the Association for Behavior Analysis International Annual Conference, Denver, CO, May, 2023.

Nist, A.N. & Shahan, T.A. Resurgence and repeated within-session progressive-interval thinning of alternative reinforcement. Paper presented at the Association for Behavior Analysis International Annual Conference, San Francisco, CA, May 2021 (virtual).

Nist, A.N. & Shahan, T.A. Resurgence and repeated within-session progressive-interval thinning of alternative reinforcement. Paper presented at the annual Utah State University Student Research Symposium, Logan, UT, April 2021 (virtual).

Nist, A.N. & Shahan, T.A. Effects of progressive-interval schedules of alternative reinforcement on resurgence (Poster). Society for the Quantitative Analysis of Behavior Annual Conference, Chicago, IL, May 2019.

Nist, A.N., Locey, M.L., & Ruff, E.M. Music and social discounting (Poster). Association for Behavior Analysis International Annual Conference, San Diego, CA, May 2018.

Nist, A.N., Locey, M.L., & Ruff, E.M. Music and social discounting (Poster). Nevada Association for Behavior Analysis Annual Conference, Reno, NV, October 2017.

Professional Journals Reviewed for

Journal of the Experimental Analysis of Behavior

Membership in Professional Organizations

Fall 2016 – Present	Nevada Association for Behavior Analysis
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Conference Attendance

October 2016	Nevada Association for Behavior Analysis Annual Conference (Reno, NV)
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October 2017	Nevada Association for Behavior Analysis Annual Conference (Reno, NV)
May 2018	Association for Behavior Analysis International Annual Conference (San Diego, CA)
February 2019	Winter Conference on Learning and Behavior (Logan, UT)
May 2019	Society for the Quantitative Analysis of Behavior Annual Conference (Chicago, IL)
May 2019	Association for Behavior Analysis International Annual Conference (Chicago, IL)
October 2019	Nevada Association for Behavior Analysis Annual Conference (Reno, NV)
March 2020	Winter Conference on Learning and Behavior (Logan, UT)
May 2021	Association for Behavior Analysis International Annual Conference (virtual)

Experiences

April 2021	**Judge, California State University Student Research Competition** California State Polytechnic University, Pomona, CA (virtual) Duties: review written research summaries, attend presentations, and provide scores and constructive feedback to undergraduate and graduate student participants.
March 2021	**Reviewer, Undergraduate Research and Creative Opportunities Grant Program,** Utah State University, Logan, UT Duties: Review, score, and provide constructive feedback for undergraduate grant submissions. Provide a decision as to whether proposal should be funded or not.
November 2020	**Reviewer, Undergraduate Research and Creative Opportunities Grant Program,** Utah State University, Logan, UT Duties: Review, score, and provide constructive feedback for undergraduate grant submissions. Provide a decision as to whether proposal should be funded or not.
July 2019	**Reviewer, Undergraduate Research and Creative Opportunities Grant Program,** Utah State University, Logan, UT Duties: Review, score, and provide constructive feedback for undergraduate grant submissions. Provide a decision as to whether proposal should be funded or not.
April 2019	**Judge, Student Research Symposium,** Utah State University, Logan, UT Duties: Attend student presentations and provide relevant, constructive feedback.

August 2017	**Liberal Arts-FIT Mentor** for NevadaFIT program at University of Nevada, Reno, NV Duties: mentor a group of seven incoming freshmen for a week-long, for class-credit, boot-camp style course. Teach time management skills and productive study habits. Answer student questions about university life, and introduce students to resources on campus. Supervisors: Krisi K Van Gorder, M.A. and Genevieve M. DeBernardis, Ph.D.
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