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The Influence of Tetrabenazine on Operant Behavior and Binge-Like Eating Model in Rats

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Abstract

Tetrabenazine (TBZ), a vesicular monoamine transporter-2 (VMAT-2) inhibitor that preferentially depletes dopamine (DA), produces depressive symptoms including motivational symptoms in humans. In rodents, it reduces selection of high-effort effort alternatives in effort-based choice tasks, but does not affect food intake or preference (Nunes et al. 2013; Yang et al. 2020). However, no studies have focused on the effects of TBZ on binge-like eating to determine if it would influence “hedonic eating”. The current study used both binge-like eating and effort-based operant tasks in rats. To assess effects on binge-like eating, non-food restricted rats (n=8) were exposed to chocolate over 12 sessions and their chocolate intake was recorded. Following the initial training, rats were tested for the effects of either vehicle or 1.0 mg/kg TBZ. There was no significant effect of 1.0 mg/kg TBZ treatment on chocolate intake. For the effects on operant behavior, food-restricted rats (n=8) were trained on a fixed-ratio (FR)5/chow feeding choice task. With this task, animals have a choice between lever pressing to obtain a relatively preferred food (Bioserve pellets) or consuming a less preferred food (lab chow) that is available in the chamber. After training, rats were tested for the effects of injections of vehicle or 1.0 mg/kg TBZ. Administration of TBZ shifted the behavior from lever pressing, the high-effort alternative, to chow intake. Unlike high-effort choice, binge-like eating behavior was not affected by the depletion of DA induced by TBZ. These results indicate that TBZ is not impairing “hedonic eating” at the dose that reduces selection of high-effort activities such as lever pressing, further validating the TBZ model of motivational dysfunction.

Introduction

Motivational dysfunction

According to the National Institute of Mental Health, a 2020 study showed that an estimated 21.0 million adults in the United States had at least one major depressive episode, representing 8.4% of all U.S. adults (NIMH). According to the Diagnostic and Statistical Manual of Mental Disorders (DSM-V; APA 2013), major depressive disorders (MDD) consist of five or more of a variety of symptoms present during a two-week period, also constituting change from the previous function. At least one of the symptoms must be a depressed mood or loss of pleasure. Other symptoms include significant weight loss when not dieting or weight gain, change in appetite, insomnia or hypersomnia, thoughts of death, suicidal ideations/attempts and psychomotor agitation or retardation nearly every day.

Despite all of these symptoms being identified, patients with MDD also suffer from motivational problems on a daily basis affecting their employment and a variety of other social functions (Stahl, 2002). The motivational problems mainly include symptoms such as loss of motivation to complete tasks, anergia, and fatigue and patients with depression are less likely to make an effort to earn a reward (Treadway et al., 2012). There are currently six selective serotonin reuptake inhibitors (SSRIs) approved by the Food and Drug Administration in the United States that are commonly prescribed to treat depression, anxiety, and other mood disorders (U.S. Food and Drug Administration). SSRIs are known to primarily increase the levels of serotonin in the brain by blocking their reuptake into neurons (Hyttel, 1994) and some of the approved SSRIs include citalopram (Celexa), escitalopram (Lexapro), fluoxetine (Prozac,

Sarafem, Symbyax), and sertraline (Zoloft), and vilazodone (Viibryd; U.S. Food and Drug Administration). While SSRIs are able to combat some symptoms of depression, including mood dysfunction, rumination, and anxiety, clinical data suggests that motivational dysfunction often remains as a debilitating symptom of this condition even with SSRI treatment (Fava et al., 2014). These findings highlight the importance of developing therapeutics for effort-based motivational symptoms in MDD and other disorders to improve level of daily function and overall patient outcomes.

A variety of other mental disorders are known to display symptoms consistent with lack of motivation. According to the DSM-V, schizophrenia is characterized by a patient who displays two or more of the following symptoms: delusion, hallucinations, disorganized speech, grossly disorganized or catatonic behavior, and negative symptoms (APA 2013). Negative symptoms are the absence of behaviors typically present in most people, for example diminished emotional expression and avolition. Avolition, or a decrease in goal directed behavior, is a core negative symptom of schizophrenia, interacting with the other negative symptom domains — anhedonia, asociality, blunted affect, alogia (Strauss et al., 2021). Therefore, addressing avolition is essential in the development of pharmaceuticals that impact the negative symptoms of schizophrenia. Another example of a neurological disorder that affects motivation is Parkinson's disease. Although Parkinson's disease is typically classified as a disease of movement, studies show that regardless of medication status, patients with Parkison's disorder chose to engage less effort than control patients for the lowest reward (Chong et al., 2015). Parkinson's patients chose to invest more effort for a reward when they were in the ON relative to OFF dopamine state, indicating that dopamine (DA) has a

role in eliminating motivational deficits by promoting the exertion of effort (Chong et al., 2015).

Overall, it is clear that motivational dysfunction is widely observed in a variety of neurological disorders. This makes it critical to investigate therapeutics that target motivational deficits in order to produce the most effective treatments possible for patients suffering from these disorders.

Effort-related choice behavior

In order to understand motivational dysfunction, it is critical to define motivation and its features. Motivation has two aspects – directional and activational. The directional aspect is a behavior directed toward or away from a particular stimulus, for example the appetite to consume food (Salamone and Correa, 2002). Activational aspects are the energetic components of motivational behavior including vigor, speed, persistence, and working to obtain food (Salamone and Correa, 2002). For example, the persistence of an animal to obtain food is evident despite the great effort to earn the reward of the pellet. A combination of directional and activational aspects are essential for motivation to drive behavior. As discussed above, motivational drive and other related functions are often inhibited as symptoms of various mental disorders, resulting in anergia, akinesia, and fatigue. Crucially, these motivational symptoms can be modeled in animals by utilizing effort-based choice tasks (Salamone et al., 2018) in order to test the effectiveness of pharmacological therapeutics.

In the literature, there are several ways to utilize animal models that can measure effort-based decision making which involve the cost-benefit analyses organisms make

during decision making processes. This process can be studied by offering the organism a choice between high-effort/high reward options and low-effort/low reward options. Some examples include T-maze choice procedures where the animals have a choice (Salamone et al., 2018) between a high food density arm which is presented with an effort-based challenge (i.e., climbing a barrier) and a low food density arm with no barrier. Under normal conditions, animals prefer to exert the effort of climbing the barrier to obtain the food from the high-density arm. Another common method is the lever pressing choice procedures with various schedules (Salamone et al., 2018). In this paradigm, animals again have a choice between a high-effort/high-reward option in which they can press the lever to obtain the highly preferred high carbohydrate pellets or a low-effort/low-reward alternative which is consuming the standard laboratory chow that is concurrently available in the chamber. For example, with a Fixed Ratio 5 (FR5)/chow feeding choice task, animals have a choice between pressing the lever five times to obtain the relatively preferred food (Bioserve pellets), or consume the lab chow in the chamber. At the baseline level, a healthy rat would prefer to press the lever and obtain the preferred pellet and eat very little chow (Salamone et al., 1991). Other examples of lever pressing choice procedures include progressive ratio (PROG)/chow feeding choice task where the work requirement gradually increases throughout the session (Randall et al., 2012). Similar to the T-maze task, these concurrent lever pressing choice tasks have been validated in many ways for their ability to assess effort-related choice behavior and numerously used in testing novel treatments.

Given the complex nature of motivational functions, it is difficult to pinpoint the mapping between behavioral processes and neural systems, since motivational

functions have also been shown to be related to motor processes, emotion, learning, and other functions of the brain (Salamone & Correa, 2012). Yet, many studies showed that specifically mesolimbic DA is necessary for certain aspects of motivational function (Salamone and Correa, 2012). A major function of the mesolimbic DA is connecting the psychological distance that separates an organism from motivationally significant stimuli. In other words, when the mesolimbic DA system is interfered with, the tendency to exert effort for food reinforcement is decreased. DA depletions or antagonism make animals more sensitive to work requirements when it comes to instrumental tasks, like lever pressing (Salamone and Correa, 2002). Some examples include DA receptor antagonists such as haloperidol and ecopipam which are reported to decrease lever-pressing and increase the consumption of concurrently available chow in effort-based choice tasks when measured with FR5/chow feeding choice tasks (Cousins et al., 1994; Sink et al., 2008). The DA antagonist haloperidol decreases lever pressing and increases chow consumption at doses of 0.1 and 0.15 mg/kg (Cousins et al., 1994). Additionally, rats treated with the DA D1 antagonist SCH39166 (ecopipam; 0.05–0.2 mg/kg) or the D2 antagonist eticlopride (0.025–0.1 mg/kg) showed substantial decreases in lever pressing and increases in chow consumption (Sink et al., 2008). These studies underline specifically that DA antagonism is leading to a low-effort bias, which could be useful to serve as a deficit model. In Salamone Lab, DA antagonism and DA depleting agents have been repeatedly used to induce a low-effort bias in effort-based choice tasks.

Tetrabenazine as a deficit model

The vesicular monoamine transporter-2 (VMAT-2), found mainly in the central nervous system, transports the neurotransmitters serotonin, dopamine, norepinephrine, and histamine into vesicles for storage. Tetrabenazine (TBZ) binds to VMAT-2 receptors and acts as a reversible high-affinity inhibitor of mono-amine uptake into the granular vesicles of the presynaptic neurons (Yero & Rey, 2008). By inhibiting this transporter, TBZ depletes DA, reducing DA levels and allowing degradation enzymes present in the synaptic cleft to break down DA. DA is broken down into inactive metabolites by the enzymes monoamine oxidase (MAO), catechol-o-methyltransferase (COMT), and aldehyde dehydrogenase (ALDH) which act in sequence (Eisenhofer et al., 2004). Once broken down by these enzymes, the inactive DA metabolites are no longer able to act to transmit signals in the neuron. Due to the role of TBZ in the depletion of DA, it has been utilized to treat Huntington's disease in order to balance the DA levels in the brain, especially in the early hyperkinetic stage of Huntington's disease when the DA levels are increased in the striatum (Chen et al., 2013). In autopsy studies of Huntington's disease patients, patients who received TBZ displayed a greater overall depletion of DA than patients not exposed to TBZ, in all regions studied, with the greatest reduction in the caudate (Pearson and Reynolds, 1988). DA depletion was also found in the limbic regions, which may explain depressive symptoms as a side effect to TBZ treatment (Pearson and Reynolds, 1988).

Given that TBZ can induce depressive symptoms in humans, it has been suggested to be used as a tool to alter effort-related choice behavior. Rat studies that utilized effort-based choice tasks, such as FR5/chow feeding choice task, reported a

low-effort bias in lever pressing and chow intake in rats treated with 0.75 mg/kg and 1.0 mg/kg of TBZ (Nunes et al., 2013), similar to the studies with DA antagonists (Cousins et al., 1994; Sink et al., 2008). The Salamone lab has repeatedly shown that TBZ depletes DA, especially at the nucleus accumbens, inducing this low-effort bias in effort-related choice behavior tasks, in which TBZ-treated rats showed a decrease in lever pressing and increase in chow consumption (Rotolo et al., 2020; 2021). These examples include studies showing a 1.0 mg/kg TBZ dose reliably leads to a low-effort bias in the effort-related behavior by causing the shift from lever pressing to chow intake. Importantly, these low-effort bias effects were reversed through treatment with modafinil, methylphenidate, A2A antagonists, and novel DA transport (DAT) blockers CE-123 and CE-158 (Salamone et al., 2016; Nunes et al., 2013; Rotolo et al., 2019; 2020). Rats who received TBZ treatment (0.75 mg/kg) 90 minutes prior to testing followed by modafinil (3.75, 7.5, 15.0, 30.0 mg/kg), a DA reuptake inhibitor, 30 minutes prior to testing showed significantly increased lever pressing relative to TBZ plus vehicle and significantly decreased chow intake relative to TBZ plus vehicle (Salamone et al., 2016). Additionally, rats who received TBZ treatment (0.75 mg/kg) 90 minutes prior to testing followed by methylphenidate (0.5, 1.0, 2.0, 4.0 mg/kg), also a DA reuptake inhibitor, 30 minutes prior to testing showed significantly increased lever pressing relative to TBZ plus vehicle and significantly decreased chow intake relative to TBZ plus vehicle (Salamone et al., 2016). Recently, two DAT blockers, (S)-CE-123 and (S, S)-CE-158, demonstrated the ability to reverse the effort-related effects of TBZ, as well as increase selection of high-effort PROG lever pressing in rats tested on PROG/chow feeding choice task (Rotolo et al., 2019; 2020). At the neuronal level, when animals are

treated with a behaviorally active dose of TBZ, there is a decrease in extracellular DA in nucleus accumbens and changes in the expression of phosphorylated DARPP-32 in accumbens medium spiny neurons, indicating reduced transmission at both D1 and D2 DA receptors (Nunes et al., 2013). However, when MSX-3, an A2A antagonist, is used there is an increase in high-effort/high reward decision making (Nunes et al., 2013). More specifically, MSX-3 was only able to counteract the effects of TBZ on pDARPP-32 (Thr34) expression, indicating that A2A receptors are colocalized with D2 and not D1 receptors (Nunes et al., 2013). These studies in combination illustrate the ability to reverse TBZ-induced DA depletion with both DA reuptake inhibitors and A2A antagonists.

Despite its effects on DA depletion, it is critical to note that TBZ does not alter primary food motivation. In rat studies, TBZ did not alter food preference in free-feeding tests (Salamone et al., 1991), and did not produce effects similar to reinforcer devaluation by pre-feeding or appetite suppressant drugs (Randall et al., 2012; 2014). Specifically, appetite suppressants including cannabinoid CB1 antagonists have been found to not increase chow intake at doses that they suppress lever pressing (Salamone et al., 2002; Sink et al., 2008). These results indicate that TBZ does not alter appetite, but rather impacts how much effort rats are willing to exert in order to receive a high-reward food. Similarly, TBZ does not affect sucrose appetitive taste reactivity, sucrose consumption or preference, in free consumption tests (Pardo et al., 2015). In this study, rats were given access to varying concentrations of sucrose (0.3%, 0.5%, and 5%) and treated with TBZ (0, 0.5, 0.75, 1 mg/kg). At all doses of TBZ treatment, there was no significant difference between intake of the 0.3%, 0.5%, and 5% sucrose

reinforcers, indicating that TBZ does not impact the preference towards a certain sucrose concentration. This enforces the idea that the low-effort bias as a result of TBZ is based on motivation to work for a certain reinforcer, not necessarily sucrose itself. Moreover, the effect of TBZ on feeding behavior and temporal characteristics of responding on effort-related choice tasks has recently been investigated. During this experiment, detailed timing of lever pressing was monitored with an event recording system and the temporal characteristics of operant behavior was observed after 1.0 mg/kg tetrabenazine or vehicle treatments. It has been shown that TBZ increases the feeding duration and total number of feeding bouts, however, does not affect feeding rate or total time spent for both lever pressing to obtain pellets and consuming chow (Ren et al., 2022). Although there was a small effect on interresponse-time (IRT) distribution within ratios, the post reinforcement-pause (PRP) distribution was bimodal and TBZ did not increase the duration of PRPs (Ren et al., 2022). This illustrates that TBZ does not significantly alter consummatory motor acts involved in chow intake, but solely alters the relative allocation of time between lever pressing and chow. These findings again emphasize the trend that TBZ mainly affects the motivation to exert effort (i.e., pressing the lever for a pellet) not the motor activity or the primary food motivation required to do so.

This research demonstrates that TBZ predominantly affects the relative allocation of lever pressing versus chow, with little impact on the overall motor activity or primary food motivation. The intersection of these findings illustrates the precision of TBZ to reduce the selection of high-effort rewards while increasing chow consumption, suggesting that its interference with DA transmission leads to animals selecting lower-

cost alternatives to obtain food, while maintaining the primary food motivation (Salamone & Correa, 2002). Overall, these studies underline that TBZ remains a validated measure to study motivational deficiency models in effort-related choice tasks.

Hedonic eating

Hedonic eating is a term that is commonly used in literature to describe the consumption of highly palatable foods by rats (Salamone et al., 2022). Previous studies have indicated that hedonic eating may involve the dopaminergic pathways emanating from the midbrain ventral tegmental area, projecting to the nucleus accumbens in the ventral striatum and other areas such as the amygdala, medial prefrontal cortex, hippocampus, and hypothalamus (Kenny, 2011). Several studies have also implicated the central DA system in a variety of human eating disorders (Bello and Hajnal, 2010). As discussed above, DA transmission within the nucleus accumbens does not significantly impact hedonic reactivity to different tastes, nor does it seem to affect primary food motivation and appetite alike (Salamone & Correa, 2012). To further test the effects of TBZ in hedonic eating, binge-like eating models can be utilized in animal models.

Binge eating disorder (BED) is defined as an excessive intake of food within a short period of time in combination with feelings of anxiety and shame when or about overeating (NIMH). These episodes must occur at least once a week over a 3 month period (DSM-5; APA 2013). BED affects approximately 2% of the US adult population, making it important to develop animal models of palatable food consumption and food seeking that may have relevance for BED and other conditions associated with

excessive food intake (NIMH). In rats, this can be modeled by exposing them to a highly palatable food (i.e., chocolate) in short intermittent periods of time (Vickers et al., 2015). Lisdexamfetamine (LDX) is commonly used to treat BED and has been shown to affect food intake and food-reinforced operant behavior, with larger effects when rats are exposed to chocolate (Presby et al., 2020). LDX is a central nervous system stimulant that increases DA levels by inhibiting its reuptake into the presynaptic neuron and thereby increasing the release of DA into the extra neuronal space (Griffiths et al., 2019). In Presby et al. (2020), three groups of female rats who were not restricted from food received different food exposure conditions over several weeks: a chocolate exposure group, a lab chow exposure group, and an empty food dish group. LDX significantly reduced intake of both chocolate and chow in the chocolate exposure group. In the lab chow exposure group, overall chow intake was reduced with LDX treatment. This study provides an increased understanding of the role of increased DA via LDX in binge-like eating behavior.

For the purpose of the present study, binge-like eating behavior is facilitated by repeated exposure to chocolate as it represents large-scale intake of a highly palatable food in non-restricted animals. The primary aim of this experiment is to gain a better understanding of the role of DA depletion by TBZ in “hedonic eating” that is modeled by utilizing highly palatable food intake in rats. TBZ will also be tested on and operant effort-based choice task to further understand its influence on effort-based choice behavior. It is hypothesized that TBZ is not reducing “hedonic eating” at the doses that induce a reduction in high-effort/high-reward selection.

Materials and Methods

Animals

Adult, male Sprague Dawley rats (Envigo, Indianapolis, IN, USA) were housed in a colony maintained at 23 °C with 12-h light/dark cycles (lights on 07:00). Rats (n = 8 for the operant training; n = 8 for binge-like eating model) weighed 275–299 g at the beginning of the study. The operant training group was initially food restricted to 85% of their free-feeding body weight for operant training with a modest weight gain throughout the experiment. Rats were fed supplemental chow to maintain weight throughout the study, with water available *ad libitum*. Animal protocols were in accordance with University of Connecticut Institutional Animal Care and Use Committee guidelines.

Development of chocolate binge eating behavior

Ground Cadbury's Dairy Milk Chocolate composed of 0.3 g fat, 0.57 g carbohydrate, and 0.073 g protein with a total of 5.34 kcal/g was utilized in the development and maintenance of binge like eating behavior. To develop binge-like eating behaviors, rats were initially exposed to 1-hour sessions in an empty feeding cage with a ceramic dish for 3 days. Following this initial exposure, the rats had 12 exposure sessions for 1 hour each in an empty feeding cage with a ceramic dish containing ground chocolate. These chocolate exposures took place on days 1, 2, 4, 5, 7, 8, 12, 14, 15, 18, 23, and 28 in which the amount of chocolate consumed was recorded after each session (Presby et al., 2020). The weights of the rats and consumed home-cage laboratory chow were recorded daily. Water in the home cage

was provided *ad libitum* throughout the experiment. After the initial training, drug experiments had started.

Behavioral procedure of operant training

The rats were trained in a fixed ratio (FR) 5/chow feeding choice task. Using Med Associates operant chambers (28 × 23 × 23 cm), rats were trained with the following schedule – 3 days of magazine training, followed by 3 days of FR 1. After, the rats were trained to lever press for the plain high carbohydrate pellets on a FR5 schedule for 5 weeks which was followed by FR5/choice training for another 5 weeks. Lab chow (5P00 ProLab RMH 3000; ScottPharma Solutions) was used during the exposure sessions, operant task, and non-food restriction phase, and was composed of approximately 14% fat, 60% carbohydrate and 26% protein with a total of 4.18 kcal/g. For the operant task, the pellets (Dustless Precision Pellets, 45 mg, Rodent Purified Diet; Bio-Serv) were composed of 5.5% fat, 59.1% carbohydrate and 18.4% protein with a total of 3.6 kcal/g. Water in the home cage was provided *ad libitum* throughout the experiment. After the initial training, drug experiments had started.

Drug treatment

TBZ (Tocris Bioscience) was dissolved in a solution of 20% dimethylsulfoxide (DMSO) and 0.9% saline prior to each drug treatment. It was then titrated with 1N HCl resulting in a pH of about 4.5. In order to formulate the vehicle (VEH) solution, 20% DMSO and 80% saline were combined with an equal amount of HCl to maintain the same volume as the TBZ solution.

Experimental design

Experiment 1

Following the completion of the binge-like eating training, rats experienced two sessions in the empty feeding cage with the ceramic dish containing chocolate for 1 hour each week. Their weights and their home cage lab chow intake were recorded Monday through Friday. A repeated measure design was used to assess the drug effects. After the completion of this phase, rats were injected with VEH or 1.0mg/kg TBZ 120 minutes prior to the beginning of the experiment which took place once a week, on Thursday or Friday. The amount of chocolate consumption was recorded after the conclusion of each exposure.

Experiment 2

A repeated measure design was used to assess the drug effects. After the completion of the operant training, another set of rats were injected with 1.0 mg/kg of TBZ or VEH 120 minutes before the beginning of the experiment. This was performed over consecutive weeks in a randomized order in which drug experiments were conducted on Thursday or Friday. For each session, the lever pressing and chow intake were then recorded after each run.

Statistical analysis

A paired sample t-test was used to compare the TBZ and VEH conditions for each binge-like eating behavior and operant task by using Statistical Package for the Social Sciences version 28 (SPSS; IBM).

Results

For the binge-like eating behavior, there was no significant difference in chocolate intake for TBZ (M = 7.075, SD = 0.500546576) and VEH (M = 6.45, SD = 0.744773455), t(7) = -0.625, p = 0.420 (**Figure 1**).

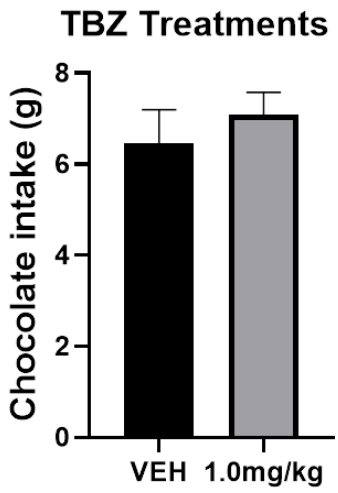

Figure 1. *The effects of tetrabenazine on chocolate intake in binge-eating behavioral model (n=8).* Mean (±SEM) chocolate intake during the 1-hour exposure session. There was no statistically significant difference between 1.0 mg/kg TBZ and VEH chocolate intake.

For the operant behavior, there was a significant difference in lever pressing for TBZ (M = 367.3750, SD = 411.98611) and VEH (M = 1416.125, SD = 261.38064), t(7) = 4.806, p = 0.002 (**Figure 2**). There was a significant difference in chow intake for TBZ (M = 5.5125, SD = 2.50681) and VEH (M =0.9500, SD = 0.89443), t(7) = -6.111, p = < 0.001 (**Figure 3**).

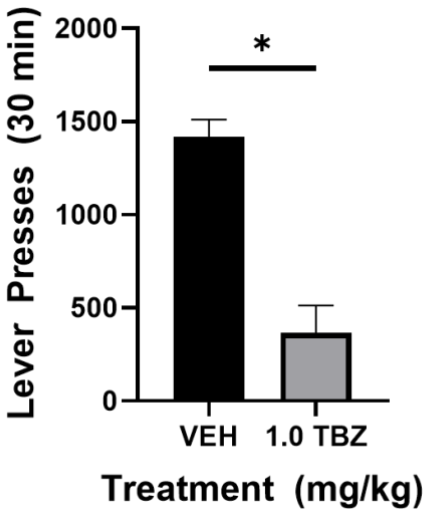

Figure 2. *The effects of tetrabenazine on lever pressing in the FR5/chow feeding choice task effort related decision-making (n=8).* Mean (±SEM) number of total lever presses during the 30-minute operant session, in which *p = 0.002: statistically significant difference between 1.0 mg/kg TBZ and VEH on number of lever presses.

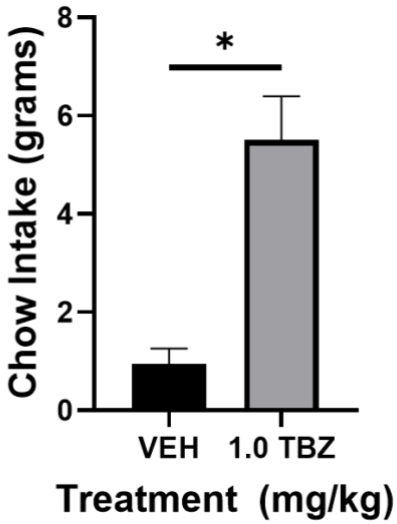

Figure 3. *The effects of tetrabenazine on chow intake in the FR5/chow feeding choice task effort related decision-making (n=8).* Mean (±SEM) chow intake during the 30-

minute operant session, in which *p = 0.002: statistically significant difference 1.0 mg/kg TBZ and VEH chow intake.

Discussion

The aim of this study was to determine if TBZ reduces “hedonic eating” at the doses that induce a reduction in high-effort/high-reward selection. As hypothesized, there was not a statistically significant difference between chocolate intake with TBZ treatment (**Figure 1**), helping us understand the effects of TBZ in hedonic eating by modeling binge-eating behavior. Treatment with TBZ resulted in a statistically significant decrease in lever pressing (**Figure 2**) and increase in chow intake (**Figure 3**). This illustrates that low-effort bias induced by impairment of DA transmission, in this case by TBZ, is not a result of lack of primary food motivation or “anhedonia”.

These findings concur with a wide variety of research that DA transmission does not affect hedonic activity or primary food motivation, it is involved in specific functions of motivation and exertion of effort (Salamone and Correa, 2012). Moreover, it has been discussed that interference with DA transmission leaves the primary food motivation and food reinforcement intact (Salamone and Correa, 2002). Previous studies have consistently shown that DA antagonism and depletion in rats does not alter their hedonic taste reactivity to sucrose, as well as sucrose preference in general (Berridge & Robinson, 1998). The findings concur with previous studies involving the effects of TBZ including pre-feeding in which animals are fed the day before of their operant run, or with appetite-suppressant drugs such as CB1 agonists (Randall et al., 2012, 2014; Salamone et al., 2002; Sink et al., 2008). In addition, TBZ has been found to not

influence the food and sucrose preferences in rats (Nunes et al., 2013; Pardo et al., 2015). In a recent study, TBZ also has shown to not alter feeding rate or time allocated to feeding during operant tasks, FR5/chow feeding choice task (Ren et al., 2022). In all of these examples it is clear that DA depletion by TBZ is not affecting “hedonic eating” or altering the primary feeding motivation. In the present study, treatment with TBZ did not change the intake of a highly palatable food, such as chocolate, that was presented for a short duration of time. In fact, TBZ animals show a slight increase in their chocolate consumption compared to vehicle condition rats, further suggesting that TBZ is not affecting hedonic eating.

Although it has been shown that TBZ does not affect primary food motivation, preference, or hedonic eating as discussed above, it does influence the exertion of effort as tested in effort-based decision-making tasks. In baseline conditions, the rats prefer to press the lever to get the highly palatable food, Bioserve pellets, and eat little chow in FR5/chow feeding choice tasks. As has been shown many times in literature, 1.0mg/kg dose of TBZ induces a low-effort bias, when tested in FR5/chow feeding choice tasks (Rotolo et al., 2020; 2021) in which TBZ treated rats have lower lever pressing but increased chow intake. In the current study, this finding was replicated and TBZ treatment of rats decreases lever pressing and increases chow intake in FR5/chow feeding choice task in comparison to the vehicle condition. It is important to note that when there is a decrease in lever pressing, there is an increase in the concurrently available chow intake in all the aforementioned studies utilizing TBZ, underlining the fact that it is not altering the primary food motivation. However, the same dose that leads to a shift in choice behavior, did not alter chocolate consumption in free-feeding animals

as measured by the chocolate intake in binge-like eating behavior model. Importantly, the fact that TBZ treatment did not significantly impact chocolate intake in the binge-eating model and only led to a low-effort bias, shows TBZ primarily affects the choice behavior. This highlights the role of TBZ treatment in inducing low-effort bias in effort-related choice tasks, making it a valid model of motivational dysfunction.

As with any scientific experiment, there were several limitations to this study. Although the use of rodent models has been essential for understanding the pathogenesis of many human diseases, including BED, it is difficult to model all features of human BED in rat models (Corwin and Buda-Levin, 2004; Perello et al., 2010). Most notably, these models measure the objective characteristics of binge-eating, the consumption of a large amount of food over a short period of time, and exclude subjective characteristics including the feeling of loss of control felt by patients with BED (Perello et al., 2014). A significant weakness in studying MDD in rats is that this is a short-term application of depression, which is very different from the long-lasting cases of human MDD (Nestler & Hyman, 2010). It is important to acknowledge these limitations when considering the results of this study.

Due to the high incidence of depression in our country and world, it is highly important that we continue to investigate the fine details of the symptoms experienced during major depressive episodes, especially motivational symptoms. The results of this study may indicate having healthy options readily available for individuals during major depressive episodes may better their overall health. By showing TBZ is not altering hedonic eating, TBZ treatment is validated as an animal model for effort-related

motivational symptoms observed in many disorders including depression. This study further allows the usage of TBZ as a motivational dysfunction model in rats.

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