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Lever Press Duration as a Measure of Frustration Motivated Response
Variation in Rat Self-administration to Investigate the Effects of Frustration on Substance Use Behavior.

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Dedication

To my husband John, who supported through this entire arduous endeavor. To those friends who were simply there to be a support network and let me know that I am cherished, especially Andrea, Jerrin, Tony, Laura, and Laura.

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Lever Press Duration as a Measure of Frustration Motivated Response Variation in Rat Self-administration to Investigate the Effects of Frustration on Substance Use Behavior

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In human studies, frustration has been identified as a potential risk factor for substance use disorders. Currently, there is little research into the role of frustration in substance use disorders despite research showing that frustration tolerance in humans is associated with a lower likelihood of developing substance use problems, better outcomes in recovery, and fewer relapses. To address this need, our studies use rat self-administration models to focus on frustration-related behavior in natural reward and addiction-related behavioral procedures.

First, to study frustration in operant responding, there is a need to establish a real-time objective measure and validate its use in predicting vulnerability to drug use. Frustration is when a subject cannot achieve a reinforcer, receives less than the anticipated reinforcer, or has to work harder to achieve a reinforcer. Therefore, the measure of frustration should increase in instances consistent with the definition of frustration. Furthermore, the operant measure of frustration should assess a form of either the approach or avoidance responses to frustration.

Increases in bar press durations are shown to be an approach strategy that can be used to measure frustration-related behavior objectively and, in some conditions, has the potential to signal a future transition to avoidance strategies. Supplementary experiments solidify that changes in bar press durations are a modification of continued approach behavior that can be used to objectively measure frustration-related behavior by satisfying nine behavioral criteria for barpress durations to measure frustration. Additional experiments established the predictive validity of identifying individual differences in frustration-like behavior as measured by bar press durations prior to drug self-administration. This work also affirms bar press durations as the optimal measure of frustration-like behavior within operant self-administration compared to the force of a bar press as a potential alternative approach response variation. Essentially, the results within this dissertation solidify bar press durations as an effective measure of frustration-like behavior to further elucidate the understanding of frustration within substance use disorders.

TABLE OF CONTENTS

List of Tables

List of Figures

List of Abbreviations

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
AAV	Adeno-Associated Virus
Aldh1a1	Aldehyde dehydrogenase 1 family member a1
AP	Anterior-Posterior coordinate
Avg.	Average
BP	Bar Press
CI	Confidence Intervals
Dur.	Duration
DV	Dorsal-Ventral coordinate
EXT	Extinction
Fen.	Fentanyl
FN	Frustrative Nonreward
FR	Fixed Ratio
g	grams
GFP	Green Fluorescent Protein
g/ml	grams/milliliter
h	hour
HCl	Hydrogen Chloride
IACUC	Institutional Animal Care and Use Committee
inf.	infusion
i.p.	intraperitoneal injection

iSNC	instrumental Successive Negative Contrast
IV	Intravenous
L	Lateral coordinate
mg/kg	milligram/kilogram
mg/kg/inf.	milligram/kilogram/infusion
min	minute(s)
ml	milliliter
mm	millimeter
MN	Memory state
n	the total number of individuals or observations in the sample
NIDA	National Institute on Drug Abuse
NIH	National Institutes of Health
oSNC	operant Successive Negative Contrast
PSAP	Point Subtraction Aggression Paradigm
RARβ	Retinoic Acid Receptor Beta
PR	Progressive Ratio
RDoC	Research Domain Criteria
s	seconds
SEM	Standard Error of the Mean
shNAc	shell of the Nucleus Accumbens
shRNA	short hairpin RNA
SNC	Successive Negative Contrast

Suc.	Sucrose
SUD	Substance Use Disorder(s)
µL	microliter
U/ml	Units/milliliter
UTMB	University of Texas Medical Branch
VI	Variable Interval
WSDR	Within-Session Dose-Response

Chapter 1 Introduction

FRUSTRATION

Frustration is an emotional response that arises from resistance or failure to fulfill a goal. The inability to achieve a goal can be due to either internal or external factors. Generally, internal causes of frustration are an individual's own perceived or real deficiencies in the ability to obtain a goal. Contrastingly, external causes of frustration are conditions outside an individual's control that impede the ability to obtain a goal. Additionally, it is external factors resulting in the omission of a reward that are the most reliable way to trigger frustration (Jeronimus et al., 2017). Furthermore, the effect of frustration on behavior varies depending on both individual differences as well as situational differences. Some examples of individual differences are the individual's tolerance to frustration and how much the individual values the reward (Berkowitz, 1989). Situational differences include the proximity of the reward, the extent of the interference to obtaining the reward, and the number of times there is interference the obtaining the reward (Berkowitz, 1989). While many components contribute to the expression of frustration within an individual; frustration ultimately provides the same function for all individuals.

Function of frustration

Frustration evolved as a result of the evolutionarily recurring situation of goal-oriented behavior not being fulfilled when expected (Jeronimus & Laceulle, 2017). This situation is commonly known as unexpected nonreward (Amsel, 1958; Daly, 1974). To endure unexpected nonreward, frustration signals that behavioral adjustments are required to remove psychological barriers or obstructions to a goal (Jeronimus & Laceulle, 2017).

Depending on whether or not the obstacle is perceived as controllable can result in different types of motivationally driven behavioral responses.

ROLE IN MOTIVATION:

Generally, motivation is understood as an internal state that drives goal-directed behaviors to initiate, continue or terminate a specific behavior at a particular time. When goal-directed behaviors are not rewarded, frustration arises and drives adjustment to the behaviors. Consequently, frustration functions as a feedback loop to regulate motivation. This effect can be observed in the modulation of motivational salience. Motivational salience is known to direct an individual's behavior towards or away from an object or situation. When a situation is deemed controllable and the goal attainable, arousal of frustration facilitates continued approach behavior and exploration of a broad range of actions resulting in learning and creativity (Wong, 1979). Continued approach behavior includes modification and exploration of different responses known as frustration-motivated response-variation (Jeronimus & Laceulle, 2017; Wong, 1979). Conversely, when the situation is considered uncontrollable and the goal unattainable, frustration facilitates termination of the previously rewarded behavior and escape from the situation (Daly, 1974; Jeronimus & Laceulle, 2017; Rosellini & Seligman, 1975). While escape from frustration does not result in obtaining the previously expected goal, it can still be reinforcing (Daly, 1974). This is due to negative reinforcement, which is when a behavior results in the removal of an aversive stimulus further strengthening the behavior. Thus, escape results in obtaining a secondary goal of removal from the frustrating situation strengthening future avoidance behavior.

However, one type of frustration response strategy does not preclude the other. There can be a transition from approach behavior to avoidance behavior when experiencing frustration. For example, frustration-motivated responding can initially start high when the goal is still perceived as attainable but after repeated exposure to additional nonrewarded

trials responding decreases (Daly, 1974). Additionally, these strategies can be employed simultaneously, such as an increase in response variation concurrent with a decrease in overall responding during extinction (Wong, 1979). Taken altogether, these observations demonstrate that an individual's motivational state can be modulated by frustration and varies as a function of controllability and the number of times a goal is thwarted.

ROLE IN AGGRESSION:

Aggression describes hostile behaviors that can enable an individual to adapt and overcome obstacles within their environment. When the environment is physically preventing a goal, these aggressive behaviors are typically intended to be destructive. However, aggressive behaviors can be channeled into practical and creative solutions to remove impediments to a goal (Wong, 1979). Additionally, when a goal is perceived as attainable and the situation as controllable, aggressive behaviors are more likely to be utilized to remove sources of frustration and persist in goal approach (Jeronimus et al., 2017). This demonstrates a general sequence of events of frustration progressing towards aggressive behavior. Consequently, this common sequence of events is the foundation of the frustration-aggression hypothesis which states "that the occurrence of aggression always presupposes the existence of frustration and, contrariwise, that the existence of frustration always leads to some form of aggression" (Dollard et al., 1939). However, this hypothesis has been reformulated to propose that frustration can lead to aggressive tendencies, but frustration does not always precede aggression (Berkowitz, 1988, 1989).

ROLE IN ANXIETY AND DEPRESSION:

Frustration can also result in nonaggressive emotional responses such as anxiety and depression. For example, when a frustrating situation is perceived as uncontrollable an individual is more likely to experience anxiety and withdraw from the situation (Jeronimus et al., 2017). Additionally, it is possible that frustration can contribute to depression when

a goal is clearly unattainable; however, it has been shown that learned helplessness from being unable to avoid a situation transfers to a decrease in escape from frustration behavior (Jeronimus et al., 2017; Rosellini & Seligman, 1975). These types of uncontrollable or unavoidable experiences can result in excessive amounts of psychological stress. To reduce stress, coping strategies are employed and an individual's ability to cope with frustrating circumstances is known as frustration tolerance. Furthermore, low frustration tolerance has been shown to correlate with more depression and anxiety symptoms. (Chang & D'Zurilla, 1996; Jeronimus et al., 2016, 2017). Thus, frustration is a potential contributor to experiencing and expressing feelings of anxiety or depression.

MEASURES OF FRUSTRATION IN HUMANS

The US National Institute of Mental Health currently classifies frustrative nonreward (FN) as a construct in the Negative Valence Systems domain of the Research Domain Criteria (RDoC) framework. This framework provides an organizational structure to investigate human constructs of mental disorders. When it comes to frustrative nonreward within this framework a person's response to frustrating situations can have broad implications for a variety of neuropsychiatric conditions. As previously discussed, many components contribute to the presence of frustration and how an individual will respond to the source of frustration. Focusing on a few of these components previous human studies of frustration were able to demonstrate that increased frustration behavior predicts an increased risk to develop anxiety, depression, substance abuse, and thought disorders (Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017). The elements these experiments concentrated on measuring were frustration tolerance and displays of aggressive behavior. These indirect measures of frustration are used because they are more readily observed and definitively described. To obtain their measurements, the frustration studies relied primarily on surveys, questionnaires, and report scales.

One study previously referenced, used the parent version of the Early Adolescent Temperament Questionnaire-Revised. This questionnaire focuses on observer reports to scale irritability. While the core of the scale captures irritability it may not reflect frustration proneness because as described previously, frustration may not always precede aggressive tendencies. Additionally, the use of an observer report is controversial in that the subject may not externalize the emotion or discreetly externalize a different emotion than the one they are currently experiencing (Mauss & Robinson, 2009).

Instead many other studies utilized self-reports to measure frustration tolerance. This method is the most common form of data collection when studying frustration. The method operates under the assumption that "you know yourself better than anyone"; and if a subject can assess themselves accurately it can obtain less observable information about motives, intentions, and past behaviors (Abernethy, 2015; Mount et al., 1994; Paulhus & Vazire, 2007). The reports used scales to assess frustration tolerance beliefs or the likelihood an individual will lose interest in a task when they cannot meet their goal (Demaria et al., 1989; Harrington, 2005; Peters et al., 1980; Wright et al., 2009). However, those scales rely on the recalling of emotions made distant in time from the assessment and thus tend to lack reliability (Mauss & Robinson, 2009; M. D. Robinson & Clore, 2002)

Conversely, self-reports of an immediate current emotional state or experience tend to be a more valid measure of an emotion (M. D. Robinson & Clore, 2002). Thus, some studies have focused on trying to capture how subjects feel in the moment when presented with a frustrating situation using think-aloud techniques (Klara Hoppmann & Hoppmann, 2007; Scime & Norvilitis, 2006). However, these analyses still have issues that are seen in all self-reports. Some of the issues are whether or not the subject interpreted the questions and instructions as they were intended, is the subject answering honestly or providing a more socially acceptable response, and how biased is their current response to their previous responses (M. D. Robinson & Clore, 2002). To supplement these drawbacks, other studies measure frustration by a combination of direct survey questions and

physiological data, such as heart rate and electrical activity in facial muscles (Douglas & Parry, 1994; Hazlett, 2003; Reynolds et al., 1999). The use of physiological data provides objective information that is more reliable, quantifiable, and consistent within the context of a subject's frustration experience.

There is also a behavioral task that is used to assess aggressive responding while experiencing frustration. The human point subtraction aggression paradigm (PSAP) is a validated behavioral measure of aggression in response to perceived provocation (Cherek, Moeller, Dougherty, et al., 1997; Cherek, Moeller, Schnapp, et al., 1997) and subjects had the option to respond in one of three ways to obtain points: the first option was to continue normal responding to earn points (nonaggressive responding), the second option was to subtract points from a fictitious person to add to their score (retaliation/aggressive responding), and the last option was to protect points from being subtracted (escape). Point subtractions were presented randomly and were attributed to a fictitious other person paired with the subject. The frequency of each type of response was assessed to evaluate the level of aggressive responding. While the focus of the task was to grade aggressive responding to provocation it was indirectly assessing responding to a frustrating situation. The source of frustration within the task is the perception that another person making it harder to achieve points. Ultimately, this study has the advantage of objectively measuring aggressive responding to provocation from a perceived frustration source.

ANIMAL MODELS OF FRUSTRATION

Animal models of frustration have also been used to study how this mental state enables organisms to adapt, survive, and reproduce. The studies investigated the influence of frustration on aggressive behavior, stress, learning, and memory when dealing with goal obstruction or replacing a lost resource. Goal obstruction leading to the arousal of aggressive behavior is the main focus of some of these experiments (Arnone & Dantzer, 1980; Capaldi, 1974; Duncan & Wood-Gush, 1971; Finch, 1942). However, the most

common studies of frustration use a rodent runway model where the rats are trained to run to goal boxes baited with a food reinforcement (Adelman & Maatsch, 1956; Amsel & Hancock, 1957; Amsel & Roussel, 1952; Amsel & Ward, 1954; Capaldi, 1974; Daly, 1974; Lambert & Solomon, 1952). After runway training, the reinforcer is removed, or the amount of the reinforcer size would be changed for subsequent trials. The earlier studies found when a reinforcer is omitted in the first goal box rats increase runway speed to the second goal box (Amsel & Roussel, 1952). Additional experiments demonstrated that runway speeds objectively measured increases in response vigor and approach behavior when the rats were frustrated (Amsel & Hancock, 1957; Amsel & Ward, 1954). Ultimately, these studies established increases in runway speeds as a measure of frustration behavior since frustration is known to occur when a goal (i.e. food reinforcement) is denied. Furthermore, other studies used increased speeds to escape a runway to measure avoidance behavior. The escape runway tasks measured the time it took to jump out of the goal box or hurdle jump to escape the goal box (Adelman & Maatsch, 1956; Daly, 1974). Thus, altogether demonstrating that runway speeds can be used to examine the effects of frustration on both approach and avoidance frustration responses.

Further experiments assessed the arousal of frustration behavior on a partial reinforcement schedule compared to a continuous schedule. On a partial reinforcement schedule, the response behavior is reinforced occasionally rather than every instance. Thus, the rats did not receive their reinforcement when expected. These studies of reward expectancy were able to show that both a partial reinforcement schedule, as well as nonreward following acquisition on a partial reinforcement schedule arouses frustration (Amsel, 1958; Daly, 1974; Scull, 1973). Additional studies focused on using successive negative contrast (SNC) to manipulate reward expectancy by decreasing reinforcement magnitude (i.e. decreased number of food pellets) after training with a greater reinforcement magnitude (Capaldi, 1972; Crespi, 1942; Flaherty, 1982). Consequently, the decrease in reward magnitude led to a decrease in speed on the runways. Altogether, these

results demonstrate runway speeds can be used as an objective measure to assess both complete denial of a reinforcer and reward expectancy.

Other rodent experiments began investigating the influence of frustration on substance use. To study substance use, self-administration within operant chambers was essential instead of the use of standard runways. The general procedure involved rats trained to self-administer food and then the drug. Afterward, the rats were put on extinction for the drug while they responded for food reinforcement. Next, the food was withheld, which increased responding for the drug, even though extinction for the drug was still in effect. This effect of when the extinction of a non-drug reinforcer reinstates a previously extinguished operant response is known as resurgence (Podlesnik et al., 2006; Quick et al., 2011). Alternatively, it has also been shown that withholding the non-drug reinforcer when the drug reinforcer is available results in increases responding for the drug and the number of drug reinforcements earned (Ginsburg & Lamb, 2018; Gipson et al., 2012). Altogether, these studies demonstrated that the loss of an alternative food reinforcer increases both drug seeking and taking (Ginsburg & Lamb, 2018; Gipson et al., 2012; Podlesnik et al., 2006; Pyszczynski & Shahan, 2013; Quick et al., 2011). Thus, these experiments reveal a causal effect of frustration on drug self-administration behavior through standard operant conditioning.

FRUSTRATION IN SUBSTANCE USE DISORDERS

There are several different environmental risk factors for developing a substance use disorder, including various psychosocial stressors. Some psychosocial stressors can be frustration-, anxiety-, or fear-provoking situations such as divorce, death, prolonged illness, or a natural catastrophe. These types of stressors create an unusual or intense level of stress that may contribute to the development of psychopathology. For example, frustration has been shown to impact an individual's vulnerability to mental disorders, illness, or maladaptive behaviors (Caspi et al., 2017; Jeronimus et al., 2016, 2017;

Jeronimus & Laceulle, 2017). Furthermore, studies demonstrated that frustration serves as a component of causal pathways toward psychopathology during adolescence (Caspi et al., 2017; Jeronimus et al., 2016, 2017). Thus, high frustration during adolescence predicts increases in aggression, anxiety, depression, substance abuse, and thought disorders during adulthood (Jeronimus et al., 2016, 2017).

Additionally, research shows that persons with substance use disorders rate higher in tests of frustration and that sensitivity to frustration correlates with number of relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). Substance use, and by extension relapse, is recognized as a way to cope with negative emotions or stressful situations as explained by the self-medication hypothesis. One component of the self-medication hypothesis suggests that engaging in substance use may be appealing as drugs can temporarily relieve negative affect states such as frustration (Khantzian, 1997; Torres & Papini, 2016). Clinical observations and empirical studies indicate that negative affect states are important psychological determinants in using, becoming dependent upon, and relapsing to addictive substances (Khantzian, 1997; Khantzian & Albanese, 2008; Weiss et al., 2009).

However, experiencing negative affect states alone does not make someone vulnerable to substance use disorders. For there to be a vulnerability to substance use disorders there needs to be some exposure to drugs combined with the inability to tolerate negative affect states. (Khantzian, 1985, 1997). For example, some studies demonstrate that frustration intolerance puts an individual at a high risk of developing a substance use disorder and relapsing (Baars et al., 2013; Ramirez-Castillo et al., 2019). Additionally, frustration can drive risky exploratory behavior, which is due to frustration-motivated response-variation encouraging exploration of a broad range of actions in an attempt to alleviate the feeling of frustration (Wong, 1979). Thus, once a vulnerable frustration-intolerant subject discovers that the use of a drug results in a reduction of frustration, it can lead to repeated substance use.

OPERANT SELF ADMINISTRATION TO STUDY SUBSTANCE USE DISORDERS

Operant self-administration relies on operant conditioning which is a type of associative learning process. Associative learning occurs when emitted behavioral responses become associated with stimuli. Then within the context of operant conditioning, the probability of the voluntary behavior occurring is modified by reinforcement or punishment. Reinforcement increases the probability of the behavior occurring again and punishment decreases the probability. The two consequences can be classified as positive or negative, where positive implies that a behavior will be followed by the presentation of a stimulus and negative implies that the behavior will result in the removal of a stimulus. Within substance use, positive and negative reinforcement play central roles in the development and maintenance of substance use disorder. The euphoria of drugs can initially function as a positive reinforcer. However, when dependence on a substance develops there is also the possibility of negative reinforcement, which occurs when a drug is self-administered to alleviate the symptoms of dependence or withdrawal (Blume, 2014; Cho et al., 2019; Edwards, 2016). Furthermore, when going through a withdrawal period, abstinence from a drug can lead to craving and relapse.

Craving tends to occur in the presence of previously neutral cues that have been associated with the drug (O'Brien et al., 1992; Stewart, 1983). Recent animal models of craving have expanded operant studies to investigate the incubation of craving. These studies demonstrate that as the duration of abstinence increases, so does the motivational impact of the drug-associated cues on operant drug seeking (Grimm et al., 2001; Lu et al., 2004; Neisewander et al., 2000; Pickens et al., 2011; Wolf, 2016). Furthermore, craving along with acquisition, escalation, and relapse of a substance of abuse can all be impacted by impulsivity (Doran et al., 2007; Moeller et al., 2001; Perry & Carroll, 2008).

Impulsivity is the tendency to act on a whim with no forethought or consideration of the consequences. Due to impulsivity having a multi-faceted impact on substance use

there is a wide assortment of behavioral models to study the separate underlying processes. Delay discounting is a commonly used operant paradigm of impulsive choice which measures the relative preference for smaller, more immediate rewards over larger, more delayed rewards (de Wit, 2009; Perry et al., 2005, 2007; Stanis et al., 2008). Other frequently used gauges of impulsivity are behavioral-inhibition tasks that focus on impulsive actions and measure the ability (or inability) to inhibit a prepotent response (de Wit, 2009; Feola et al., 2000; Moschak et al., 2012). Overall these previous studies demonstrated that increased impulsivity predicts drug self-administration and the reciprocal, that drug self-administration increases impulsive behaviors.

On the opposite of the behavior spectrum of impulsivity within substance use disorders tends to be habit. It is suggested that habit can contribute to compulsive drug use which is driven by excess goal-directed motivation for the drug (Vandaele & Ahmed, 2020). Hence, habit within substance use is generally studied as a resistant behavior to changes in the value of a reinforcer (Panlilio & Goldberg, 2007; Root et al., 2009). Drug reinforcers within operant self-administration were devalued by pairing self-administration with an aversive intraperitoneal injection of lithium chloride. When rats did not reduce goal-directed self-administration responding, the responding was considered to be more habitual and less controlled by the value of the reinforcer.

While these three characteristics of substance use have been extensively examined, frustration has recently begun to be studied within operant-self administration. The acquisition of operant self-administration enables subjects to build expectations of what may happen in the future given their behavior. Expectations involving reinforcements not being met can lead to frustration. When studying the effect of frustration on substance use there are currently a limited number of rodent models. The models demonstrated that frustration from loss of an alternative food reinforcer increases drug seeking and taking (Ginsburg & Lamb, 2018; Gipson et al., 2012; Podlesnik et al., 2006; Pyszczynski &

Shahan, 2013; Quick et al., 2011). However, this model of resurgence has only scratched the surface of frustration in substance use.

Frustration impacts motivation as well as both approach and avoidance behavior. It is important to understand how these components of frustration can contribute to substance use (see above). Thus, expanding on the operant and runway behavioral tasks is necessary. Furthermore, it is important to develop a model to identify frustration intolerance prior to substance use to study vulnerability to substance use disorder. Therefore, to study frustration in operant responding there is a need to establish a real-time objective measure similar to the runway experiments and to validate its use in predicting vulnerability to drug use. Ultimately, the operant measure of frustration will be assessing a form of either the approach or the avoidance frustration responses. Provided that the measure of frustration is a continued approach strategy, when the reinforcement is denied or made more difficult to achieve during operant self-administration there will be an increase in the measure if the reinforcement is still perceived as obtainable or decrease if the reinforcement is perceived as unobtainable. The result would be reversed should the measure be an avoidance response strategy. However, there is the caveat that it is impractical to speculate about an animal subject's perception of goal obtainability. Therefore more broadly, when an animal subject is confronted by a situation where the reinforcement has been denied or made more difficult to achieve during operant self-administration there will be a change in the frustration response behavioral measure. Additionally, motivation is known to be a factor in substance use disorders and moreover, frustration can influence motivation. Thus, the intensity of the frustration response measured should validly predict motivation for future substance use.

Chapter 2 Lever Press Duration as a Measure of Frustration in Sucrose and Drug Reinforcement

It has been established that the operant measure of frustration must assess a form of either the approach or the avoidance frustration strategies. In our research we focus on changes in bar press durations as a modification of the previously rewarded response approach strategy. We focus on this strategy because the alternatives are more limited in their ability to measure frustration in operant responding. The avoidance strategies are escape from the source of frustration or termination of the previously rewarded behavior. Measuring escape behavior is impractical as there is no option to escape the operant chamber during self-administration. Alternatively, termination of the previously rewarded behavior is feasible and commonly measured in extinction and progressive ratio schedules of operant self-administration. However, the measure is obtained at the end of a session and our goal is to establish a measure that can evaluate frustration in real-time since immediate current emotional state tends to be a more valid measure of an emotion in humans (M. D. Robinson & Clore, 2002). On the other hand, the standard approach strategies are modifications of the previously rewarded response or exploration of different responses. Measuring the exploration of alternative behaviors during operant self-administration is a possibility. However, this type of measure is reliant on an observer subjectively identifying and counting what may be alternative behaviors. Preferably, the measure of frustration would be an objective measure because it will be more reliable, easily quantifiable, and consistent. These criteria are more easily met by measuring the modification of the previously rewarded operant response; bar pressing in the case of our operant chambers. One easily quantifiable and objectively measurable modification to the behavior of bar pressing is the duration at which the bar is held. This measure also has the advantage of being assessed throughout the operant self-administration session with every

response. Therefore, this measure provides an evaluation of frustration related behavior with minimal temporal delay after the frustrating event. Thus, bar press durations as a modification of the previously rewarded response stands as the optimal way to measure continued approach response. Consequently, in the following chapter, we examine changes in bar press durations as a candidate for a real-time objective measure of frustration.

This chapter contains information from the published manuscript, Lever-press duration as a measure of frustration in sucrose and drug reinforcement, Tileena E. S. Vasquez, Ryan J. McAuley, Nikita S. Gupta, Shyny Koshy, Yorkiris Marmol-Contreras, Thomas A. Green (2021), Psychopharmacology 10.1007/s00213-020-05742-2.

INTRODUCTION

Historically, research into substance use disorders (SUDs) has focused predominantly on 3 facets of addiction: craving, impulsivity, and habit. Here we focus on a fourth facet of SUD-related behavior, that of frustration. While frustration is a psychological construct well known to all, little attention has been paid to the role of frustration in SUDs. Is high frustration a positive force because it facilitates extinction of drug seeking or is frustration a negative force because it perpetuates compulsivity and relapse? The answers to these questions can only come when we are able to quantify frustration in real time.

Research into the role of frustration specifically regarding substance use disorders is sparse, but a number of studies suggest that persons with substance use disorders rate higher in tests of frustration (i.e., lower frustration tolerance) and that sensitivity to frustration correlates with number of relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). Rodent models are also sparse but generally support the human research. For example, frustration from loss of an alternative food reinforcer increases drug seeking and taking (Ginsburg & Lamb, 2018; Podlesnik et al., 2006; Pyszczynski & Shahan, 2013; Quick et al., 2011).

While frustration with addiction have garnered little attention, there is a very rich history of studying frustration in the runway task with regard to extinction. Rodents placed in a state of frustration showed faster speeds in running down a runway to a goal box baited with a food reward, allowing for the study of fundamental aspects of extinction (Adelman & Maatsch, 1955; Amsel & Roussel, 1952; Capaldi, 1974). The current project expands upon these techniques with a lever-press operant response procedure.

We operationally define frustration in rats (and humans) as a state where the subject is unable to achieve a reinforcer (e.g., extinction), receives less of a reinforcer than anticipated (incentive downshift), or has to work harder to achieve a reinforcer (e.g., progressive ratio schedule). Whereas a number of non-reinforced bar presses has been used as a model of craving in operant procedures, we offer evidence below that duration of bar presses represents a measure of frustration. We show several conditions where bar press durations increase when rats are in a state of frustration (i.e., not sated): during cued extinction, before "breaking" in a progressive ratio procedure, when the fixed ratio requirement is increased (e.g., FR1 to FR3 or FR3 to FR5), during the loading phase of cocaine self-administration, and for the small number of rats that escalate fentanyl self-administration. It is important to note that although bar press durations can only be measured when rats are seeking a reinforcer (i.e., pressing the bar), the results below show that bar press durations (i.e., frustration) are not isometric measures to number of bar presses representing seeking (i.e., craving).

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats were obtained at 225–250g. Measures were taken to minimize pain or discomfort following surgical procedures. Except during food regulation, rats were pair-housed throughout the experiments and maintained in a controlled environment (temperature, 22 °C; relative humidity, 50%; and 12h light/dark cycle, lights

on 0600h) in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC)–approved colony and procedures were approved by the UTMB Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals.

Fentanyl operant responding

After 1 week of acclimating to the colony room, rats were implanted with indwelling intrajugular silastic catheters as described previously (Crofton et al., 2017; Zhang et al., 2016). Before surgery, rats were anesthetized with a ketamine/xylazine mixture (100 and 10 mg/kg, i.p). To maintain catheter patency, catheters were flushed daily with 0.1 ml of heparinized (10 U/ml) saline with ticarcillin (0.067 g/ml). Following 1-week recovery from surgery, animals were placed in the operant chambers to self-administer either fentanyl (0.0032 mg/kg/infusion: n = 18; NIDA drug supply program) or saline (n = 17). Animals began fentanyl self-administration on a continuous schedule (FR1) of reinforcement until the fentanyl rats were responding consistently for 4 days (greater than 10 infusions and less than a difference of 10 infusion variability in daily intake). Each session lasted 3h where a single response on the active lever resulted in a 0.1 ml intravenous infusion delivered over 5.8s, concurrent with the illumination of two circular cues lights located above the levers. Each infusion was followed by a 20s timeout period during which the cue lights were illuminated, the house light was extinguished, and responding was recorded but animals could not earn more fentanyl or saline. Throughout the session, responding on the inactive lever was recorded but had no consequences. After stabilization on FR1, the response requirement for the next session was increased to FR3, followed by an increase to FR5 until they were responding consistently for 5 days. Then between-session cued extinction consisted of 2h with cue lights delivered under the normal FR5 schedule but no drug delivery. To prevent full withdrawal, the extinction session was immediately followed by 1h of fentanyl self-administration at an FR5 schedule. For the

next 3 sessions, the rats were placed on a progressive ratio (PR) schedule in which each successive fentanyl injection required an increasing number of lever-press responses according to the following semi-logarithmic progression, 1, 2, 4, 6, 9, 12, 15, 20, etc. The session continued until the rats went 1h without obtaining a reinforcement, or up to a maximum of 6h.

In a separate set of experiments, rats were initially placed on a regulated diet for 6 days until rats reached 85% of free-feed body weight. Rats were allowed to perform a single lever press (FR1) to receive sucrose pellets for 2h with an unconditional pellet provided every 10 min until they self-administered 100 pellets first training session. Rats that showed slower learning (e.g., did not reach 100 pellets) were placed in the operant chambers again until they could self-administer a combined 100 pellets across the session. For successive sessions, the FR schedule was incremented daily until an FR5 session. Next, the rats were then returned to ad libitum feeding for 1 week before surgery. The rats then underwent catheter surgery and catheter patency was maintained as described above. Following 1-week recovery from surgery, animals were placed in the operant chambers to self-administer (0.0032 mg/kg/infusion fentanyl: n = 40). Animals began fentanyl self-administration on a continuous schedule (FR1) of reinforcement for 6 sessions and the number of infusions each rat could obtain was capped at 30. Each session lasted up to 3h where a single response on the active lever resulted in the same sequence of events as described above. Throughout the session, responding on the inactive lever was recorded but had no consequences. Then, rats were then allowed to self-administer for the next 6 sessions uncapped. The next session, 10 of the rats underwent within-session extinction, where for the first 60 min rats were able to obtain fentanyl at FR1 and then the following 3h rats were still given cues, but not fentanyl.

Cocaine operant responding

Rats were initially injected with shRNA adeno-associated viral vectors expressing a control hairpin or a hairpin directed at Aldh1a1 in the nucleus accumbens shell. The vector had no effect on the number of bar presses nor bar press durations (Appendix B Supplemental Table for Chapter 2); thus, the rats were combined into one group for the purpose of these studies. Rats were initially placed on a regulated diet and trained to lever press for sucrose pellets using the methods as described above. For the following sessions, the FR schedule was incremented daily until an FR5 session. The rats were then returned to ad libitum feeding for 1 week before surgery. The rats then underwent catheter surgery and catheter patency was maintained as described above.

Following 1-week recovery from surgery, rats were placed in the operant chambers to self-administer a low dose of cocaine (0.2 mg/kg/infusion: n = 17; NIDA drug supply program) on a continuous schedule (FR1) of reinforcement for 5 days for assessment of acquisition. The dose was then increased to 0.5 mf/kg/infusion for 5 days. Each session lasted 3h where a single response on the active lever resulted in a 0.05 ml intravenous infusion delivered over 2.6s, concurrent with the illumination of a circular cues light located above the levers. Each infusion was followed by a 20s timeout period where the animals could not earn more cocaine. Throughout the session, responding on the inactive lever was recorded but had no consequences. The following 8 days, rats underwent within-session dose-response (WSDR) where every 30 min the dose was halved starting at 0.5 mg/kg/infusion. Then, for 7 sessions the rats were placed on within-session extinction, as described above for fentanyl self-administration. Following within-session extinction, for the next 7 sessions the rats were placed on a progressive ratio (PR) schedule with the same progression and session duration as described above. The first 4 days for PR, the rats self-administered 0.125 mg/kg/inf followed by 3 days of 0.5 mg/kg/inf.

Sucrose operant responding

Rats were initially placed on a regulated diet and trained to lever press for sucrose pellets using the methods described above. After 4 sessions of stable responding on FR1, the response requirement for the next session was increased to FR3, followed by an increase to FR5 the subsequent 4 sessions. Then, between-session cued extinction consisted of 2h with cue lights delivered under the normal FR5 schedule but no sucrose delivery. The extinction session was immediately followed by 15 min of sucrose self-administration at an FR5 schedule to prevent extinction from affecting the next session. For the next 3 sessions, the rats were placed on a progressive ratio (PR) schedule with the same progression and session duration as described above.

Statistical analysis of behavior

Significance between only two conditions was analyzed using a Student's t test. One-factor repeated measures analyses of variance (ANOVA) was used for comparing data across multiple conditions. If conditions for sphericity were not met, the Greenhouse-Geisser statistic was used. All data are expressed as mean ± SEM. Statistical significance was set at $p < 0.05$. Rats not completing a given experiment were not considered in that analysis.

RESULTS

Maintenance responding

In fentanyl self-administration, rats administering fentanyl had decreasing average bar press durations across the last 4 days of FR1 and the same for the first 4 days of FR5 (FR1, $F(2.161, 32.422) = 13.043$, $p = < 0.001$; FR5, $F(3, 42) = 9.130$, $p < 0.001$, Figure 2.1 D). For rats administering sucrose, there was a strong trend of decreasing average bar press durations across the last 4 days of FR1 and as well as for the 4 days of FR5 (FR1, $F(2.102,37.838) = 3.002$, $p = 0.059$; FR5, $F(1.923,34.617) = 2.658$, $p = 0.086$, Figure 2.1 A). This demonstrates that as rats acclimate to the current schedule, they exhibit lower bar

press durations. Rats also demonstrated a significant increase in bar press durations when the schedule increased from FR1 to FR3 (fentanyl, $t(14) = -6.800$, $p < 0.001$, Figure 2.1 D; sucrose, $t(18) = -3.700$, $p = 0.002$, Figure 2.1 A) and from FR3 to FR5 for fentanyl self-administration ($t(14) = -3.055$, $p = 0.009$, Figure 2.1 D). There was only a slight trend for an increase in bar press durations when the schedule increased from FR3 to FR5 for sucrose self-administration ($t(18) = -1.541$, $p = 0.141$, Figure 2.1 A). When the schedule requirement is increased, the rats must work harder to achieve the same number of reinforcers, and this leads to an increase in bar press durations. Using a representative rat for both the sucrose and fentanyl self-administration, we show that the rolling average (averaged across 10 bar presses) on the last day of FR1, FR3, and the first day FR5 remain consistently higher than the previous session (Figure 2.1 C and F). This is also true with group average of the rolling averages (Figure 2.1 B and E).

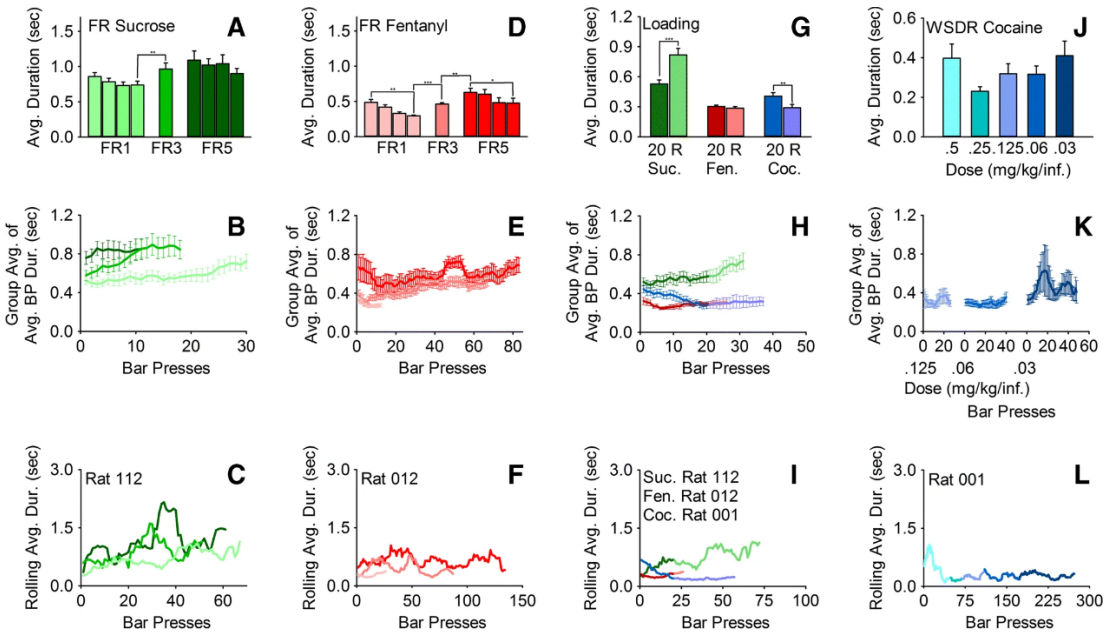


Figure 2.1: Conditions that increase average bar press durations during maintenance responding.

A. Average bar press durations (seconds ± SEM) of sucrose rats during the last four sessions of FR1, the FR3 session, and the four FR5 sessions. **B.** Group average (seconds ± SEM) of the rolling averages of bar press durations (10 presses) for sucrose rats during the last session of FR1, the FR3 session, and the first FR5 session. The number of bar presses shown for each session was limited by the rat with the lowest number of responses. **C.** Rolling average bar press durations of representative sucrose Rat 112 during the last session of FR1, the FR3 session, and the first FR5 session. **D.** Average bar press durations of fentanyl rats during the last four sessions of FR1, the FR3 session, and the first four FR5 sessions. **E.** Group average of the rolling averages of bar press durations for fentanyl rats during the last session FR1, the FR3 session, and the first FR5 session. **F.** Rolling average durations of representative fentanyl Rat 12 during the last session of FR1, the FR3 session, and the first FR5 session. **G.** Average bar press durations of sucrose, fentanyl, and cocaine rats for the first 20 bar presses versus the rest of the session during the last session of FR1. **H.** Group average of the rolling averages of bar press durations of sucrose, fentanyl, and

Figure 2.1: Conditions that increase average bar press durations during maintenance responding.

cocaine rats during the last session FR1. Fentanyl rats that had no points for the rolling average during the rest of the session were excluded from the data analysis **I.** Rolling average durations of representative sucrose Rat 112, fentanyl Rat 12, and cocaine Rat 1 during the last session of FR1. **J.** Average bar press durations of cocaine rats for each dose self-administered (descending order) during within-session dose-response (WSDR). **K.** Group average of the rolling averages of cocaine rats during WSDR for doses 0.125, 0.06, 0.03 mg/kg/inf. Doses 0.5 and 0.25 mg/kg/inf. were excluded since there were too few data points for the rolling average. **L.** Rolling average bar press durations of representative cocaine Rat 1 during the WSDR session.

Extinction responding

Rats demonstrate a significant increase in average bar press durations during extinction compared to the previous reinforced responding, using either within- or between-session procedures ($t(18) = -6.983$, $p < 0.001$, Figure 2.2 A; $t(14) = -4.6$, $p < 0.001$, Figure 2.2 D; $t(8) = -8.358$, $p < 0.001$, Figure 2.2 G; $t(10) = -5.343$, $p < 0.001$, Figure 2.2 J). Using representative rats, we show that the rolling average gradually increases across the extinction portion of each of the session while the rolling average remains consistent across the previous reinforced responding (Figure 2.2 C, F, I, and L). This is also shown with group average of the rolling average (Figure 2.2 B, E, H, and K).

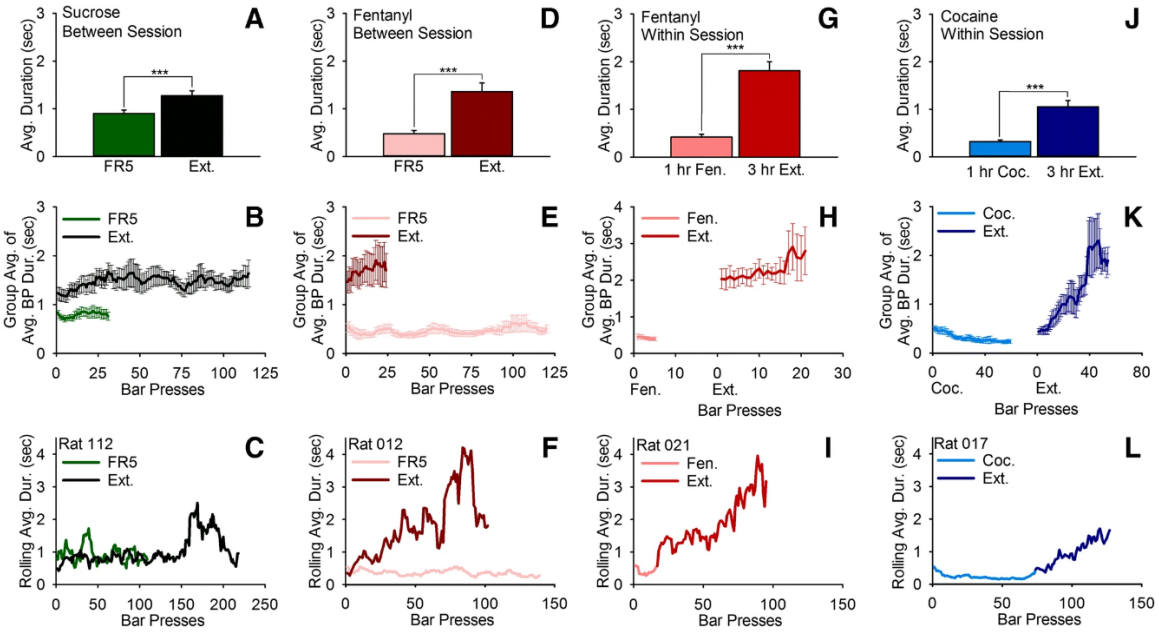


Figure 2.2: Extinction increases average bar press durations.

A. Average bar press durations (seconds ± SEM) of sucrose rats during between-session cued extinction and the prior session of FR5. **B.** Group average (seconds ± SEM) of the rolling averages of bar press durations (10 presses) for sucrose rats during between-session cued extinction and the prior session of FR5. **C.** Rolling average bar press durations of representative sucrose Rat 112 during between-session cued extinction and the prior session of FR5. **D.** Average bar press durations of fentanyl rats during between-session cued extinction and the prior session of FR5. **E.** Group average of the rolling averages of bar press durations for fentanyl rats during between-session cued extinction and the prior session of FR5. **F.** Rolling average bar press durations of representative fentanyl Rat 12 during between-session cued extinction and the prior session of FR5. **G.** Average bar press durations of fentanyl rats during within-session cued extinction. **H.** Group average of the rolling averages of bar press durations for fentanyl rats during within-session cued extinction. **I.** Rolling average bar press durations of representative fentanyl Rat 21 during within-session cued extinction. **J.** Average bar press durations of cocaine rats during within-session cued extinction. **K.** Group average of the rolling averages of bar press

Figure 2.2: Extinction increases average bar press durations.

durations for cocaine rats during within-session cued extinction. **L.** Rolling average bar press durations of representative cocaine Rat 17 during within-session cued extinction.

PR responding

Average bar press durations are significantly higher for the PR schedule than for FR schedules (sucrose, $t(18) = -8.535$, $p < 0.001$; fentanyl, $t(13) = -2.868$, $p = 0.013$; cocaine 0.125 mg/kg/inf., $t(11) = -5.376$, $p < 0.001$; cocaine 0.5 mg/kg/inf., $t(12) = -4.718$, $p < 0.001$; data not shown). Cumulative records of representative rats show an increased number of long bar presses as rats approach breakpoint (Figure 2.3 B, E, and H; Appendix A Supplemental Figures for Chapter 2: Figures 3, 7 and 10). As rats must work harder to achieve a reinforcer, rolling averages show increases in durations before breaking for fentanyl and cocaine, but not consistently with sucrose (Figure 2.3 C, F, and I; Appendix A Supplemental Figures for Chapter 2: Figures 3, 7, and 10). This is confirmed with the group average of the rolling average for fentanyl and cocaine (Figure 2.3 A, D, and G). Data from FR1 responding are included in panels A, D, and G merely for comparison purposes.

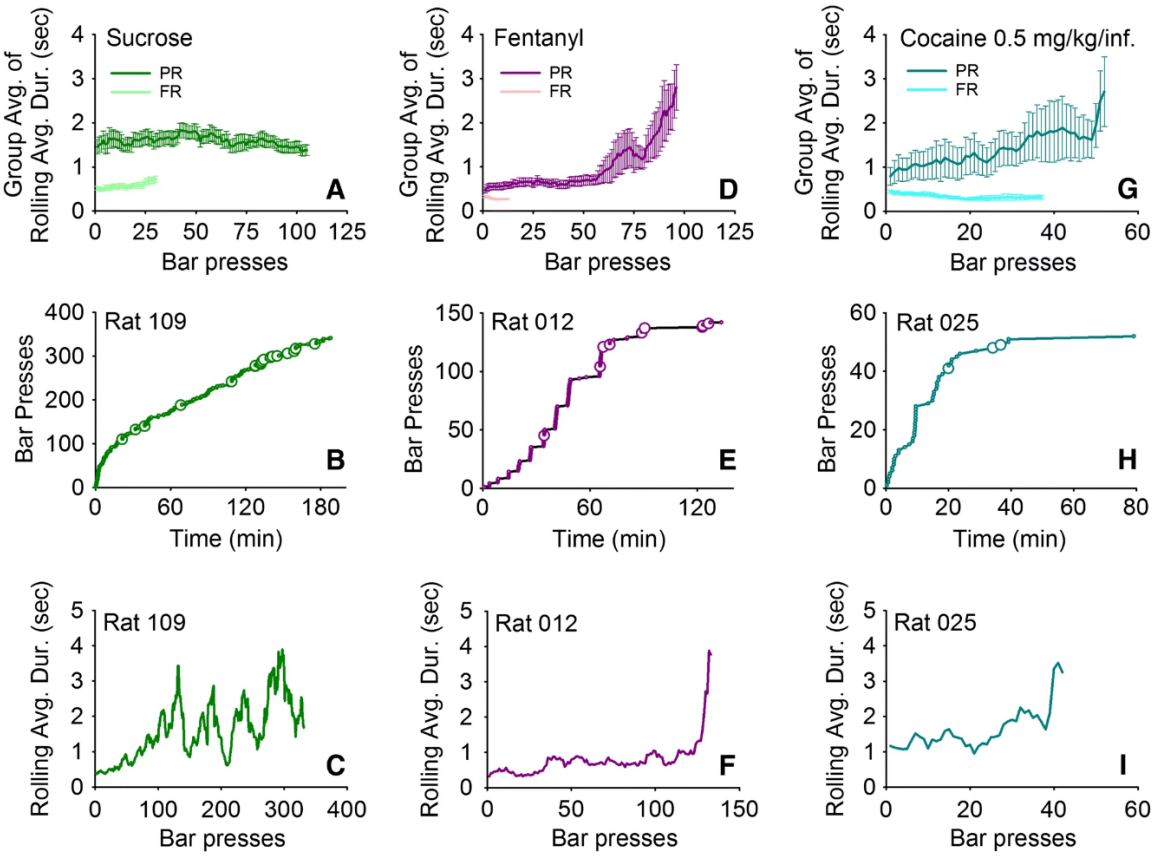


Figure 2.3: Progressive ratio increases bar press durations.

A. Group average (seconds ± SEM) of the rolling averages of bar press durations (10 presses) for sucrose rats during PR. **B.** Cumulative record (bar presses over time) for sucrose PR where bar press durations greater than 5s are represented as the larger circles of representative sucrose Rat 109. **C.** Rolling average bar press durations of representative sucrose Rat 109. **D.** Group average of the rolling averages of bar press durations for fentanyl rats during PR. **E.** Cumulative record for fentanyl PR where bar press durations greater than 2s are represented as larger circles of representative fentanyl Rat 12. **F.** Rolling average bar press durations of representative fentanyl Rat 12. **G.** Group average of the rolling averages of bar press durations for cocaine rats during high-dose PR. **H.** Cumulative record for high-dose PR cocaine where bar press durations greater than 5s are represented as the larger circles of representative cocaine Rat 25. **I.** Rolling average bar press durations of representative cocaine Rat 25 Fentanyl escalation.

Fentanyl escalation

Two rats self-administering fentanyl escalated intake across maintenance sessions (FR1; Figure 2.4 D). Interestingly, those two rats had the longest average bar press durations (Figure 2.4 E) and had spikes in the rolling average of long bar press durations demonstrated in Figure 2.4 F. There were no sucrose or cocaine self-administration rats that demonstrated this escalation (Figure 2.4 A, B, C, G, H, and I).

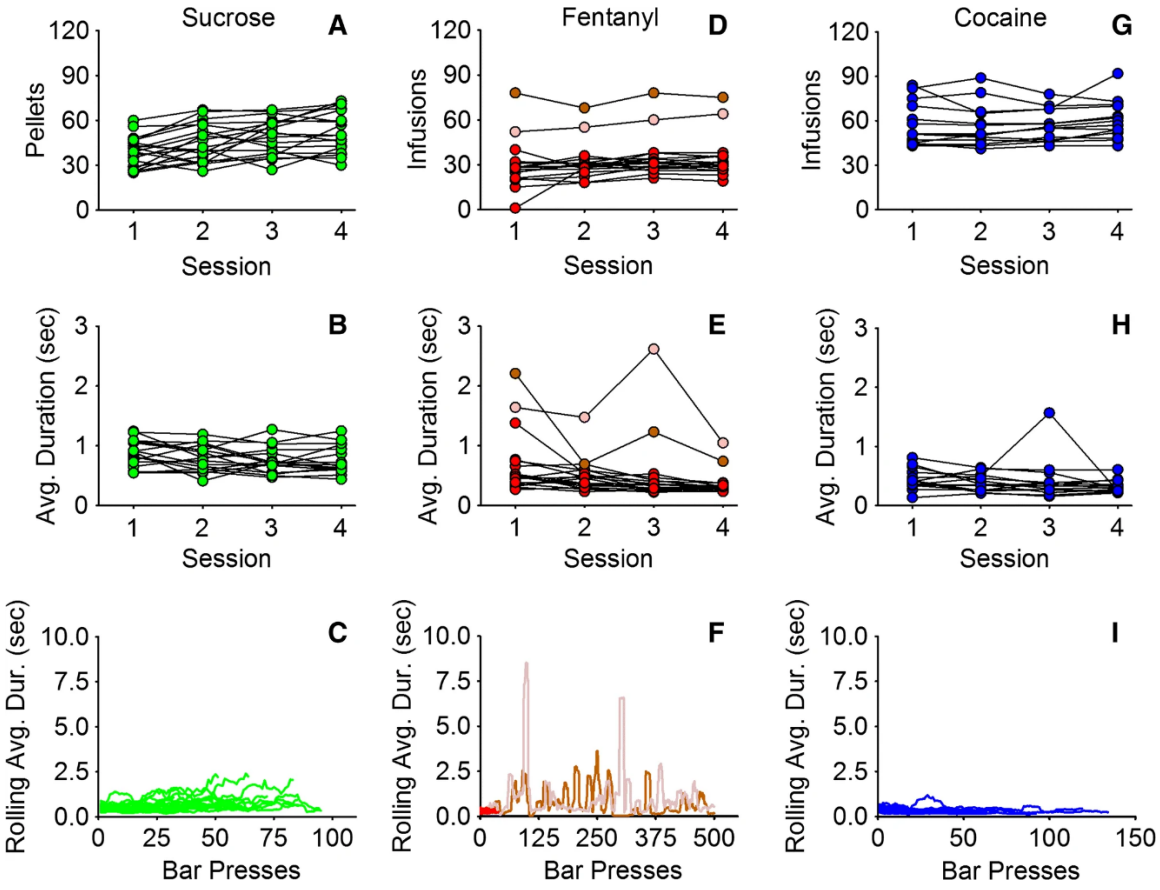


Figure 2.4: Fentanyl escalation increases bar press durations.

A. Number of pellets for each sucrose rat during the last four sessions of FR1. **B.** Average bar press durations for each sucrose rat during the last four sessions of FR1. **C.** Rolling average durations (10 presses) for each sucrose rat during the last session of FR1. **D.** Number of infusions for each fentanyl rat during the last four sessions of FR1. **E.** Average bar press durations for each fentanyl rat during the last four sessions of FR1. **F.** Rolling average durations for each fentanyl rat during the last sessions of FR1. **G.** Number of infusions for each cocaine rat during the last four sessions of FR1. **H.** Average bar press durations for each cocaine rat self-administering during the last four sessions of FR1. **I.** Rolling average durations for each cocaine rat during the last four sessions of FR1.

Bar press durations vs. Number of bar presses

The hypothesis of this project is that duration of bar presses is a measure of frustration-related behavior that is distinct from the number of bar presses (i.e., taking or seeking). To determine if durations are isometric with bar presses, we determined correlations under maintenance responding (taking), extinction, and reinstatement (seeking). There were no statistically significant correlations in cocaine self-administration, and FR1 maintenance, extinction, and progressive ratio for sucrose and fentanyl($R = 0.283$, $p = 0.241$, Figure 2.5 A; $R = 0.417$, $p = 0.076$, Figure 2.5 C; $R = 0.088$, $p = 0.720$, Figure 2.5 D; $R = 0.110$, $p = 0.685$, Figure 2.5 E; $R = 0.300$, $p = 0.278$, Figure 2.5 G; $R = 0.290$, $p = 0.337$, Figure 2.5 H; $R = 0.101$, $p = 0.731$, Figure 2.5 I; $R = 0.021$, $p = 0.930$, Figure 2.5 J; $R = 0.563$, $p = 0.071$, Figure 2.5 K; $R = 0.166$, $p = 0.588$, Figure 2.5 L). Number of bar presses negatively correlated with average bar press durations in FR5 maintenance for both sucrose and fentanyl ($R = 0.461$, $p = 0.047$, Figure 2.5 B; $R = 0.579$, $p = 0.024$, Figure 2.5 F). This indicates that number of bar presses are not equivalent with the average bar press duration. Thus, duration is an independent measurement that represents a behavioral construct distinct from craving, which is typically measured with number of bar presses.

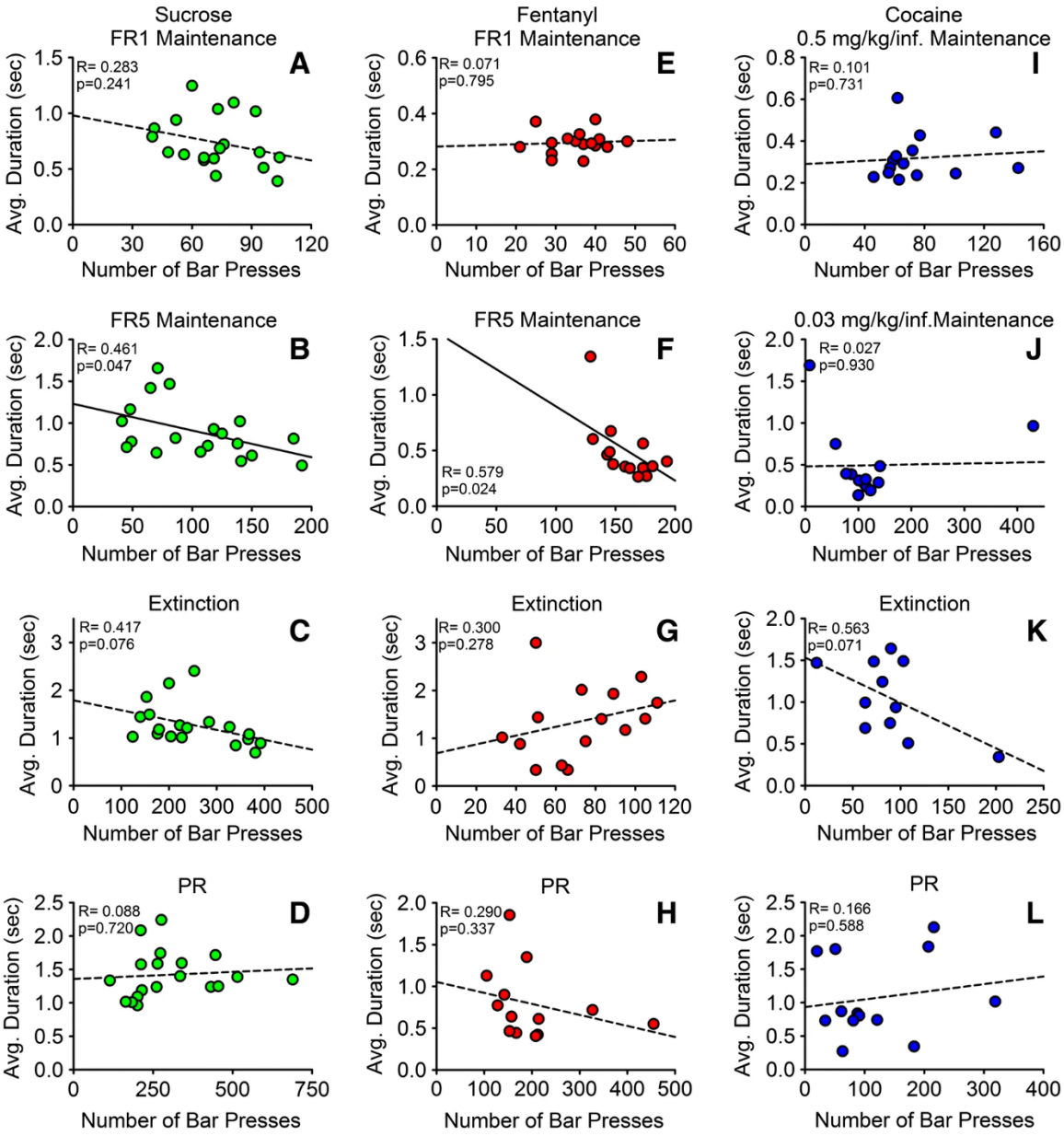


Figure 2.5: Number of bar presses does not correlate with bar press durations.

A. Simple linear regression was assessed to investigate the relationship between number of bar presses vs. average bar press durations of sucrose rats during the last session of FR1. **B.** Number of bar presses vs. average bar press durations for sucrose rats during the last session of FR5. **C.** Number of bar presses vs. average bar press durations for sucrose rats during between-session cued extinction. **D.** Number of bar presses vs. average bar press durations for sucrose rats during PR. **E.** Number of bar presses vs. average bar press

Figure 2.5: Number of bar presses does not correlate with bar press durations.

durations of fentanyl rats during the last session of FR1. **F.** Number of bar presses vs. average bar press durations for fentanyl rats during the last session of FR5. **G.** Number of bar presses vs. average bar press durations for fentanyl rats during the between-session cued extinction. **H.** Number of bar presses vs. average bar press durations for fentanyl rats during PR. **I.** Number of bar presses vs. average bar press durations of cocaine rats during last session of FR1 at 0.5 mg/kg/inf. **J.** Number of bar presses vs. average bar press durations for cocaine rats during the WSDR for dose 0.03 mg/kg/inf. **K.** Number of bar presses vs. average bar press durations for cocaine during within-session cued extinction. **L.** Number of bar presses vs. average bar press durations for cocaine during PR.

DISCUSSION

Frustration arises from the perceived resistance to the fulfillment of one's will/goal: when a goal is more difficult to achieve (e.g., increasing FR values and progressive ratio) or when that goal is denied or blocked (e.g., extinction). In the case of rat intravenous drug self-administration, the goal is achieving or maintaining desired brain levels of the drug. In the case of sucrose pellet self-administration, the goal is both hunger and hedonic satiation. Our results offer strong evidence that bar press durations are a representative measure of frustration-related behavior. We found that rats lengthened bar presses at any time brain levels of fentanyl or cocaine were below satiety: increases in the FR requirement, during extinction, and prior to terminating responding in progressive ratio (for fentanyl and cocaine). Since the overall increase in bar press durations is observed in self-administration of three different reinforcers, this suggests that this measure can be generalized to many other reinforcers. Cocaine additionally produced increased durations during the loading phase of a session as rats were building up to satiety levels, and fentanyl-escalating rats also exhibited a profound increase in durations.

When looking at rolling averages, a clear picture emerged as average durations increased throughout the session when rats underwent an extinction protocol, yet in most rats the durations spiked either shortly or immediately before breaking during PR testing for fentanyl or cocaine. However, during PR testing, there were some fentanyl or cocaine rats that showed a general rise in durations across the session (similar to extinction) rather than a spike just before breaking (Appendix A Supplemental Figures for Chapter 2: Figures 5-7, 9 and 10). Regardless, every rat showed elevated average durations in PR compared to average durations for FR responding. It should be noted that for sucrose PR responding, rats displayed longer average bar press durations than FR but there was no spike just before breaking. This is likely due to a fundamental difference in reinforcers (sucrose vs. drug) and may represent a species-typical response. Rats are by nature foraging creatures, and

when hungry, exhausting a local food supply (e.g., eating all of the seeds from under a particular bush) is the successful fulfillment of a goal rather than a frustrating event. Thus, breakpoint for sucrose pellets may be seen by rats as successfully exhausting a food supply and thus not a frustrating event. However, more research would be needed to draw firm conclusions.

Rats increase average bar press durations in each instance where they meet the above definition of frustration. Importantly, the definition of frustration is different from craving or wanting (measured by number of presses), so the question must be asked if longer bar press durations are merely another measure of craving proportional to number of presses. Figure 2.5 shows that there are no significant positive correlations between number of responses and average durations in any of our experiments, suggesting that these two constructs are not isometric, even though durations can only be measured while a rat is pressing. In a couple of cases (FR5 sucrose and fentanyl self-administration), there is a significant negative correlation between number and duration, but never a positive correlation. This too suggests that these measures are not isometric. It should be noted, however, that appropriate data for power analyses for durations are lacking, so the lack of significant correlation in the other conditions may be underpowered to definitively make any absolute claims.

Interestingly, as the FR requirement for fentanyl self-administration increased the durations also increased, but repeated sessions of FR5 showed a waning as the rats habituated to the new schedule across sessions.

We observed an increase in bar press durations when the rats were required to perform more responses throughout the session in order to obtain the reinforcer (i.e., PR) but relatively less so for decreasing doses within a session, which also necessitates more responses for the same amount of drug but reinforced with every response. However, rats were able to maintain satiety drug levels throughout the within-session dose-response whereas rats do not maintain satiety levels under PR.

It is unclear at this point why cocaine self-administration produces an increase in durations during the loading phase of self-administration, yet fentanyl did not (Figure 2.2 E and F). The cocaine loading effect was mirrored in the within-session dose-response as well (Figure 2.2 G and H). This could be idiosyncratic to the class of drug (pure stimulant vs. opioid) or to the relative reinforcing effect of the doses chosen.

Perhaps the most interesting finding is that the two rats escalating fentanyl self-administration displayed a high number of long bar presses (i.e., increased durations), which would suggest that these rats, despite extreme intake, are still unable to reach satiation. This is supported by the number of presses during the 20s timeout period during/after the infusion, which are rare in non-escalating rats (i.e., a few up to < 25 per session) but reach 200 or more per session in the two escalating rats. We consistently find that 10–15% of rats escalate responding in this way, which compares well to the 8–12% of humans who develop opioid use disorders (Vowles et al., 2015).

The current procedures can expand upon the rich history of the study of frustration behavior in runways. For example, in 1951, Amsel and Roussel tested the hypothesis that frustration is a motivational state, and that the magnitude of frustration would increase with the duration of time in the frustrating situation (Amsel & Roussel, 1952). They found no evidence that frustration varied with time in the condition, but the runway task only studied a limited number of trials and the time varied from 5 to 30s in the frustrating condition. Our results show that bar press durations progressively increase across time in the extinction sessions, on the order of several minutes to a few hours in the frustrating condition.

Although Capaldi does not address frustration specifically in his sequential hypothesis of instrumental learning (Capaldi, 1967), our extinction data fit with his idea of non-reinforced memory state (MN). While Capaldi hypothesized that subjects could track or indeed “count” the number of non-reinforced trials in a theoretical measure of salience

of MN, our extinction data can act as a rough quantification of such (Capaldi & Miller, 1988). However, more research would be needed to fully validate this assertion.

Our primary conclusion is that bar press durations are a good surrogate measure of frustration in rat operant responding. This measure provides a functional readout during drug taking as well as drug seeking. Future studies will determine exactly what role frustration plays in substance use disorders, but for the time being, we know that increased bar press durations signify that a rat's goals (drug delivery) are not being met and that a spike in bar press durations can predict extinction of drug seeking. Thus, this manuscript adds frustration as a 4th major facet of addiction-related behavior, adding to craving, impulsivity, and habit.

End of published manuscript Vasquez et al (2021), Psychopharmacology 10.1007/s00213-020-05742-2.

In relation to the frustration response strategies, increases in bar press durations is a continued approach strategy of response modification. During operant self-administration, when a rat was confronted by a situation where the reinforcement had been denied or made more difficult to achieve, there were subsequent increases in bar press durations. Additionally, the rewarded response within our operant chambers is bar pressing and increases in bar press durations were a quantifiable modification to that rewarded response. Accordingly, increases in bar press durations are a continued approach response to frustration that met the previously stated requirements of a real-time, easily quantifiable, and objective measure of frustration-related behavior.

While bar press durations provided a measure of the approach response to frustration it should still provide insight into the employment of the alternative strategy: avoidance. Should the behaviors of each strategy be in direct conflict, there will be a decrease in one strategy and an increase in the other. For example, when responding

initially starts high when the goal is still perceived as attainable but after repeated exposure to additional nonrewarded trials the responding decreases (Daly, 1974). Thus, demonstrating a transition from a continued approach response to an increase in the avoidance strategy. Alternatively, if the behaviors are not in direct conflict, one response strategy will not always preclude the other. Such as an increase in response variation concurrent with a decrease in overall responding during extinction (Wong, 1979). Thus, it is possible to observe transitions between or simultaneous increases in approach and avoidance strategies. Furthermore, should termination of responding be considered a viable measure of the avoidance response strategy to frustration in operant self-administration, then the spikes in bar press durations observed towards the end of extinction and progressive ratio sessions may be signaling a transition from the approach strategy to the avoidance strategy (Appendix A Supplemental Figures for Chapter 2: Figures 2, 3, 5-7, 9, 10). The reasoning is that the spikes in bar press durations are occurring contiguously to terminating responding for the reinforcer during extinction and progressive ratio self-administration. Overall, increases in bar press durations are an approach strategy that can be used to objectively measure frustration-related behavior and, in some conditions, has the potential to signal a future transition to avoidance strategies.

Chapter 3 Individual Differences in Frustrative Nonreward Behavior for Sucrose in Rats Predict Motivation for Fentanyl Under Progressive Ratio

There is a need to develop a model to objectively identify frustration intolerance prior to substance use to study vulnerability to substance use disorders. The previous chapter established that bar press durations have been shown to have the advantages of being a real-time, easily quantifiable, and objective measure of frustration-related behavior. Furthermore, the previous chapter revealed that rats with high levels of responding for the drug fentanyl also have the highest bar press durations (Figure 2.4). These findings provide preliminary evidence of the concept that individual differences in frustration levels are related to substance use behavior. However, in order for bar press durations to be predictive of drug taking or seeking, the individual differences in frustration levels needs to be observed and measured prior to drug exposure. Thus, the goal is to demonstrate that frustration-like behavior as measured by bar press durations for sucrose possesses the ability to predict future drug seeking and taking behavior.

This chapter contains information from the published in manuscript, Individual Differences in Frustrative Nonreward Behavior for Sucrose in Rats Predict Motivation for Fentanyl Under Progressive Ratio, Tileena E. S. Vasquez, Poonam Shah, Jessica Di Re, Fernanda Laezza, and Thomas A. Green (2021), eNeuro 10.1523/ENEURO.0136-21.2021.

INTRODUCTION

Historically, animal models of substance use disorders have focused on facets such as craving, impulsivity, or habit. We propose an animal model to study another facet of substance use disorder-related behavior: that of frustration. Previous research showed that

rats increase lever-press durations under conditions of frustration for drug or sucrose reward (Vasquez, McAuley, et al., 2021). Our data show that this measure of frustration is a robust, replicable, and sensitive surrogate for frustration behavior.

While there is little research into the role of frustration in substance use disorders specifically, a few studies highlighted that persons with substance use disorders rate higher in tests of frustration and that sensitivity to frustration correlates with number of relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). The limited number of rodent models generally support the human research, demonstrating frustration from loss of an alternative food reinforcer increases drug seeking and taking (Ginsburg & Lamb, 2018; Gipson et al., 2012; Podlesnik et al., 2006; Pyszczynski & Shahan, 2013; Quick et al., 2011), likely an expression of negative urgency (Gipson et al., 2012).

Moreover, previous studies that exclusively focused on frustration used speeds running down a runway as a measure of frustration behavior by utilizing food solely as the reinforcement (Adelman & Maatsch, 1955; Amsel & Roussel, 1952; Capaldi, 1974). Additional studies investigated arousal of frustration and associated cues in nonreinforced or noncontinuous reinforcement conditions, referring to these conditions as instances of nonreward (Amsel, 1958; Daly, 1974). The current project expands upon these techniques with a lever-press operant response procedure to study the effects of frustration in drug self-administration.

In addition to substance use disorders, frustration is a significant component of many other neuropsychiatric conditions ranging from conduct disorder to personality disorders to mood disorders (Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017). The US National Institute of Mental Health currently classifies frustrative nonreward (FN) as a construct in the Negative Valence Systems domain of the Research Domain Criteria (RDoC) framework. An organism's appropriate response to a frustrating situation (i.e. being unable or having to work harder to fulfill a goal) is an important aspect of normal

behavior, and inappropriate responses to frustration can be a component of a neuropsychiatric condition.

The objective of this project was to develop a FN operant task, based loosely on the human point subtraction aggression paradigm (PSAP), that can be used as a tool to identify rat's individual differences in frustration-like behavior during self-administration of sucrose pellets with the hypothesis that those individual differences can predict a rat's drug seeking or taking prior to exposure to the drug. The human PSAP is a validated behavioral measure of aggression in response to perceived provocation and (Cherek, Moeller, Dougherty, et al., 1997; Cherek, Moeller, Schnapp, et al., 1997) subjects had the option to respond in one of three ways to obtain points: the first option was to continue normal responding to earn points (nonaggressive responding), the second option was to subtract points from a fictious person to add to their own score (retaliation/aggressive responding), and the last option was to protect points from being subtracted (escape). Rats are incapable of comprehending instructions of a conspecific in the next cage stealing points, but it has been shown that rats are capable of knowing how close they are to receiving a reinforcer through conditioning of reward expectancy (Amsel, 1958; Daly, 1974).

The current study is the first lever-press operant-based paradigm for quantifying FN in rats. Herein we show that the task is consistent at baseline across days, responsive to reward size, and that low, medium, and high frustration behavior for sucrose reward predict early breaking on a progressive ratio schedule for intravenous fentanyl. The breakpoint during progressive ratio is when the response output falls below a predefined level and is commonly used to evaluate the reinforcing efficacy of abused drugs (Cain & Bardo, 2010). Breakpoint is generally defined as the last ratio in effect when the rat fails to meet the response output requirements for that ratio. However, our data utilize an alternative measure of breakpoint: the total number of reinforcing events during the session, which is slightly different than the last ratio (D. C. S. Roberts, 2010).

Additionally, prior research shows that manipulations of retinoic acid signaling alter drug taking and seeking (Crofton et al., 2021; Zhang et al., 2016), thus we hypothesized that overexpression of retinoic acid receptor beta in the nucleus accumbens shell would alter FN and/or fentanyl taking/seeking, but this hypothesis was not supported by the data.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague-Dawley rats were obtained from Envigo at 225-250g. Except during food regulation, rats were pair-housed throughout the experiments and maintained in a controlled environment (temperature, 22°C; relative humidity, 50%; and 12 h light/dark cycle, lights on 0600 h) in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Procedures were approved by the UTMB Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals.

Sucrose operant responding: Initial training

Rats (n=20) were initially placed on a regulated intake diet for 6 days until rats reached 85% of free-feed body weight. Rats were then placed in operant chambers where achieving the response requirement on the active lever resulted in the extinguishing of the house light, the illumination of two circular cues lights located above the levers for 5 seconds, delivery of a banana-flavored sucrose pellet (45 mg; Bio-Serv). The illumination of the two cue lights signaling the delivery of the reinforcer also serve to signal a time-out during which responding during the 5 seconds was recorded but animals could not earn more sucrose. Throughout the session, responding on the inactive lever was recorded but had no consequences.

FR RESPONDING

During the first session rats were allowed to perform a single lever press (FR1) to receive sucrose pellets for 2 hours with an unconditional pellet provided every 10 minutes until they self-administered 100 pellets. Rats that showed slower learning (e.g., did not reach 100 pellets) were placed in the operant chambers again until they could self-administer a combined 100 pellets across the sessions. Next, rats were placed on an FR1 schedule for 15 minutes and after 4 sessions on FR1, the response requirement for the next session was increased to FR3 (three lever presses to receive the reinforcer), followed by an increase to FR5 the subsequent 4 sessions.

CUED EXTINCTION

The protocol consisted of 2 hours with cue lights delivered under the normal FR5 schedule but no sucrose pellet delivery. The extinction session was immediately followed by 15 minutes of maintenance sucrose self-administration at an FR1 schedule to prevent extinction from affecting the next session.

PROGRESSIVE RATIO

For the next 3 sessions the rats were placed on a progressive ratio (PR) schedule in which each successive reinforcement required an increasing number of lever-press responses according to the following semi-logarithmic progression, 1, 2, 4, 6, 9, 12, 15, 20, etc. The session continued until the rats went 1 hour without obtaining a reinforcement, or up to a maximum of 6 hours.

The frustrative nonreward sucrose task

The FN task is an operant lever-press procedure based loosely on the point subtraction aggression paradigm (PSAP) in humans (Cherek, Moeller, Dougherty, et al., 1997; Cherek, Moeller, Schnapp, et al., 1997). The training procedure consists of a

compound schedule where the two cue lights and the house light are illuminated at the beginning of the trial, and rats press the active lever 2 times to turn off the left cue light (leaving two lights on), 2 more times to turn off the right cue light, 2 more to turn off the house light, and 2 more for delivery of the reward (FN8 = 8 presses for a reward), when all lights are again illuminated and the rat can begin the next trial. There was no time-out, and the next trial began immediately, therefore there was no illumination of the two circular cue lights concurrent with the delivery of the reinforcement. Each bar press during a trial was recorded as a point to be added to the rats score for the trial. Once the rat achieved the required score (e.g. FN8 required a score of 8 points per trial) the points were reset for the next trial. Thus, the more points the rat has, the less light in the chamber. For training, each session the number of presses was incremented until 5 points per step (FN12, FN16, and then FN20). Thus, the FN20 procedure requires 20 lever presses per reward (FN20 no frustration; 5 presses to turn off each light). Light cue contingencies for the FN task were different from the FR tasks (sucrose and drug) to help the rats discriminate between the two tasks. For data analysis, 5 rats were removed from the study due to non-acquisition of the FN task.

DETERMINING SENSITIVITY TO REWARD MAGNITUDE

Rats were first given "FN20 no frustration" throughout the first session with only 1 pellet per trial. For the next session, the first 5 trials were 1 pellet and the subsequent trials were 4 pellets (incentive upshift). The next session was 4 pellets per trial throughout, and the last session was 4 pellets for the first 5 trials and 1 pellet for all remaining trials (incentive downshift). Only data from after the 6th trial were analyzed.

ADDING THE FRUSTRATION COMPONENT

To introduce a frustration element, when the rat presses for the 18th point of the FN20, instead of incrementing the score by 1 point, the computer can deduct 7 points as

programmed, bringing the point level from 17 to 10, turning on the house light to signal deducted points. The rat must continue to press to make up the lost points. For the "FN20 low frustration" condition, 7 points are deducted every other trial (i.e. 27 presses for the deduction trial). For the "FN20 medium frustration" condition, 7 points are deducted twice every other trial (i.e. 34 responses for deduction trial). For the "FN20 high frustration" condition, 7 points are deducted three times each trial (41 responses for every trial), and the "FN20 extreme frustration deducts 7 points 26 times for each trial, requiring 202 presses for each reward. One session of "FN20 no frustration" intervened between each of the FN20 Low, Medium, and High condition to maintain stable responding. A "frustration score" was calculated as the average lever press duration of each frustration session (FN Low, Medium, High, and Extreme) divided by the average lever press duration of that subject's "no frustration" condition (FN20 none).

Fentanyl operant responding

After one week of free feed in the colony room, rats were injected bilaterally into the nucleus accumbens shell (shNAc) with 1 μL of adeno-associated viral vector expressing GFP or one expressing GFP plus the retinoic acid receptor beta (RARβ). Coordinates were AP=1.3, L = 2.4 from bregma and DV = -6.7 mm from dura (Zhang et al., 2014). The shNAc was targeted as the repetitive activation of the shNAc by drugs of abuse results in strengthening of stimulus-reward and stimulus-response associations (Di Chiara, 1998, 2002; di Chiara et al., 2004). Additionally, retinoic acid signaling is the most enhanced shNAc pathway with RARβ being one transcript that was identified as a strong target (Crofton et al., 2021; Zhang et al., 2016). It is also suggested that the shNAc plays a role in goal-oriented behavior (Mannella et al., 2013); and it is generally understood that the experience of frustration occurs when the goal is denied or made more difficult to achieve. Therefore, the original hypothesis was that decreasing RARβ in the shNAc would alter drug seeking and frustration behavior. However, this hypothesis was not supported by

the data as there was no significant effect on fentanyl self-administration behavior. Thus, when analyzing these data for number of infusions and bar press durations the animals were collapsed into one group.

After one week of recovery, rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg, i.p.) and implanted with indwelling intrajugular silastic catheters as described previously (Crofton et al., 2017; Zhang et al., 2016). To maintain catheter patency, catheters were flushed daily with 0.1 ml of heparinized (10 U/ml) saline with ticarcillin (0.067g/ml). Following 1-week recovery from catheter surgery, animals were placed in the operant chambers to self-administer fentanyl HCl (0.0032 mg/kg/infusion; NIDA Drug Supply Program).

FR RESPONDING

Animals began fentanyl self-administration on a continuous schedule (FR1) of reinforcement until they were responding consistently for 4 days (greater than 10 infusions per session and less than a difference of 10 infusion variability in daily intake). Each session lasted 3 hours where a single response on the active lever resulted in a 0.1 ml intravenous infusion delivered over 5.8 seconds, concurrent with the illumination of two circular cues lights located above the levers. Each infusion was followed by a 20s time-out period during which the cue lights remained illuminated, the house light was extinguished, and responding was recorded but animals could not earn more fentanyl. The cued time-out period was extended to 20s from the 5s during sucrose operant responding to prevent rats from potentially overdosing. Throughout the session, responding on the inactive lever was recorded but had no consequences.

CUED EXTINCTION

After stabilization on FR1, the response requirement for the next three sessions was a between-session cued extinction procedure consisting of 3 hours with cue lights was

delivered under the normal FR1 schedule but with no drug delivery. To prevent full withdrawal, the extinction session was immediately followed by 1 hour of maintenance fentanyl self-administration at an FR1 schedule.

PROGRESSIVE RATIO

The next 3 sessions the rats were placed on a progressive ratio (PR) schedule in which each successive fentanyl injection (0.0032 mg/kg/inf) required an increasing number of lever-press responses according to the following semi-logarithmic progression, 1, 2, 4, 6, 9, 12, 15, 20, etc (Green et al., 2002). The session continued until the rats went 1 hour without obtaining a reinforcer, or up to a maximum of 6 hours.

Statistical analysis of behavior

For estimation based on confidence intervals (CIs), we directly introduced the raw data in https://www.estimationstats.com/ and downloaded the results and graphs for the permutation t-tests in which 5000 bootstrap samples were taken; the confidence interval is bias-corrected and accelerated (Ho et al., 2019; Manouze et al., 2019). The P values reported are the likelihoods of observing the effect sizes, if the null hypothesis of zero difference is true. For each permutation P value, 5000 reshuffles of the control and test labels were performed. The effect sizes and CIs are as: effect size [CI width lower bound; upper bound]. Simple linear regression was used to assess correlations. The alpha level was set at $p<0.05$. There was no correction for multiple comparisons. Rats not completing a given experiment were not considered in that analysis. Frustrative nonreward data analysis, 5 rats were removed from due to non-acquisition, making the N for this analysis 15. Additionally, 1 rat was removed from fentanyl self-administration analysis for unstable responding during acquisition, making the final N for the study 14.

RESULTS

Consistency of FN20 Responding

There were strong positive correlations when comparing among session average lever press durations during training and stabilization sessions for FN responding with no frustration trials (Figure 3.1A). A representative scatterplot of FN20 DAY4 vs FN20 DAY6 is shown in Figure 3.1B ($R=0.784$, $p=0.001$). This demonstrates that responding is surprisingly stable across FN no frustration sessions. The exceptions were from comparisons from early training or those that had the greatest number of intervening days (e.g. FN12 DAY2 vs FN20 DAY14, $R=0.16$, $p=0.590$). Active:inactive lever press ratio was >10:1 for all rats.

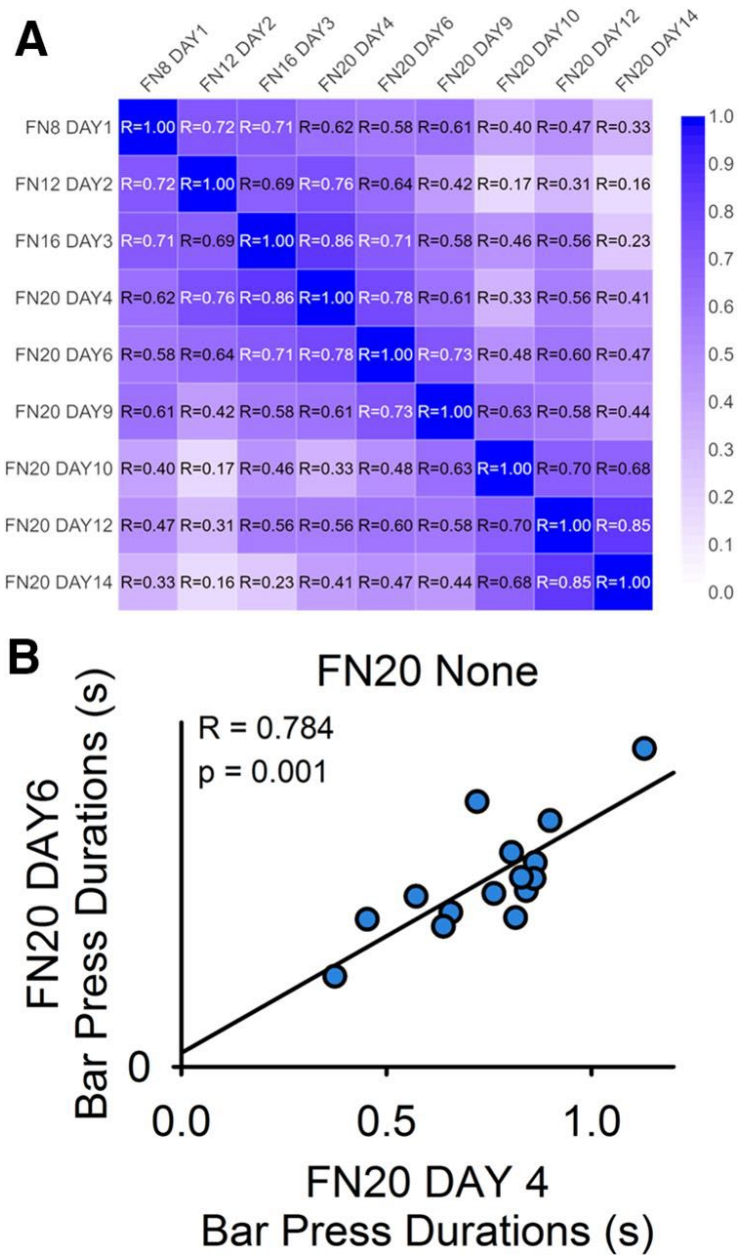


Figure 3.1: Frustration level is consistent across FN sessions.

A. Correlation matrix with heat map of the correlation coefficients (R) from simple linear regression analyses to investigate average lever press durations compared across multiple sessions of FN training and stabilization (i.e., no frustration trials). Blue represents strong positive correlation and white represents no correlation. **B.** Representative simple linear regression analysis of average lever press durations during FN20 day 4 versus FN20 day 6.

Sensitivity to reward magnitude

Rats demonstrated a significant decrease in average lever press durations when the reward size was changed mid-session from 1 pellet to 4 pellets (incentive upshift) compared to the previous session of 1 pellet throughout. The paired mean difference between 1 pellet and upshift is -0.133 [95.0% CI -0.245, -0.044]. The P value of the two-sided permutation t-test is 0.0214 (Figure 3.2A). There was also a significant increase in average lever press durations when the reward size was changed mid-session from 4 pellets to 1 pellet (incentive downshift) compared to the previous session of 4 pellets throughout. The paired mean difference between 4 pellet and downshift is 0.164 [95.0% CI 0.098, 0.257]. The P value of the two-sided permutation t-test is 0.0002 (Figure 3.2B).

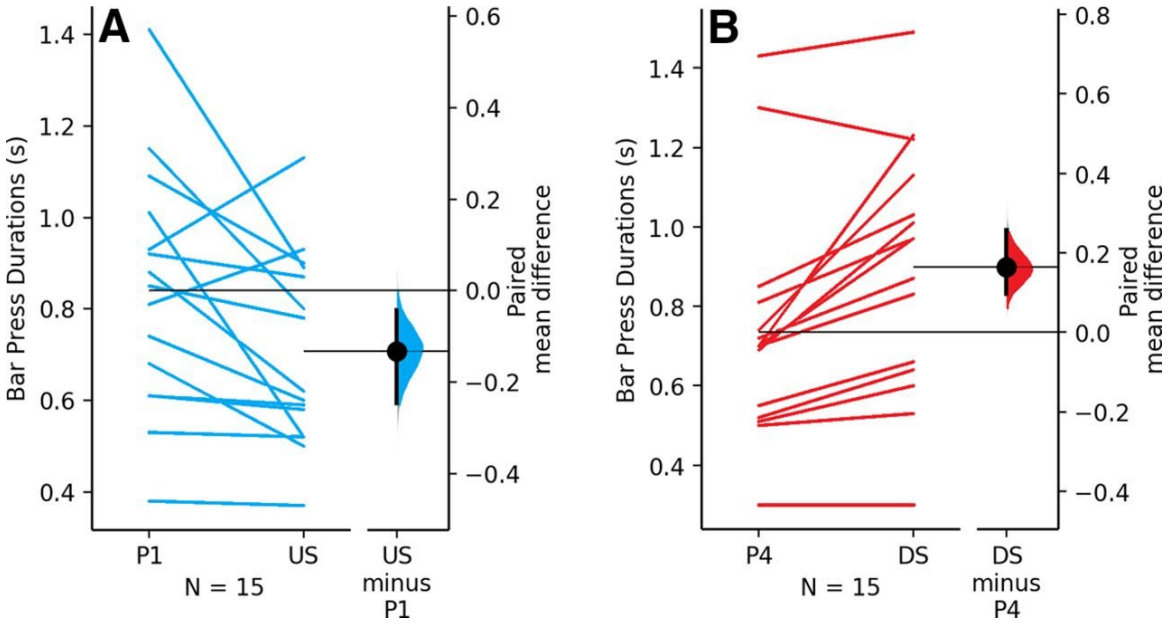


Figure 3.2: Frustration level is sensitive to reinforcer size.

A. The paired mean difference between average lever press durations (seconds) during the sucrose self-administration session for one pellet throughout the session (P1) and incentive upshift (one pellet for the first five reinforcers and four pellets for all subsequent reinforcers; US) is shown in the above Gardner–Altman estimation plot. Both groups are plotted on the left axes as a slope graph: each paired set of observations is connected by a line. The paired mean difference is plotted on a floating axes on the right as a bootstrap sampling distribution. The mean difference is depicted as a dot; the 95% CI is indicated by the ends of the vertical error bar. **B.** The paired mean difference between average lever press durations (seconds) for four pellet throughout the session (P4) and incentive downshift (four pellet for the first five reinforcers and one pellets for all subsequent reinforcers; DS) is shown in the above Gardner–Altman estimation plot.

Individual differences in sucrose FN frustration responding vs. PR fentanyl infusions

The goal of this project was to determine if seeking or taking of fentanyl could be predicted by an individual rat's frustration-like behavior for sucrose pellets prior to exposure to the drug. Thus, frustration scores for conditions of FN Low, Medium, High, and Extreme frustration were used to quantify each rat's frustration score, and these were compared to their number of reinforcements during fentanyl self-administration sessions. Frustration scores for sucrose Low, Medium, High, Extreme Frustrative Nonreward, Extinction, and Progressive Ratio were compared to the number of fentanyl reinforcements earned during FR1, Extinction, and Progressive Ratio. Of these comparisons there were only significant strong correlations for Low, Medium, and High Frustration Scores for Progressive Ratio fentanyl infusions (averaged across 3 sessions; Figure 3.3E). The statistically significant negative correlations of Low, Medium, and High Frustration Scores with average PR fentanyl infusions were $R = 0.561$, $p = 0.046$ for Low (Figure 3.3A), $R = 0.567$, $p = 0.043$ for Medium (Figure 3.3B) and $R = 0.576$, $p = 0.039$ for High (Figure 3.3C). Extreme Frustration scores, however, did not significantly correlate with average PR fentanyl infusions ($R = 0.162$, $p = 0.596$, Figure 3.3D). Additionally, Extinction and PR scores also did not significantly correlate with average PR fentanyl infusions (Extinction Score, $R = 0.187$, $p = 0.459$; PR Score, $R = 0.054$, $p = 0.832$, data not shown).

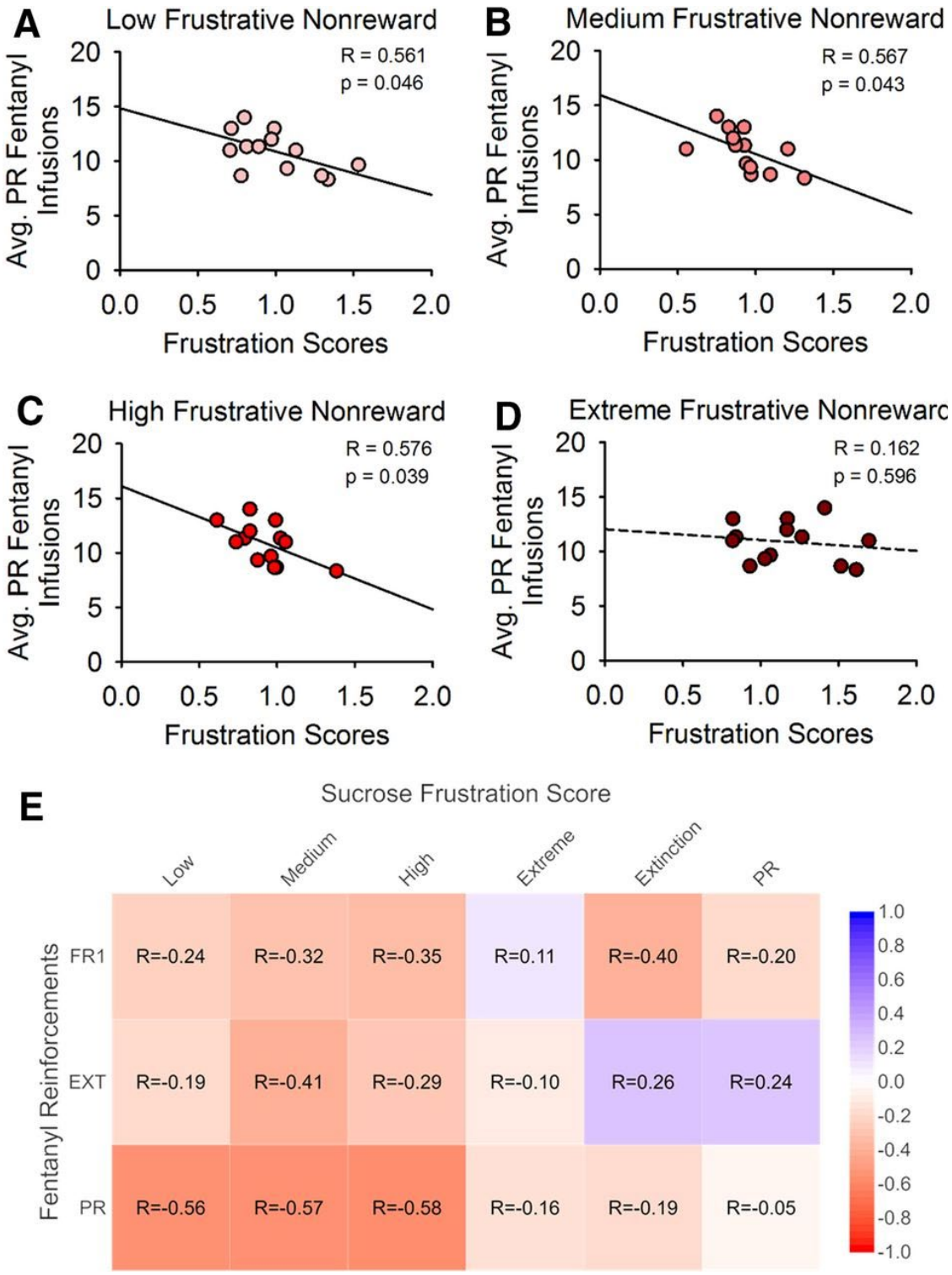


Figure 3.3: Frustration scores predict break point for PR.

A. Simple linear regression was used to investigate the relationship between frustration score during low FN sucrose self-administration and the average number of infusions

Figure 3.3: Frustration scores predict break point for PR.

during fentanyl PR. **B.** Relationship between frustration score during medium FN sucrose self-administration and the average number of infusions during fentanyl PR. **C.** Relationship between frustration score during high FN sucrose self-administration and the average number of infusions during fentanyl PR. **D.** Relationship between frustration score during extreme FN sucrose self-administration and the average number of infusions during fentanyl PR. **E.** Correlation matrix with a heat map of the correlation coefficients (R) from simple linear regression analyses to investigate relationship of frustration scores during the sucrose FN tasks, extinction, and PR with the average number of fentanyl infusions during FR1, EXT, and PR. Blue represents strong positive correlation, white represents no correlation, red represents strong negative correlation.

DISCUSSION

High frustration has been shown to predict an increased risk to develop anxiety, depression, substance abuse and thought disorders (Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017). This animal study expands upon the role of frustration in substance use disorders by creating a frustrative nonreward task constructed using the same concept as the human Point Subtraction Aggression Paradigm. The frustrative nonreward task demonstrates that individual differences in a rat's frustration level are consistent throughout baseline FN conditions and are sensitive to reward magnitude. Most interestingly, this study of frustrative nonreward demonstrates that individual differences in FN sucrose pellet self-administration can be used to predict a rat's motivation for intravenous fentanyl self-administration under a PR schedule. Accordingly, rats with higher frustration scores during Low, Medium, and High sucrose FN conditions (but not Extreme FN, Extinction, or PR for sucrose) obtain fewer infusions of fentanyl during progressive ratio. Interestingly, these data also demonstrate that in order to be able to predict a rat's intake of fentanyl during PR using frustration scores, the frustration difficulty during the FN task must not be extreme. This is likely a ceiling effect where nearly all animals show high frustration scores, thus washing out individual differences in frustration scores.

In a longitudinal human study by Jeronimus et al., 2017, data showed that high frustration in adolescence predicted increases in externalizing symptoms of psychopathology like drug use, suggesting that frustration behavior is a risk factor for substance use disorders. Our data would predict the opposite. However, it is important to understand that "frustration" is not a unitary phenomenon. The type of frustration of Jeronimus' study was parental report of a child's irritability and aggression. The type of frustration measured by lever press durations is related to extinguishing responding for a reinforcer. Recent research demonstrated that lever press durations can be used as a

measure of frustration level (Vasquez, McAuley, et al., 2021), and rats exhibited their longest lever press durations late in extinction sessions or shortly before breaking under a PR schedule for drug. Correlations of frustration scores for sucrose FN tasks vs. PR fentanyl infusions in Figure 3.3 demonstrate that breaking under a PR procedure for fentanyl can be predicted by individual differences in FN sucrose responding.

The schedules used for incentive upshift and downshift in FN responding were inspired by changes in running speed down a runway in Capaldi's runway paradigm (Capaldi, 1974). Our data showed that durations were sensitive to reward magnitude as incentive upshift decreased durations and downshift increased durations.

This study shows that individual differences in frustration behavior for sucrose predict subsequent early breaking on a PR schedule for IV fentanyl. This builds upon a significant foundation on individual differences research typified most clearly by Piazza and colleagues showing that high locomotor responders during exposure to a novel environment take amphetamine more readily than low responders (Piazza et al., 1989). Of relevance to the current project, two studies showed that high sucrose intake during free access predicted amphetamine and cocaine taking (DeSousa et al., 2000; Gosnell, 2000). The current study found no such link with operant sucrose intake failing to predict fentanyl intake, but rather frustration to sucrose responding predicting fentanyl early breaking.

It should be noted that the Low, Medium, and High FN scores predicting PR breaking is correlational and should be further investigated in a causal fashion. Future studies will affect neurobiological aspects of frustration to determine the underlying mechanisms of the effect of frustrative nonreward on motivation in substance abuse related behavior in rats.

Our conclusion is that a rat's frustration level is a consistent trait and that increased sensitivity to frustration can be used to predict a rat's motivation to seek fentanyl. Thus, these frustrative nonreward tasks provide a novel tool to assess individual differences in rats' frustration levels that can be used in future studies of frustration/frustrative nonreward

as an important factor for substance use disorders in addition to craving, impulsivity, and habit.

End of Published manuscript Vasquez et al (2021), eNeuro 10.1523/ENEURO.0136-21.2021.

The introduction to this dissertation has established that an individual's motivational state drives goal-directed behaviors to initiate, continue or terminate a specific behavior. Thus, motivation can play a role in the employment of the approach and avoidance response strategies to frustration. To confirm that the new behavioral measure could be utilized to explore this connection between motivation and approach/avoidance responses to frustration, it was necessary to further scrutinize the measure for within subject variability since changes in the approach response is the basis for the detection of frustration-like behavior. Therefore, it was essential to demonstrate that the increases in bar press durations are not utilized as a continued approach response to frustration until the rat is faced with a frustrating situation. Accordingly, the rats maintained a baseline level of frustration, as measured by bar press durations, when not performing the frustration tasks. Then once the rats engage in the FN sucrose self-administration tasks, the changes in bar press durations are demonstrating modification of continued approach behavior as a response to frustration. Furthermore, rats that exhibited larger changes in the approach response during the FN sucrose self-administration tasks later demonstrated lower motivational states through a quicker employment of an avoidance frustration response during the Progressive Ratio schedule for fentanyl by breaking responding sooner. Moreover, it has been shown in a human study that experiencing frustration is also associated with decreased motivation, which can lead to a spillover effect causing motivation to recede in subsequent tasks (Fang et al., 2020). Therefore, the increased approach frustration response observed during the FN sucrose self-administration tasks are

associated with a spillover effect that impacts motivation, leading to faster employment of the avoidance frustration response strategy during progressive ratio.

Chapter 4 Bar Press Durations as a Measure of Frustration in Rats: Investigating Performance Factors

The results from our previous studies have led us to the conclusion that increases in bar press durations are modifications of the previously rewarded response strategy that is employed when rats are frustrated. This was through robust and replicable demonstrations of bar press durations increasing in instances that are consistent with the definition of frustration (Vasquez, McAuley, et al., 2021). This is further supported by establishing that bar press durations possess some relevance to neuropsychiatric conditions for which frustration is known to be important and are stable in non-frustrating conditions (Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021). However, there are remaining factors to consider in order to reinforce the certainty of this conclusion.

INTRODUCTION

There can be many different factors (such as psychological states, physical conditions, and environmental situations) that have the potential to impact goal-directed behavior (Simpson & Balsam, 2016). Therefore, since frustration arises from the perceived resistance to the fulfillment of one's will/goal, we need to ensure that the observed changes in bar press durations are a result of changes in internal psychological state and not in a physical or environmental condition. Within our previous studies, there is the possibility for fatigue as a physical condition to impact bar press durations. The highest increases in bar press durations were observed when rats approached the end of Extinction and Progressive (PR) schedules. These results have the potential to reflect the increased effort expended to achieve the reinforcer or exhaustion due to the duration session. To control for this within an operant context, the amount of work to achieve a reinforcer needs to remain constant while the reward expectancy is manipulated. Thus, we developed an operant task analogous to the instrumental successive negative contrast (iSNC) task used

to evaluate the effect of performance factors within a runway (Capaldi, 1972). We term our task the operant successive negative contrast (oSNC), which utilizes the Frustrative Nonreward (FN) behavioral procedure from Vasquez, Shah, et al., 2021.

Within our experiments, there is the potential for bar press durations to reflect the impact of environmental changes on goal-directed behavior. Particularly, when manipulation of the environmental situation results in changes to secondary cues. This is because cues associated with rewards, such as food or drugs, can serve as secondary reinforcers and acquire motivational value (Berridge, 2004). Within our operant chamber experiments, cue lights serve as secondary reinforcers. Therefore, to ensure changes in bar press durations are a result of changes in frustration level and not changes in the environmental cues; we manipulated whether or not the cue lights were present during the progression to the primary reinforcers in a FN task.

Frustration, as an internal psychological state, results from the presence (or perceived presence) of an impediment to obtaining a goal. Thus, when the source of frustration is removed, the internal state of frustration is expected to subside. In an operant setting, it would therefore be expected that bar press durations would decrease once the rats are no longer subjected to a frustration task. Thereby demonstrating a relief of frustration which further strengthens the supposition that bar press durations are a measure of frustration.

A measure of frustration should be sensitive enough to detect continuous changes in the psychological state. This is because emotional states are more dynamic than static (Kuppens et al., 2017). Thus, to further validate that we are measuring a psychological state it is important to show that increases in bar press durations are a more graded rather than static response to frustration. Previous experiments support this though demonstrating that bar press durations gradually increase as the reinforcement becomes more difficult to achieve (during PR) or continuously denied (during Extinction) (Vasquez, McAuley, et al., 2021). However, when the previous experiments gradually decreased the dose of cocaine

to make achieving the same blood concentration gradually more difficult (during Within-session Dose-Response), these data were not conclusive of each step of increased difficulty increasing the degree of frustration (Vasquez, McAuley, et al., 2021). Therefore, to come to a decisive conclusion our study applied the same WSDR protocol to fentanyl self-administration and added more doses for cocaine responding.

Finally, it is essential to further substantiate that the effect of frustration on bar press durations is replicable, robust, and applicable to both sexes. The effect needs to remain consistent across a variety of conditions to be an effective method of measuring frustration during self-administration. The previous experiments show that increases in bar press durations are similar across sucrose, fentanyl, and cocaine for Extinction and PR in male rats (Vasquez, McAuley, et al., 2021). Thus, we sought to demonstrate the rigor and reproducibility of this frustration effect with the addition of one more factor, sex. It is crucial to study this measure of frustration in female rats as this could reveal sex differences in the measure of frustration or impacts of frustration on drug seeking and taking. Therefore, overall the experiment would confirm the robustness of bar press durations as a measure of frustration-like behavior by replicating the increase in average durations across the board for the different factors of reinforcer, schedule, and sex.

EXPERIMENTAL PROCEDURES

Animals

Sprague-Dawley rats were obtained from Envigo (Houston, TX) at 225-250g for males and 150g-175g for females; age matched 7-8 weeks. Rats were housed in an AAALAC-approved facility and conformed to the NIH Guide for the Care and Treatment of Laboratory Animals. Procedures were approved by the University of Texas Medical Branch's Institutional Animal Care and Use Committee (IACUC).

Statistics

Data were analyzed by RM one-way ANOVA, RM two-way ANOVA, or Mixed-model analysis if values were missing. Geisser-Greenhouse correction was employed when sphericity is not assumed. Post hoc analyses used Tukey's multiple comparisons test. T-tests were also utilized along with Welch's correction when appropriate. All statistics were performed using Graphpad 9 software. Rats not completing a given experiment were not considered in that analysis. One-tailed tests were used for oSNC duration measures, as a clear directional hypothesis was generated during the inception of the study based on literature of the iSNC (Amsel & Roussel, 1952; Capaldi, 1957)

Operant Successive Negative Contrast

TRAINING

A total of 16 male rats were singly housed and food regulated to 85% of free feed body weight. Rats were randomly assigned to either the 1-pellet or 4-pellet reinforcer condition (n = 8). The rats were then trained to press a bar for banana-flavored sucrose pellets (45 mg, Bio-Serv). Then they were given 1 session on FR1 at 15-min/session for 1 pellet reinforcer. The next two sessions were FR3 and FR5. For the rest of the training sessions, rats were divided into groups to self-administer either 1 pellet or 4 pellets. Rats were then trained on the FN procedure (Vasquez, Shah, et al., 2021)), starting with FN8 (8 presses [i.e. points] per reinforcer). For this task, the trial starts with both cue lights and the house light illuminated. After 2 bar presses, one cue light extinguishes; two more bar presses, and the second light extinguishes. After another 2 more presses, the house light extinguishes leaving the chamber in darkness. Finally, after two more presses (8 total), all three lights are illuminated and the sucrose pellet(s) dispensed, starting the next trial. For FN12, three bar presses were required per step, and so on until FN20, which required five presses per step. All FN sessions lasted 60 minutes and were under the FN no-frustration condition (i.e. no points were deducted). Rats first underwent 3 sessions at FN8, then proceeded to 3 sessions each at FN12 and FN16. Then the rats were then tested under FN20

for 9 sessions. Average bar press durations were used as a measure of frustration-related behavior, as with the previously published work (Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021).

oSNC

At the beginning of FN training, rats were randomly assigned to receive either 4 sucrose pellets per reinforcer or 1 pellet throughout training. For the negative contrast session, the previous 4-pellet group was switched to 1 pellet reinforcer. Only durations for responses after the first reinforcer were assessed, as rats are unaware of the downshift until they receive the first reinforcer. Curiously, both groups gradually decreased responding under the FN20 schedule from an average of 12.6 reinforcers on Session 1 of FN20 down to 4.8 reinforcers on Session 9, trending toward extinction. The lack of responding could not be attributed to satiation as rats will easily consume more than 100 pellets on an FR1 schedule (Zhang et al., 2014). Nor could this be due to exhaustion, as rats are capable of pressing more than 1000 times in an hour (Green et al., 2002). Even so, the previous 4-pellet group was switched to 1 pellet reinforcer to make an effort to examine negative contrast. Unfortunately, due to continued low borderline extinction responding the experiment did not provide sufficient data to produce decisive results. Subsequently, the rats were then dropped from FN20 to FN12 and placed back in their previous reinforcement group (1 vs. 4 pellet reinforcer) for 4 sessions to increase the number of earned reinforcers. The negative contrast was examined again at FN12, with all rats receiving 1 pellet/reinforcement for 6 sessions. To mitigate individual differences in baseline durations, data were analyzed as a frustration index (i.e. change score) by dividing by the average of the final 2 sessions before changing the reinforcer size. Thus, a score of 1 in the frustration index represents durations at the level of baseline responding. This approach is taken from the published literature (Vasquez, Shah, et al., 2021).

FN vs FR

It is important to know if any change in cues presented within a schedule (with no incentive downshift) would cause an increase in durations. This is because cues associated with rewards, such as food or drugs, can serve as secondary reinforcers and acquire motivational value (Berridge, 2012; T. E. Robinson et al., 2014; Saunders & Robinson, 2013). This might suggest that when the cue lights are removed/changed within a schedule there is the possibility of observing increases in bar press durations. Therefore, half of the rats were maintained on an FN12 while the other half was switched from FN12 to FR12 for 7 sessions. To counterbalance the rats in this study, half of each of the 4- and 1-pellet groups from the prior experiment were switched to FR12 (all rats were on 1-pellet reinforcement). The importance of the switch from FN12 to FR12 is that there is a change in when the cues are presented as a secondary reinforcement (cue light signals every 3 bar presses in FN vs. every 12 bar presses in FR) but no change in the amount of work to obtain the primary reinforcement.

Relief from Frustration

Previous research conducted by (Vasquez, McAuley, et al., 2021) demonstrated increases in bar press durations measure frustration-related behavior. Since bar press durations are a measure of frustration, then durations should decrease when frustration is removed. Thus, to model the relief from frustration effect, rats' extinction sessions were immediately followed by FR5 maintenance.

Sucrose & Fentanyl

We analyzed the male and female average bar press durations data from the extinction sessions and the subsequent FR5 maintenance sessions. For data analysis, rats not earning more than 3 reinforcers during the FR5 maintenance sessions were excluded. The reasoning for setting the constraint to 3 reinforcers is because we cannot conclude that

a rat is aware of a change in the availability of the reinforcement prior to that first reinforcer on the FR5 schedule. Altogether, 3 male and 4 female rats met the criteria and were excluded from both the sucrose and fentanyl self-administration data analysis.

Within Session Dose Response: Replicability and Extension

Male rats were singly housed, and food regulated to 85% of free feed body weight. They were then trained to press a bar for banana-flavored sucrose pellets (45 mg, Bio-Serv) within a sound-attenuating operant chamber (Med-Associates). After self-administration for sucrose pellets, the rats were returned to free feed. After one week of free feed in the colony room, rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg, i.p.) and implanted with indwelling intrajugular silastic catheters as described previously (Crofton et al., 2017; Zhang et al., 2016). To maintain catheter patency, catheters were flushed daily with 0.1 ml of heparinized (10 U/ml) saline with ticarcillin (0.067g/ml). Following a 1-week recovery from catheter surgery, animals were placed in the operant chambers to self-administer either cocaine HCl (0.5 mg/kg/infusion) or fentanyl HCl (32 μg/kg/infusion; NIDA Drug Supply Program). Rats had maintenance responding and extinction training prior to 3 sessions of within-session dose-response (WSDR). WSDR began with the full dose and every 30 minutes the dose was halved for a duration of 4 hours for cocaine self-administration and 2 hours and 30 minutes for fentanyl self-administration. Average bar press durations were averaged for each dose over the 3 days of WSDR and were used as a measure of frustration-related behavior.

COCAINE EXPERIMENT

Data were collected from a study analyzing the effects of an shRNA vector on cocaine operant self-administration. The data analyzed are from the 10 rats that were injected bilaterally into the nucleus accumbens shell (shNAc) with 1 μL of adeno-associated control viral vector expressing GFP and a non-targeted short hairpin RNA

(Crofton et al., 2017; Zhang et al., 2016, 2019). The injection occurred simultaneously with catheter implantation. Coordinates were AP=1.3, L = 2.4 from bregma and DV = -6.7 mm from dura (Zhang et al., 2014). For statistical analysis, 1 rat was removed because the catheter was compromised. Additionally, only bar press durations starting at the second dose of .25 mg/kg/inf. were analyzed due to the loading effect shown previously for cocaine (Vasquez, McAuley, et al., 2021).

Cocaine Replication Experiment

Data was collected from another study analyzing the effects of a different shRNA vector on cocaine operant self-administration. The data analyzed are from the 10 rats that were injected bilaterally with the control viral vector into the shNAc following the same protocol as previously described. The injection occurred simultaneously with catheter implantation. Data analysis was conducted in the same manner as explained previously, and 1 rat was removed earlier in the study for unstable responding prior to WSDR.

Fentanyl Extension Experiment

Control data was collected from a different study analyzing the effects of another shRNA vector on fentanyl operant self-administration. The data analyzed are from 10 rats that were injected bilaterally into the shNAc with the control viral vector. The injection occurred prior to sucrose self-administration and was conducted in the same manner as explained previously. Additionally, all doses were analyzed because fentanyl self-administration does not have the same loading phase effect as cocaine self-administration on bar press durations (Vasquez, McAuley, et al., 2021). For statistical analysis, 3 rats were removed earlier in the study due to compromised catheters.

Determining Sex Equitability

The experiment was conducted with a total of 20 rats (10 male and 10 female) to investigate if increases in bar press durations are replicable and robust, and applicable to both sexes. Previous work only used male in the experiments establishing bar press durations as a measure of frustration (Vasquez, McAuley, et al., 2021). Hence, it is important to establish that this measure of frustration is equally applicable for both sexes and investigate any potential sex differences.

SUCROSE

Rats were food regulated to 85% of free feed body weight and trained to press a bar for banana-flavored sucrose pellets (45 mg, Bio-Serv). After 4 sessions of stable responding on a continuous reinforcement schedule (FR1), the response requirement for the next session was increased to FR3 for 4 sessions. This was then followed by FR5 for 4 sessions. Then 3 sessions of between-session cued extinction which consisted of 4 hours with cue lights delivered under the normal FR5 schedule but no sucrose delivery. The extinction sessions were immediately followed by 15 minutes of sucrose self-administration at an FR5 schedule to prevent extinction from affecting the next session. For the next 3 sessions, the rats were placed on a progressive ratio (PR) schedule in which each successive sucrose reinforcement required an increasing number of lever-press responses according to the following semi-logarithmic progression, 1, 2, 4, 6, 9, 12, 15, 20, etc. The session continued until the rats went 1 hour without obtaining a reinforcement, or up to a maximum of 6 hours.

FENTANYL

After one week of free feed in the colony room, rats underwent catheter surgery and catheter patency was maintained as previously described. Following a 1-week recovery from catheter surgery, animals were placed in the operant chambers to self-administer fentanyl HCl (32 μg/kg/infusion). Rats self-administered fentanyl on FR1 until stably

responding for 4 sessions. This was followed by FR3 for 4 sessions and subsequently FR5 for 4 sessions. Then rats underwent 3 extinction sessions that lasted 3 hours with 1 hour of fentanyl self-administration afterward to prevent full withdrawal. Finally, the rats were given 3 sessions of PR. Two female rats died during catheter surgery. For statistical analysis, rats unable to complete a session due to compromising the catheter leash or escaping the operant chamber through the entry point for the catheter leash were not considered in the session's data analysis. During fixed ratio, no more than 1 rat was excluded per session. One female rat was removed from both extinction and progressive ratio data analysis. Another female and three male rats were removed from the progressive ratio analysis.

Robust Increases in Bar Press Durations

Additionally, previous research demonstrated robust increases in bar press durations across three different types of reinforcers (Vasquez, McAuley, et al., 2021). To further assess the robustness of this effect we further analyzed our male and female data across the two reinforcers sucrose and fentanyl. Individual average durations for the FR5 maintenance schedule were plotted against the average durations for either extinction or progressive ratio schedules. The scatterplots of individual data points are shown as falling above or below a 1-to-1 ratio threshold (Figure 4.3). Individual data points falling above the 1-to-1 threshold indicate the extinction/progressive ratio schedule resulted in increased bar press durations compared to the FR5 maintenance schedule. Individual data points falling below the threshold indicate the extinction/progressive ratio schedule resulted in decreased bar press durations. Therefore, this analysis demonstrates which schedule results in longer bar press durations compared to another schedule.

RESULTS

Operant Successive Negative Contrast

FN12 oSNC

Once the rats were stable under the FN12 schedule receiving 1- or 4-pellet reinforcer, there was no significant difference in mean number of reinforcers, although there was a trend for the 4 pellet group to have fewer ($t(14) = 2.09$, $p = 0.059$). Additionally, there was no significant difference in non-normalized bar press durations at baseline between the 1- and 4-pellet groups ($t(14) = 1.34$, $p = 0.20$). For the oSNC session where both groups receive only 1 pellet, there was a significant increase in bar press durations of the 4-pellet reinforcement rats compared to the 1-pellet rats ($t(14) = 1.78$, $p = 0.049$, one-tailed, Figure 4.1). Thus, confirming an oSNC.

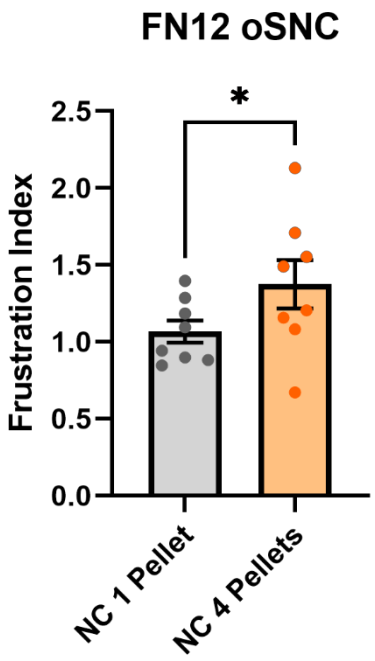


Figure 4.1: Operant Successive Negative Contrast.

Average bar press durations during the session when the 4-pellet (NC 4 Pellet) group switched to 1 pellet reinforcer compared to the 1-pellet group (NC 1 Pellet) that remained on 1 pellet reinforcer.

FN vs FR Schedules

FN12 to FR12

Before the reinforcement schedule was changed for half of the rats from FN12 to FR12, there were no significant differences between groups at baseline (FN12) for non-normalized bar press durations ($t(14) = 0.21$, $p = 0.83$) nor number of reinforcers ($t(14) = 1.55$, $p = 0.14$). Then, the session the switch was made there were no significant differences between frustration scores ($t(8.009) = 0.04072$, $p = 0.97$, Figure 4.2) nor number of reinforcers ($t(14) = 1.78$, $p = 0.097$). Thus, these results offer no evidence of a contrast effect based simply on changing schedule.

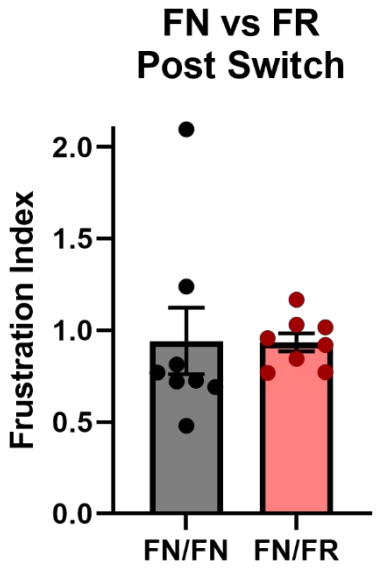


Figure 4.2: Schedule Change of FN 12 to FR 12.

Average bar press durations during the session when one group was switched from FN to the FR schedule (FN/FR) compared to the other group that remained on FN schedule (FN/FN).

Relief from Frustration

Sucrose

Both males and females show decreased bar press durations during FR self-administration of sucrose compared to the prior extinction session (Males, $t(6) = 2.459$, $p = 0.0492$, Figure 4.3A; Females $t(5) = 2.994$, $p = 0.0303$, Figure 4.3B). Thus, bar press durations decrease when the goal of the sucrose reinforcer is made available again after being denied.

Fentanyl

Females demonstrated a significant decrease and males had a trend towards a decrease in bar press durations during FR self-administration of fentanyl compared to extinction responding immediately prior (Females, $t(5) = 7.117$, $p = 0.0008$, Figure 4.3C; Males, $t(6) = 2.160$, $p = 0.0741$, Figure 4.3D). Of the 7 males subjects, one's bar press durations increased during FR5 from extinction, which may have been a contributor to the lack of significance. Another contributor is that the group size was limited to 7 of the 10 males due to unresponsiveness during FR5 self-administration after extinction, which impacts the power to detect significance. Nonetheless, altogether these data support a relief of frustration effect and demonstrate the transient nature of frustration.

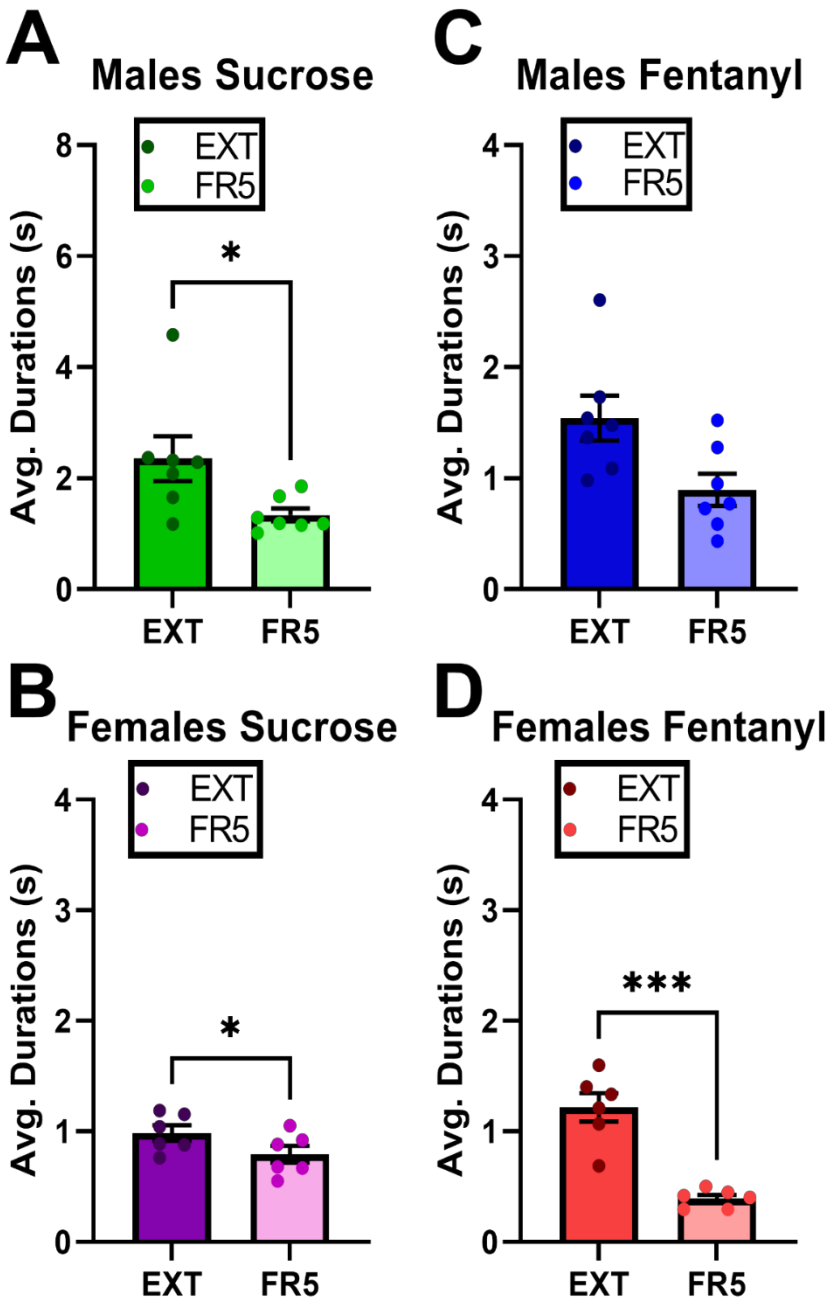


Figure 4.3: Relief from Frustration for Male and Female Rats.

A-D. Average bar press durations during between-session cued extinction and the FR5 schedule immediately afterwards for rats self-administering sucrose (A. males and B. females) and fentanyl (C. males and D. females).

Within Session Dose Response

Cocaine

When analyzing the 3 day average bar press durations for the within-session dose-response for cocaine self-administration for this study there were several significant differences observed across doses ($F(1.357, 10.86) = 16.24$, $p = 0.001$, Figure 4.4A). The Tukey's post-hoc analysis revealed many of the multiple comparisons to be significantly different. However, for clarity within Figure 4.4A, we show select comparisons where the average bar press durations for 0.25 mg/kg/inf. (highest dose measured) are significantly lower than the four smallest doses (0.03125 mg/kg/inf. $p = 0.0012$; 0.015625 mg/kg/inf. $p = 0.0138$; 0.0078125 mg/kg/inf. $p = 0.0476$ and 0.00390625 mg/kg/inf. $p = 0.0044$). Additionally, durations for the lowest dose are significantly greater than all other doses (data not shown). These results suggest that frustration as measured by average bar press durations increase as the dose decreases.

Cocaine Replication

For this replication study of within-session dose-response cocaine self-administration there are significant differences observed across doses ($F(1.545, 10.82) = 10.91$, $p = 0.0028$, Figure 4.4B). However, the post-hoc analysis revealed that average bar press durations for the highest dose were only significantly lower than the smallest dose ($p = 0.0102$, Figure 4.4B). As for the highest dose compared to the 3 other smallest doses, there was a trend for durations to be lower than the second smallest dose ($p = 0.0614$) and no significant differences for the third ($p = 0.2156$) and fourth ($p = 0.7765$) smallest doses. Still, durations for the smallest dose are significantly greater than all the other doses (data not shown). Additionally, these data replicate the absence of significant differences seen in Vasquez, Shah, et al., 2021 for the four largest doses starting at the 0.25 mg/kg/inf. Thus,

taken together these data may suggest that changes in frustration level become more easily detectable at lower doses of a substance.

FENTANYL

For within-session dose-response fentanyl self-administration there were significant differences observed across doses ($F(2.032, 12.19) = 13.72$, $p = 0.001$, Figure 4.4C). The post-hoc analysis revealed the average bar press durations for highest dose to be significantly lower than the second highest dose ($p = 0.007$, Figure 4.4C) and smallest dose ($p = 0.005$, Figure 4.4C). Once again, durations for smallest dose are significantly greater than all the other doses (data not shown). Altogether these results demonstrate that as the doses decrease the bar press durations increase, suggesting frustration level is sensitive to changes in dose.

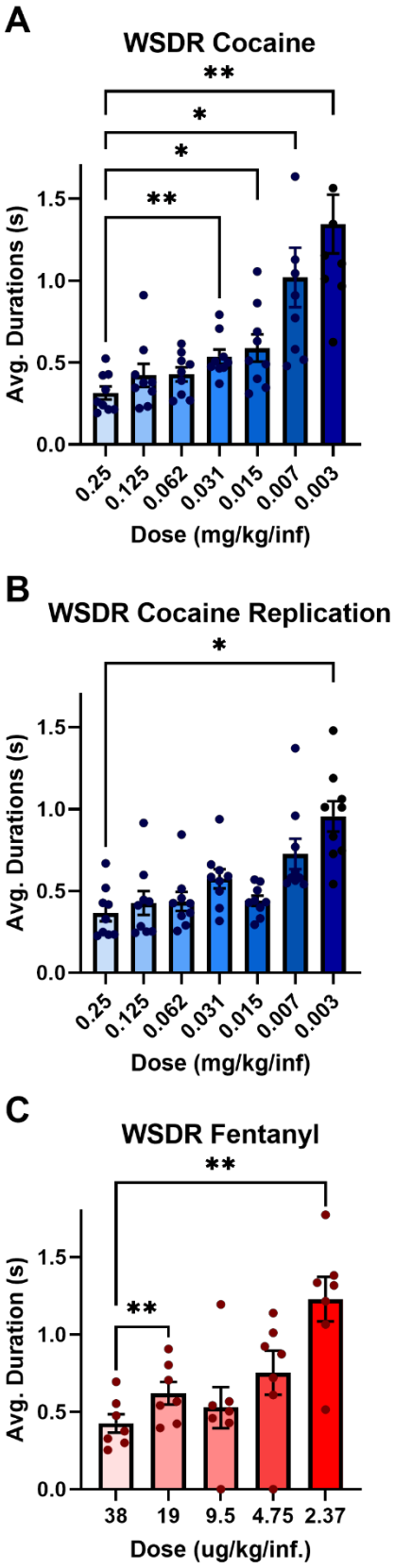

A
WSDR Cocaine
**
*
*
**
Avg. Durations (s)
1.5
1.0
0.5
0.0
0.25
0.125
0.062
0.031
0.015
0.007
0.003
Dose (mg/kg/inf)
B
WSDR Cocaine Replication
*
Avg. Durations (s)
1.5
1.0
0.5
0.0
0.25
0.125
0.062
0.031
0.015
0.007
0.003
Dose (mg/kg/inf)
C
WSDR Fentanyl
**
**
Avg. Duration (s)
1.5
1.0
0.5
0.0
38
19
9.5
4.75
2.37
Dose (ug/kg/inf.)

Figure 4.4: Increases in Average Bar Press Durations During Within-Session Dose-Response.

A and B. Average bar press durations (seconds ±SEM) for two separate studies of cocaine self-administration for each dose (descending order) during within-session dose-response (WSDR). **C.** Average bar press durations of rats self-administering fentanyl for each dose during WSDR.

Sex Equitability of Bar Press Durations

FIXED RATIO

For both males and females self-administering sucrose as well as females self-administering fentanyl there were no significant changes in average bar press durations across any of the four days for each FR schedule (Figure 4.5A, B & D). However, in fentanyl self-administration for male rats, there was a significant decrease in average bar press durations within an FR schedule ($F(1.695, 15.26) = 4.645$, $p = 0.0312$). The difference lay between the first stable day of FR1 compared to the fourth day of FR1 ($p = 0.0433$, Figure 4.5C). This replicates what was previously seen in Vasquez, McAuley, et al., 2021. However, this difference was not observed for FR3 ($p = 0.5730$) nor FR5 ($p = 0.8456$). These results suggest that male rats self-administering fentanyl decrease frustration like behavior as they acclimate to a continuous self-administration schedule.

Furthermore, there is an interaction effect seen between the schedule and day for males self-administering fentanyl ($F(2.281, 18.63) = 4.855$, $p = 0.0170$). There is a significant increase in average bar press durations for second and third days of FR3 compared to the fourth stable day of FR1 (Day 2, $p = 0.0330$, Figure 4.5C; Day 3, $p = 0.0416$, data not shown). However, there were no significant increases in durations from the fourth day of FR3 to any day of FR5. These data indicate that changing the reinforcement schedule from continuous to partial increases frustration like behavior males.

Additionally, for male rats these data reveal schedule changes in fentanyl self-administration have more of an impact on frustration-like behavior than schedule changes in sucrose pellets, confirming the preliminary findings of Vasquez, McAuley, et al., 2021. Moreover, these data may suggest that male rats are more sensitive to changes in continuous drug reinforcement schedules than females, because female rats do not show the same changes in bar press durations.

EXTINCTION

Both males and females increased bar press durations during extinction responding for both sucrose and fentanyl compared to the previous FR5 sessions (Sucrose, Males, $t(9) = 4.538$, $p = 0.0014$, Figure 4.5E; Sucrose, Females $t(9) = 9.121$, $p < 0.0001$, Figure 4.5F; Fentanyl, Males, $t(8) = 3.533$, $p = 0.0077$, Figure 4.5G; Fentanyl, Females $t(7) = 3.585$, $p = 0.009$, Figure 4.5H). These data replicate and expand upon the previous study's results (Vasquez, McAuley, et al., 2021). This suggests that bar press durations as a measure of frustration when the reward is ***denied*** is equally applicable in both male and female rats.

PROGRESSIVE RATIO

Both males and females increased bar press durations during PR self-administration of sucrose compared to the previous FR5 sessions (Males, $t(9) = 5.204$, $p = 0.0006$, Figure 4.5I; Females $t(9) = 5.694$, $p = 0.0003$, Figure 4.5J). For males bar press durations also increased during PR self-administration of fentanyl compared to the previous FR5 session ($t(8) = 2.827$, $p = 0.0222$, Figure 4.5K). However, these data were unable to reflect the same effect for female rats self-administering fentanyl ($t(5) = 2.024$, $p = 0.0989$, Figure 4.5L). This may be a result of a deficiency in the number of subjects to significantly power the results. Nonetheless, the average durations still show a trend in the expected direction. Thus, overall these data also replicate and expand upon the previous study's results (Vasquez, McAuley, et al., 2021). Altogether, this suggests that bar press durations as a measure of frustration when the reward is ***made more difficult to achieve*** is equally applicable in males and females.

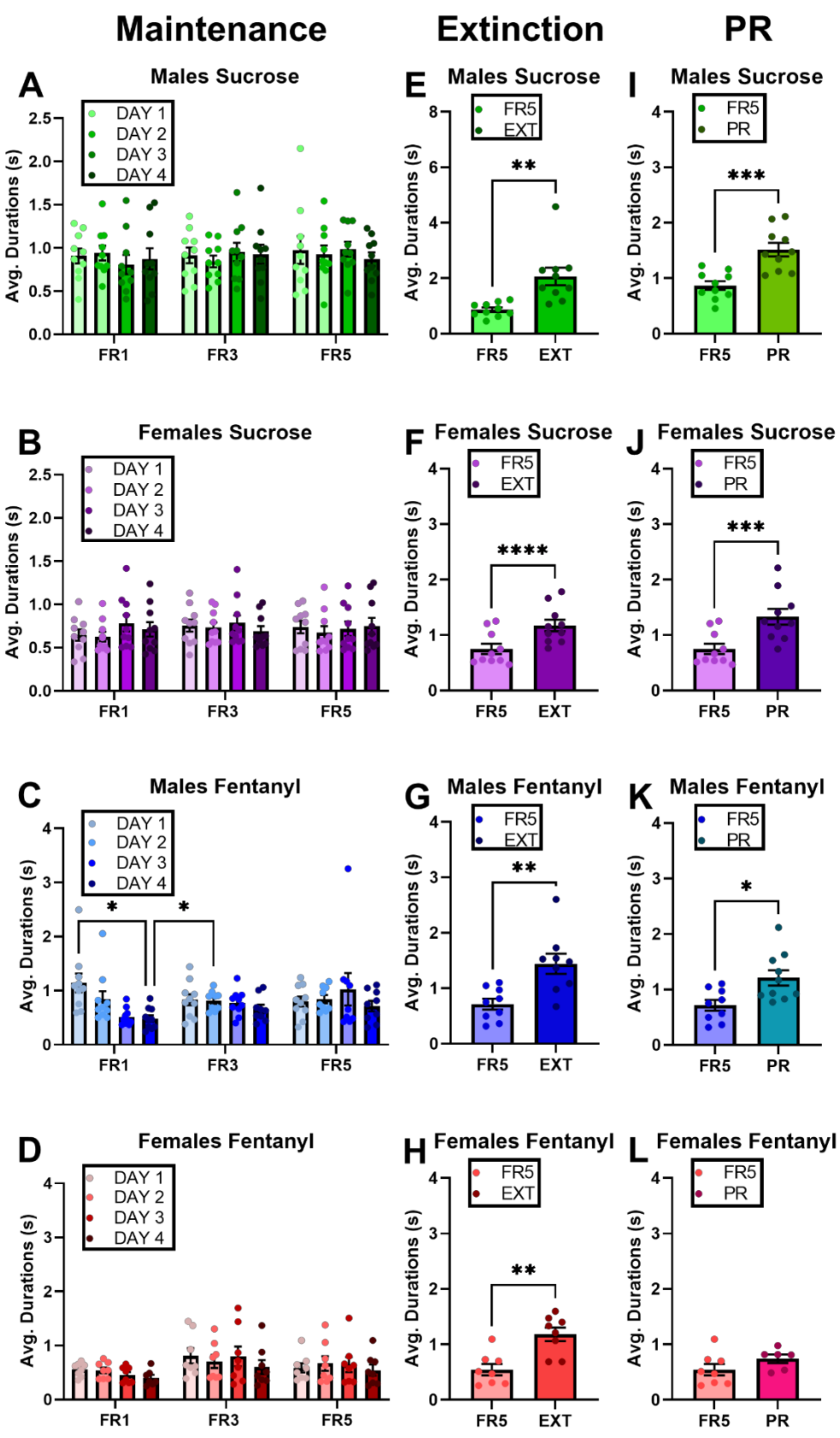

Maintenance
Extinction
PR
A
Males Sucrose
DAY 1
DAY 2
DAY 3
DAY 4
Avg. Durations (s)
FR1
FR3
FR5
E
Males Sucrose
FR5
EXT
**
I
Males Sucrose
FR5
PR
***
B
Females Sucrose
F
Females Sucrose
****
J
Females Sucrose
C
Males Fentanyl
*
*
G
Males Fentanyl
**
K
Males Fentanyl
*
D
Females Fentanyl
H
Females Fentanyl
**
L
Females Fentanyl

Figure 4.5: Replication of Increases in Bar Press Durations for Male and Female Rats.

A-D. Average bar press durations (seconds ±SEM) of rats during the last four sessions of FR1, the four FR3 sessions and the four FR5 sessions for sucrose (A. males and B. females) and fentanyl (C. males and D. females) self-administration. **E-H.** Average bar press durations during between-session cued extinction and the prior session of FR5 for rats self-administering sucrose (E. males and F. females) and fentanyl (G. males and H. females). **I-L.** Average bar press durations during progressive ratio and the prior session of FR5 for rats self-administering sucrose (I. males and J. females) and fentanyl (K. males and L. females).

How Robust are Increases in Bar Press Durations?

SUCROSE

Individual males and females had longer bar press durations during extinction and PR compared to their previous FR5 sessions (Figure 4.6A, B, E & F). There was the exception of a single female demonstrating slightly longer bar press durations during FR5 than PR.

FENTANYL

Additionally, a majority of males and females had longer bar press durations during extinction and PR compared to their previous FR5 sessions (Figure 4.6C, D, G &H). However, there were 1 to 2 individual rats that would demonstrate longer bar press durations during FR5 compared to both extinction and PR for both males and females.

Overall, these results suggest that increases in bar press durations is a robust measure through replicating the increase in average durations across the board for 3 factors: sex, reinforcer, and schedule. Moreover, these scatterplots demonstrate a within-group variability that can be utilized to evaluate individual differences.

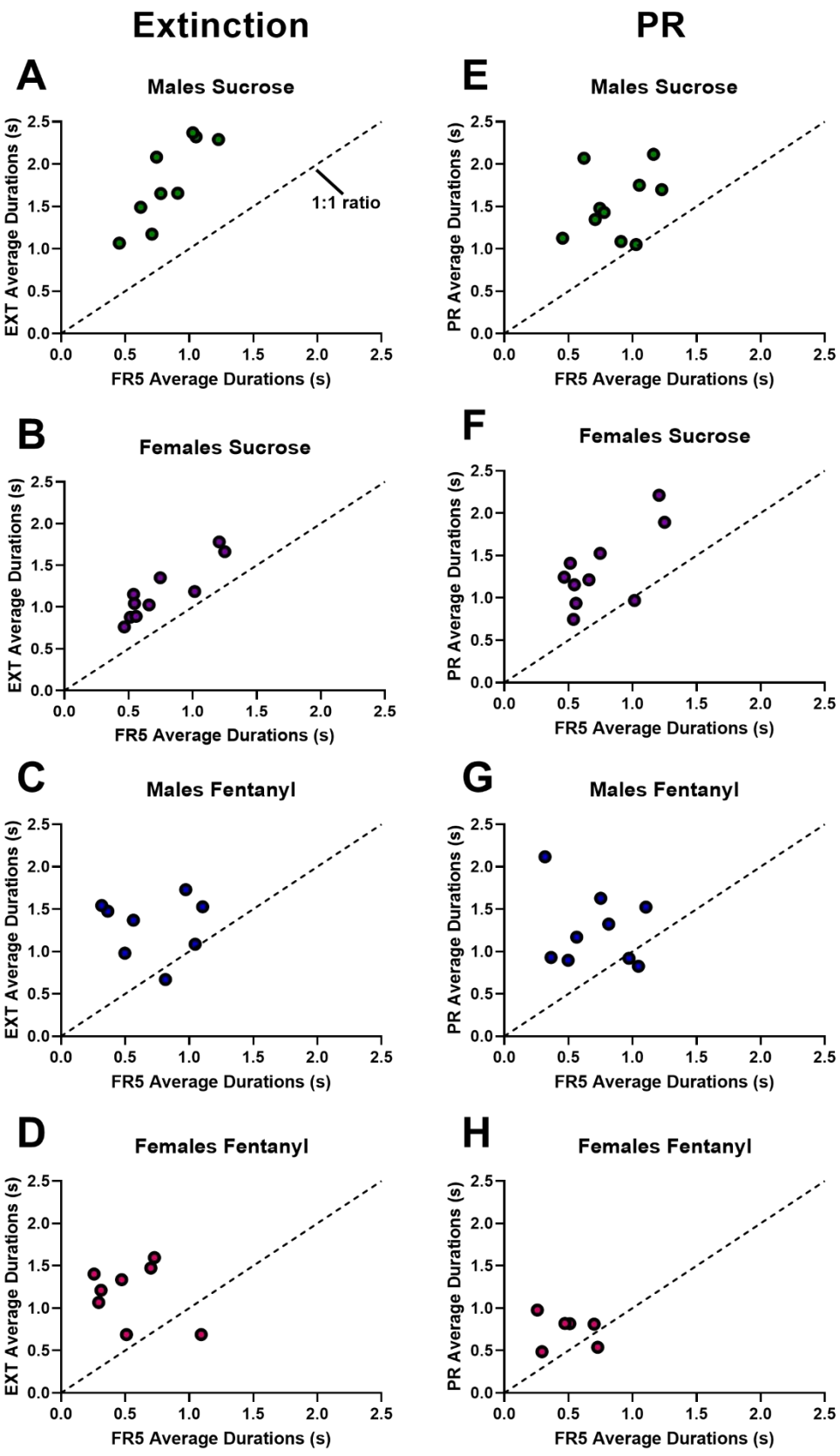

Extinction
PR
A
Males Sucrose
EXT Average Durations (s)
FR5 Average Durations (s)
1:1 ratio
E
Males Sucrose
PR Average Durations (s)
FR5 Average Durations (s)
B
Females Sucrose
EXT Average Durations (s)
FR5 Average Durations (s)
F
Females Sucrose
PR Average Durations (s)
FR5 Average Durations (s)
C
Males Fentanyl
EXT Average Durations (s)
FR5 Average Durations (s)
G
Males Fentanyl
PR Average Durations (s)
FR5 Average Durations (s)
D
Females Fentanyl
EXT Average Durations (s)
FR5 Average Durations (s)
H
Females Fentanyl
PR Average Durations (s)
FR5 Average Durations (s)
0.0
0.5
1.0
1.5
2.0
2.5

Figure 4.6: Robust Within Group Increase in Bar Press Durations for Male and Female Rats.

A-D. Scatter plots of average bar press durations for each rat during between-session cued extinction vs the prior FR5 schedule for rats self-administering sucrose (A. males and B. females) and fentanyl (C. males and D. females). Above the dashed line is the threshold for longer bar press durations during extinction and below are longer bar press durations for FR5. **E-H.** Scatter plots of average bar press durations for each rat during between-session cued extinction and the prior FR5 schedule for rats self-administering sucrose (E. males and F. females) and fentanyl (G. males and H. females). Above the dashed line is the threshold for longer bar press durations during progressive and below are longer bar press durations for FR5.

DISCUSSION

Frustration is a significant component of many neuropsychiatric conditions. Some human studies suggest that frustration behavior is a risk factor to develop anxiety, depression, or even substance use disorders (Baars et al., 2013; Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017; Ramirez-Castillo et al., 2019). Frustration can also be involved in conduct and personality disorders (Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017). To begin making steps towards identifying frustration's role in susceptibility to these disorders there is the need of a reliable, versatile, and translatable way to measure frustration behavior.

Early animal studies of frustration laid the foundation to developing measures of frustration related behavior. Goal obstruction leading to the arousal of aggressive behavior was the main focus of some of these studies (Arnone & Dantzer, 1980; Capaldi, 1974; Duncan & Wood-Gush, 1971; Finch, 1942). Others focused on using goal obstruction within a runway model where the rats are trained to run to goal boxes baited with a food reinforcement (Adelman & Maatsch, 1955; Amsel & Roussel, 1952; Capaldi, 1974; Daly, 1974). Within those studies the objective measure of frustration was determined to runway speeds. Our previous research has adapted these concepts of measuring of frustration for use in operant chambers to allow for the study of frustration in substance use. One study of drug self-administration rats has recently demonstrated that lever press durations can be used as a real time measure of frustration level (Vasquez, McAuley, et al., 2021). Furthermore, a rat's motivation to seek fentanyl can be predicted by increased sensitivity to frustration, as measured by bar press durations (Vasquez, Shah, et al., 2021). Thus, presenting that bar press durations have the potential to contribute to development of a human model to objectively identify frustration intolerance.

Another previous study came to the opposite conclusion that durations are not a measure of frustration, in spite of showing similar observations. Their operant experiments

utilized probability of not reinforcing the first bar press with a sucrose pellet within a trial (Gharib et al., 2001; S. Roberts & Gharib, 2006). Their analysis demonstrated increases durations that lasted until the end of the trial when the first bar press did not result in a reinforcer (~195s trial duration). Thus, they concluded the long-lasting increase in bar press durations was triggered by the omission of expected food. However, they were not confident this is a frustration effect because they believed the long-lasting increase in bar press durations lasted longer than what was observed in a runway experiment of frustration. In runway experiments, when a reinforcer is omitted in the first goal box rats increase runway speed to the second goal box (Amsel & Roussel, 1952). When the rats were confined to the first goalbox for 15 seconds the increase in runway speeds persists. However, when the rats were confined to the first goalbox for 90 seconds the increase in runway speeds disappears (McKinnon & Amsel, 1964). Therefore, the authors of the operant chamber study presume that the increase in bar press duration should only last until shortly after reinforcement omission. However, it needs to be noted that the procedures of the two experiments are not comparable. In the runway experiment, the rats are unable to immediately continue to perform the previously reinforced response of running for 90 seconds after reinforcer omission, while in the operant chamber experiment the rats are allowed to continue performing the reinforced response of bar pressing. Still being allowed to perform the previously reinforced response does not make it apparent that the reinforcement is no longer available, which facilitates prolonging the display of frustration related behavior.

An additional experiment within the previous study added 120s of variable interval 100s (a bar press is reinforced on average around 100s into the trial; VI 100) on top of the probability of not reinforcing the first bar press within a trial (Gharib et al., 2001; S. Roberts & Gharib, 2006). They expected increases in durations similar to the first experiment; and stated that if the durations are measuring frustration, any omission of expected food should produce a duration increase. They did see a small increase in response duration within the

first couple of bar presses after the expected food reinforcer was not delivered. However, since it was not as big an increase as the first experiment, this convinced them that durations were not measuring frustration. However, it is important to note that in their methods they stated that they capped the durations they included in their analysis to 2.9 seconds, thus artificially deceasing any significant increase/variation in durations. Furthermore, their data show that when the rats did receive their reinforcement there was a large decrease in the durations, which is in line with our relief from frustration concept. Thus, their results might suggest that their VI 100 with the no reinforcement 20% of the time is a frustrating schedule and once the reinforcement is received there is relief from the frustration.

Two more experiments utilized different signals to indicate that the reinforcement would be delivered 100%, 50% or 25% of the time (Gharib et al., 2004; S. Roberts & Gharib, 2006). They found that with the lower probability signals, the rats had higher bar press durations. Thus, they concluded that lower reward expectancy increases the variation of bar press duration. Therefore, they are lowering reward expectancy through unexpected nonreward by only providing the reinforcement 50% or 25% of the time on the lower probability signals. Then, since we know frustration is elicited when a goal-pursuit is not fulfilled at the expected time in the behavioral sequence known as unexpected nonreward (Amsel, 1958; Daly, 1974; Jeronimus & Laceulle, 2017), these studies are measuring frustration behavior with bar press durations by creating situations of unexpected nonreward.

Overall, bar press durations are a versatile measure of frustration related behavior with the advantages of being a real-time, objective and easily quantifiable. However, fundamental behavioral neuroscience concepts provide 8 criteria that bar press durations as a measure of frustration needs to fulfill. Fulfilling these nine criteria is accomplished through the results of this research in coordination with two previous manuscripts Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021. The nine criteria along with their

supporting evidence are listed in Appendix B Supplemental Table for Chapter 4 and discussed below:

1. Durations should increase when rats are frustrated.

This is the principal component, and all other criteria will build upon this factor. Frustration has been defined as occurring when the will or goal is denied or made more difficult to achieve. Thus, to satisfy this criteria, it has been previously demonstrated that increases in bar press durations occur when the subject is unable to achieve a reinforcer through extinction or has to work harder to achieve a reinforcer through progressive ratio (Vasquez, McAuley, et al., 2021). Another instance of making a goal harder to achieve is through WSDR, where the goal of maintaining desired brain levels of a drug is harder to achieve by progressively decreasing doses. The WSDR data within a previous study showed a trend towards increasing bar press durations; however the results were not conclusive (Vasquez, McAuley, et al., 2021). Our study applies this protocol to fentanyl self-administration and adds more doses for cocaine responding. These data demonstrated bar press durations increase as the doses progressively decrease in WSDR (Figure 4.4). Thus, further supporting the criterion that durations should increase when rats are frustrated.

2. When frustration is removed, durations should return to normal.

Since bar press durations increase in instances of frustration, this would imply that when the impediment to achieving the goal is removed there should be a subsequent decrease in bar press durations. The results of our study do support that bar press durations decrease once the goal that was previously denied is made available once again. This effect is observed in both males and females self-administering sucrose and fentanyl (Figure 4.3).

3. Durations should be a dynamic measure of frustration.

Since emotional states are more dynamic than static (Kuppens et al., 2017) a measure of frustration should be sensitive enough to detect continuous changes. The previous study by Vasquez, McAuley, et al., 2021 provided considerable evidence that bar press durations meet this requirement by demonstrating that bar press durations progressively increase as the reinforcement becomes more difficult to achieve (during PR) or is continuously denied (during Extinction). The results within this study provide additional evidence by using WSDR to make achieving the same blood concentration increasingly more difficult, which results in bar press durations increasing in stages as the dose decreases (Figure 4.4). This demonstrates that each step of increased difficulty increases the degree of frustration, further supporting that bar press durations measure frustration as a dynamic emotional state.

4. Durations should be stable over time.

This new behavioral measure must be scrutinized for variability, as changes in durations are the basis for detection of frustration-like behavior. The previous study by Vasquez, Shah, et al., 2021 demonstrated that durations are extremely stable across time, usually with a day-to-day correlation R value of ~0.75 to ~0.9. Thus, at a baseline frustration state, bar press durations are consistent within an individual subject.

5. Frustration effect should be robust.

The effect needs to remain consistent across a variety of conditions to be an effective method of measuring frustration during self-administration. Previous experiments show that increases in bar press durations are similar across sucrose, fentanyl, and cocaine for extinction and PR (Vasquez, McAuley, et al., 2021). Additionally, the results of our study validate this criteria through replicating the increase in average durations across the board for factors of reinforcer, sex, and schedule (Figures 4.5 & 4.6). Moreover, the scatterplots shows that even as so many rats increase bar press durations, there exists

within-group variability to assess individual differences (Figure 4.6). Thus, these studies have the rare advantage of having a large effect size despite significant individual variability.

6. Frustration effect should be replicable.

The previous study demonstrated an increase in bar press durations when the goal is made harder to achieve and during extinction (Vasquez, McAuley, et al., 2021). Our results showed the same increases average bar press durations for both males and females during extinction responding and PR self-administration for sucrose and fentanyl (Figure 4.5). Additionally, this increase in average bar press durations is resilient in that the effect is still observable in this study with a lower number of subjects.

Our data appear to replicate what was previously seen with decreasing bar press durations within the 4 days of FR1 for fentanyl self-administration in males. The rest of these data do not replicate the previously observed changes in bar press durations for males self-administering fentanyl on an FR schedule. However, the lack of effect could be a consequence of this study possessing roughly half the number of subjects in each group than were in the previous study. Regardless, it appears changes in the FR schedule effecting frustration level in males self-administering fentanyl are not as robust as extinction and PR.

7. Frustration effect should not be isometric with other concepts.

Bar press durations should not be isometric with number of bar presses, otherwise bar press durations would be redundant measure of drug taking or seeking. Previously published research shows that number of bar presses are not equivalent to bar press durations (Vasquez, McAuley, et al., 2021). Thus, durations are a measure of another element of self-administration other than drug seeking and taking behavior.

8. The effect should not be a function of performance variables.

Frustration is based on expectation not living up to reality. When expectations are not met during self-administration for rats there are clear increases in durations. However, this effect is observed when a goal requires more bar presses or appears to require more bar presses. Hence, it is important to know if durations are measuring frustration or simply reflecting increases the amount of work needed to achieve (or try to achieve) a reinforcer.

Previous runway frustration tasks, having a similar predicament using runways speeds, employed a successive negative contrast (iSNC) procedure to address this concern. In this procedure there were two groups that received different reinforcer sizes (i.e. 12 pellets vs 1 pellet). The two groups worked under the exact same physical conditions and performed similarly in runway speeds. The group initially on 12 pellets was shifted to receive only 1 or 2 pellets (i.e. incentive downshift) while the other group continued to only receive 1 pellet. The group that experienced incentive downshift increased latency to reach the goal box beyond that of rats trained for the smaller reward (Lerma-Cabrera et al., 2019; Sabariego et al., 2013). The advantage of this procedure is that after incentive downshift, both groups remained working under the exact same physical conditions, meaning that any differences could not be a function of performance variables.

Within an operant context, the amount of work to achieve a reinforcer needs to remain constant while the reward expectancy is manipulated. Thus, we developed an analogous operant task to evaluate negative contrast, what we term the operant successive negative contrast (oSNC), using the FN behavioral procedure. The current project assessed frustration in a condition where one group of rats was trained on an FN12 procedure for a 4-pellet reinforcer while the other group was trained with a 1-pellet reinforcer. The two groups of rats had comparable bar press durations while pressing under the same schedule (FN12). When the 4-pellet group was downshifted to 1 pellet reinforcer, both groups were working under the same contingencies, yet the frustration score for the previous 4-pellet group was higher than that of the 1-pellet group (Figure 4.1). Therefore, we draw the

conclusion that the effect on bar press durations is a function of frustration rather than amount of effort or fatigue.

Cues associated with rewards, such as food or drugs, can serve as secondary reinforcers and acquire motivational value (Berridge, 2004). This might suggest that when the cues are removed there is the possibility to observe an increase in bar press durations. Interestingly, the FN12 procedure utilizes cue lights throughout the progression to the reinforcer. However, when these cues are removed by switching half of the rats to FR12 there was no change in bar press durations compared to rats remaining on the FN12 schedule (Figure 4.2). Thus, any differences in bar press durations observed is chiefly a result of changes in the primary reinforcer rather than a change in the secondary reinforcers.

9. The effect should have some relevance to neuropsychiatric conditions for which frustration is known to be important.

Research in humans shows that persons with substance use disorders rate higher in tests of frustration and that sensitivity to frustration correlates with number of relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). One study demonstrated that bar press durations could be used as an antecedent predictor of motivation for a drug reinforcement (Vasquez, Shah, et al., 2021). Another study provides additional evidence by demonstrating that approximately 10-15% of rats self-administering fentanyl escalate intake shortly after acquisition and have increased bar press durations (Vasquez, McAuley, et al., 2021). This is comparable to human statistics where 8-12% of people using opioids develop an opioid use disorder (Vowles et al., 2015). Thus, bar press durations can be used to investigate frustration in substance use disorders.

These nine criteria for bar press durations as a measure of frustration have been satisfied by several operant paradigms. The studies within this chapter have provided evidence for several of the criteria through demonstrating that durations are similar in males and

females, sensitive to dose, and not merely a function of performance variables. Thus, the major conclusion is that durations are a robust and replicable measure of frustration-related behavior.

Previous chapters have provided evidence that the changes in bar press durations are demonstrating modification of continued approach behavior as a response to frustration. The conditions and paradigms within this chapter's experiments replicate the evidence as well as provide additional data to support this interpretation. Most importantly, the results of the oSNC paradigm demonstrate that the increases in bar press durations are occurring through continued engagement in approach behavior and not potential changes in the physical condition of fatigue. The removal of cue lights in the FN task demonstrates that the modifications to bar press durations are not dependent on changes in environmental cues that function as secondary reinforcers, but rather are a goal-oriented continued approach behavior dependent on changes to the availability of the primary reinforcer. The findings also demonstrated that the modification to bar press durations is a dynamic reflection of frustration as an emotion. This is demonstrated when the modification of increased bar press durations subsides when the previously denied goal is made obtainable again. Additionally, the bar press durations dynamically increase in vigor with each step of increasing difficulty of achieving the same end goal (steady blood concentration of drug) during the WSDR experiments. Overall, these experiments solidify that changes in bar press durations are a modification of continued approach behavior that can be used to objectively measure frustration-related behavior.

Chapter 5 Investigating Bar Press Force as a Comparable Measure of Frustration to Bar Press Durations in Rats

Increased bar press durations have been presented throughout this dissertation as a modification of a previously rewarded response that can be used to measure frustration. To increase the probability of obtaining the reward, subjects could also engage in other modifications to bar pressing. Thus, there could be alternative modifications to bar presses that can be used as potential measures of frustration behavior. One accessible alternative to consider is the force of a bar press.

INTRODUCTION

While appropriate responses to a frustrating situation are an important aspect of normal behavior, inappropriate responses to frustration can be a component of neuropsychiatric conditions ranging from conduct disorder to personality disorders, mood disorders, and substance use disorders (Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017). Therefore, there is the need to investigate frustration responses to then subsequently elucidate their relationship with the development and presence of psychological disorders. Initial animal models used to investigate frustration behavior observed changes in the time spent performing a previously rewarded behavior when the animal was subjected to a frustrating situation. For example, some studies measured frustration using speeds to reach a goal box at the end of a runway utilizing food as reinforcement (Adelman & Maatsch, 1955; Amsel & Roussel, 1952; Capaldi, 1974). Additional studies have expanded this measure to operant sucrose pellet and drug self-administration, demonstrating in multiple ways that frustration increases lever-press durations under frustrating conditions (Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021). Thus, in the pursuit of obtaining a goal, it is suggested that frustration can function as a motivator to increase the intensity of a response immediately following nonreward of the previously rewarded response (Amsel

& Hancock, 1957; Amsel & Roussel, 1952). This increase in "response vigor" following frustrative nonreward is termed the frustration effect (Amsel & Hancock, 1957). Hence, this frustration effect might suggest increases in the intensity of other variables within a behavioral response. Thus, our experiments sought to investigate the force of a bar press as another measure of frustration-related behavior. Our experiments measured force in addition to barpress durations during operant self-administration utilizing the methods from Vasquez, McAuley, et al., 2021.

Our previous research demonstrated that the barpress durations increase across different types of frustration and for multiple different reinforcers, such as sucrose, cocaine, and fentanyl. Taking this into consideration, this study aimed to examine force as a measure of frustration. Frustration in rats has been defined as a state where the subject is unable to achieve a reinforcer (e.g. extinction) or has to work harder to achieve a reinforcer (e.g. progressive ratio schedule) (Adelman & Maatsch, 1955; Vasquez, McAuley, et al., 2021). Thus, the overall hypothesis of this study was that frustration would result in a comparable or greater increase in the force of a barpress compared to barpress durations. Indicating the force of a barpress could be used as another, potentially more sensitive, measure of frustration behavior in operant sucrose and drug self-administration.

Three hypotheses posed by other researchers is whether barpress could be considered "microaggressions", "displacement behaviors", or merely represent increased variability of responses to frustration. The microaggression hypothesis is interesting in light of the strong link between frustration and aggression. One would expect increases in force during frustration if this were the underlying cause of long barpresses. The second two options would not necessarily predict increased force during frustration.

The current study quantifies the force and duration of a bar press during operant-based frustration paradigms utilizing behavioral protocols from Vasquez, McAuley, et al., 2021.

EXPERIMENTAL PROCEDURES

Animals

8 Male Sprague-Dawley rats were obtained from Envigo at 225-250g. Except during food regulation, rats were maintained in a controlled environment (temperature, 22°C; relative humidity, 50%; and 12 h light/dark cycle, lights on 0600 h) in an Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC). Procedures were approved by the UTMB Institutional Animal Care and Use Committee and conform to the NIH Guide for the Care and Use of Laboratory Animals.

Statistics

Statistical significance was assessed by one-way repeated-measures analysis of variance (ANOVAs) followed by Bonferroni's post-hoc tests, via GraphPad 9 software. If conditions for sphericity were not met, the Greenhouse-Geisser statistic was used. Data are conveyed within Tables 5.1-5.4. Simple linear regression was used to assess correlations. The alpha level was set at $p < 0.05$. There was no correction for multiple comparisons. Two rats were unable to complete the fentanyl PR schedules due to compromised catheters and were not considered in the analysis of those data.

Sucrose Operant Responding

TRAINING

Rats were singly housed and placed on a regulated intake diet for 6 days until rats reached 85% of free-feed body weight. Rats were then placed in operant chambers equipped with force levers (Med-Associates, St. Albans, VT, Product Number: ENV-118M) in a sound-attenuating box. The threshold for a barpresses was set to ≥ 5g to avoid unintentional movement onto the lever being counted as a barpress and the threshold for the termination of a barpress is set at ≤ 2g. Achieving the response requirement on the active lever resulted in the extinguishing of the house light, the illumination of two circular

cues lights located above the levers for 5 seconds, and the delivery of a banana-flavored sucrose pellet (45 mg; Bio-Serv). The illumination of the two cue lights signaling the delivery of the reinforcer also serves to signal a time-out during which responding during the 5 seconds was recorded but animals could not earn more sucrose. Throughout the session, responding on the inactive lever was recorded but had no programmed consequences. During the first session, rats were allowed to perform a single lever press (FR1) to receive sucrose pellets for 3 hours with a non-contingent pellet delivered every 10 minutes until they self-administered 100 pellets. Rats that showed slower learning (e.g., did not reach 100 pellets) remained in the operant chambers for another session until they could self-administer a combined 100 pellets across the sessions.

FR Responding

Rats were placed on an FR1 schedule (5 second light cues) for 15 minutes for 10 sessions. This represented the non-frustrating condition.

Cued Extinction

The protocol consisted of 3 hours with cue lights delivered under the normal FR1 schedule but no sucrose pellet delivery. There were 3 cued extinction sessions with 1 intervening session of 15 minutes of maintenance sucrose self-administration at an FR1 schedule between each extinction session to reinstate the operant response.

Progressive Ratio

Next the rats were placed on a progressive ratio (PR) schedule in which each successive reinforcement required an increasing number of lever-press responses according to the following semi-logarithmic progression, 1, 2, 4, 6, 9, 12, 15, 20, etc. (Green et al., 2002). The session continued until the rats went 1 hour without obtaining a reinforcement, or up to a maximum of 6 hours. There were 3 PR sessions that also had 1

intervening session of 15 minutes of maintenance sucrose self-administration at an FR1 schedule between each PR session.

Fentanyl Operant Responding

TRAINING

After one week of free feed in the colony room, rats were anesthetized with ketamine (100 mg/kg i.p.) and xylazine (10 mg/kg, i.p.) and implanted with indwelling intrajugular silastic catheters as described previously (Crofton et al., 2017; Zhang et al., 2016). To maintain catheter patency, catheters were flushed daily with 0.1 ml of heparinized (10 U/ml) saline with ticarcillin (0.067 g/ml). Following a 1-week recovery from catheter surgery, animals were placed in the operant chambers to self-administer fentanyl HCl (0.0032 mg/kg/infusion; NIDA Drug Supply Program).

FR RESPONDING

Animals began fentanyl self-administration on a continuous schedule (FR1) of reinforcement until they were responding consistently for 4 days (no greater than a 14-infusion difference between sessions). Each session lasted 1 hour where a single response on the active lever resulted in a 0.1 ml intravenous infusion delivered over 5.8 seconds, concurrent with the illumination of two circular cues lights located above the levers. Each infusion was followed by a 20 second time-out period during which the cue lights remained illuminated, the house light was extinguished, and responding was recorded but animals could not earn more fentanyl. The cued time-out period was extended to 20 seconds from the 5 seconds during sucrose operant responding to prevent rats from potentially overdosing. Throughout the session, responding on the inactive lever was recorded but had no consequences.

Cued Extinction

After stabilization on FR1, the following sessions utilized a between-session cued extinction procedure consisting of 3 hours with cue lights delivered under the normal FR1 schedule but with no drug delivery. To prevent drug withdrawal, the extinction session was immediately followed by 1 hour of maintenance fentanyl self-administration at an FR1 schedule (4 hours total session time). Additionally, each of the 3 cued extinction sessions had 1 intervening session of 1 hour of maintenance fentanyl self-administration at an FR1 schedule.

Progressive Ratio

The rats were then placed on a PR schedule. The session continued until the rats went 1 hour without obtaining a reinforcer, or up to a maximum of 4 hours. There were 3 PR sessions that had 1 intervening session of 1 hour of maintenance fentanyl self-administration at an FR1 schedule between each PR session.

Results

Average Durations, Max Force and Sum Force

Previous research has suggested that frustration increases the intensity of a response (Amsel & Hancock, 1957). Within operant self-administration, the response is a barpress and previous research demonstrates that barpress durations increase during frustration tasks (Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021). However, the force of a barpress is another potential variable that may increase in intensity during frustrating conditions.

Therefore, the force exerted during frustrating conditions was examined as a potential measure of frustration-related behavior similar to barpress durations. Accordingly, we examined the average max force alongside barpress durations during self-administration. Barpress durations during cued extinction and PR were then compared to

previous reinforced responding on an FR1 schedule. The hypothesis was that increases in max force would be comparable to or more sensitive than increases in barpress durations.

Our experiment comparably replicated the previous study's increases in average barpress durations during extinction and PR. Durations for FR1, extinction, and PR were averaged across three days for both sucrose and fentanyl. There were significant increases in barpress durations for both extinction and PR compared to the previously reinforced FR1 schedule with one exception (Table 5.1; Figure 5.1A & B): there was no significant increase in barpress durations during fentanyl self-administration for PR compared to the FR1 responding. Nevertheless, there was a trend in increased bar press durations and the lack of significance is possibly due to fewer subjects that completed fentanyl PR self-administration (Table 5.1).

However, there were no significant changes in the three-day average max force for sucrose or fentanyl during cued extinction and PR compared to the previous FR1 responding (Table 5.1, Figure 5.1C & D).

While barpress durations can detect changes in frustration-related behavior; an additional hypothesis was that max force in combination with barpress durations (i.e., total sum force) would provide the most sensitive measure of frustration-related behavior. Thus, we examined the sum of the force over the duration of barpresses. While we did see a significant change in sum force for sucrose self-administration during PR compared to FR1 responding (Table 5.1, Figure 5.1E) this was a result of the increases in barpress durations that we saw in Figure 5.1A. No other significant differences were seen in sum force (Table 5.1, Figure 5.1E & F).

Average Durations, Max Force and Sum Force				
	Sucrose		Fentanyl	
	Statistic	p value	Statistic	p value
Durations	F(1.448, 10.14) = 15.27	p = 0.0015	F(1.133, 6.801)	p = 0.0086
FR1-EXT		p = 0.0021		p = 0.0016
FR1-PR		p = 0.005		p = 0.0617
Max Force	F(1.372, 9.606) = 0.1606	p = 0.7739	F(1.198, 7.191) = 0.9524	p = 0.3797
FR1-PR				
FR1-EXT				
Sum Force	F(1.796, 12.57) = 10.10	p = 0.0029	F(0.8533, 5.120) = 4.741	p = 0.0823
FR1-PR		p = 0.0096		
FR1-EXT		p = 0.0719		

Table 5.1: Average Durations, Max Force and Sum Force

ANOVA results followed by Bonferroni's post-hoc test results when appropriate from comparing FR1 Average Durations, Max Force and Sum Force across PR and EXT schedules.

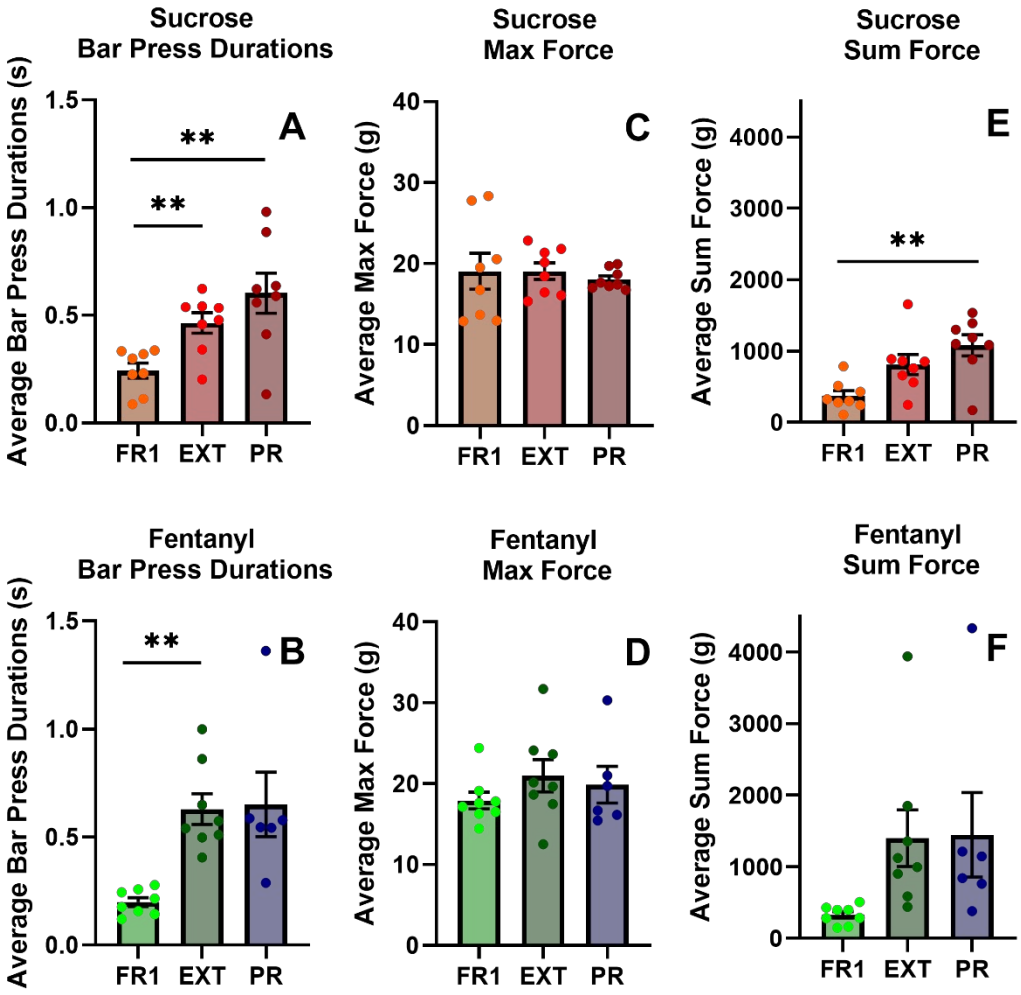


Figure 5.1: Max and Sum Force Are Not Comparable to Barpress Durations.

Three-session average for FR1, Extinction, and PR of **A.** barpress durations (seconds ± SEM) of rats self-administering sucrose and **B.** rats self-administering fentanyl. **C.** Barpress max force (grams ± SEM) for sucrose and **D.** fentanyl. **E.** Barpress sum force (grams ± SEM) for sucrose and **F.** fentanyl.

Duration, Max Force, and Sum Force for Cued Extinction

As the initial analysis looked at three-day averages, we decided to separate the days and assess if there are any differences in the individual sessions of extinction when compared to the last session of FR1. The primary reason for this analysis was to determine if averaging the three days may be diminishing any significance in max force or sum force during one of the days, for example, an effect on Session 1 that washed out with all three sessions averaged. Our data reliably demonstrated increases in barpress durations for each day of cued extinction compared to the last day of FR1 for both sucrose and fentanyl self-administration (Table 5.2, Figure 5.2A & B). Nevertheless, there still was no significant difference in the individual sessions compared to the final day of FR1 for both max force (Table 5.2, Figure 5.2C & D) and sum force (Table 5.2, Figure 5.2E & F).

Durations, Max Force and Sum Force for Cued Extinction				
	Sucrose		Fentanyl	
	Statistic	p value	Statistic	p value
Durations	F(1.263, 8.842) = 5.543	p = 0.0378	F(2.596, 17.31)	p < 0.0001
FR1-EXT DAY 1		p = 0.0069		p = 0.0018
FR1-EXT DAY 2		p = 0.009		p = 0.0035
FR1-EXT DAY 3		p = 0.0446		p = 0.0273
Max Force	F(1.430, 10.01) = 0.5148	p = 0.5534	F(1.323, 8.823) = 1.253	p = 0.3101
FR1-EXT DAY 1				
FR1-EXT DAY 2				
FR1-EXT DAY 3				
Sum Force	F(1.117, 7.822) = 1.939	p = 0.2039	F(1.384, 9.228) = 5.722	p = 0.0321
FR1-EXT DAY 1				p = 0.0538
FR1-EXT DAY 2				p = 0.1281
FR1-EXT DAY 3				p = 0.1705

Table 5.2: Durations, Max Force and Sum Force for Cued Extinction

ANOVA results followed by Bonferroni's post-hoc test results when appropriate from comparing the last session of FR1 Average Durations, Max Force and Sum Force across all three sessions of Cued Extinction.

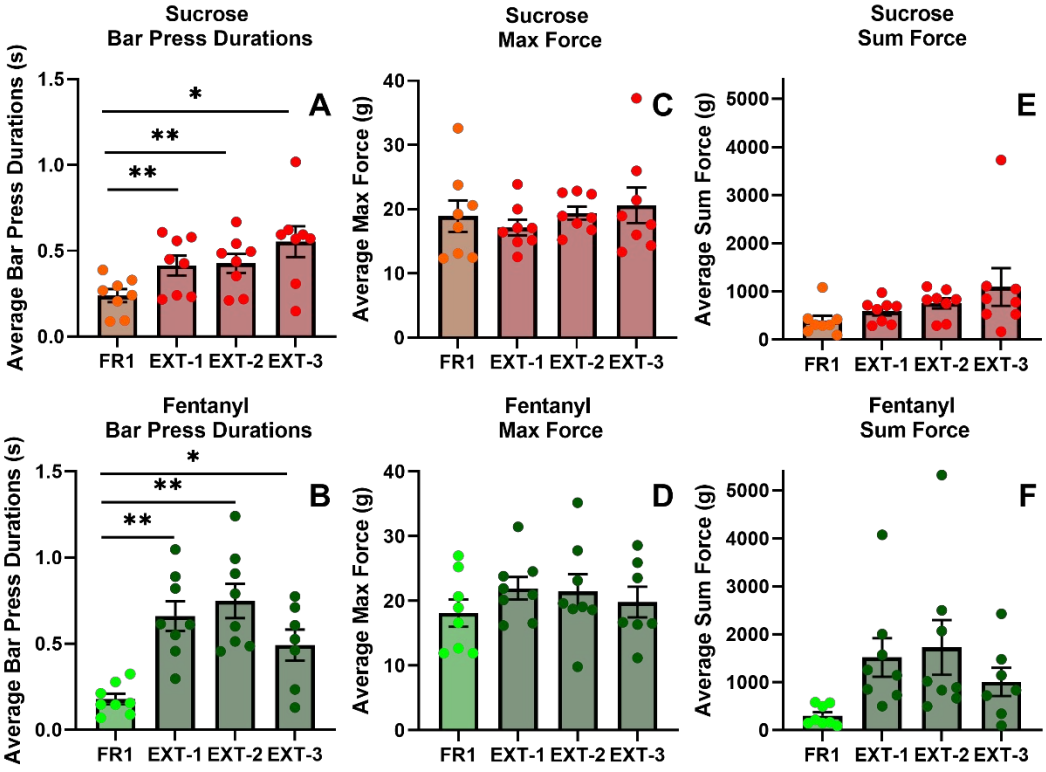


Figure 5.2: Max and Sum Force Are Not Analogous to Barpress Durations During Individual Extinction Sessions.

The three separate sessions of Extinction compared to the last session of FR1 for average **A.** barpress durations (seconds ± SEM) of rats self-administering sucrose and **B.** rats self-administering fentanyl. **C.** Barpress max force (grams ± SEM) for sucrose and **D.** fentanyl. **E.** Barpress sum force (grams ± SEM) for sucrose and **F.** fentanyl.

Duration, Max Force, and Sum Force for Progressive Ratio

When performing the same analysis of the individual days of PR we produced received similar results to what was seen with cued extinction. Dependably, these data demonstrated significant increases in barpress durations for all three days of PR compared to FR1 for sucrose self-administration and the second day of fentanyl (Table 5.3, Figure 5.3A & B) self-administration. Once again, while day one and day three (Table 5.3, Figure 5.3B) of fentanyl self-administration for PR were trending towards increases in barpress durations the possible explanation for the lack of significance is possibly due to fewer subjects that completed the fentanyl self-administration PR schedule. However, for max force (Table 5.3, Figure 5.3C & D) and sum force (Table 5.3, Figure 5.3E & F) there was no significant difference in the individual days compared to the last day of FR1 except for the sucrose sum force for Session 1 of PR. This one day might be contributing to the difference in sum force observed in Figure 5.1E however the increase in durations observed in Figure 5.3A is the major contributor to the difference seen in this analysis of sum force.

Durations, Max Force and Sum Force for Progressive Ratio				
	Sucrose		Fentanyl	
	Statistic	p value	Statistic	p value
Durations	F(2.421, 16.94) = 5.896	p = 0.0085	F(1.389, 6.943) = 6.328	p = 0.0338
FR1-PR DAY 1		p = 0.0234		p = 0.0526
FR1-PR DAY 2		p = 0.0194		p = 0.0409
FR1-PR DAY 3		p = 0.0381		p = 0.1357
Max Force	F(1.920, 13.44) = 0.1554	p = 0.8497	F(1.397, 6.985) = 0.1511	p = 0.7888
FR1-PR DAY 1				
FR1-PR DAY 2				
FR1-PR DAY 3				
Sum Force	F(1.992, 13.94) = 2.897	p = 0.0889	F(1.131, 5.657) = 2.780	p = 0.1494
FR1-PR DAY 1				
FR1-PR DAY 2				
FR1-PR DAY 3				

Table 5.3: Durations, Max Force and Sum Force for Progressive Ratio

ANOVA results followed by Bonferroni's post-hoc test results when appropriate from comparing the last session of FR1 Average Durations, Max Force and Sum Force across all three sessions of Progressive Ratio.

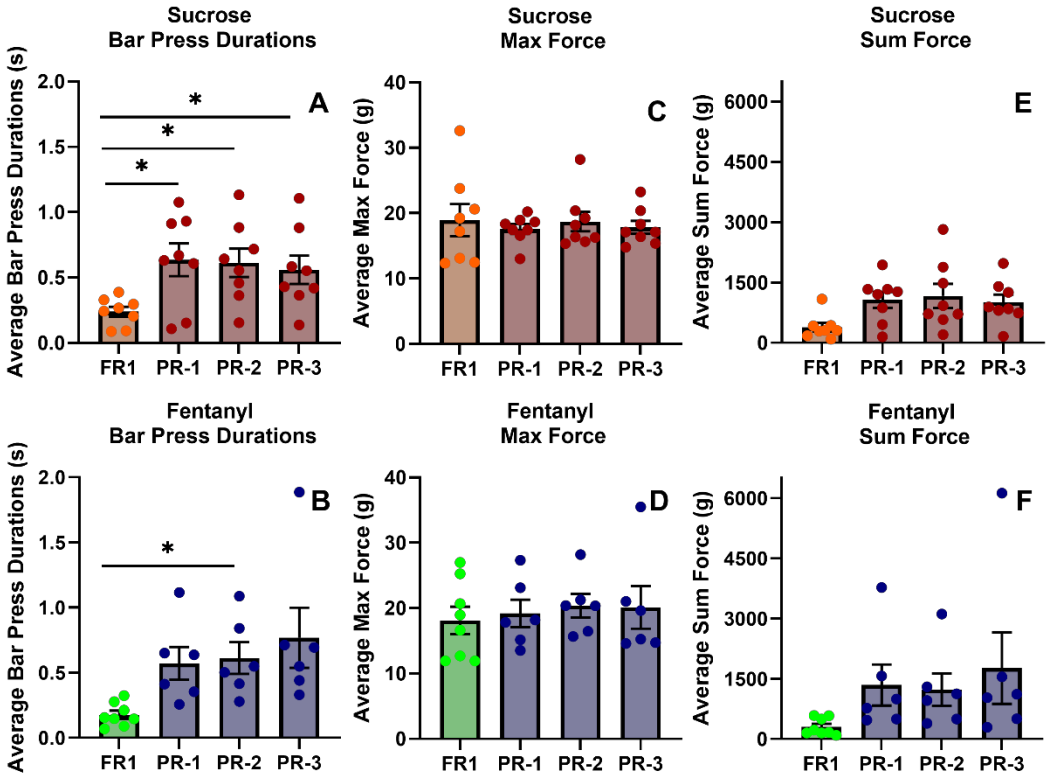


Figure 5.3: Max and Sum Force Are Not Analogous to Barpress Durations During Individual Progressive Ratio Sessions.

The three separate sessions of PR compared to the last session of FR1 for average **A.** barpress durations (seconds ± SEM) of rats self-administering sucrose and **B.** rats self-administering fentanyl. **C.** Barpress max force (grams ± SEM) for sucrose and **D.** fentanyl. **E.** Barpress sum force (grams ± SEM) for sucrose and **F.** fentanyl.

Percentage of Barpress Force Above 60, 40, and 20 Grams

The previous force analyses may not have been as sensitive a measure of frustration as we expected but changes in the number of high-force bar presses may be a better reflection of frustration-related behavior. Therefore, another factor taken into consideration was that the percentage of barpresses above a force threshold may increase for cued extinction and PR compared to FR1 sessions. These data were analyzed for percentages of barpresses above three force thresholds (20g, 40 g, and 60g) for the three-day average and individual day comparisons. Nevertheless, even when performing this analysis there were no significant changes in percentages for the three-day averages, (Table 5.4, Figure 5.4A-C & J-L), individual days for cued extinction (Table 5.4, Figure 5.4D-F &M-O), or individual days for PR (Figure 5.4G-I & P-R).

Percentage of Barpress Force Above 60, 40, and 20 Grams												
	Percent Bar Press Force Above 60g				Percent Bar Press Force Above 40g				Percent Bar Press Force Above 20g			
	Sucrose		Fentanyl		Sucrose		Fentanyl		Sucrose		Fentanyl	
	Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value	Statistic	p value
Averages of 3 Days	F(1.101, 7.706) = 0.1427	p = 0.7397	F(0.9945, 5.967) = 0.7586	p = 0.4167	F(1.128, 7.893) = 0.03796	p = 0.8764	F(1.335, 8.012) = 0.8805	p = 0.4081	F(1.371, 9.598) = 0.7420	p = 0.4524	F(1.661, 15.77) = 1.901	p = 0.1854
FR1-EXT												
FR1-PR												
Indivdual Cued Extinction Days	F(1.231, 8.616) = 0.9743	p = 0.3710	F(1.316, 8.770) = 1.414	p = 0.2787	F(1.381, 9.669) = 0.5052	p = 0.5525	F(1.541, 10.27) = 0.9423	p = 0.3969	F(1.547, 10.83) = 0.5809	p = 0.5341	F(1.735 , 11.56) = 0.9453	p = 0.4039
FR1-EXT DAY 1												
FR1-EXT DAY 2												
FR1-EXT DAY 3												
Indivdual Progressive Ratio Days	F(1.731, 12.12) = 0.4293	p = 0.6326	F(1.546, 7.729) = 0.2626	p = 0.7215	F(1.583, 11.08) = 0.1245	p = 0.8387	F(1.628, 8.139) = 0.001267	p = 0.9961	F(1.779, 12.45) = 0.4704	p = 0.6137	F(1.354, 6.771) = 0.1881	p = 0.7501
FR1-PR DAY 1												
FR1-PR DAY 2												
FR1-PR DAY 3												

Table 5.4: Percentage of Barpress Force Above 60, 40, and 20 Grams

For the Percentage of Barpresses with a Barpress Force Above 60, 40, and 20 Grams: ANOVA results from comparing three-day averages for FR1 to extinction, and PR; the last session of FR1 to all three sessions of Cued Extinction; and the last session of FR1 to all three sessions of Progressive Ratio.

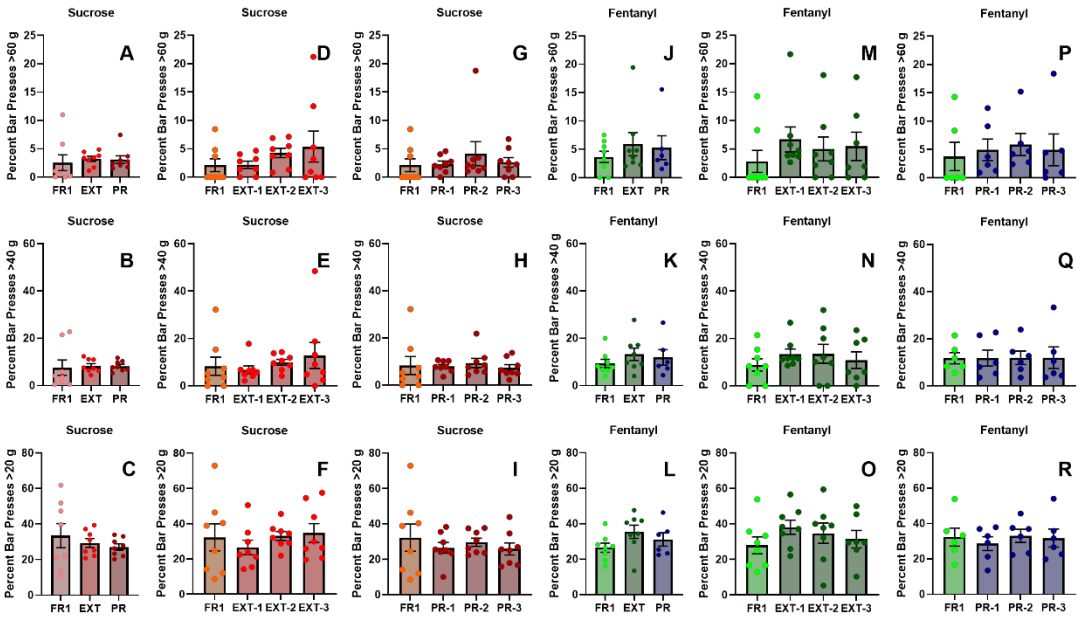


Figure 5.4: Percentage of Barpresses with Force Above 60, 40, and 20 Grams.

For rats self-administering **A-I.** sucrose and **J-R.** fentanyl data are represented as percentages of average barpress force (% ± SEM) above thresholds 20g **(bottom row of graphs)**, 40g **(middle row of graphs)**, and 60g **(top row of graphs)**. **A-C.** and **J-L.** show the three-day averages for FR1, extinction, and PR. **D-F.** and **M-O.** show individual sessions of extinction compared to the last session of FR1. **G-I.** and **P-R.** show individual sessions of PR compared to the last day of FR1.

Correlations of Durations vs. Max Force of Barpresses for FR1, Extinction, and PR

Even though the max force does not appear to show any significant changes when the rat is frustrated, there may a relationship between the max force and the duration of a bar press. Thus, we wanted to determine if there was a relationship between the two variables and the strength of that relationship. Furthermore, the slope of the correlation will reveal which of the two variables is a more sensitive measure of frustration. Our results show there were weak to moderately strong positive correlations when comparing durations and max force of barpresses for all rats during FR1, Extinction, and PR (Appendix A Supplemental Figure for Chapter 5). Representative scatterplots of Rat 18 durations vs. max force for sucrose (FR1, EXT, and PR; Figure 5.5A) and fentanyl self-administration (Figure 5.5B) are shown in Figure 5.5. While these ranged from weak to moderately strong positive correlations, the crucial observation is that the majority of slopes of the correlations decreased during extinction and PR compared to FR1 for both sucrose and fentanyl self-administration (Appendix A Supplemental Figure for Chapter 5). Representative Rat 18 slopes for sucrose were FR1 m = 7.000, EXT m = 1.172, and PR m = 5.854 (Figure 5.5A), and for fentanyl were FR1 m = 62.997, EXT m = 11.678 and PR m =4.968 (Figure 5.5B).

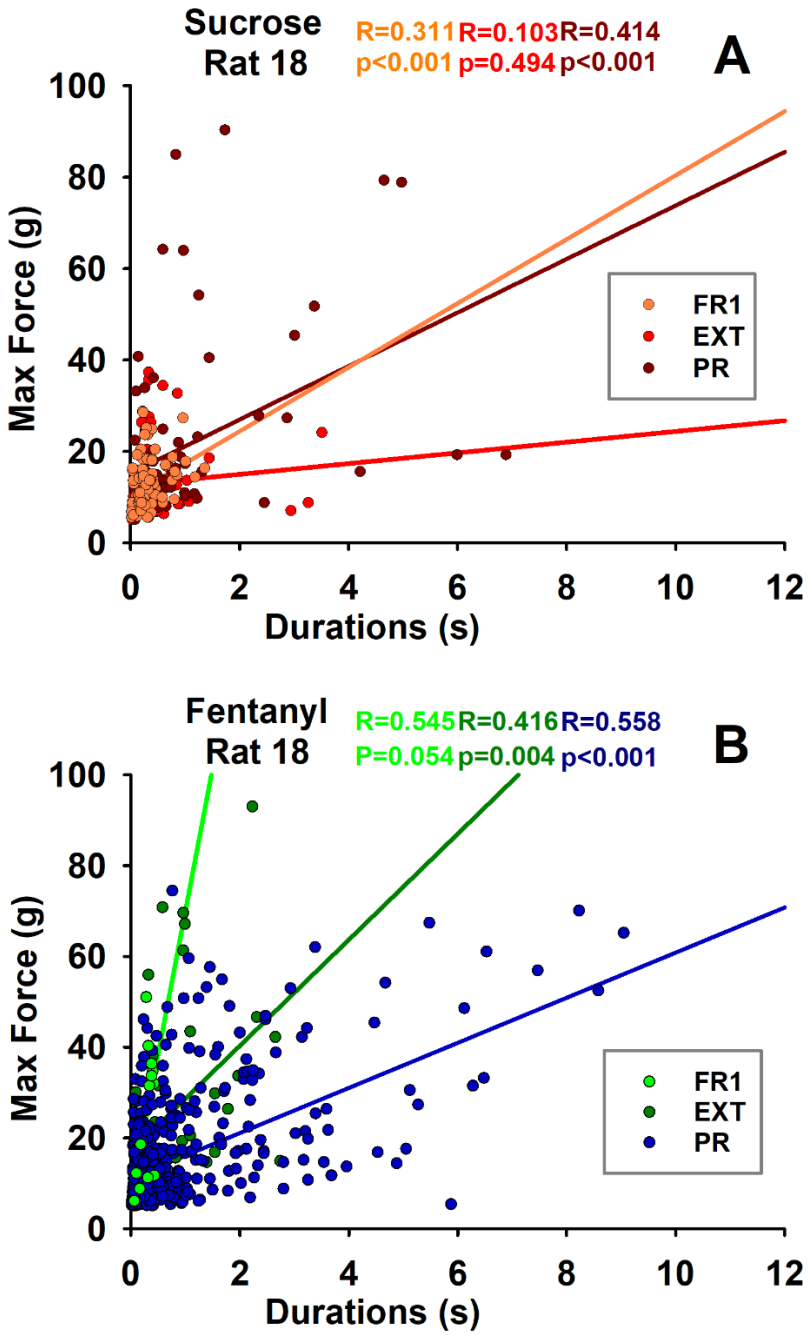


Figure 5.5: Barpress Durations Often Correlate with Force, but Frustration Increases Only Duration.

Simple linear correlation of representative Rat 18 to investigate the slopes and relationship between barpress durations (s) vs. max force (g) of barpresses for the last days of FR1, extinction, and PR for **A.** sucrose and **B.** fentanyl self-administration.

DISCUSSION

This current study replicated the increases in average barpress durations seen in previous research during extinction and PR for both sucrose and fentanyl (Vasquez, McAuley, et al., 2021; Vasquez, Shah, et al., 2021). However, when examining the force of barpresses, these data did not demonstrate the hypothesized increases in max force. Together, these results suggest that max force is an insufficient measure of frustration compared to barpress durations.

When examining the sum of the force over the duration of barpresses there were trends in increasing sum force. However, barpress durations were determined to be the major contributor to the observed trends. Therefore, the increases seen in bar press durations during frustration self-administration tasks become diluted when factoring in the force of barpresses. That is, the increased variability in max force makes sum force too variable. Thus, sum force is too an inadequate measure of frustration-related behavior.

To test the possibility that average force may be a less sensitive measure than the number of high-force presses, the percentage of barpresses above set force thresholds for cued extinction and PR compared to FR1 sessions were analyzed. The percentages of the force of barpresses above three thresholds (20g, 40g, and 60g) were used for the three-day average and individual day comparisons. Ultimately, these analyses demonstrated no significant changes in any of the factors. Thus, these results further support that the force of barpresses is ineffective as a measure of frustration-related behavior.

One question we had at the outset was if longer duration barpresses were merely a function of higher force, meaning that it takes longer to press with more force than to press with less. This could mean that force would be a more sensitive measure than duration. Interestingly, some data does support the first idea, but not the second. In all cases, there is a positive correlation between force and duration, particularly at forces just above the threshold (Appendix A Supplemental Figure for Chapter 5). However, during times of

frustration, the slope of that correlation tends toward longer barpresses rather than greater force (5 of 8 sucrose cases and 7 of 8 fentanyl cases; Appendix A Supplemental Figure for Chapter 5). Thus, duration is a much more sensitive measure than force.

It should be noted that although the increases in duration are convincing and do replicate our previous work, the magnitude of the increase was considerably less and variability was greater in this study (Vasquez, McAuley, et al., 2021). It is likely due to the previous non-force levers having a noticeable click that can be felt as tactile feedback upon successful pressing whereas the force levers have no such tactile feedback. This could become more important as the rats press repeatedly with no reward during extinction and later in progressive ratio.

One important question about long barpresses is whether longer barpresses represent a microaggression. It should be noted at the outset that frustration and aggression have been commonly and intimately associated with one another even before the frustration-aggression hypothesis proposed by Dollard et al., 1939. The frustration-aggression hypothesis has strived to describe this association by suggesting that frustration leads to some form of aggression and that aggression always presupposes the existence of frustration (Dollard et al., 1939). Berkowitz has since reformulated the frustration-aggression hypothesis to point out that failure to obtain a desired goal is not as necessary for aggression as it is for frustration (Berkowitz, 1989). One human study investigated the effect of frustration on aggressive behavior using the force exerted on a telephone when terminating a call (Kulik & Brown, 1979). Kulik and Brown's study demonstrated that the amount of force used is a reliable measure of aggression aroused in the presence of frustration in humans. Thus, if one assumes that an act of aggression would be associated with increased force, the current results argue against longer barpresses being a microaggression.

Altogether these data support barpress duration as a more suitable sensitive measure of frustration-related behavior in rats than the force of a barpress. Although the force has

not provided further insight into the role of frustration per se in rat self-administration, it still has the potential to investigate the possible role of aggression-related behavior in other aspects of self-administration and substance use disorders.

Ultimately these results do not disqualify changes in the force of a bar press from being a modification of a previously rewarded response. They simply suggest, that in response to frustration, increases in force are not as sensitive of a modification to continued approach behavior as increases in bar press durations. Thus, confirming bar press durations as a modification of the previously rewarded response is the present optimal way to measure the continued approach response strategy of frustration.

Chapter 6 Conclusions and Perspectives

SUMMARY

In this dissertation, I have investigated the use of bar press durations as a measure of frustration-related behavior during operant self-administration to study the effect of frustration on substance use behavior. My experiments have presented data needed to determine and validate the use of bar press durations as a measure of frustration-like behavior (Chapter 2 and 4). Additional experiments established the predictive validity of identifying individual differences in frustration-like behavior as measured by bar press durations prior to drug self-administration (Chapter 3). This work also affirmed bar press durations as the optimal measure of frustration-like behavior within operant self-administration compared to force of a bar press as a potential alternative (Chapter 5). Thus, the work I have presented within this dissertation solidifies bar press durations as an effective measure of frustration-like behavior to further elucidate the understanding of frustration within substance use disorders.

Prior to these experiments, there was a lack of a method to measure frustration-like behavior during operant self-administration. Although there are previous operant self-administration studies of frustration, these studies required the use of two different types of reinforcers to induce frustration through loss of alternative reinforcement. These studies also relied on differences in the number of either responses or reinforcers at the conclusion of a session (Ginsburg & Lamb, 2018; Gipson et al., 2012; Podlesnik et al., 2006; Pyszczynski & Shahan, 2013; Quick et al., 2011). While this loss of alternative reinforcement paradigm provides insight into frustration driving seeking of other reinforcers; without the presence of an additional reinforcer there is no way to observe frustration arousal within other operant tasks. This led to the need to establish and validate a way to measure frustration-like behavior in real time during multiple different self-

administration tasks. Subsequently, we discovered that bar press durations can be observed throughout a single session to study the effect of frustration (Appendix A Supplemental Figures for Chapter 2). Furthermore, utilizing bar press durations to observe frustration-like behavior is not constrained to only using the loss of alternative reinforcement paradigm but can be used to study several situations that arouse frustration during self-administration.

There are various self-administration tasks in which frustration can arise and it is necessary to demonstrate that we can observe changes in bar press durations during those tasks to validate bar press durations as a measure of frustration. For example within operant self-administration denial of reinforcement or partial reinforcement impacts expectation and leads to frustration. This is reflected in an increase in bar press durations during extinction and progressive ratio (Chapter 2 Figures 2.2 and 2.3; Chapter 4 Figures 4.5 and 4.6). Additionally, the impact of frustration on expectation can be observed through increases in bar press durations when there are changes in reinforcement size such as operant successive negative contrast and within-session dose-response (Chapter 3 Figure 3.2; Chapter 4 Figures 4.1 and 4.4). These observations of increases in bar press durations during frustration are also observed in different types of reinforcers as well as for both males and females (Chapter 2 Figures 2.2 and 2.3; Chapter 4 Figure 4.5). While it is imperative that we demonstrate increases in bar press duration during tasks that arouse frustration, it is also significant that we demonstrated a decrease in frustration related behavior through a decrease in bar press durations when the previously denied reinforcement was made available once again (Chapter 4 Figure 4.3). Furthermore, it was necessary to rule out some potential alternatives to bar press durations, such as force of a bar press and number of bar presses, as they may have been more sensitive measures of frustration-like behavior (Chapter 2 Figure 2.5; Chapter 5 Figures 5.1-5.5). Thus, measuring increases in bar press durations provide the opportunity to broadly study the effect of frustration during operant self-administration.

Frustration is a significant component of many neuropsychiatric conditions including substance use, however there is a shortage of predictive models to study the effect of frustration on substance use disorders. There are a few human studies that show persons with substance use disorders rate higher in tests of frustration and that sensitivity to frustration correlates with number of relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). Therefore, there was a need to demonstrate that frustration-like behavior as measured by bar press durations possesses the ability to predict future drug seeking and taking behavior. Chapter 3 highlights that increased frustration-like behavior (as measured by bar press durations) during frustrative nonreward tasks for sucrose pellet responding correlate with fewer infusions for intravenous fentanyl (Chapter 3 Figure 3.3). Consequently, demonstrating motivation for IV fentanyl can be predicted before drug exposure by examining frustration in a sucrose task. These results additionally suggest that higher frustration-like behavior prior to IV self-administration results in a decrease in motivation for IV fentanyl. This is the opposite of what we initially expected because the previous human studies demonstrate that higher levels of frustration result in an increased vulnerability to substance use and relapses (Baars et al., 2013; Ramirez-Castillo et al., 2019). Contrarily, it has been shown in a human study that experiencing frustration is also associated with decreased motivation, which can lead to a spillover effect causing motivation to recede in subsequent tasks (Fang et al., 2020). Thus, suggesting frustration is not just a motivator for drug seeking but a potential demotivator under different circumstances.

The work presented here demonstrates bar press durations can be used as a tool to measure frustration arousal and investigate the relationship between frustration and substance use behavior. This has been accomplished by recording the increases in bar press durations when eliciting frustration via extinction, progressive ratio, within-session dose-response, operant successive negative contrast and a novel frustrative nonreward task. The study utilizing the novel frustrative nonreward task contributed to the understanding of

frustration in relation to motivation and the effect on future drug seeking behavior. Altogether, this work highlights the appeal of using bar press durations to study frustration as it relates to susceptibility to substance use because this measure can be applied to many other frustration arousing operant self-administration tasks, both well established and novel.

SIGNIFICANCE

Frustration provokes adjustment to goal-directed behaviors when the behaviors are not rewarded. When frustrated, there are generally two ways the goal-directed behaviors are adjusted: variation of continued approach behavior or the initiation of avoidance behavior. The variation of approach behaviors tends to be a more immediate response to frustration if overcoming the source of frustration is deemed to be within one's control and the goal attainable (Jeronimus & Laceulle, 2017; Wong, 1979). Initiation of avoidance behavior arises in response to a frustration source that has been deemed uncontrollable or the goal unattainable (Daly, 1974; Jeronimus & Laceulle, 2017; Rosellini & Seligman, 1975). The experiments outlined within this dissertation have increased our understanding of the two general responses to frustration and, more importantly, how these responses to frustration can impact drug self-administration.

Variation of approach behaviors increases the probability of obtaining a denied reward. A collection of studies that focused on responses to frustration observed adjustments in the 'intensity, vigor, or strength' of the previously rewarded response. For example, increases in runways speeds, decreases in latency to initiate a response, and performing the behavior with increased variability in frequency and duration (Amsel & Hancock, 1957; Amsel & Roussel, 1952; Amsel & Ward, 1954; Skinner, 1938). Other studies documented subjects engaging in behaviors different from the previously rewarded responses when in the presence of frustration-arousing stimuli. Daly observed that some rats would initially nudge the food cup, sit, sniff around, or scratch at doors when experiencing frustrative

nonreward (Daly, 1974). Older studies of extinction by B. F. Skinner describe that an organism will increase the scope of responses to increase the likelihood of reinforcement (Skinner, 1938). Thus, two types of variation in approach behavior are modification of the previously rewarded response or exploration of different responses. Furthermore, the arousal of frustration facilitates modification and exploration of different responses when a situation is deemed controllable and the goal attainable (Jeronimus & Laceulle, 2017; Wong, 1979). Ultimately, these adjustments in approach behavior are standard strategies employed when the subject experiences unexpected nonreward and continues pursuing the denied reinforcement.

This dissertation presents evidence to assert that increases in bar press duration function as a modification of the previously rewarded response. The previous experiments observing response variation described increases in the 'vigor' of a response following extinction and frustrative nonreward (Amsel & Hancock, 1957; Amsel & Roussel, 1952; Amsel & Ward, 1954). The self-administration experiments within this dissertation parallel those described increases in vigor. During frustrative nonreward, the previous experiments show increases in runways speeds and faster response initiation, while our experiments show increases in bar press durations (Chapter 2 Figures 2.1-2.3; Chapter 3 Figure 3.2; Chapter 4 Figures 4.1, 4.4-4.6; Appendix A Supplemental Figures for Chapter 2: Figures 2, 3, 5-7, 9 and 10). However, the previous studies required the completion of a rewarded and a nonrewarded session to determine the effect of frustration. Interestingly, the method of measuring bar press durations has the benefit of observing changes in frustration levels throughout a single session. For example, the bar press durations gradually increase during a self-administration session that continually arouses frustration (Chapter 2 Figures 2.2 and 2.3; Appendix A Supplemental Figures for Chapter 2: Figures 2, 3, 5-7, 9 and 10). Thus, bar press durations directly measure the frustration response strategy: modification of the previously rewarded response.

The avoidance response to nonreward is employed when the subject recognizes the inability to control or influence the availability of a goal. However, when experiencing frustrative nonreward, it may not be immediately apparent that the goal is unachievable. Thus, there may still be an expression of response variation prior to transitioning to avoidance behavior. In an escape from frustration experiment, rats engaged in less variation of exploration behavior and increased the escape behavior of hurdle jumping (Daly, 1974). After additional nonrewarded trials, the hurdle jump to escape became the dominant response (Daly, 1974). Thus, the expression of frustration-motivated avoidance behavior may require repeated exposure to additional nonrewarded trials. Additionally, increases in response variation can occur simultaneously with decreases in responding before eventually terminating the behavior during extinction (Wong, 1979). Ultimately, escape and termination of the previously rewarded behavior are two types of avoidance responses to frustration. These avoidance responses are utilized because the subjects experience unexpected nonreward and consider the situation uncontrollable or the goal unattainable (Daly, 1974; Jeronimus et al., 2017; Rosellini & Seligman, 1975). In conclusion, while the employment of avoidance strategies may require experience to recognize that the goal is no longer attainable, it has the potential to be employed in conjunction with continued approach strategies.

In response to frustration, bar press durations are a continued approach strategy that can be employed in conjunction with the avoidance strategy: termination of responding. As previously discussed, when a goal is unattainable, exploration of response variation can occur simultaneously with decreases in responding before eventually terminating the behavior (Wong, 1979). The bar press duration data presented within this dissertation corroborates that approach and avoidance responses to frustration can be employed contiguously. The rolling average and cumulative record data demonstrate that the bar press durations continue to increase as the number of responses decrease throughout extinction and progressive ratio. (Chapter 2 Figures 2.2 and 2.3; Appendix A Supplemental

Figures for Chapter 2: Figures 2, 3, 5-7, 9 and 10). Furthermore, the spike in bar press durations observed towards the end of extinction and progressive ratio sessions could function as an early indicator of the transition from the approach strategy to the avoidance strategy (Appendix A Supplemental Figures for Chapter 2: Figures 2, 3, 5-7, 9, 10). In conclusion, increases in bar press durations are an approach strategy that could signal a future transition to avoidance strategies in real-time.

Approach and avoidance responses to frustration have the potential to impact substance use. Substance use may be appealing as drugs temporarily relieve aversive affect states (Khantzian, 1997). This suggests that substance use has the potential to provide relief from the affect state of frustration. Furthermore, clinical studies indicate that negative affect states contribute to the use, relapse, and dependence on addictive substances (Khantzian, 1997; Khantzian & Albanese, 2008; Weiss et al., 2009). Therefore, substance use may be a frustration-motivated exploration behavior used to overcome frustrative nonreward. Some clinical interventions use contextual extinction learning to reduce craving and relapse in substance abuse (Kaplan et al., 2011). Kaplan discusses the potential effectiveness of repeated exposure to contextual stimuli in inhibiting conditioned responses in the absence of the substance of abuse (Kaplan et al., 2011). Contextual extinction is repeatedly denying a goal causing frustrative nonreward within the same context. Therefore, frustration is a contributor to the development of the newly learned avoidance behavior of terminating the conditioned response. Thus, experience with employing avoidance responses to frustration has the potential to be protective against substance use and relapse.

When examining frustration-related behavior in rats during self-administration, increases in bar press durations provide insight into how approach and avoidance responses to frustration can impact drug self-administration. The results demonstrated that more significant changes in the approach response of bar press durations during previous exposure to frustrative nonreward tasks predict sooner termination of responding for the

drug fentanyl during progressive ratio (Chapter 3 Figure 3.3). This result was unexpected, as previous human studies found that lower frustration tolerance predicted increased substance use (Baars et al., 2013; Ramirez-Castillo et al., 2019). However, the FN tasks repeatedly expose the subject to frustration. As previously discussed, repeated exposure to frustrative nonreward can drive a transition from continued approach responses to avoidance responses. The progressive ratio schedule also assesses effort-related motivation (Hailwood et al., 2018; Hodos, 1961). Plus, repeatedly experiencing frustration can lead to a spillover effect causing motivation to recede in following tasks (Fang et al., 2020). Therefore, the increased approach frustration response observed during the FN sucrose self-administration tasks is associated with a decreased motivation spillover effect, leading to faster employment of the avoidance frustration response strategy during PR. Thus, bar press durations provide insight into the interaction between the approach and avoidance responses to frustration that can be applied in future studies of substance use disorders and disorders with a frustration component.

FUTURE DIRECTIONS

Now that we have established bar press durations as a measure for frustration-like behavior during operant self-administration, this measure can be utilized broadly within studies that possess a frustration component. The advantage of collecting the duration of the conditioned behaviors during operant self-administration is that there would be no need to reform the protocols of other models of substance use disorders and neuropsychiatric conditions. Additionally, this measure can provide additional insight into functional circuits involved in processing frustration and the subsequent impact on substance use through manipulating related neurobiology. Overall utilizing bar press durations as a measure of frustration-like behavior has wide-ranging applicability that can be readily implemented in operant self-administration paradigms.

In future studies, it would be essential to determine the interaction of frustration with other components of substance use, such as impulsivity, craving, and habit. Impulsivity is the tendency to act on a whim with no forethought or consideration of the consequences. In delay discounting, impulsive choice measures the relative preference for smaller, more immediate rewards over larger, more delayed rewards (de Wit, 2009; Perry et al., 2005, 2007; Stanis et al., 2008). It is conceivable that individuals experiencing frustration might engage in variations to their behavior and make more impulsive decisions that will relieve frustration. Thus, higher frustration levels could correlate with a preference for smaller, more immediate rewards, suggesting a potential relationship between frustration and impulsivity. Craving is an intense desire for a substance or activity. Some studies of craving demonstrate that as the duration of abstinence increases, so does the motivational impact of the drug-associated cues on operant drug seeking (Grimm et al., 2001; Lu et al., 2004; Neisewander et al., 2000; Pickens et al., 2011; Wolf, 2016). During the extinction experiments within this dissertation, the frustration level progressively increases throughout the session. As the abstinence period is a longer time frame of extinction, the frustration level may also increase during that period. Thus, abstinence could be operating as a source of frustration that ultimately contributes to the development of craving. Habit is a general tendency to perform a behavior regularly. Within substance use, habit is studied as a resistant behavior to changes in the value of a reinforcer (Panlilio & Goldberg, 2007; Root et al., 2009). Within the experiments studying habit, the changes in the value of the reinforcer could result in expectations not being met and thus causing frustration. However, in these experiments, the reinforcer is not entirely denied. Therefore, the subject could continue to engage in approach behavior rather than transitioning to avoidance behavior to alleviate the frustration. Thus, once a habit has been established, either the subject may not be experiencing frustration to the intensity necessary to transition termination of the behavior, or engaging in continued approach behavior has become the dominant response to frustration. Ultimately, the interactions between performance in the

self-administration models of these three elements of substance use disorder and frustration remain unknown. Therefore, measuring bar press durations during the self-administration models can be used to uncover, establish, and analyze the interactions. Obtaining this information would provide a better understanding of the relationship between these substance use behaviors that could lead to a more holistic understanding of an individual's behavioral susceptibility to drug use.

Additional prospective studies can measure bar press durations in coordination with manipulation of the neurobiology of frustration and substance abuse to identify the neurobiological roots of frustration. While it is well established that the mesolimbic pathway functions in reward-related learning and thus plays a role in substance use, the functional circuits involved in processing frustration during substance use remain unexplored. It has also been suggested that the amygdala influences the motivational mechanism that drives approach behavior during frustration (Henke & Maxwell, 1973). Furthermore, the basolateral amygdala functions in motivation, fear, aggression, and reward (Bertsch et al., 2020; Tovote et al., 2015). However, the amygdala's role in frustration has yet to be investigated in relation to substance use and drug self-administration. Nonetheless, there are neural circuits that interestingly play a role in the two general behavioral responses to frustration: approach and avoidance behavior. Activation of the ventral hippocampus-basolateral amygdala-medial prefrontal cortex circuit impacts an animal's subsequent approach or avoidance behavior (Jacinto et al., 2016). Additionally, basolateral amygdala projections to the ventral tegmental area can affect the appropriate balance of avoidance and approach behavior (Tovote et al., 2015). Accordingly, manipulating these neurocircuits may provide insight into the impact of approach and avoidance frustration responses on subsequent drug self-administration behavior. To help bridge the gap in understanding which neurobiological factors are associated with frustration in substance use disorders, bar press durations can be used to measure frustration behavior post manipulation of pathway-specific neuronal activity.

Consequently, future exploration of neurobiological activity within brain regions associated with frustration in substance use could lead to developing neuronal pathway-specific pharmacotherapeutics for substance use disorder.

Other potential studies could investigate a correlation between frustration-like behavior and performance during behavioral tasks designed to assess anxiety-, depression- and aggression-like behavior. This is important to examine because it has been suggested that frustration plays a role in several neuropsychiatric conditions, such as mood disorders like depression and anxiety, as well as mental illnesses with increased aggressive behaviors (Castellanos & Tannock, 2002; Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017; Scime & Norvilitis, 2006). Additionally, it is understood that uncontrollable or unavoidable experiences can result in frustration, anxiety, depression, and excessive amounts of psychological stress (Armfield, 2006; Daly, 1974; Doyle-Portillo & Pastorino, 2016; Jeronimus & Laceulle, 2017; Newman et al., 2013; Rosellini & Seligman, 1975). Furthermore, displays of aggressive behavior have been used to measure frustration tolerance in humans and are more likely to occur to remove sources of frustration if the goal is perceived as attainable and the situation controllable (Jeronimus et al., 2017; 2016; Jeronimus and Laceulle, 2017). Thus, the relationships between frustration and anxiety-, depression- and aggression-like behavior can provide insight into several neuropsychiatric disorders.

Displays of anxiety-related and frustration-related behaviors during stressful situations depend on the circumstances. In stressful situations where an animal is caught between performing two or more conflicting behaviors, the animal will engage in displacement behavior (Troisi, 2002). Displacement behaviors are actions irrelevant to the behavioral context where the animal engages in neither approach nor avoidance behavior (Breed & Moore, 2016). For example, self-grooming is when an animal has the desire to approach an object while at the same time being fearful of that object (Breed & Moore, 2016). Consequently, displacement behaviors are commonly observed in uncontrollable

situations and induce anxiety (Armfield, 2006; Newman et al., 2013; Troisi, 2002). Conversely, while some of our self-administration experiments study frustration, we observe the rats simultaneously engage in approach and avoidance behavior. For instance, during progressive ratio and extinction, the rats gradually decrease bar pressing while pressing the bar for more extended periods of time (Chapter 2 Figures 2.2 and 2.3; Appendix A Supplemental Figures for Chapter 2: Figures 2, 3, 5-7, 9, and 10). The rats can engage in both behaviors simultaneously because the behaviors are not in direct conflict. Furthermore, frustration is also known to result in variations of continued approach behavior or initiation of avoidance behavior (Daly, 1974; Jeronimus & Laceulle, 2017; Rosellini & Seligman, 1975; Wong, 1979). Thus, frustration-like behavior may be an inverse of anxiety-related displacement behavior because there is no conflict between displaying approach and avoidance behaviors when experiencing stress. Moreover, in Daly's experiments, she observed that before displaying escape behavior, some rats would initially nudge the food cup, sit, sniff around, or scratch at doors when experiencing frustrative nonreward (Daly, 1974). Therefore, some rats may display anxiety-related displacement behavior prior to avoidance behavior. Accordingly, anxiety-like behavior in some instances may precede frustration-like behavior. However, it is also possible that when a frustrating situation is perceived as uncontrollable, an individual may subsequently display anxiety-like behavior and withdraw from the situation (Jeronimus et al., 2017). Altogether, depending on the situation, frustration behavior may be inversely related to anxiety. In contrast, in other situations, anxiety-related behavior may predict future displays of frustration-related behavior and vice versa. However, more research is needed to confirm these relationships and how they apply to anxiety-related neuropsychiatric disorders.

Approach or avoidance behavior resulting from frustration could contribute to an individual's experience and expression of depression. The frustration resulting when a goal is unattainable leads to decreased approach behavior and is suggested to contribute to

depression (Jeronimus et al., 2017). Concerning bar press durations, lower bar press durations should predict subsequent performance on depression-like behavioral tasks. However, learned helplessness from being unable to avoid a situation transfers to a decrease in escape from frustration behavior (Rosellini & Seligman, 1975). Thus, it may be possible for individual differences in escape from frustration behavior to predict subsequent displays of learned helplessness and depression behavior. Ultimately, understanding this relationship may provide further insight into how frustration behavior (approach or escape) relates to the development of clinical depression.

While frustration may not always precede aggression, some individuals may be susceptible to frustration that leads to aggressive tendencies. Human research on frustration generally focuses on using aggressive behaviors to measure frustration, while animal studies of frustration describe increases in the intensity, vigor, or strength of the previously rewarded response (Amsel & Hancock, 1957; Amsel & Roussel, 1952; Amsel & Ward, 1954; Jeronimus et al., 2016, 2017; Jeronimus & Laceulle, 2017; Skinner, 1938). Additionally, when persisting in goal approach, aggressive behaviors are more likely to remove sources of frustration that are perceived to be within one's control (Jeronimus et al., 2017). Therefore, long bar press durations and force of a bar press could be micro-aggressive measures of approach behavior in response to frustration. By this reasoning, we should have seen similar increases in the force of bar presses compared to the duration of bar presses during frustration self-administration tasks, but this was not the case (Chapter 5 Figures 5.1-5.4). However, just because there were no similar increases does not mean neither were measuring micro-aggressive behavior. The results from Chapter 5 (Appendix A Supplemental Figure for Chapter 5) revealed that some rats have strong positive correlations between force and duration of barpresses, while the majority are weak correlations. Since there is the potential for frustration to presuppose some form of aggression, the force of a bar press may be a form of aggression response dependent on an individual's sensitivity to frustration. Thus, the individual differences in the correlation

between duration and force may suggest that some rats that display increased frustration behavior are more likely to display increased aggressive behavior. Studying this interaction will further elucidate individual differences in when frustration leads to aggressive tendencies and what role frustration may play in mental illnesses with increased aggression (i.e., intermittent explosive disorder, oppositional defiant disorder, and attention deficit disorder).

Altogether this suggests that identifying excessive frustration-like behavior may provide insight into neuropsychological disorders. Thus, using bar press duration as a measure of frustration behavior during self-administration could predict performance in anxiety-, depression- and aggression-behavioral paradigms. This could lead to a greater understanding of how an individual's frustration experience contributes to mood disorders or mental illnesses and subsequently improve personalized cognitive-behavioral therapies for those conditions.

Overall this dissertation has successfully identified and validated the use of bar press durations as a distinguished measure of frustration-like behavior during operant self-administration that lays a foundation to investigate ways to manipulate frustration-like behavior and the subsequent effect of frustration on future goal-directed behaviors.

Appendix A Supplemental Figures

Chapter 2: Real Time Measure of Frustration Behavior for Individual Rats

FIGURE 1: SUCROSE LOADING PHASE

Rolling average bar press durations for each rat self-administering sucrose for the first 20 bar presses versus the rest of the session during the last session of FR1.

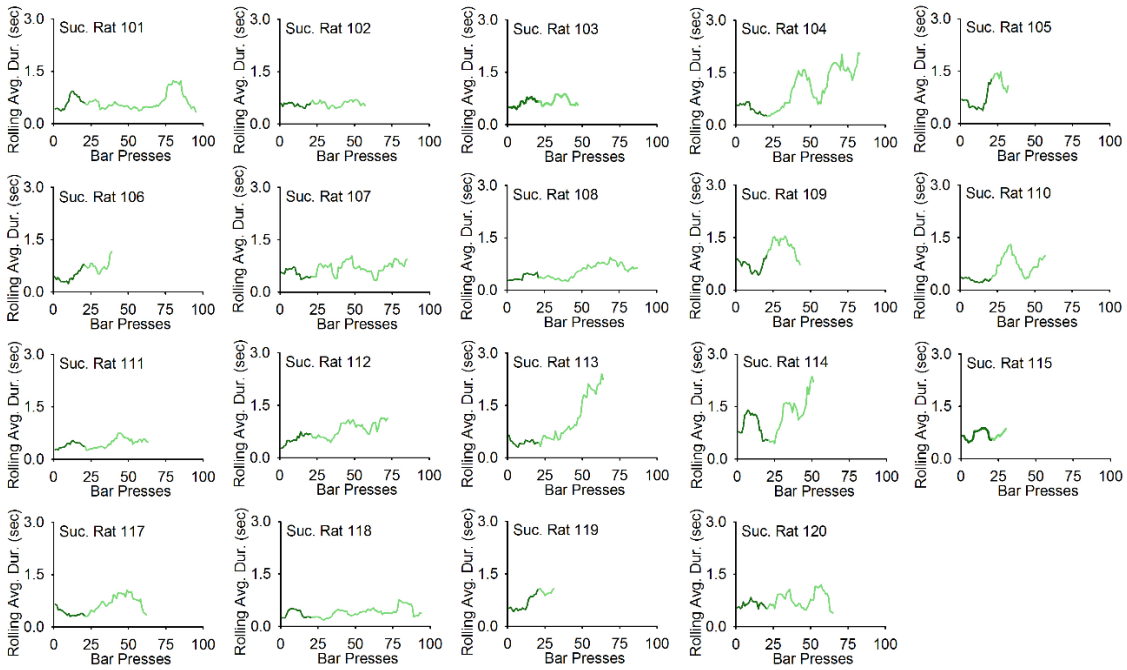


FIGURE 2: SUCROSE BETWEEN-SESSION EXTINCTION

Rolling average bar press durations of each rat self-administering sucrose during between-session cued extinction and the prior session of FR5.

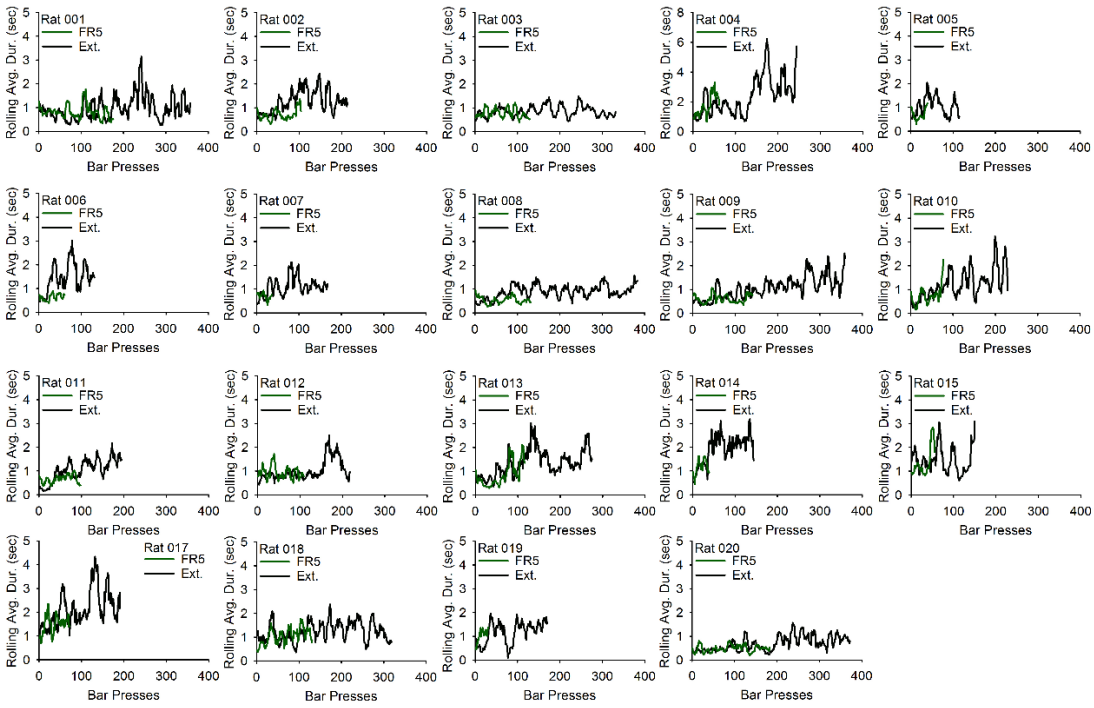


FIGURE 3: SUCROSE PROGRESSIVE RATIO

Rolling average bar press durations of each rat self-administering sucrose during Progressive Ratio.

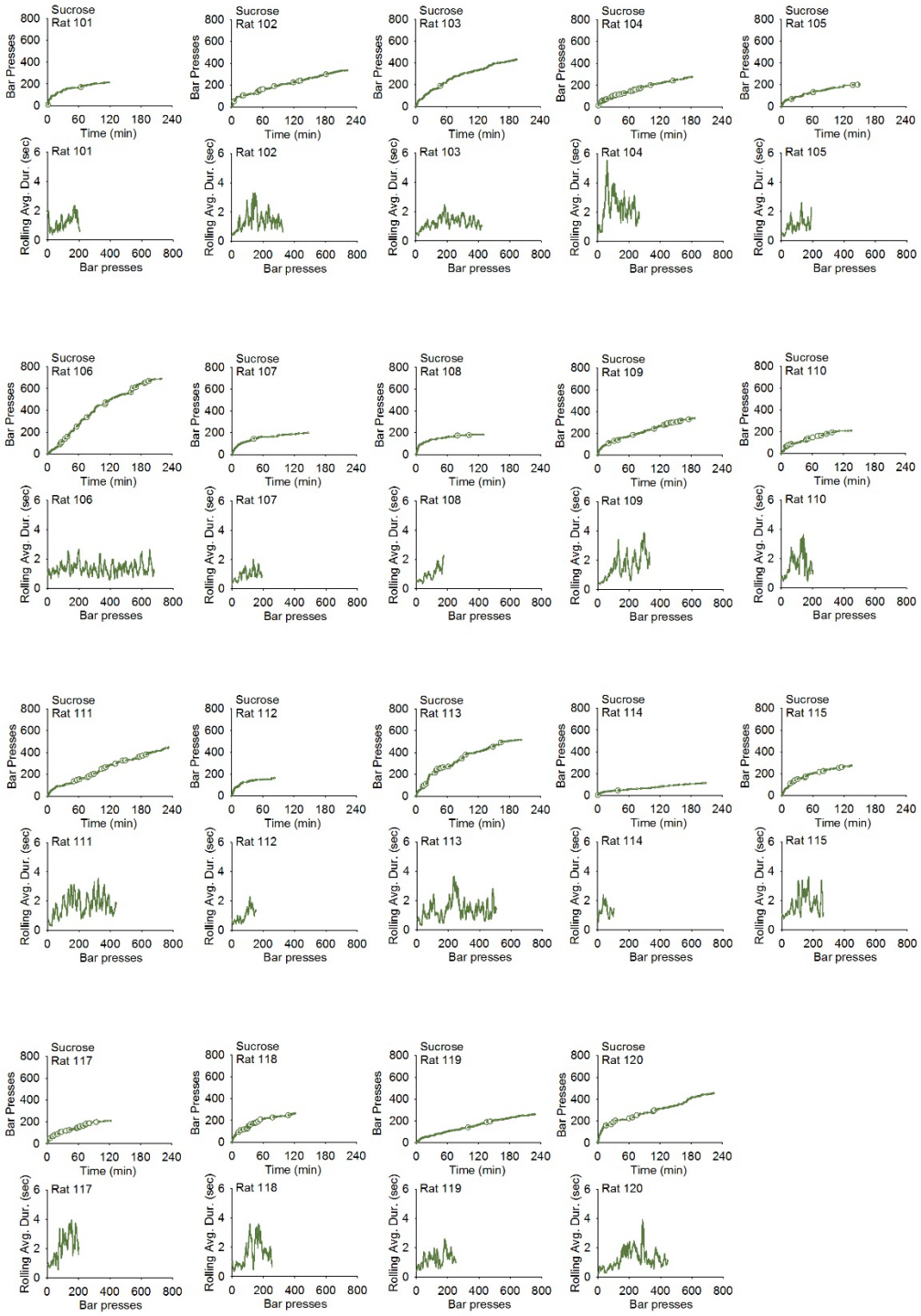


FIGURE 4: FENTANYL LOADING PHASE

Rolling average bar press durations for each rat self-administering fentanyl for the first 20 bar presses versus the rest of the session during the last session of FR1.

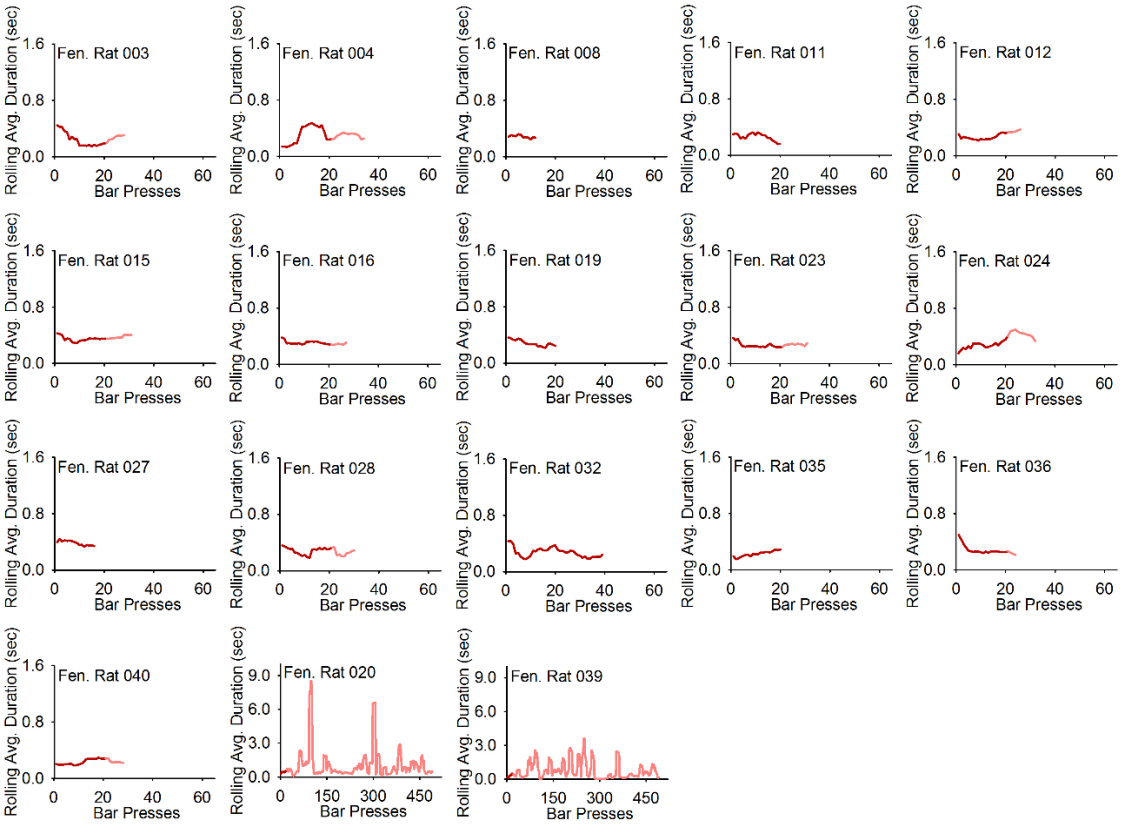


FIGURE 5: FENTANYL BETWEEN-SESSION EXTINCTION

Rolling average bar press durations of each rat self-administering fentanyl during between-session cued extinction and the prior session of FR5.

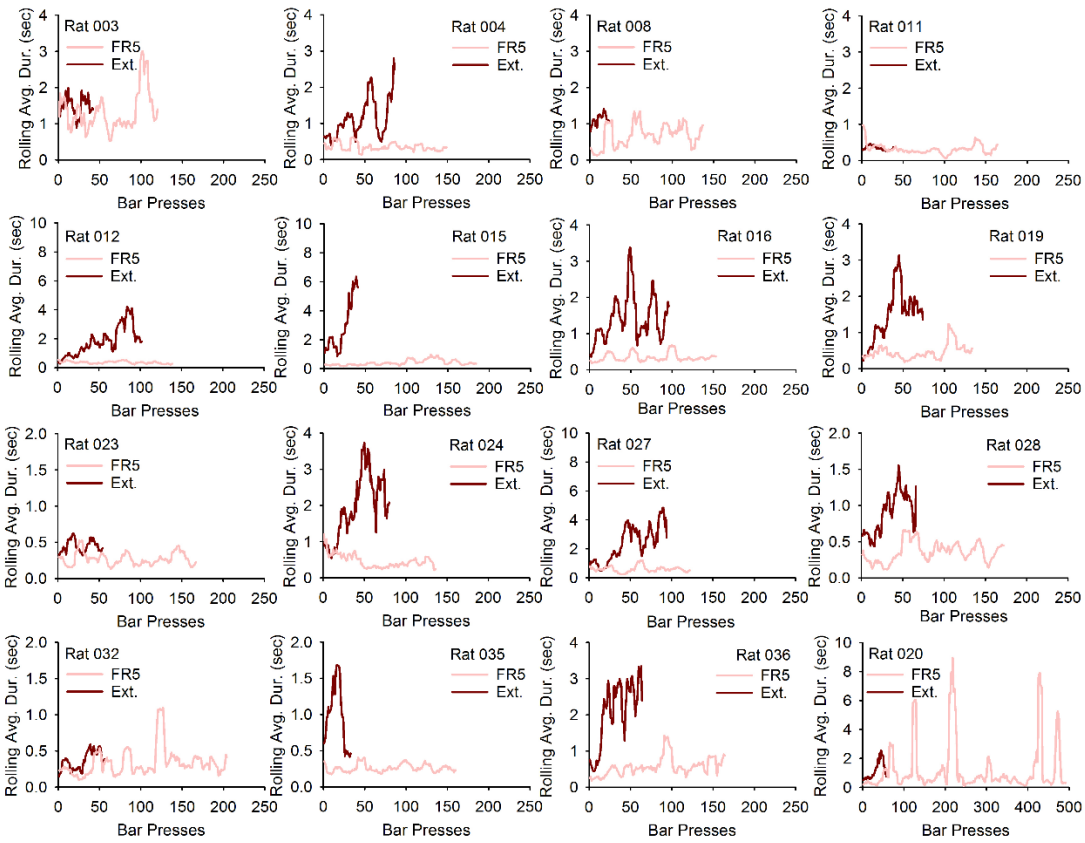


FIGURE 6: FENTANYL WITHIN-SESSION EXTINCTION

Rolling average bar press durations of each rat self-administering fentanyl during within-session cued extinction.

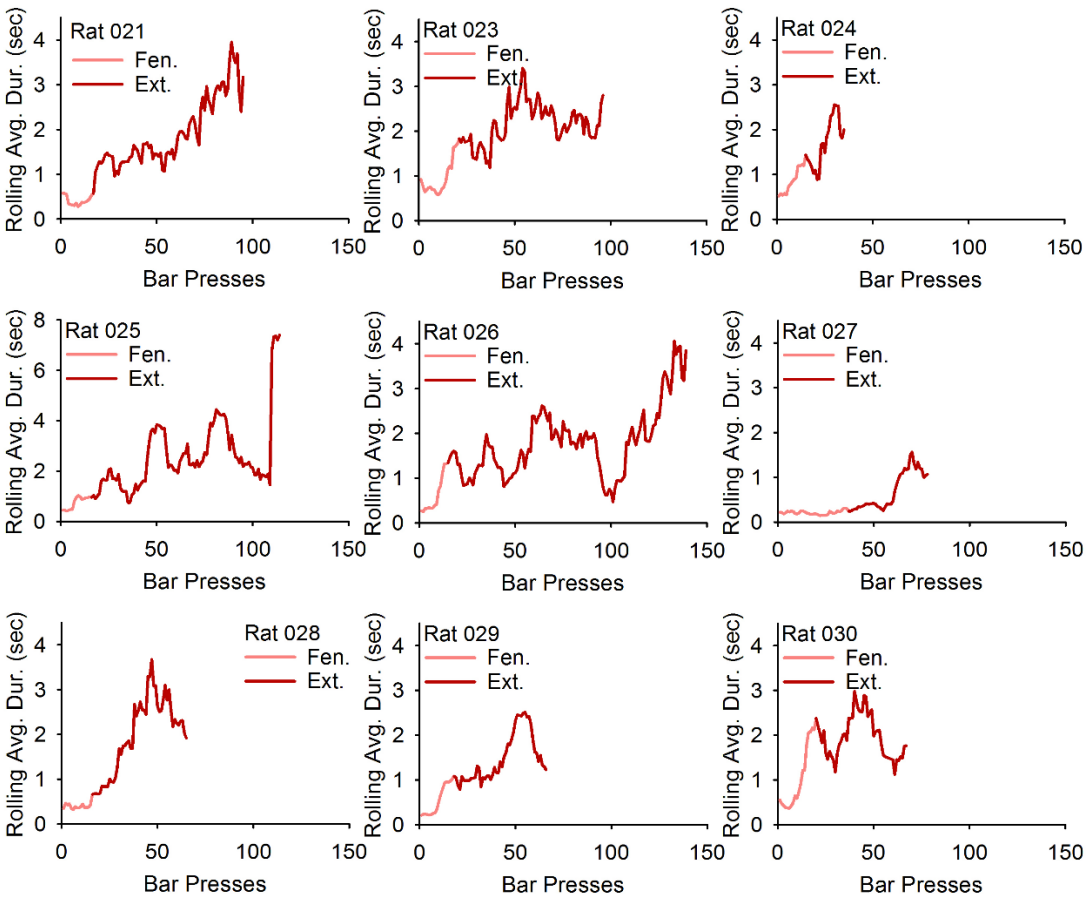


FIGURE 7: FENTANYL PROGRESSIVE RATIO

Rolling average bar press durations of each rat self-administering fentanyl during Progressive Ratio.

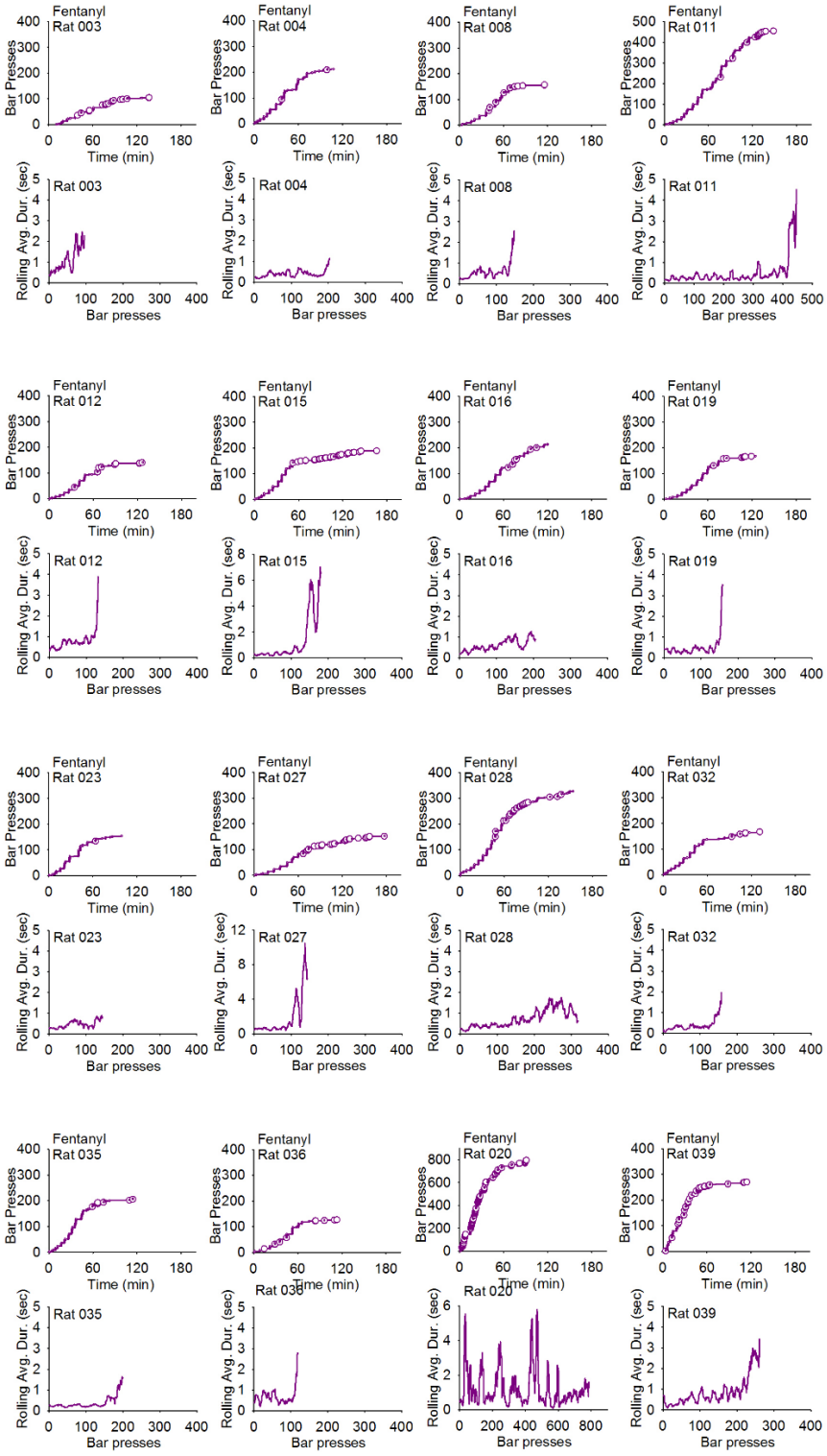


FIGURE 8: COCAINE LOADING PHASE

Rolling average bar press durations for each rat self-administering cocaine for the first 20 bar presses versus the rest of the session during the last session of FR1.

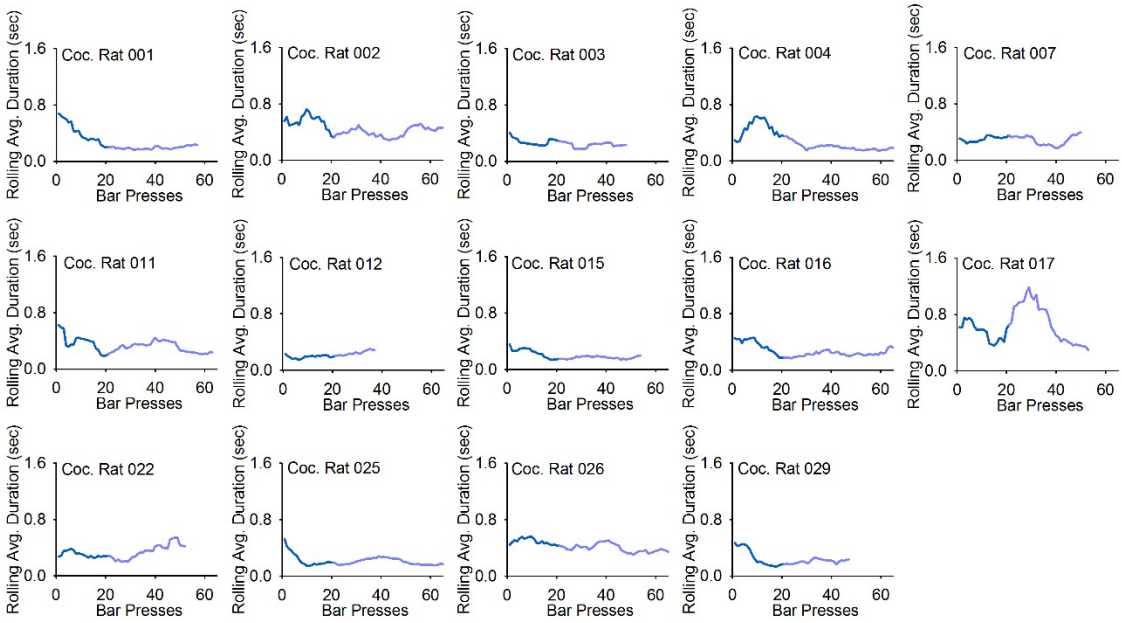


FIGURE 9: COCAINE WITHIN-SESSION EXTINCTION

Rolling average bar press durations of each rat self-administering cocaine during within-session cued extinction.

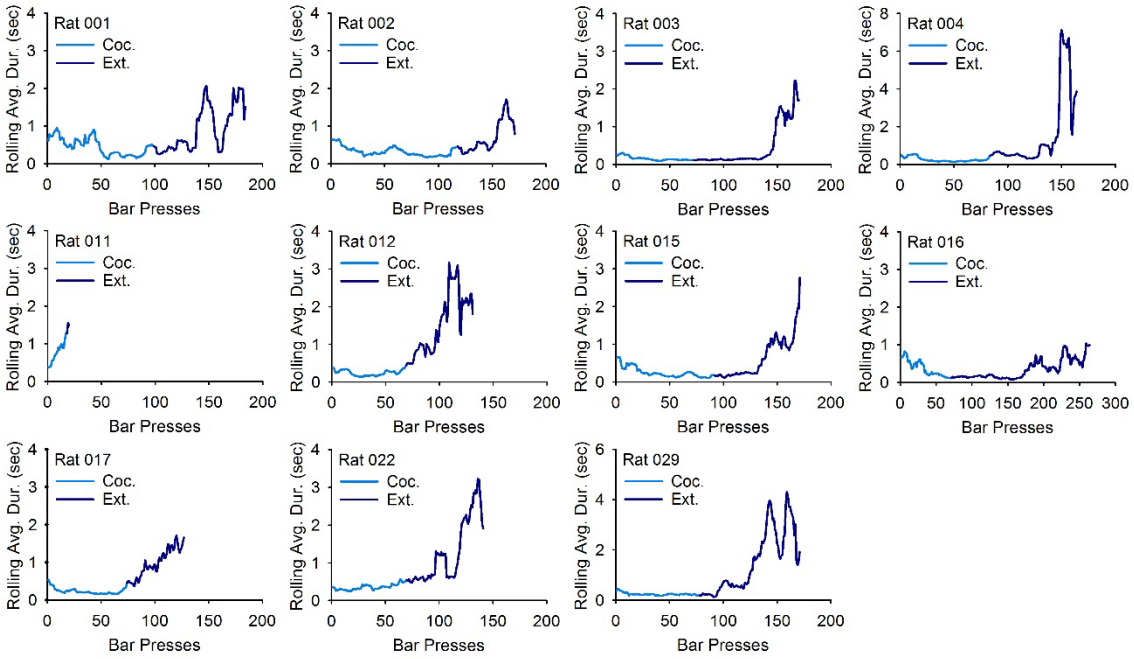


FIGURE 10: COCAINE PROGRESSIVE RATIO

Rolling average bar press durations of each rat self-administering high dose cocaine during Progressive Ratio.

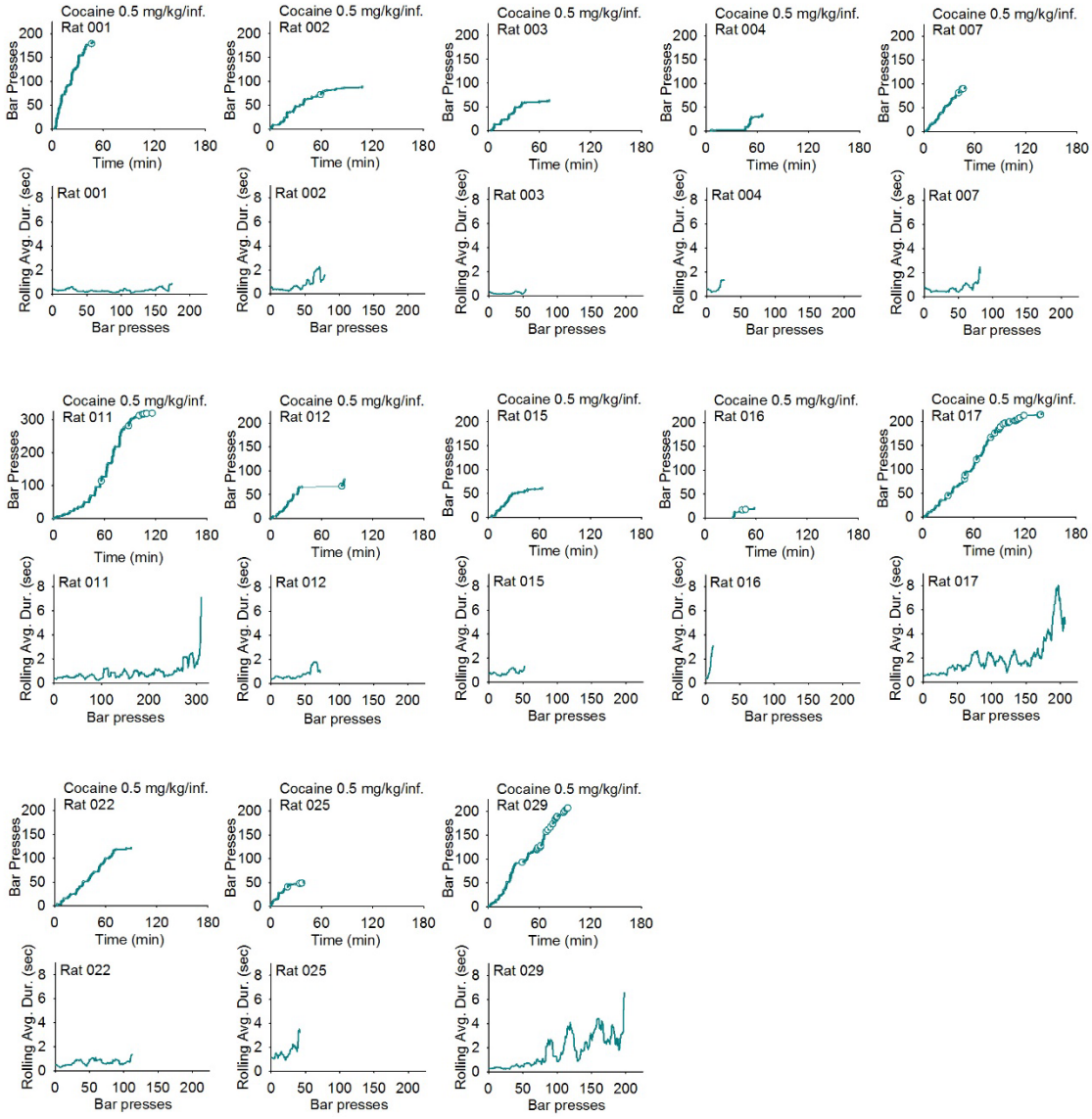
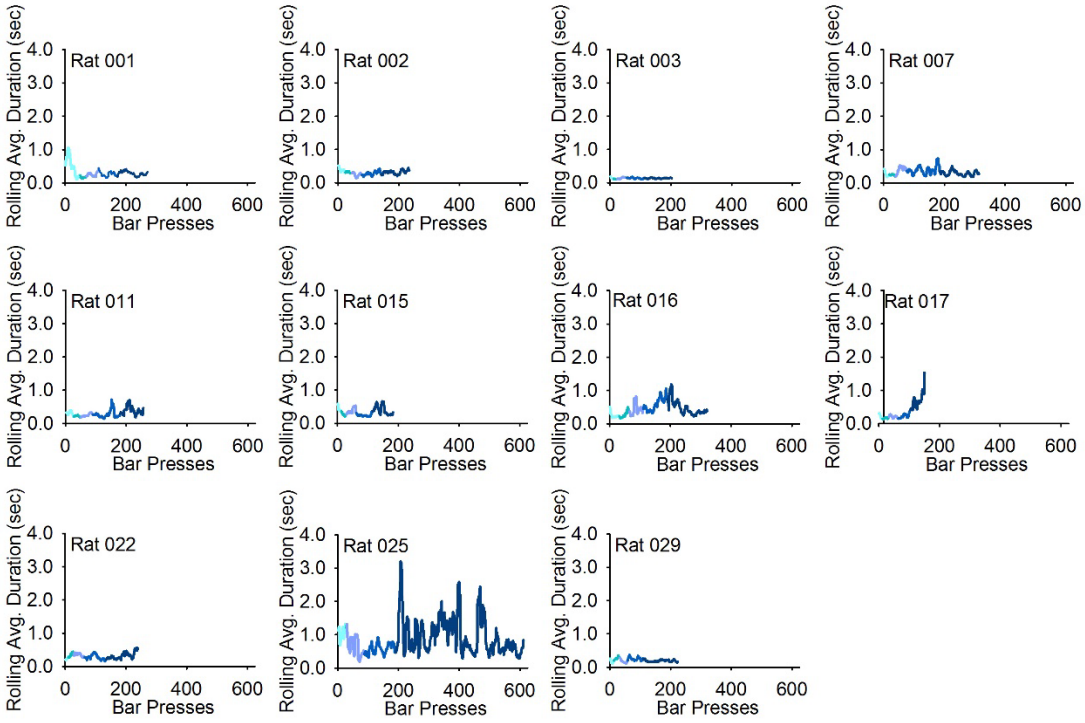


FIGURE 11: COCAINE WITHIN-SESSION DOSE-RESPONSE

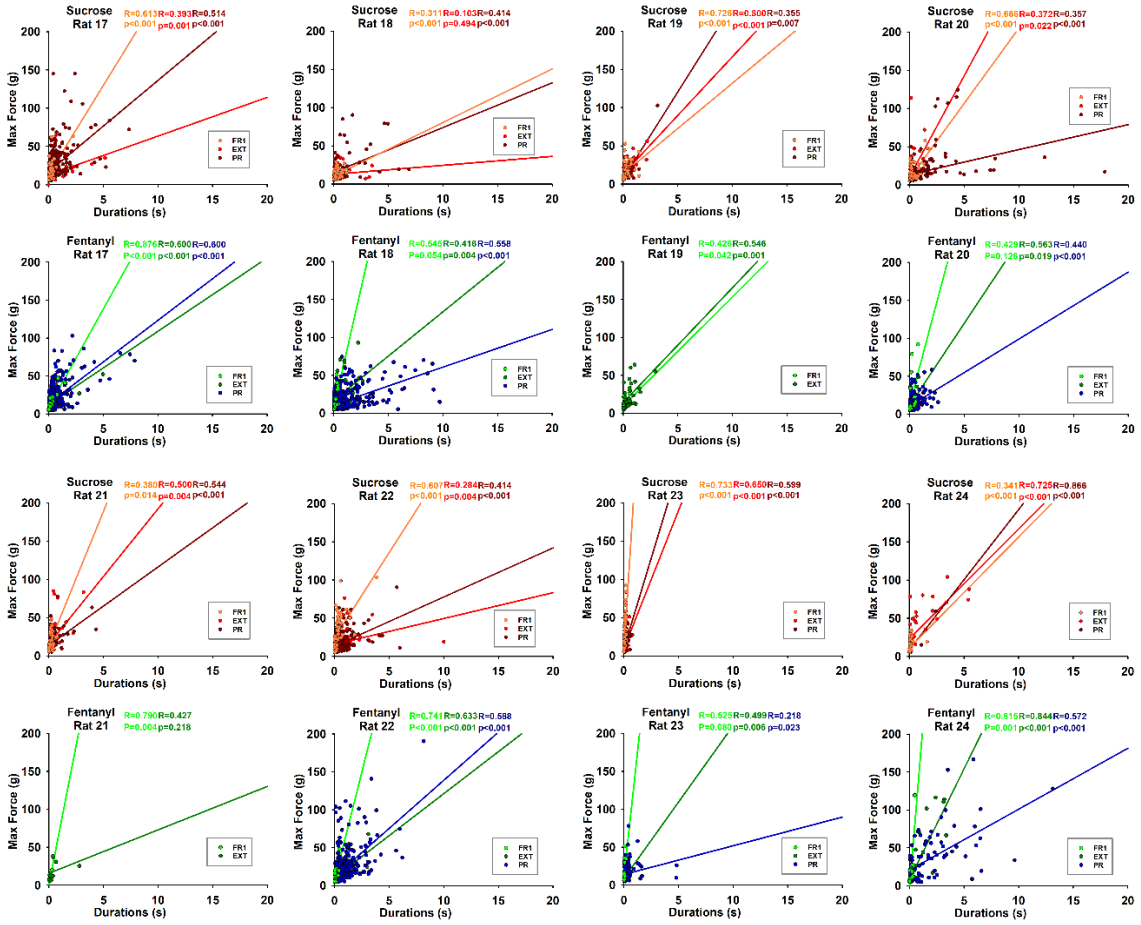
Rolling average bar press durations of each rat self-administering fentanyl during within-session dose-response.



Chapter 5: Correlations of Durations vs. Max Force of Barpresses for Individual Rats

CORRELATIONS FOR FR1, EXTINCTION, AND PR

Rolling average bar press durations for each rat self-administering sucrose for the first 20 bar presses versus the rest of the session during the last session of FR1.



Appendix B Supplemental Table

Chapter 2: Contol vs. Aldh1a1 AAV

COCAINE SELF ADMINISTRATION DATA FOR CONTOL VS. ALDH1A1 AAV

Rats were initially injected with shRNA adeno-associated viral vectors expressing a control hairpin or a hairpin directed at Aldh1a1 in the nucleus accumbens shell. The vector had no significant effect on the number of bar presses nor bar press durations. Thus, the rats were combined into one group for statistical analysis.

Cocaine Self Administration Control vs. Aldh1a1 AAV				
	Bar Presses		Average Bar Press Durations	
	Statistic	p value	Statistic	p value
Sucrose FR5	t(12) = 0.533	p = 0.604	t(12) = -1.402	p = 0.186
Maintenance	F (1,12) = 1.931	p = 0.190 Main	F (1 ,12) = 0.954	p = 0.348 Main
	F (2.038, 24.450) = 0.953	p = 0.401 Day	F (1.585, 19.022) = 1.418	p = 0.263 Day
	F (2.038, 24.450) = 0.485	p = 0.625 Interaction	F (1.585, 19.022) = 0.716	p = 0.471 Interaction
DAY 6				
DAY 7				
DAY 8				
DAY 9				
Within-Session Extinction DAY 1	F (1,9) = 3.549	p = 0.092 Main	F (1,9) = 0.989	p = 0.346 Main
	F (1,9) = 1.625	p = 0.234 Time	F (1,9) = 25.512	p = 0.01* Time
	F (1,9) = 4.184	p = 0.071 Interaction	F (1,9) = 0.124	p = 0.733 Interaction
1st Hour Cocaine				
2nd-4th Hours Extinction				
Progressive Ratio 0.5 mg/kg/inf. DAY 1	t(11) = -1.906	p = 0.083	t(11) = 0.789	p = 0.455
Within-Session Dose-Response DAY 8	F (1,9) = 0.718	p = 0.419 Main	F (1,9) = 0.457	p = 0.516 Main
	F (1.079, 9.713) = 14.466	p = 0.03* Dose	F (4, 36) = 2.970	p = 0.032* Dose
	F (1.079, 9.713) = 1.656	p = 0.230 Interaction	F (4, 36) = 0.502	p = 0.735 Interaction
0.5 mg/kg/inf.				
0.25 mg/kg/inf.				
0.125 mg/kg/inf.				
0.06 mg/kg/inf.				
0.03 mg/kg/inf.				

Chapter 4: Nine Criteria

NINE BEHAVIORAL NEUROSCIENCE CRITERIA FOR BARPRESS DURATIONS TO BE A MEASURE OF FRUSTRATION

The nine criteria along with the location of supporting evidence within this dissertation.

Satisfied Nine Criteria			
Criteria	Evidence Source		
	Chapter 2	Chapter 3	Chapter 4
1. Durations should increase when rats are frustrated	Figures 2.1 - 2.3		Figure 4.4
2. When frustration is removed, durations should return to normal			Figure 4.3
3. Durations should be a dynamic measure of frustration	Figures 2.2 & 2.3		Figure 4.4
4. Durations should be stable over time		Figure 3.1	
5. Frustration effect should be robust	Figures 2.2 & 2.3		Figures 4.5 & 4.6
6. Frustration effect should be replicable	Figures 2.1-2.3		Figure 4.5
7. Frustration effect should not be isometric with other concepts	Figure 2.5		
8. The effect should not be a function of performance variables			Figures 4.1 & 4.2
9. The effect should have some relevance to neuropsychiatric conditions for which frustration is known to be important	Figure 2.4	Figure 3.3	

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