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Restoring Effort-Related Functions in Models of Depression Symptoms: Reversing Fatigue Symptoms Induced by Catecholamine Depleting Agent Tetrabenazine with the Adenosine A2A Antagonist MSX-3

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Restoring Effort-Related Functions in Models of Depression Symptoms: Reversing Fatigue Symptoms Induced by Catecholamine-Depleting Agent Tetrabenazine with the Adenosine A_{2A} Antagonist MSX-3

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II. Thesis Abstract

Motivational symptoms related to effort expenditure have been associated with major depression and other disorders that afflict millions of individuals worldwide. In an effort to identify potential therapeutic agents and characterize the underlying biochemical mechanisms related to these behaviors, recent research has utilized animal models to study and characterize such behavior. Previous work in the Salamone lab produced evidence that rats with impaired dopamine (DA) transmission show changes in response allocation in tasks that measure effort-related choice behavior, which are characterized by a decrease in selection of the high-effort choice but increased selection of the low-effort alternative. The present work was undertaken to determine if pharmacological manipulations associated with depression-like symptoms can induce effort-based dysfunctions in rats, as measured by the concurrent fixed ratio (FR)5/chow feeding choice task. The present set of experiments were performed to determine if the effects of tetrabenazine (TBZ), a VMAT-2 inhibitor that preferentially depletes DA, upon effort-related choice behavior can be reversed by adenosine A_{2A} antagonism, to effectively restore normal behavior. Behavioral measures included both the number of lever presses and the amount of freely available lab chow that was consumed. The behavioral effect of MSX-3, an adenosine A_{2A} antagonist, was compared against the well-established antidepressant bupropion. The data collected indicated that the effects of tetrabenazine on effort-related choice had a dose-dependent reversal when co-administered with MSX-3, as well as with the antidepressant bupropion. Thus pharmacological manipulations that produce depressive-like symptoms in humans produced effort-related choice impairments in rodents, which was then reversed by a putative antidepressant MSX-3 and the well-established

antidepressant bupropion. The data obtained from these experiments support that adenosine A_{2A} antagonists could ultimately be used to promote the development of novel treatments and therapeutics that target effort-related symptoms of depression, such as apathy, psychomotor slowing, and fatigue in humans.

III. Introduction

Motivational symptoms related to effort expenditure, including psychomotor slowing, apathy, anergia and fatigue, have been associated with major depression and other disorders, including Parkinsonism and multiple sclerosis (Tylee et al. 1999; Stahl 2002; Demyttenaere et al. 2005; Salamone et al. 2006; Treadway and Zald 2011). The idea that motivated behaviors have an energetic or activational component is a recurring theme in the literatures of psychology, psychiatry, and neurology (Salamone and Correa 2002; Salamone et al. 2005, 2007, 2009). The vigor or persistence of work output in stimulus-seeking behavior is recognized to be a fundamental aspect of motivation, and organisms continually make effort-related decisions based upon cost/benefit analyses, allocating behavioral resources into goal-directed behaviors based upon differential assessments of motivational value and response costs (Salamone and Correa 2002). Effort-related behaviors have been described as being characterized by a high degree of effort, activity, vigor, and persistence (Salamone 2010b). In some psychiatric or neurological patients, there is a self-reported lack of energy, rather than a physical inability to perform a given task. Such individuals make statements like "my battery is run down", and thus are less likely to voluntarily choose to participate in high-effort activities (Friedman et al. 2007). The severity of these psychomotor or "energy"-related symptoms in major depression is correlated with problems with social function, employment, and treatment outcomes (Tylee et al. 1999; Stahl 2002). These effort-related symptoms are a core aspect of depression that affects millions of individuals around the globe.

Because of the clinical significance of these effort-related symptoms, animal models have been developed to identify potential therapeutic agents and characterize the underlying

biochemical mechanisms related to these behaviors. In fact, over the past several years, the Salamone lab has devised behavioral paradigms using rats to assess the role of various neurotransmitters, including dopamine (DA) and adenosine, both of which play critical roles in exertion of effort and effort-related decision-making. Such behavioral procedures involve effort-related tests that enable the rat to choose between high-effort alternatives that result in more highly valued rewards or low-effort alternatives that give the animal less valued rewards (Salamone and Correa 2002; Salamone et al. 1991, 1996, 2002, 2003, 2005, 2006, 2007, 2009a,b). Some studies have used an operant concurrent lever pressing/chow-feeding task (Salamone et al. 1991, 2002), in which the high effort choice requires the rat to press a lever to receive a carbohydrate-rich pellet with a high nutritional value, or to choose the option of approaching and consuming concurrently available lab chow, which requires little to no effort to obtain. Under non-drug conditions, rats pressing on FR1 or FR5 schedules typically acquire most of their food by lever pressing, while consuming only small amounts of lab chow.

Previous studies with this task and related procedures have shown that DA, particularly in the nucleus accumbens, is a critical component in the brain circuitry that regulates behavioral actions and effort-related processes (Nunes et al., 2010). Rats with impaired DA transmission (i.e. by injection of DA antagonists or depleting agents) show changes in response allocation in tasks that measure effort-related choice behavior, which are characterized by a decrease in selection of the high-effort choice but increased selection of the low-effort alternative (Salamone et al., 1991, 1997, 2003, 2005, 2006, 2007). This evidence that impaired DA transmission reduces the exertion of effort, and thus alters effort-related decision-making, suggests that this animal model has relevance for studies of effort-

related dysfunction in humans (Salamone and Correa 2002; Salamone et al. 1991, 1996, 2002, 2003, 2005, 2006, 2007, 2009a,b).

The use of this behavioral paradigm as a measure of effort-related choice behavior has been validated in several ways. First, the pharmacological conditions that produce the shift in choice behavior did not alter food intake or preference in free-feeding choice tests (Salamone et al. 1991; Koch et al. 2000; Farrar et al. 2008). This indicates that dopaminergic manipulations on effort-based choice are not explained by changes in appetite, food consumption or preference, or discrimination of reward magnitude, which could account for the decreased lever presses (Salamone et al. 1991, 1994, 2002; Sink et al. 2008; Randall et al. 2012). Additionally, the effects of DA antagonists do not resemble pre-feeding or appetite suppressant drugs (Salamone and Correa, 2009). These data were supported by a recent study demonstrating that catecholamine-depleting agent tetrabenazine did not alter food intake or preference in parallel free-feeding choice studies (Nunes et al. 2013), substantiating that the shift in behavior seen in the reversal experiments is due to dopaminergic manipulations rather than the food itself. Thus, these studies indicate that despite the fact that lever pressing is decreased by accumbens DA antagonism or depletions, the rats show a compensatory reallocation of behavior and select a new path to an alternative food source, i.e. one that involves lower work-related response costs.

Tests involving operant behavior have been used to demonstrate that low doses of DA antagonists, as well as DA depletions or antagonism in nucleus accumbens, reduce the exertion of effort and alter effort-related choice, biasing animals towards low-effort alternatives (Salamone and Correa 2002; Salamone et al. 1991, 1996, 2002, 2003, 2005, 2006, 2007, 2009). One such drug that has been shown to affect DA transmission is

tetrabenazine (TBZ). TBZ functions as an inhibitor of one subtype of the vesicular monoamine transporters (VMAT-2), and thus blocks storage of monoamines, particularly the neurotransmitter DA (Adam et al., 2008). TBZ has its greatest impact is upon DA in striatal areas (Pettibone et al. 1984). TBZ is commonly used to treat Huntington's disease, and in some patients TBZ has been shown to produce depressive side effects that include psychomotor slowing, fatigue, and anergia (Guay 2010; Frank 2010). Clinical research has shown that TBZ can produce depression as a side effect in humans (Kenney et al. 2007), and studies with animal models have used TBZ as an agent for the pharmacological induction of depression-like behavioral effects (Skolnick et al. 2006). Effort-related effects of TBZ in rats were assessed using the concurrent lever pressing/chow feeding choice task (Salamone et al. 1991, 2002; Nunes et al. 2010). The following experiments presented in this paper hypothesized that systemic administration of TBZ affects effort-related choice behavior, producing a dose-dependent decrease in lever pressing with a concurrent increase in freely available chow on the FR5 lever pressing/chow-feeding task. We then examined if putative and well-characterized antidepressant drugs are able to reverse the effects of TBZ in animal models.

Moreover, strong evidence implicates that DA interacts with the purine neuromodulator adenosine to regulate effort-related functions. Indeed anatomical data shows the adenosine A_{2A} receptor subtype is co-localized with DA D2 receptors same medium spiny neuron (Fink et al. 1992; Ferré 1997; Hillion et al. 2002; Fuxe et al. 2003) and on enkephalin-positive neurons (Svenningson et al. 1999) in the striatum and nucleus accumbens. The interactions between adenosine A_{2A} and DA D2 receptors have important implications in the motivational-related effects of DA antagonists. Indeed,

several studies have substantiated that adenosine A_{2A} antagonists reverse the effort and motivational-related effects of DA antagonists (Farrar et al. 2007, 2010; Worden et al. 2009; Mott et al. 2009; Salamone et al. 2009a). Adenosine A_{2A} antagonists have been shown to have psychomotor stimulant properties, and to reverse many of the behavioral effects of DA D2 antagonists such as decreases in operant lever pressing and effort-related choice behavior (Randall et al. 2011, Font et al. 2008, Mingote et al. 2008). Based on this preclinical research, it has been suggested that adenosine A_{2A} antagonists could be useful as anti-parkinsonian drugs, antidepressants, or as a treatment for motivational or effort-related symptoms such as psychomotor retardation, anergia, apathy and fatigue, which are core symptoms of depression and other psychiatric disorders in humans (Ferré et al. 1997; Svenningsson et al. 1999; Wardas et al. 2001; Morelli and Pinna 2001; Hodgson et al. 2009; Salamone et al. 2008a,b., El Yacoubi et al. 2003; Hanff et al. 2010, Hodgson et al. 2009, Salamone et al. 2007, 2010, Marin, 1996; Demyttenaere et al. 2005; Salamone et al. 2006; Friedman, 2009).

With the growing clinical interest in adenosine A_{2A} antagonists, developing and testing novel adenosine A_{2A} antagonists is becoming a primary research priority (Le Witt et al. 2008; Pinna 2009; Hodgson et al. 2009; Salamone, 2010a). In particular, MSX-3 is one such adenosine A_{2A} antagonists that evidence indicates could be useful in treating such effort-related symptoms in humans **(**Farrar et al. 2007, 2010; Worden et al. 2009; Mott et al. 2009; Salamone et al. 2006, 2009b). Thus, the goal of the experiments outlined in this paper was to determine if the effects of TBZ upon effort-related choice behavior can be reversed by adenosine A_{2A} antagonism. I hypothesize that MSX-3 can reverse the effects of TBZ and restore normal behavior. In essence, this was a "reversal experiment"

similar to those previously conducted in the Salamone lab (e.g. Nunes et al. 2010), as the aim of this project was to reverse the drug-induced lack of activation and effort. This pattern of results would suggest that MSX-3, or mechanistically similar drugs, could be useful for treating effort-related symptoms in humans. These experiments are part of a larger project that will assess the effects of a novel treatment strategy for depression (adenosine A_{2A} antagonism). Such studies could change current thinking about the neurobiology of motivational impairments in major depression, and could promote the development of novel treatments that are specifically targeted towards the effort-related symptoms of depression such as anergia, psychomotor slowing, and fatigue.

Moreover, the effects of MSX-3 were compared with those of the well-characterized antidepressant bupropion. Bupropion, more commonly known by its trade name Wellbutrin, is a widely used antidepressant (Milea et al. 2010). This pharmaceutical was selected because some clinical evidence suggests that blockade of DA uptake may be relatively effective at treating effort-related symptoms such as anergia and fatigue in depressed people (Rampello et al. 1991; Stahl 2002; Demyttenaere et al. 2005; Pae et al. 2007). Bupropion exerts its action through the D1 D2 receptor blockade, acting as an inhibitor of dopamine reuptake by occupying the DA transporter (DAT). This drug has been shown to produce antidepressant-like effects in animals tested on tasks such as the forced swim and tail suspension tests (Yamada et al. 2004; Bourin et al. 2005; Kitamura et al. 2010). Furthermore, these results are consistent with human clinical data indicating that bupropion is relatively effective for treating psychomotor retardation and fatigue symptoms of depression (Fabre et al. 1983; Pae et al. 2007). Thus, bupropion was selected for this experiment because some clinical evidence

suggests that blockade of DA uptake may be relatively effective at treating effort-related symptoms such as anergia and fatigue in depressed people (Rampello et al. 1991; Stahl 2002; Demyttenaere et al. 2005; Pae et al. 2007).

To summarize, it was hypothesized that TBZ would produce effort-related impairments in rats tested on the concurrent fixed ratio 5 (FR5)/chow-feeding choice task and that these motivational impairments can be reversed by the putative anti-depressant MSX-3, as well as the well-established antidepressant bupropion. The data collected serves as a critical piece to a larger project involving the neurobiology of motivational impairments in clinical depression and other medical conditions, which could ultimately promote the development of novel treatments and therapeutics that target effort-related symptoms of depression, such as apathy, psychomotor slowing, and fatigue in humans.

IV. Materials & Methods

a. Animals

Adult male, drug-naïve, Sprague-Dawley rats (Harlan, Indianapolis, IN, USA) were in a colony maintained at 23°C with 12-hour light/dark cycles (lights on at 07:00h). Rats (n=X) weighed 290-340 grams at the beginning of the study and were initially food deprived to 85% of their free-feeding body weight for operant training. Water was available ad libitum in the home cages. For most baseline days, rats did not receive supplemental feeding; however, over weekends and after drug tests, rats usually received supplemental chow in the home cage. Despite the food restriction, rats were allowed modest weight gain throughout the experiment. All animals were approved by University of Connecticut Institutional Animal Care and Use Committee, and followed NIH guidelines.

b. Pharmacological Agents and Dose Selection

Tetrabenazine (3R, 11bR)-rel-1,3,4,6,7,11 b-hexahydro-9, 10 dimethoxy-3-(2-methylpropyl)-2H-benzo[a]quinolizin-2-one)) and bupropion (1-(3-Chlorophenyl)-2-[1, 1-dimethylethyl)ammino]-1-propanone hydrochloride) were obtained from Tocris Bioscience (Ellisville, MO, USA). TBZ was dissolved in a 10% dimethyl sulfoxide (DMSO) solution mixed with saline and pH-adjusted with 1N HCl to bring the final solution to a pH of 3.5. The 10% DMSO solution used to dissolve the TBZ served as the vehicle control. Doses of TBZ used were based on previous data and pilot studies. Bupropion was dissolved in 0.9% saline. MSX-3 ((E)-phosphoric acid mono-[3-[8-[2-(3-methoxyphenyl)vinyl]-7-methyl-

2,6-dioxo-1-prop-2-ynyl-1,2,6,7-tetrahydropurin-3-yl]propyl] ester disodium salt) was provided by Christa Müller at the Pharmazeutisches Institut, Universität Bonn, in Bonn, Germany. MSX-3 (free acid) was dissolved in 0.9% saline, and pH was adjusted by titrating with microliter quantities of 1.0 N NaOH until the drug was in solution. The final pH was usually 7.5±0.2 and was not allowed to exceed 7.8.

For the dose response study, doses were selected based upon pilot experiments and previous studies (Nunes et al. 2010). Saline injections were used as the "Vehicle" (Veh), or control. The test rats received the following treatments: Veh, 0.25 mg/kg TBZ, 0.5 mg/kg TBZ, 0.75 mg/kg TBZ and 1.0 mg/kg TBZ. For the reversal experiments with MSX-3, rats received Veh/Veh, TBZ/Veh, TBZ/0.5 mg/kg MSX-3, TBZ/1.0 mg/kg MSX-3 and TBZ/2.0 mg/kg MSX-3. For the bupropion (BU) reversal study, rats received Veh/Veh, TBZ/Veh, TBZ/5 mg/kg BU, TBZ/10 mg/kg BU, and TBZ/15 mg/kg BU. For both the MSX-3 and BU reversal studies, a dose of 0.75 mg/kg of TBZ was used.

c. Behavioral Procedures

Behavioral sessions were conducted in operant conditioning chambers (28x23x23cm, Med Associates; Figure 1). Rats were initially trained to lever press on a continuous reinforcement schedule for five days to obtain 45-mg high-carbohydrate pellets (Bio-serv, Frenchtown, NJ, USA). This behavioral procedure is a FR1 schedule in which the rat earns one pellet per lever press. Thirty-minute sessions five days per week were used for all operant behavior tests. The rats

were then shifted and trained on the FR5 schedule (30-min sessions, 5 days/week) for 4 weeks, after which they were trained on a concurrent FR 5/choice procedure for several weeks. In this procedure, weighed amounts of laboratory chow (Laboratory Diet, 5P00 Prolab RHM 3000, Purina Mills, St. Louis MO, USA; typically 15-20 g, three or four large pieces) were concurrently available on the floor of the operant chamber during the FR 5 sessions (Worden et al. 2009; Nunes et al. 2010). After the session, rats were immediately removed from the chambers, and food intake was determined by weighing the remaining food (including spillage). Rats were trained until they attained stable levels of baseline lever pressing and chow intake (i.e. consistent responding over 1200 lever presses per 30 min), after which drug testing began. For most baseline days, rats did not receive supplemental feeding; however, over weekends and after drug tests, rats usually received supplemental chow in the home cage. On baseline and drug treatment days, rats normally consumed all the operant pellets that were delivered from lever pressing during each session.

d. Experimental Procedures

Rats were trained on the concurrent FR5/chow-feeding procedure (as described above) before testing began, and each experiment employed different groups of rats. The experiments used a repeated measures within-groups design; with each rat receiving all combined intraperitoneal (i.p.) drug treatments in their particular experiment in a randomly varied order (one treatment per week, with none of the treatment sequences repeated across different animals in the same experiment).

Baseline (i.e. non-drug) sessions were conducted four additional days per week. Behavioral measures included both the number of lever presses and the amount of freely available lab choice that was consumed. The specific treatments and testing times for each experiment are listed below.

Experiment 1: *Effects of systemic administration of the catecholamine-depleting agent TBZ on the concurrent FR5/chow–feeding procedure*

All animals were trained until stable baseline performance was achieved (i.e. lever presses consistently over 1200 per session). All animals (n=8) received i.p. injections of the following doses of TBZ: 10% DMSO vehicle, 0.25, 0.5, 0.75, 1.0 mg/kg. Experiment 1 used a within-groups design, with all rats receiving all drug treatments in a randomly varied order. Baseline training (i.e. non-drug) sessions were conducted four additional days per week. All injections were given 90 minutes before the beginning of the testing session.

Experiment 2: *Effects of systemic administration of the catecholamine-depleting agent TBZ on the concurrent FR5/chow–feeding procedure: reversal with MSX-3*

All animals were trained until a stable baseline performance was achieved (i.e. lever presses over 1200). All animals (n=8) received i.p. injections of the following doses of TBZ plus MSX-3: 10% DMSO vehicle (90 min before testing) plus saline vehicle (20 min before testing), 0.75 mg/kg TBZ (90 min) plus saline vehicle (20 min), 0.75 mg/kg TBZ (90 min) plus 0.5 mg/kg MSX-3 (20 min), 0.75 mg/kg TBZ (90 min) plus 1.0 mg/kg MSX-3 (20 min), and 0.75 mg/kg TBZ (90

min) plus 2.0 mg/kg MSX-3 (20 min). Experiment 2 used a within-groups design, with all rats receiving all drug treatments in a randomly varied order. Baseline training (i.e. non-drug) sessions were conducted four additional days per week.

Experiment 3: *Effects of systemic administration of the catecholamine-depleting agent TBZ on the concurrent FR5/chow–feeding procedure: reversal with bupropion*

All animals were trained until a stable baseline performance was achieved (i.e. lever presses over 1200). All animals (n=11) received i.p. injections of the following doses of TBZ plus bupropion: 10% DMSO vehicle (90 min before testing) plus saline vehicle (30 min before testing), 0.75 mg/kg TBZ (90 min) plus saline vehicle (30 min), 0.75 mg/kg TBZ (90 min) plus 5.0 mg/kg bupropion (30 min), 0.75 mg/kg TBZ (90 min) plus 10.0 mg/kg bupropion (30 min), and 0.75 mg/kg TBZ (90 min) plus 15.0 mg/kg bupropion (30 min). Experiment 3 used a within-groups design, with all rats receiving all drug treatments in a randomly varied order. Baseline training (i.e. non-drug) sessions were conducted four additional days per week.

e. Statistical Analyses

The total number of lever presses and gram quantity of chow intake from the 30-minute sessions were analyzed with repeated measures of analysis of variance (ANOVA). For the food preference study, total quantity of Bio-serve pellets and chow were analyzed with factorial ANOVA. When the overall ANOVA was

significant, non-orthogonal planned comparisons using the overall error term were used to compare each treatment with the TBZ vehicle control group (Keppel, 1991). For these comparisons, a level was kept at 0.05 because the number of comparisons was restricted to the number of treatments minus one. With this analysis, each condition that combined TBZ plus MSX-3 or bupropion was compared with the TBZ vehicle condition using planned comparisons.

V. Results

Experiment 1: Effects of systemic administration of the catecholamine-depleting agent TBZ on the concurrent FR5/chow–feeding procedure

Systemic administration of TBZ significantly decreased lever pressing and produced a concurrent increase in the consumption of the freely available lab chow, as shown in Figure 2. In the standard 30-minute operant procedure, a rat who received a Veh injection pressed the lever an average of 1442.125 times, while the consuming only an average 1.825 grams of lab chow. Upon administration of TBZ in doses of 0.25, 0.5, 0.75, and 1.0 mg/kg, lever pressing decreased from 1257.25, 1066.875, 364.375, and 157.125 respectively, while chow consumption concurrently increased from 3.4875, 3.7625, 6.325 and 6.7125 grams, respectively. ANOVA revealed a significant effect of dose on lever pressing ($F(4, 28) = 45.9$, $p < 0.001$). There was also an overall significant effect of drug treatment on chow intake ($F(4,28 = 33.8$, $p < 0.001$). Planned comparisons were performed and showed that the two highest doses of TBZ significantly decreased lever pressing and increased the consumption of the freely available lab chow relative to control ($p < 0.05$).

Experiments 2 and 3: Effects of systemic administration of the catecholamine-depleting agent TBZ on the concurrent FR5/chow–feeding procedure: Reversal with MSX-3 and bupropion

The results of experiment 2 are shown in Figure 3. A rat injected with both TBZ and the A_{2A} antagonist MSX-3, showed a clear reversal of lever pressing/chow-consumption occurred, as the dosage of MSX-3 increased. Using MSX-3 doses of 0.5, 1, and 2 mg/kg, lever pressing increased from 747.25, 983.0 and 1275.625 lever presses respectively, while chow consumption decreased from 4.412, 3.462, and 2.425 grams, respectively. There was an overall significant effect of drug treatment on lever pressing ($F(4, 28 = 26.8, p < 0.001$). There was also an overall significant effect of drug treatment on chow intake ($F(4, 28 = 40.5, p < 0.001$). Planned comparisons were performed and showed that TBZ suppressed lever pressing and increased chow intake, and that all doses of MSX-3 were able to attenuate the effects of TBZ both on lever pressing and as well as the consumption of the freely available lab chow relative to the TBZ control ($p < 0.05$).

As shown in Figure 4, the antidepressant bupropion was able to attenuate the behavioral effects of TBZ. Using bupropion doses of 5, 10, and 15 mg/kg, lever pressing increased from 196.5, 777.1 and 1003.1 respectively, while chow consumption decreased from 6.325, 4.46, and 2.52 grams, respectively. There was an overall significant effect of drug treatment on lever pressing ($F(4, 40 = 19.4, p < 0.001$), and also an overall significant effect of drug treatment on chow intake ($F(4, 40) = 46.3, p < 0.001$). Planned comparisons showed that, as in the previous two experiments, 0.75 mg/kg TBZ decreased lever pressing and increased chow intake. In addition, the two highest doses of bupropion significantly reversed lever pressing reductions produced by TBZ, as well as

decreased the consumption of the freely available lab chow relative to the TBZ group ($p < 0.05$). When the rat was injected with both TBZ and the well-established antidepressant bupropion, a clear reversal of lever pressing/chow-consumption occurred (Figure 4), as was seen in the reversal experiment with MSX-3 (Figure 3).

VI. Discussion

The present study was conducted to determine the effort-related motivational effects of the VMAT-2 inhibitor tetrabenazine, and the ability of the novel adenosine A_{2A} antagonist MSX-3 and the DA reuptake inhibitor bupropion to reverse these motivational impairments. The behavioral experiments performed employed the concurrent FR5/chow feeding task to provide a measure of effort-related choice behavior (Salamone et al. 1991, 1997; Nunes et al. 2010). Previous studies have consistently shown that DA D1 or D2 antagonism shifts choice behavior in rodents tested on this task, suppressing food-reinforced lever pressing, while concurrently increasing chow intake (Salamone et al., 1991, 2002; Cousins et al., 1994; Sink et al., 2008). In the dose range tested, tetrabenazine treatment exerted a selective effect on the tendency to work for food, as demonstrated by a reduction in food-reinforced instrumental behavior, but nevertheless left the rats directed towards the acquisition and consumption of food. In response to this condition, tetrabenazine-treated rats with reduced levels of lever pressing selected an alternative path to obtain food (i.e. to approach and consumption of the concurrently available chow). This conclusion is consistent with recent studies demonstrating that tetrabenazine also reduced selection of the high effort/high reward option in rats tested on a T-maze barrier choice task (Yohn et al. 2012) and a progressive ratio/chow feeding choice procedure (Salamone et al. 2012).

Next, the adenosine A_{2A} antagonist MSX-3 and the catecholamine reuptake blocker bupropion were assessed for their ability to reverse the behavioral task performance effects of tetrabenazine. Previous work has indicated that adenosine A_{2A} antagonists produce behavioral effects in animals that are consistent with antidepressant actions (Hodgson et al. 2009; Hanff et al. 2010). Bupropion (Wellbutrin) is a widely used antidepressant (Milea et al. 2010) that has been shown to produce antidepressant-like effects in animals tested on tasks such as the forced swim and tail suspension tests (Yamada et al. 2004; Bourin et al. 2005; Kitamura et al. 2010). In the present studies, the selective adenosine A_{2A} antagonist MSX-3 was able to fully reverse the effects of tetrabenazine on FR5/chow-feeding choice performance, increasing lever pressing and decreasing chow intake in tetrabenazine-treated rats. These results are consistent with previous research demonstrating that adenosine A_{2A} antagonists reverse the effects of DA D2 family antagonists on effort-related choice behavior (Farrar et al. 2007, 2010; Salamone et al. 2009; Worden et al. 2009; Mott et al. 2009; Nunes et al. 2010; Santerre et al. 2012). Bupropion also reversed the effort-related effects of TBZ. As bupropion has known antidepressant actions in humans, these results serve to validate the hypothesis that tests of effort-related choice behavior can be used to assess some of the motivational effects of antidepressant drugs. Furthermore, these results are consistent with human clinical data indicating that bupropion is relatively effective for treating psychomotor retardation and fatigue symptoms of depression (Fabre et al. 1983; Pae et al. 2007). Nevertheless, these studies need to be carried out in large number of animals, across a broad range of conditions, in order to determine the feasibility of using these behavioral and

pharmacological procedures together as potential models of depression-like effects in rats, and ultimately in clinical trials.

In summary, the catecholamine-depleting agent TBZ alters effort-related choice behavior, reducing food-reinforced lever pressing on the FR5 procedure and producing a psychomotor slowing effect that biases animals towards the selection of consumption of the freely available chow. The adenosine A_{2A} antagonist MSX-3 was able to reverse the effort-related impairments produced by TBZ, as lever pressing was restored and concurrent consumption of the freely available lab chow was reduced, effectively restoring normal behavior in the catecholamine-depleted rats. As was the case with MSX-3, co-administration of the well-established antidepressant bupropion reversed the effort-related impairments produced by TBZ, increasing lever pressing and concurrently decreasing chow intake. This data supports the hypothesis that adenosine A_{2A} antagonists may be beneficial for the clinical treatment of motivational impairments, such as psychomotor slowing, anergia, and fatigue that are seen in depression and other disorders. These experiments represent a novel combination of behavioral and pharmacological methods that could ultimately be employed as animal models of effort-related symptoms of depression, which could foster the developments of new treatments and help to change the way researchers and clinicians think about the relation between depression, psychomotor retardation, and central neurotransmission. Further research should be pursued in this field in an effort to develop novel therapeutics and treatment strategies for the millions of individuals affected by these motivational symptoms around the globe.

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VIII. Figure Captions

Figure 1: A standard operant chamber, with dimensions 28x23x23cm. Lever and freely available chow are present in the chamber throughout the duration of the 30-minute sessions. The lights are shut off and the door to the testing room is closed to limit external disturbances during testing.

Figure 2: In a standard 30-minute operant procedure, a rat who received a "vehicle", or control, injection pressed the lever an average of 1442.125 times, while the consuming only an average 1.825 grams of lab chow. Upon administration of catecholamine-depleting agent TBZ, in doses of 0.25, 0.5, 0.75, and 1.0 mg/kg, lever pressing decreased from 1257.25, 1066.875, 364.375, and 157.125 respectively, while chow consumption concurrently increased from 3.4875, 3.7625, 6.325 and 6.7125 grams, respectively. This demonstrates the effort-related psychomotor slowing effect of TBZ.

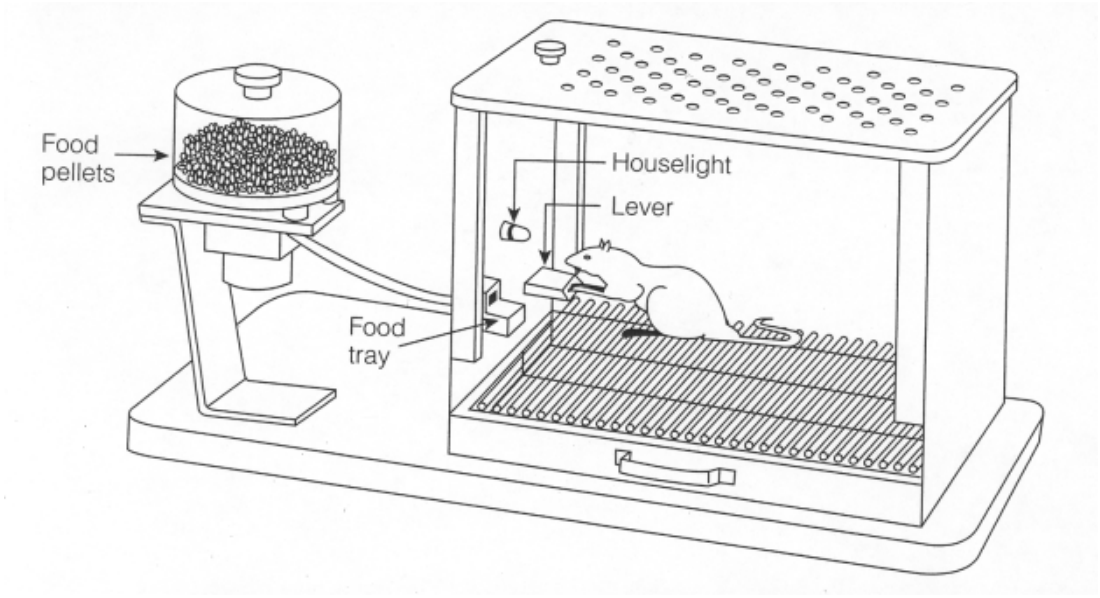
Figure 3: When the rat was injected with both TBZ and the A_{2A} antagonist MSX-3, a clear reversal of lever pressing/chow-consumption occurred, as the dosage of MSX-3 increased. Using MSX-3 doses of 0.5, 1, and 2 mg/kg, lever pressing increased from 747.25, 983.0 and 1275.62 respectively, while chow consumption decreased from 4.412, 3.462, and 2.425 grams, respectively. This demonstrates the reversal dose-response curve with the co-administration of TBZ and MSX-3, specifically how MSX-3 restored normal behavior in catecholamine-depleted animals.

Figure 4: When the rat was injected with both TBZ and the well-established antidepressant bupropion, a clear reversal of lever pressing/chow-consumption occurred, as was seen in the reversal experiment with MSX-3 (Figure 3). Using bupropion doses of 5, 10, and 15 mg/kg, lever pressing increased from 196.5, 777.1 and 1003.1 respectively, while chow consumption decreased from 6.325, 4.46, and 2.52 grams, respectively. The results demonstrate the overall significant effect of drug treatment on lever pressing and on chow consumption as attenuated by the behavioral effects of TBZ and subsequent reversal of behavior after administration of bupropion.

IX. Figures

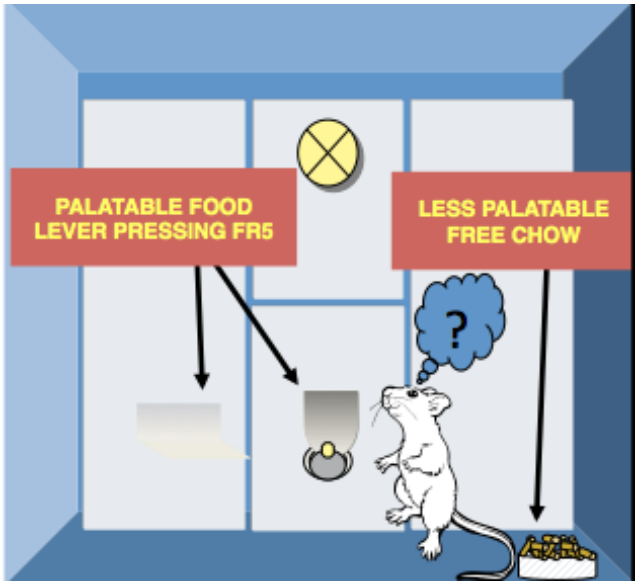
a. Figure 1: Models of Behavioral Procedures

i. Figure 1a: Standard Operant Chamber



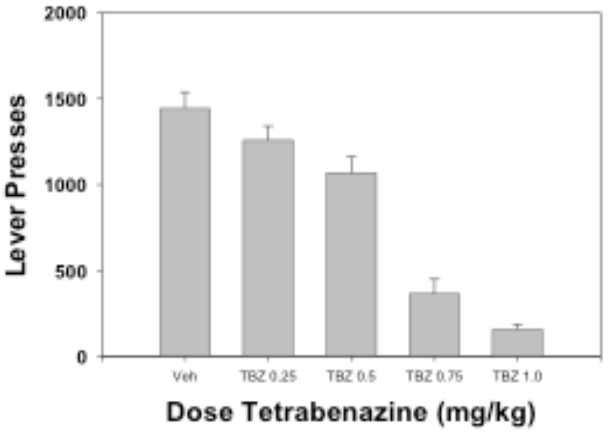
(Operant Conditioning Chamber [Google Image])

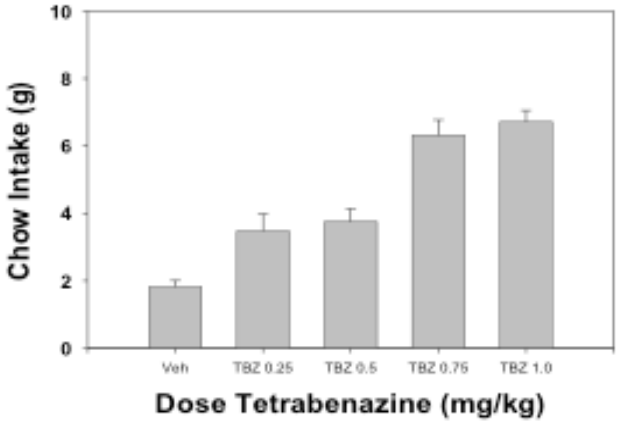
ii. Figure 1b: Use of Operant Chamber to Model Effort-Related Decision-Making Behaviors



b. Figure 2: Tetrabenazine Dose Response study

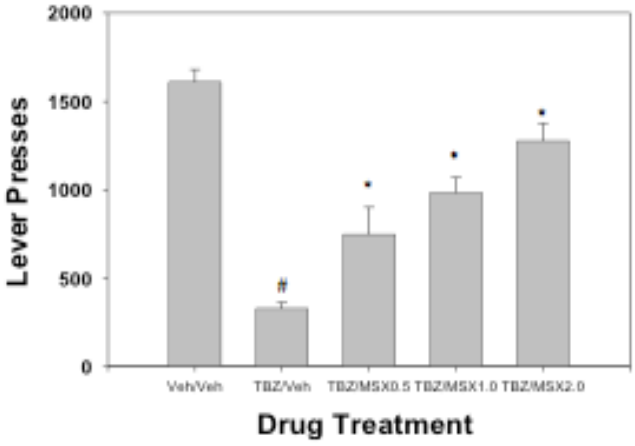
*Mean (±SEM) number of lever presses (left) and chow intake (right) with varying doses of TBZ), * p<0.01 significantly different from vehicle*

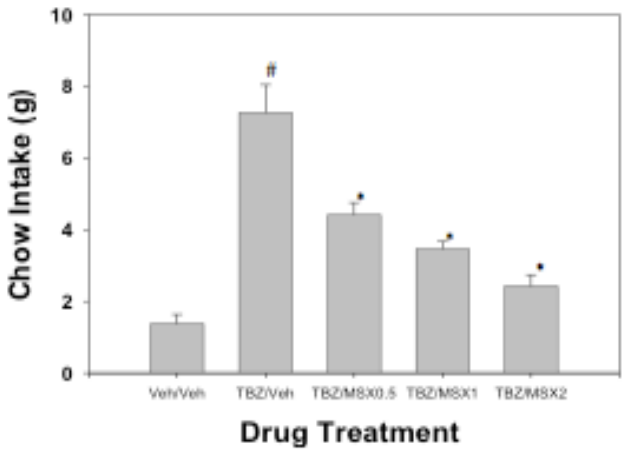




c. Figure 3: Tetrabenazine Reversal with MSX-3

*Mean (±SEM) number of lever presses (left) and chow intake (right) after treatment with TBZ (0.75mg/kg) AND various doses of MSX-3: 0.5mg/kg 1.0mg/kg and 2.0mg/kg. # p<0.01 significantly different from vehicle, * p<0.01 significantly different from TBZ/Veh*





d. Figure 4: Tetrabenazine Reversal with Bupropion

*Mean (±SEM) number of lever presses (left) and chow intake (right) after treatment with TBZ (0.75mg/kg) AND various doses of bupropion: 5mg/kg, 10mg/kg, 15mg/kg. # p<0.01 significantly different from vehicle, * p<0.01 significantly different from TBZ/Veh*