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Improving the Bioavailability of Curcumin: A New Formulation

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Abstract

Mesolimbic dopamine (DA), particularly in the nucleus accumbens, is involved in regulating behavioral activation and effort-related processes. Interference with DA transmission can lead to the production of symptoms such as anergia, psychomotor slowing, and fatigue that are seen in depression and other related disorders. Considerable evidence indicates that interference with accumbens DA effects response allocation in effort related choice procedures, biasing animals towards the lower effort alternative. Previous studies have shown that administration of the vesicular monoamine transporter-2 (VMAT-2) inhibitor tetrabenazine (TBZ) effects response allocation in the FR5/choice procedure causing a decrease in lever pressing and a compensatory increase in chow consumption. These alterations induced by TBZ are consistent with DA depletions and administration of DA D_1 or D_2 family antagonists. The effort-related impairments induced by TBZ can be attenuated through co-administration of MSX-3, an adenosine A_{2A} antagonist, bupropion, a catecholamine reuptake inhibitor, and deprenyl, a MAO-B inhibitor. The present studies investigated the ability of curcumin to reverse the TBZ-induced shifts in effort-related choice behavior. Curcumin is a natural dietary metabolite, found in turmeric, which has been successfully used in antidepressant screening paradigms such as forced swim and tail suspension tests. Additionally, human clinical trials have shown the efficacy of curcumin as an adjunct medication. Curcumin modulates levels of neurotransmitters, such as DA, serotonin (5-HT), and to a lesser extent norepinephrine (NE), by acting as an MAO-A/B inhibitor. Curcumin has low bioavailability due to poor absorption, rapid metabolism, and fast elimination. Prior studies have enhanced curcumin's bioavailability through addition of excipients, however, earlier methods implemented gavage feeding which produces a stress response as the animals are restrained, and does not mimic human ingestion. Therefore, the purpose of the current feeding

study was to mimic human administration (i.e., tablets) in rodents by studying the ability of rats to ingest food pellets formulated with curcumin. In these studies, different formulations that included the excipient neusilin were offered to rats. Additionally, the present study was to determine if curcumin that was orally administered by ingestion could reverse the effort-related effects of TBZ. Two different formulations of curcumin and neusilin were tested in a feeding study to observe consumption; the formulations crystalline (CRYS) and coground (CGR) differed in the way that they were milled. The results of these studies demonstrated clearly that rats readily consumed CRYS curcumin pellets, but did not consume the CGR pellets. Additionally, crystalline pellets were administered in the FR5/chow choice procedure at two different time courses and investigated for their ability to attenuate deficits induced by TBZ. When administered three hours before testing, ingestion of 160 mg/kg CRYS curcumin significantly reversed the effort-related effects of TBZ. These studies demonstrate that orally ingested curcumin in rats can exert motivational effects that are consistent with an antidepressant action.

1. Introduction

Dopamine (DA) systems have been linked to several neurological and psychological disorders including depression, schizophrenia, and multiple sclerosis. There are four major DA pathways, however, research has placed particular emphasis on the motivational functions of the mesolimbic DA system, which originates in the ventral tegmental area (VTA) and projects to the nucleus accumbens (NAc). Substantial evidence indicates that NAc DA is involved in effort-related dysfunctions that are seen in depression and related disorders (Salamone and Correa, 2002, 2012; Salamone et al. 2007, 2009).

In a complex environment, organisms are often separated from significant stimuli. Organisms must exert considerable effort in order to overcome work-related obstacles and gain access to motivationally relevant stimuli and do so through cost/benefit analyses (Salamone and Correa, 2002). Evidence has shown that NAc DA is involved in the regulation of behavioral activation (i.e., vigor, persistence, and response speed) and effort-related processes, such as overcoming work-related response costs in instrumental behavior (Barbano and Cador, 2007; Phillips et al., 2007; Robbins and Everitt, 2007; Salamone et al., 1997, 2005, 2007). Interference with NAc DA transmission effects the relative allocation of behavior in animals responding on tasks that assess effort-based choice behavior.

Effort-based decision-making is studied using tasks that offer a choice between high effort options leading to highly valued reinforces vs. low effort/low reward options. In animal studies, tasks include a T-maze barrier choice task that uses a vertical barrier to provide the effort-related challenge (Salamone et al., 1994; Mott et al., 2009; Pardo et al., 2012) and operant procedures that offer animals a choice between responding (i.e., lever pressing) on ratio schedules for preferred reinforces vs. approaching and consuming a less preferred food (Salamone and Correa, 2002; Treadway et al., 2012; Randall et al., 2012). Under baseline or control conditions, when ratio requirements are relatively low (i.e., FR1 or FR5), trained rats will receive most of their food from lever pressing and consume only a small quantity of the concurrently available lab chow (Salamone et al., 1991, 1997). Systemic or local administration of low-to-moderate doses of DA D_1 or D_2 family antagonists and NAc DA depletions produce a shift of response allocation; significantly decreasing lever pressing and increasing chow consumption (Nunes et al., 2010; Salamone et al., 1991, 2002; Sink et al., 2008; Worden et al., 2009). Recently our laboratory has studied the effects of the reversible VMAT-2 (vesicular

monoamine transporter-type 2) inhibitor tetrabenazine (TBZ). TBZ blocks storage and depletes monoamines, but has its greatest impact on striatal DA (Pettibone et al., 1984; Tanra et al., 1995). TBZ is currently prescribed for Huntington's disease, and is known to produce depressive side effects in some patients, including motivational symptoms, such as psychomotor slowing, anergia, and fatigue (Frank et al., 2009, 2010). Similar to the effects produced by DA antagonists, TBZ can alter effort-related choice behavior, biasing animals towards the lower effort alternative (Nunes et al., 2013; Randall et al., submitted; Yohn et al., submitted).

The concurrent FR5/chow feeding task has been validated in several ways and it has been shown that interference with NAc DA neurotransmission does not affect total food intake or preference (Nunes et al., 2013; Salamone et al., 1991) or reinforcer devaluation by prefeeding (Salamone et al., 1991). Additionally, the effects observed with TBZ or DA receptor blockade differ substantial from appetite manipulation (Salamone et al., 1993; Sink et al., 2008). Taken together, these validation studies demonstrate that interference with NAc DA alters response allocation, biasing animal towards low-effort alternatives when an alternative food source can be obtained with minimal work.

Tests of effort-related choice behavior may have utility as preclinical models of motivational symptoms that are seen in a variety of disorders (Salamone and Correa, 2012). Human pathologies that involve activational impairments can be maladaptive. The severity of effort-related symptoms is related to problems with social function, employment, and response to treatment (Stahl 2002; Tylee et al., 1999). Moreover, human studies of effort-related decision making have shown that patients with major depressive disorder (Treadway et al., 2012) and schizophrenics with a preponderance of negative symptoms (Gold et al., 2013) have impairments in exertion of effort during reward seeking. Motivational symptoms in depression and other

related disorders are highly resistant to treatment (Fava et al., 2013; Stahl 2002;). Therefore, it is important to develop novel adjunct treatments for the motivational dysfunctions. Reversal studies (e.g., co-administration of another compound) are used as pre-clinical assessment of potential treatments. Co-administration of the adenosine A_{2A} antagonist, MSX-3, was able to attenuate the shifts in behavior induced by TBZ (Nunes et al., 2013). Additionally, the widely used antidepressant drug bupropion, a catecholamine uptake blocker, and l-deprenyl, a monoamine oxidase-B (MAO-B) inhibitor, also reversed the effort-related effects of TBZ (Nunes et al., 2013; Randall et al., IN PRESS).

Curcma longa lin is a plant distributed throughout tropical and subtropical regions, and is highly cultivated throughout Asian countries, especially India and China. Curcumin is a natural dietary alkaloid from this plant and is found in turmeric, a spice used for flavoring and coloring in foods, such as curry. Curcumin has been used for medicinal purposes for thousands of years in India, China, and Indonesia (Kulkarni et al., 2009). Curcumin has been shown to possess antidepressant-like effects in animal models commonly employed to screen antidepressants, such as the forced swim and tail suspension test (Kulkarni et al., 1985; Xu et al., 2005;). For instance, in these paradigms co-administration of curcumin with DA depleting agents decreased immobility time (Kulkarni et al., 2009). In addition, curcumin has been shown to be effective in human-clinical trials as an adjunct medication for depression (Kulkarni et al., 2008). Neurochemical studies have shown that the antidepressant-like effect of curcumin are likely to be due to its action of inhibition on the MAO-A and MAO-B enzymes (Kulkarni et al., 2005). In general, inhibitors of the monoamine oxidase enzyme cause an increase in monoamines and monoaminergic activity. Previous studies have shown that curcumin dose dependently inhibited MAO-A activity, whereas MAO-B inhibitory activity was only observed at higher doses (i.e., 40

or 80 mg/kg; Kulkarni et al., 2008). Although curcumin has high efficacy and safety, it has low bioavailability. It has poor absorption, rapid metabolism, and rapid elimination.

Previous studies have enhanced curcumin's bioavailability through addition of bioavailability enhancers, and have administered curcumin orally via gavage (Kulkarni et al., 2008). Gavage administration causes a stress response as the animals are restrained (Brown et al., 2000) and therefore causes a change in homeostasis. Stress induced alterations include changes in gastric secretion and mobility, changes in heart rate and increases in plasma glucocorticoids (Brown et al., 2000; Kent et al., 1983). The purpose of the current study is to enhance the bioavailability of ingested curcumin through addition of the excipient neusilin. Neusilin is a synthetic magnesium aluminometasilicate (MAS) that can be easily pressed into solid forms (e.g., pellets). The current study administered curcumin and neusilin as ingestible pellets so as to mimic human administration (i.e. tablets). Two different types of pellets were administered to animals trained on the feeding study task, a crystalline (CRYS) and a coground (CGR) formulation. These pellets differed from one another in the way that they were milled. Based upon the results of the feeding study, which showed that rats readily ingested the CRYS pellets, the CRYS pellet formulation was co-administered with the VMAT-2 inhibitor TBZ at different time points prior to determine if curcumin could reverse the effects of TBZ on effort-related choice as measured by performance on the FR5/chow choice task.

2. Materials and Methods

2.1 Animals

Adult male, drug-naïve, Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, IN, USA) were housed in a colony maintained at 23°C with 12-h light/dark cycles (lights on at 0700

hours). The rats (n=25) weighed 275-299 grams at the beginning of the study and were food-deprived to 85% of their free-feeding body weight for the experiment. Rats were fed supplemental chow to maintain the 85% free-feeding body weight throughout the course of the study with ad libitum water available in their home cages. Despite food restriction, rats were allowed modest weight gain throughout the experiment. Animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee and followed NIH guidelines.

2.2 Experimental Procedures

2.2.1 Feeding Study: Rats (n = 16) were run in clean, clear tubs (16" x 8" x 8") for a maximum of 15 minutes. Rats were initially trained on 10 sucrose pellets, 45 mg each (Bioserve, Frenchtown, NJ, USA). Next, rats were exposed to one pellet of either the crystalline or coground formulation. After adequate consumption, all rats were given doses of 160 mg/kg of both the crystalline and coground pellets but the order in which they were given was varied across animals in regard to which dose was received as the first and second exposure. Weighed amounts of pellet formulations (i.e. crystalline or coground) were administered in a dish adhered to the tub for the 15 minute session. At the end of the session rats were immediately removed from the tubs and the amount of pellets consumed was determined by weighing the remnants. Behavioral observations of latency to begin eating, explorations, grooming, and time to finish were made. A behavioral profile was determined using video monitoring and the taste reactivity test. Hedonic taste reactivity was characterized by rhythmic tongue protrusions along the midline, lateral tongue protrusions, and paw-licks. Aversive taste reactivity was characterized by gapes, face washing, chin rubs, forelimb flails, and head shakes (Grill et al., 1978).

2.2.2 Concurrent FR5/chow-choice procedure: Behavioral sessions were conducted in operant conditioning chambers (28x23x23 cm^3, Med Associates, Georgia, VT, USA) during the light period. Rats (n = 9) were initially trained to lever press on a continuous reinforcement schedule (30 minute sessions, during 5 days/week) to obtain 45mg pellets, (Bioserve, Frenchtown, NJ, USA), and then were shifted to the FR5 schedule (30 minute sessions, 5 days/week) and trained for several additional weeks until reaching a predetermined baseline number of lever presses (i.e., consistent responding ≥ 1,200 lever presses). Animals needed to consistently reach baseline criteria for the course of approximately one week before being introduced to the concurrent FR5/chow-feeding choice procedure. In this task, weighed amounts of laboratory chow (Laboratory Diet, 5P00 Prolab RHM 3000, Purina Mills, St. Louis, MO, USA; typically 20-25 grams, four-five large pieces) were concurrently available in the chamber during the 30 min FR5 session. Rats also received feeding study training and were trained to consume 10 Bioserve sucrose pellets on baseline days 2 to 3 hours prior to running. Before inclusion in the experiment, all animals were exposed to 10 (highest dose, 160 mg/kg) crystalline to insure consumption. On experimental testing days, when TBZ was administered, the rats were administered 80 mg/kg and 160 mg/kg of the crystalline pellet formulation 2 to 3 hours prior to running. At the end of the FR5/chow choice session, rats were immediately removed from the chambers, lever pressing totals were recorded, and amount of chow consumed was determined by weighing the remaining food and spillage. Rats were trained until reaching and maintaining stable levels of baseline lever pressing and chow intake. Once animals achieved baseline rates experimental testing began.

2.3 Pharmacological Agents and Dose Selection

Curcumin: CRYS curcumin was administered in the form of pellets in doses of 80 mg/kg and 160 mg/kg for the operant study; these doses of curcumin were based on previous

studies. CRYS pellets contained 7 mg curcumin, 28 mg neusilin, 15 mg 1% sodium starch glycolate (a tablet disintegrating agent to assure the release of curcumin), 4 mg Mg sterate (lubricant), and 15 mg sucrose. The crystalline pellet was ground curcumin mixed with neusilin in a crystal lattice. The coground pellet contained the same ingredients but the curcumin and neusilin were not ground prior to being mixed together. The total weight of the four doses were as follows: CRYS80 dose was 488 mg, CGR80 was 497 mg, CRYS160 was 538 mg, CGR160 was 539 mg. Doses of CRYS and CGR pellets were adjusted based on weight of the animal so that doses could be administered as mg/kg. Pellets were pressed weekly by the University of Connecticut, School of Pharmacy (Storrs, CT, USA).

Tetrabenazine (9,10-dimethoxy-3-(2-methylpropyl)-1,3,4,6,7, 11b hexahydrobenzo[a]quinolizin-2-one), the VMAT-2 inhibitor, was purchased from Tocris Bioscience (Bristol, UK). Tetrabenazine was dissolved in a vehicle solution of 0.9% saline (80%) and DMSO (20%). 1N HCl /mL volume was then added to adjust the pH and get the drug completely into solution. The final pH of the tetrabenazine solution was 3.5. The saline with 20% DMSO vehicle solution was administered as the vehicle control. The 0.75 mg/kg dose of tetrabenazine which was used for the FR5/chow choice task was based on extensive pilot work done in our laboratory.

2.4 Experiments

Different groups of rats were used for each experiment. All experiments used a within-groups design in which each rat received all doses of drug or vehicle treatments in their particular experiment in a randomly varied order (one treatment per week; no treatment sequence repeated across different animals in the experiment). Baseline training sessions (i.e.: non-drug) were conducted four days per week.

2.4.1 Experiment 1: Free Consumption of Curcumin, CRYS160 and CGR160

Rats were trained before drug testing as described above. Rats (n=16) received either 160 mg/kg CRYS or CGR pellet formulations for the first exposure and then were crossed-over into the condition not initially received for the second exposure. All rats were tested for 15 minutes. The observer recorded latency to begin and finish and exploratory or grooming behavior.

2.4.2 Experiment 2: Ability of Curcumin to Reverse the Effects of TBZ on the Concurrent FR5/Chow Choice Procedure Administration 2 hours Prior

Trained rats (n=4) received the following treatments; Vehicle plus 10 sucrose pellets (VEH/VEH), tetrabenazine plus 10 sucrose pellets (TBZ/sucr), tetrabenazine plus 80.0 mg/kg crystalline pellet (TBZ/80), and tetrabenzine plus 160.0 mg/kg crystalline pellet (TBZ/160). All animals had pellets administered two hours prior to testing and were allowed to eat for fifteen minutes. Remnants of the pellets were collected and weighed to determine the total dose of curcumin administered. Next, rats received vehicle or 0.75 mg/kg TBZ injections 90 minutes prior to testing.

2.4.3 Experiment 2: Ability of Curcumin to Reverse the Effects of TBZ on the Concurrent FR5/Chow Choice Procedure Administration 3 hours Prior

For the 3 hour condition, trained rats (n=5) received the following treatments; Vehicle plus 10 sucrose pellets (VEH/VEH), tetrabenazine plus 10 sucrose pellets (TBZ/sucr), tetrabenazine plus 80.0 mg/kg crystalline pellet (TBZ/80), and tetrabenzine plus 160.0 mg/kg crystalline pellet (TBZ/160). All animals had pellets administered three hours prior to testing and were allowed to eat for fifteen minutes. Remnants of the pellets were collected and weighed to determine the total dose of curcumin administered. Next, rats received vehicle or 0.75 mg/kg TBZ injections 90 minutes prior to testing.

2.5 Statistical Analysis

In experiment 1, the amount of pellet consumption for both formulations and in relation to which formulation was received as a first or second exposure was analyzed with repeated measures analysis of variance (ANOVA) with a mixed factor effect. For the effect size calculations partial eta-squared was used ($\eta\rho^2$). In experiments 2 and 3, total number of lever presses and gram quantity of chow intake from the 30 min session were analyzed using repeated measures ANOVA. A computerized statistical program (SPSS 21.0 for Windows) was used to perform all analyses. When there was a significant ANOVA, non-orthogonal planned comparisons using the overall error term were used to assess the differences between each treatment and the control condition. The number of comparisons was restricted to the number of treatments minus one (Keppel, 1991).

3. Results

Experiment 1: Free Consumption of Curcumin, CRYS160 and CGR160

Repeated measures ANOVA revealed a significant overall effect depending on which formulation (i.e. crystalline or curcumin) is first administered (Figure 1; [$F(1,1) = 41.917$; $p<0.001$]). The effect size for which formulation is first administered indicates a strong effect size ($\eta\rho^2 = 0.75$). There was a significant effect in regard to exposure week for the two formulations (i.e. first vs. second week exposure) (Figure 1; [$F(1,1) = 182.625$; $p<0.001$]). Thus, there was a significant effect in regard to the order in which the formulations were administered between the first and second week. The order in which the formulations were administered displayed a strong effect size ($\eta\rho^2 = 0.85$). There was also a significant formulation by exposure

interaction, [F(1,1) = 81.973; p<0.01]. Thus, there was a significant effect in the ability of animals to cross-over in the second week to the pellet formulation not originally received in the first week. The inability of animals to cross-over in the second week to the pellet formulation not originally received is displayed by a strong effect size ($\eta\rho^2 = 0.93$).

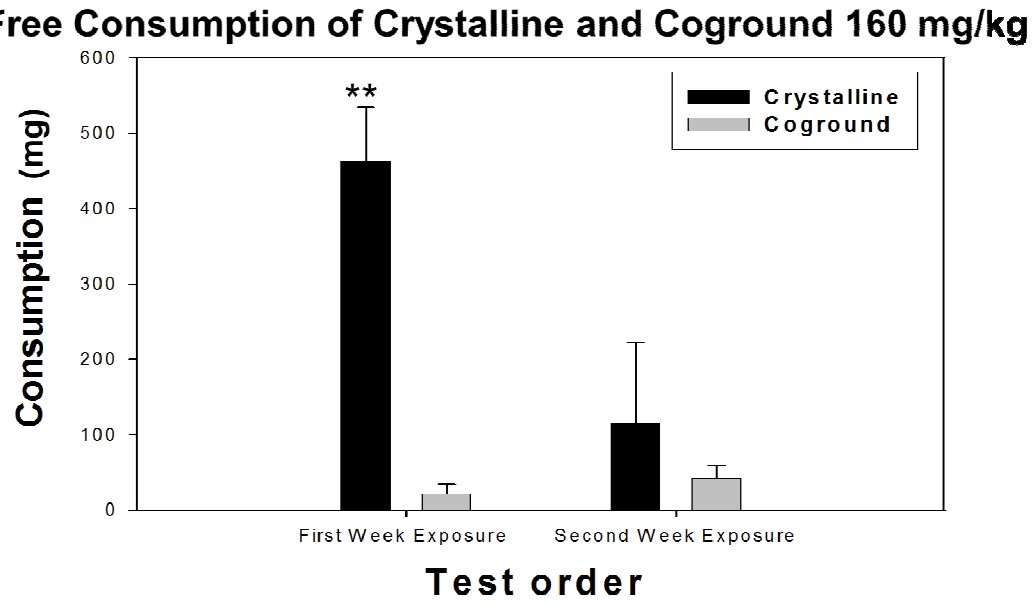

Figure 1: Free consumption of 160 mg/kg crystalline or coground curcumin mean (±SEM). Repeated measures ANOVA revealed that there was a significant effect on the formulation (crystalline or coground) first administered [F(1,1)=41.917; p<0.001] . Initial exposure to crystalline curcumin produced a significant effect on total quantity consumed relative to coground, ** p<0.001. There was also a significant effect of exposure week (first vs. second; [F (1,1)=182.625; p<0.001]) and formulation by exposure interaction [F(1,1)=81.973; p<0.001].

Experiment 2: Ability of Crystalline Curcumin to Reverse the Effects of TBZ on the Concurrent FR5/Chow Choice Procedure Administered 2 hours Prior

The effects of the VMAT-2 inhibitor TBZ were not significantly attenuated by crystalline curcumin administered 2 hours prior to testing. Repeated measures ANOVA showed that there was an overall significant effect of drug treatment on lever pressing (Figure 2; [F(3,9)=16.2777; p<0.01]). Non-orthogonal planned comparisons revealed that TBZ significantly lowed lever presses relative to vehicle control (p<0.01). There was no significant overall effect of 80 or 160

mg/kg crystalline curcumin on lever pressing compared to TBZ-treated animals ($p>0.05$). The overall treatment effect for chow consumption was also statistically significant (Figure 2; [$F(3,9)=13.96$; $p<0.01$]). Administration of TBZ increased chow consumption relative to vehicle-vehicle conditions (planned comparisons, $p<0.01$). Non-orthogonal planned comparisons revealed that there was not a significant decrease in chow consumption with either 80 or 160 mg/kg crystalline curcumin compared to TBZ-treated animals ($p>0.05$).

Experiment 3: Ability of Crystalline Curcumin to Reverse the Effects of TBZ on the Concurrent FR5/Chow Choice Procedure Administered 3 hours Prior

The MAO-A/B inhibitor, crystalline curcumin, produced a partial reversal of the effects of the VMAT-2 inhibitor, TBZ, in animals tested on the FR5/chow feeding choice task (Figure 2). Repeated measures ANOVA revealed that there was an overall significant effect of drug treatment on lever pressing (Figure 2; [$F(3,15)=7.77$; $p<0.002$]). Planned comparisons showed that TBZ produced a significant reduction in lever pressing compared to vehicle control conditions ($p<0.01$). Co-administration of 160 mg/kg crystalline curcumin three hours prior to testing significantly increased lever pressing compared to TBZ plus vehicle treated animals (planned comparisons, $p<0.01$). There was also a significant overall effect of drug treatment on chow intake [$F(3,15)=4.41$, $p<0.021$]. TBZ significantly increased chow consumption compared to vehicle-treated control animals (planned comparisons, $p<0.01$). Co-administration of 160 mg/kg crystalline curcumin plus TBZ significantly decreased chow consumption relative to TBZ-vehicle (planned comparisons, $p<0.05$). Thus co-administration of 160 mg/kg crystalline curcumin with TBZ significantly increased lever pressing and decreased chow consumption compared to TBZ-vehicle treated animals.

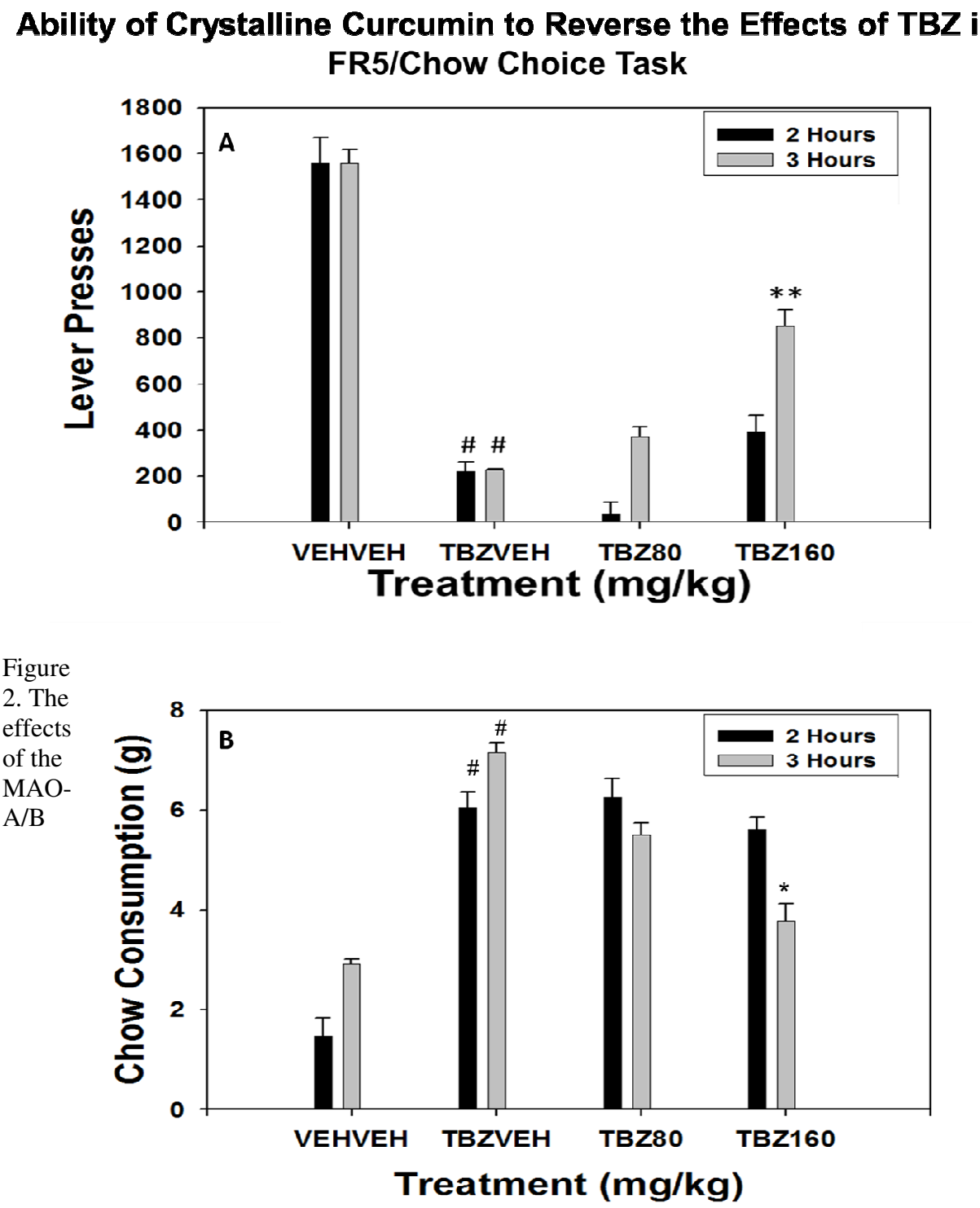

Figure 2. The effects of the MAO-A/B inhibitor crystalline curcumin on TBZ-induced changes in performance on the FR5/chow choice task. Rats received IP injections of vehicle or 0.75 mg/kg TBZ 90 minutes prior to testing. Additionally, rats were administered crystalline curcumin 2 or 3 hours. (A) Mean (±SEM) number of lever presses (FR5 schedule) during the 30 minute session. (B) Mean (±SEM) gram quantity of chow intake. TBZ significantly decreased lever pressing and increased chow

consumption relative to vehicle (# $p<0.01$). Administration of 160 crystalline curcumin to TBZ-treated rats 3 hours prior to testing significantly increased lever pressing and decreased chow consumption relative to treatment with TBZ alone (* $p<0.05$; ** $p<0.01$).

4. Discussion

Previous studies have shown that the reversible VMAT-2 inhibitor TBZ blocks the storage of and depletes, monoamines, with its greatest effects on striatal DA (Pettibone et al., 1984; Tanra et al., 1995). Moreover, TBZ also affects DA-related signal transduction in a manner consistent with reduced accumbens D_1 and D_2 receptor transmission (Nunes et al., 2013). Additionally, postmortem tissue studies of humans receiving clinical doses of TBZ reported that the only significant depletions of DA were in the caudate and hippocampus (Guay, 2010). Together these studies indicate that the effects of TBZ on effort-related choice are largely due to actions on DA. In summary, TBZ alters effort-related choice behavior, reducing food-reinforced lever pressing and biasing animals toward selection of the freely available chow. Together with the present study, the ability of TBZ to affect effort-based decision making is consistent with other manipulations that interfere with NAc DA neurotransmission. Similar to DA depletions or administration of DA D_1 or D_2 family antagonists, TBZ induced shifts in effort-related choice behavior are not due to changes in primary food motivation or food preference (Nunes et al., 2013) or resemble appetite suppressants effects (Randall et al., IN PRESS; Sink et al., 2008).

The shift in behavior induced by TBZ can be successfully attenuated through co-administration of the adenosine A_{2A} antagonist MSX-3, the catecholamine uptake blocker bupropion, and the MAO-B inhibitor l-deprenyl (Nunes et al., 2013; Randall et al., submitted). The results of the present study indicate that 160 mg/kg of CRYS curcumin three hours prior to testing produced a partial reversal of TBZ effects. However, administration of CRYS curcumin

two hours prior to testing did not produce a significant effect. Prior studies have found that curcumin orally administered (via gavage feeding) remains active in the system 90-180 minutes after administration. Since very little curcumin is absorbed from the gut and small intestine, future studies should investigate additional lead times of CRYS curcumin as they may differ from lead times of IP or orally administered curcumin. In sum, the present study is consistent with previous studies showing that curcumin produces antidepressant-like effects in animal models (Kulkarni et al., 2008). Furthermore, these results are consistent with clinical data indicating that curcumin is effective at improving depressive-like symptoms (Sanmukhani et al., 2013). Taken together, these studies suggest that curcumin could further potentiate current antidepressants.

Additionally the current study (i.e., feeding study) was conducted to examine two new formulations of curcumin pellets. Each of the formulations contained the bioavailability enhancing agent neusilin but differed in the way that they were milled. Neusilin is a multifunctional excipient with the capacity to enhance the oral bioavailability of poorly water-soluble drugs and allows for oral solid dosage forms (Cha et al., 2012). In the crystalline (CRYS) pellet formulation curcumin and neusilin were milled independently and after separate milling the compounds were then milled again together. While in the coground (CGR) pellet formulation, both neusilin and curcumin were milled together. Interestingly, there was significant and uniform consumption of the CRYS pellet upon initial and first test exposure. The CGR pellets, however, were not readily consumed during the initial exposure. Animals displayed aversive-like behavior when exposed to these pellets. For example, animals displayed gaping behavior, which is considered an aversive reaction of taste reactivity seen in rodents (Grill et al., 1978). Gaping includes lowering of the mandible while the corners of the mouth pull back

posteriorly and dorsally forming a triangular shaped open mouth. Gapes differ from yawning in that they occur in bursts (2-6 Hz) and yawns produce an elliptical shape of the mouth (Grill et al., 1978). In addition, during the initial exposure to the CGR formulation, 80% of the rats were observed twitching and sneezing, and rejecting partially eaten pellets, thus providing further evidence that there may be an aversion to this this formulation. In studies of rat behavior there is initial acceptance of a bait that has not been previously experienced by the rat; thus, refusal of the bait without previous experience is rare (Rzoska, 1953). However, rats initially exposed to the CGR formulation in week one exhibited bait rejection as aversive taste reactivity. In 89% of rats initially exposed to the CGR formulation, rats would ingest the pellet, chew the pellet, and then expel the pellet out of their mouth. Such behavior was not seen when rats were consuming the baseline of 10 sucrose or CRYS pellets.

During the second week exposure of the feeding study there was not significant consumption of either pellet formulation, indicating that the animals, after being initially exposed to either the crystalline or coground formulation, were unable to be crossed-over to the other formulation not originally received in the initial exposure. The inactive excipient neusilin has a chalky, granular texture (Cha et al., 2012). It is probable that the aversion to the CGR formulation, during both the initial and second exposure trials, may be due to the accentuation of the texture of neusilin as the pellets are formulated with both the curcumin and neusilin being milled together. The inability of the animals to switch between formulations, during the second week exposure, was most drastic in animals initially exposed to the CGR formulation. These animals displayed aversive-like behavior when attempting to consume the CRYS pellets. These animals, being initially exposed to the CGR, could have developed a textile aversion to the pellets and were thus unable to cross-over and consume the CRYS pellets in the second

exposure. This is supported by substantial evidence of bait-shyness seen in typical rat behavior. Rats given initial exposure to a food (i.e. bait), with no previous experience of the bait, accepted it and a majority of the rats consumed the bait in its entirety. A second exposure to a different bait, but in the same base or in the same form, did not exhibit the same ready acceptance seen with the initial bait exposure. This is defined as bait-shyness, or a cautious attitude towards food that was previously experienced with unpleasant effects. Bait-shyness can manifest in a range of attitudes from strict refusal of the bait to various grooming behaviors instead off consuming the bait (Rzoska, 1953). Future studies should investigate a new formulation of more palatable curcumin pellets. One possibility is to use SMEDDS (self-microemulsifying drug delivery system) which is a drug delivery system that is efficient at increasing absorption of drugs taken by mouth (Setthacheewakul et al., 2010). By creating smaller and more palatable pellets, perhaps with a chocolate or sugar coating, future studies can ensure that rats will consume the pellet in its entirety.

In summary, TBZ induced alterations in effort-related choice behavior can be attenuated through co-administration of the curcumin three hours prior to testing. The effects produced by curcumin are very similar to the MAO-B inhibitor l-deprenyl. Moreover, these results are consistent with antidepressant actions assessed by traditional behavioral models. Interestingly, this is one of the first studies to mimic human pill ingestion in rodents. Animals readily ingested the CRYS pellet formulation but displayed aversive-like behaviors while in the presence of the CGR formulation. This research could have implications for developing novel compounds for the treatment of the motivational dysfunctions seen in depression, schizophrenia, and other disorders.

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