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The Development and Evaluation of Novel DA Transport Inhibitors and their Effects on Effort-Related Motivation: A Review

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Abstract

Depression is a debilitating disorder that can cause motivational deficits such as psychomotor retardation, anergia, apathy, and fatigue. Recent research indicates that these motivational deficits, and potential pathways of therapeutic intervention, can be studied in animal models involving rats and mice. Treatments with the VMAT-2 inhibitor tetrabenazine (TBZ) and cytokine interleukin-1ß (IL-1ß) can create a low-effort bias and impair effort-related motivation (Nunes et al. 2013, 2014). A number of high-affinity DA transport inhibitors such as d-amphetamine, methylphenidate, and cocaine can restore extracellular DA, albeit with the cost of undesirable effects such as high abuse liability. These observations have led researchers to identify a number of molecules that fit the profile of "atypical" dopamine binding, which leads to a longer duration of extracellular DA and minimizes side effects. In this review, the binding affinities and dose-response behavioral outputs for respective FR5/Chow and PROG/Chow feeding procedures have been compiled for eight DAT blockers: bupropion, GBR12909, lisdexamfetamine, PRX-14040, modafinil, (*S*)-CE-123, (*S*, *S*)-CE-158, and methylphenidate. Regression analyses between measures of DAT affinity and minimum significant dose in behavioral studies suggests a strong linear relationship between binding affinity and potency in terms of the ability of drugs to reverse the effects of TBZ the FR5/chow procedure. However, there was a variable relationship in terms of the ability of drugs to enhance lever pressing in rats tested on the PROG/choice procedure.

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Introduction

Motivated behavior is recognized to have directional aspects (i.e., behavior is directed towards and away from stimuli), but it also has activational aspects related to behavioral activation and exertion of effort in work output. Organisms often make effort-based decisions as they weigh the costs and benefits of the various options in the environment. One of the ways that effort-related aspects of motivation can be assessed is through tests of effort-based choice, in which animals are given a choice between a preferred reinforcer that can only be obtained by high exertion of effort, vs. a low effort/low reward option. Studies have shown that the vesicular monoamine transport (VMAT-2) inhibitor tetrabenazine (TBZ) or the cytokine interleukin-1β (IL-1β) can cause profound motivational impairments and shift choice behavior in rats, producing a low-effort bias (Nunes et al., 2013; Yohn et al. 2016). TBZ acts by blocking vesicular storage and depleting dopamine (DA), as assessed by both pre- and postsynaptic markers of DA transmission (Nunes et al. 2013). In fact, the effects of TBZ in humans can even result in the emergence of negative symptoms such as fatigue, pointing to its potential in creating a challenge for rats to exert physical effort when presented with a choice (Chitnis and Karunapuzha, 2009; Frank, 2010; Chen et al., 2012; Rotolo et al., 2020). In response to TBZ administration, rats demonstrate a decreased selection of the high effort options and increased selection in low effort/low energy options. This is possibly related to the induction of depression-like symptoms. Using an FR5 or PROG chow feed task model, the behavioral outputs of several 5-HT selective serotonin reuptake inhibitors (SSRI) and DA transport (DAT) inhibitors can be studied for a potential reversal of the effects of TBZ (Yohn et al. 2016).

Rats have become a useful model organism to test the effects of commonly prescribed antidepressants that are SSRIs, such as Lexapro (*S*-citalopram) and Prozac (fluoxetine), which

are typical treatments of depression and generalized anxiety. Recent FR5 and PROG chow feed task trials with (*S*)-citalopram and fluoxetine, however, have yielded no significant reversal of the effects of TBZ (Salamone et al. 2018). In fact, these serotonin transport inhibitors even have the tendency to exacerbate symptoms and produce further impairments in level pressing such as fatigue (Yohn et al., 2016). These data point to the inefficiency of common market SSRIs in battling motivational symptoms in patients. Since SSRIs selectively inhibit serotonin reuptake pathways, research suggests that the dopamine transporter is associated with a critical pathway in cost/benefit analysis and effort-related decision making (Nunes et al., 2013; Yohn et al. 2016).

A number of DAT inhibitors have been tested by Salamone and colleagues (Nunes et al. 2013; Randall et al. 2015; Yohn et al. 2016; Rotolo et al. 2019, 2020, 2021). For the present study, a select number of reversal agents that directly act on the nucleus accumbens core DA transporter were investigated for their respective binding affinities and dose-response outputs (Salamone and Correa, 2002, 2012; Salamone et al, 2003, 2007; Nunes et al, 2013). These DAT inhibitors (bupropion, GBR12909, lisdexamfetamine, PRX-14040, modafinil, (S)-CE-123, (S, S)-CE-158, and methylphenidate) are all highly selective for DAT binding relative to the serotonin transporter, and some are selective vs. norepinephrine transport binding as well. Affinity more specifically refers to the strength of the tendency of a neurotransmitter or ligand to bind to its protein target, whether a receptor or a transporter. Intrinsic quantitative measures such as Ki, Km, Kapp or IC50 can represent affinity as the concentration of a ligand that yields 50% of maximal transporter binding. Transporter binding is central to determining drug affinity for drugs that act on the uptake process, yet potency in terms of behavioral effects incorporates other factors such as metabolism, penetration into the tissue of interest, and the duration of action. The doses at which a drug has its effect and its molecular affinity are typically correlated as long as

these other factors are relatively equal, so the present study seeks to identify a link for each DAT inhibitor on both FR5 and PROG operant procedures. After compiling DAT binding affinity (nM) values and calculating lowest significant doses (mg/kg) for behavioral effects in rats tested on FR5 and PROG procedures, a statistical regression analysis was conducted to determine the line of best fit and the Pearson product-moment correlation coefficient, the proportion of variability accounted for, and the standard error of estimate around the regression line. These analyses can be used to further clarify the relation between binding to the DAT as measured *in vitro* and the behavioral effects of these drugs.

Materials and Methods

Animals

For the behavioral studies (Nunes et al. 2013; Randall et al. 2015; Yohn et al. 2016; Rotolo et al. 2019, 2020, 2021), protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee (IACUC). Adult male Sprague Dawley rats (Indianapolis, IN, USA) were housed at 23 °C during 12 hour light/dark cycles (e.g. Yohn et al., 2016). The animals generally weighed approximately 300 grams at the beginning of each study. Although initially, food was restricted to 85% of their body weight, these rats were later fed supplemental lab chow and provided water *ad libitum* to allow gradual growth. Animal protocols followed National Institute of Health (NIH) guidelines (e.g. Nunes et al., 2013).

Concurrent FR5/Chow-choice Procedure

The concurrent FR5/chow-choice procedures were conducted in operant conditioning chambers, where rats were first trained to lever press with continuous reinforcement. These sessions were generally 30 minutes over 5 days, after which the rats were shifted to an FR5 schedule (30 min sessions, 5 days/week). After training for several weeks until reaching a baseline target for lever presses, the rats were finally introduced to the concurrent FR5/chow feeding choice procedure. This procedure is conducted in the operant chamber by making a low-reward lab chow and a high-carbohydrate reinforcer accessible via lever pressing during the 30-minute session. While both options are available, the rats are able to choose their behavior towards either the chow or the high carb pellet. Before starting the session, it was ensured that the weighted chow pieces would not fall through the floor of the chamber (e.g. Rotolo et al., 2019). At the start of each session, it was confirmed that the pieces of weighed chow were larger than the spaces between the bars that make up the floor of the chamber, so they could not fall through. After each session completed, each rat was removed from the chamber while the number of lever presses and consumed chow weight (calculated from spillage) was recorded (e.g. Yohn et al., 2016).

PROG/Chow Feeding Choice Task

In contrast to the concurrent FR5 procedure, the lever-pressing option will end in the PROG/chow-feeding choice procedure within 2 minutes if the rat fails to obtain a reinforcer, meaning that the chow will become the only option after this interval (Salamone et al., 2018). This “time-out” would result from the deactivation of the response lever for the rest of the session when the 2-minute interval completed without a completed ratio (Rotolo et al., 2019). For this procedure, rats were first trained on a continuous reinforcement FR1 schedule followed

by a shift to a PROG schedule, where the ratio from the FR1 was gradually increased. After several weeks of training on the PROG schedule, weighted amounts of lab chow become available in the chamber during PROG/chow feeding choice task sessions (generally 30 minutes each). Similar to the FR5 procedure, the number of lever presses and amount of chow consumed were recorded at the end of each session. After training on the concurrent FR5/chow-choice and PROG/chow feeding choice procedures for several weeks, novel drug testing was introduced respectively.

Dose Selection

Novel atypical molecules obtained by Salamone Lab were generally dissolved in dimethyl sulfoxide (DMSO) and saline solution with the DMSO/saline solution serving as vehicle control, or in physiological saline. Tetrabenazine was received from Tocris Bioscience (Ellisville, MO) and titrated with extremely small quantities of 1.0 N HCl to achieve an overall pH of 4.5. The dosage of TBZ was typically calculated to be 0.75 mg/kg-1.0 mg/kg TBZ based on extensive piloting and previous work in the laboratory. Doses of reversal agents were also determined based on previous research and rodent runaway studies (Sink et al., 2008; Esumi et al., 2013).

Statistical Analysis

DAT affinity values were extensively searched through neuropsychopharmacology literature using the electronic database PubMed, as listed in **Table 1**. These data were calculated via *in vitro* binding or inhibition assays (Cao et al., 2016; Zhang et al., 2017). In each behavioral

pharmacology paper, rats received vehicle or 0.75mg/kg TBZ approximately 90 minutes before testing followed by intraperitoneal injections of vehicle or the DAT blocker at varying times before behavioral testing, depending upon the drug (Salamone et al., 2016; Rototol et al. 2019, 2020, 2021). Statistical analyses were used in each paper to identify the doses of a DAT blocker plus TBZ that significantly increased lever pressing relative to TBZ plus vehicle ($P<0.05$). The potency values for the regression analyses for each DAT inhibitor were calculated by identifying the lowest significant dose based on the minimum dosage that significantly differed from TBZ plus vehicle. A complete list of binding affinity (nM) and lowest significant doses (mg/kg) for all eight DAT blockers used in the TBZ studies is depicted in **Table 2**. GraphPad Prism 8.4.1 was used to compute a regression analysis determining a linear relationship between affinity and potency for both concurrent FR5 and PROG chow choice procedures (**Figure 1**). Recently synthesized atypical DAT inhibitor CT-005404 (Chronos Therapeutics) was excluded from this study as doses were administered orally rather than intraperitoneally (Rotolo et al., 2020). Modafinil was studied for its effects on FR5/chow feeding choice performance but never for its ability to reverse choice-induced shifts of TBZ on the PROG/chow feeding procedure (Salamone et al., 2016). For this reason, it was included in the DAT vs. FR5 statistical analysis (n=8) but excluded from the DAT vs. PROG calculations (n=7).

Results

Bupropion

The commonly prescribed antidepressant bupropion was extensively studied in the Nunes et al. 2013 study with IP injections of Vehicle/Vehicle, 0.75 mg/kg TBZ/Veh, or TBZ coadministered

with doses of bupropion (5.0, 10.0, or 15.0 mg/kg doses). Compared to the administration of TBZ alone, catecholamine uptake inhibitor bupropion was able to significantly reverse the effects of tetrabenazine. The highest doses of bupropion increased lever presses over a 30-minute session and decreased intake of freely available laboratory chow.

In the Yohn et al. 2016 study, however, the selective DA D1 antagonist SCH 39166 (ecopipam) and DA D2 antagonist haloperidol were capable of blocking its reversal of TBZ depressive effects. Although these antagonists had no effects when administered alone, the coadministration of either 0.05 mg/kg of ecopipam or 0.05 mg/kg haloperidol significantly reduced the efficacy of bupropion.

GBR12909

Originally developed as a potential treatment for cocaine addiction, GBR12909 shows a much lower efficacy than cocaine and atypical binding properties (Yohn et al., 2016). More than a 100-fold lower in NET and SERT affinity (Anderson, 1989), GBR12909 is one of many benztropine analogs that have been associated with increased DA levels in nucleus accumbens for a longer duration than cocaine. In an experiment in the Yohn et al. 2016 study, trained rats received the following treatments of TBZ vehicle plus GBR12909 vehicle: 0.75 mg/kg TBZ + GBR12909 vehicle, 0.75 mg/kg TBZ + 1.25 mg/kg GBR12909, 0.75 mg/kg TBZ + 2.5 mg/kg GBR12909, and 0.75 mg/kg TBZ + 5.0 mg/kg GBR12909. The results showed that the highest doses of GBR12909 + TBZ vehicle (2.5 and 5.0 mg/kg of GBR12909) were statistically significant ($P<0.05$) from the TBZ vehicle, leading to a significantly lower chow consumption. Additionally, when administered without TBZ on a concurrent FR5/chow-choice procedure, the highest doses of GBR12909 yielded significantly higher lever presses than NET inhibitor

desipramine and SERT inhibitor fluoxetine. The results of this study demonstrate that GBR12909 successfully attenuates TBZ-induced shifts in rat behavior, and it is a more powerful reversal agent than norepinephrine and serotonin transport inhibitors.

LDX

Lisdexamfetamine (LDX) is a novel descendant of d-amphetamine which has been approved for the treatment of attention deficit hyperactivity disorder (ADHD) and binge eating disorder in adults (Weisler et al. 2009; FDA News Release 2015). While d-amphetamine is known to cause sudden bursts in extracellular catecholamine levels, LDX leads to a much longer duration of these neurotransmitter levels, especially high concentrations of dopamine in the nucleus accumbens. The ability of LDX to increase effort in rats treated with cytokine interleukin-1ß, TBZ, and TBZ + CIT was studied on a concurrent FR5/chow-feeding procedure. IL-1ß is associated with depressive symptoms in humans and has shown to reduce the selection of high effort alternatives and increase consumption of lab chow in rat models. In the Yohn et al. 2016 study of LDX, the ability of LDX to ameliorate the effects of IL-1ß was experimented with the following dosage: IL-1β-vehicle + LDX vehicle, 4.0 μg/kg IL-1β- + LDX vehicle, 4.0 μg/kg IL-1β- +0.09375 mg/kg LDX, 4.0 μg/kg IL-1β- + 0.1875 mg/kg LDX, 4.0 μg/kg IL-1β- + 0.375 mg/kg LDX, and 4.0 μg/kg IL-1β- + 0.75 mg/kg LDX. The treatment of 0.09, 0.375 and 0.75 mg/kg of LDX with IL-1ß significantly increased lever pressing and decreased chow consumption compared to IL-1ß alone. Similarly, the ability of LDX to attenuate the behavioral effects of TBZ treatment was investigated on the same procedure with the following dosage: TBZ vehicle + LDX vehicle, 0.75 mg/kg TBZ + LDX vehicle, 0.75 mg/kg TBZ + 0.09375 mg/kg LDX; 0.75 mg/kg TBZ + 0.1875 mg/kg LDX, 0.75 mg/kg TBZ + 0.375 mg/kg LDX, and

0.75 mg/kg TBZ + 0.75 mg/kg LDX. Similar to the results of the IL-1ß experiment, LDX successfully attenuated the effects of TBZ with the coadministration of 0.1875, 0.375, and 0.75 mg/kg LDX being statistically significant ($P<0.05$). In all three treatments, LDX plus TBZ significantly increased lever pressing and decreased chow consumption, indicating a full reversal.

PRX-14040

Novel DA update inhibitor PRX-14040 was introduced as a transporter with low molecular weight and Log P values yet an optimal atypical DA selectivity. While PRX is able to bind to both dopamine and norepinephrine transporters, it shows a 28-fold higher selectivity for the dopamine transporter (Yohn et al., 2016). The Yohn et al. (2016) PREXA study analyzed the ability of PRX to both reverse the TBZ shifts in behavior on the FR5/chow choice task and increase lever pressing in the PROG/chow choice task. On the FR5 procedure, trained rats received the following dosage: TBZ vehicle + PRX vehicle, 0.75 mg/kg TBZ + PRX vehicle, 0.75 mg/kg TBZ + 1.25 mg/kg PRX, 0.75 mg/kg TBZ + 2.5 mg/kg PRX, 0.75 mg/kg TBZ + 5.0 mg/kg PRX, and 0.75 mg/kg TBZ + 10.0 mg/kg PRX. Overall, PRX yielded a complete reversal of the effects of TBZ with the co-administration of 2.5, 5.0, and 10.0 mg.kg PRX significantly increasing the number of lever presses and increasing chow consumption relative to the TBZ vehicle group ($P<0.0001$). In the PROG/chow choice task, trained rats were IP injected with doses of 5.0, 10.0, 20.0, and 40.0 mg/kg PRX an hour before testing. The results revealed that the administration of 10.0, 20.0 and 40.0 mg/kg PRX significantly increased the total number of lever presses, achieved the highest ratio relative to vehicle treatment ($F(4.36) = 12.075$), and decreased consumption of lab chow ($P<0.05$). PRX also yielded the highest reversal effect size

(partial ε2) for TBZ-induced reversal of suppression in lever pressing and increase in chow intake relative to bupropion, modafinil and methylphenidate. The unique DA binding selectivity of PRX, compared to other drugs such as BUP, MOD, and MET, improves its candidacy as a treatment of motivational dysfunction.

Modafinil

Modafinil, or MOD, is a wakefulness agent that has already been used in clinical studies to treat depressive-like symptoms such as fatigue and anergia. The Rotolo et al. 2019 study compared modafinil to its analog (S)-CE-123. While MOD demonstrates improvement in fatigue and reversal of low-effort bias, it requires higher doses on the FR5 procedure than analogs with highly selective DA binding. Despite its low affinity in the reversal of depressive symptoms, MOD demonstrates a low abuse liability. As mentioned earlier, modafinil was not tested for its potential to reverse effects of TBZ on the PROG procedure.

(*S*)-CE-123

Novel atypical DA transport inhibitor (S)-CE-123 is a synthesized analog of modafinil with a highly selective affinity for DAT. The Rotolo et al. 2019 study has not only demonstrated its ability to reverse TBZ-induced shifts in rats, but also its enhancement of cognitive flexibility and reduction of impulsivity and undesirable effects. In the study, trained rats were administered the following combinations of (S)-CE-123 and TBZ: TBZ vehicle + (S)-CE-123 vehicle, 1.0 mg/kg TBZ + (S)-CE-123 vehicle, 1/0 mg/kg TBZ + 6.0 mg/kg (S)-CE-123, 1.0 mg/kg TBZ + 12.0 mg/kg (S)-CE-123, and 1.0 mg/kg TBZ + 24.0 mg/kg (S)-CE-123. The coadministration of 24.0 mg/kg (S)-CE-123 significantly increased total lever presses and decreased chow consumption.

Although the administration of (S)-CE-123 alone had no effect on lever pressing or chow consumption, it significantly increased the DA levels in the nucleus accumbens core ($P<0.05$).

(*S, S*)-CE-158

(*S, S*)-CE-158 is a novel atypical analog of modafinil, similar to its stereoisomer, thiazole-based (S)-CE-123, in its specificity of its DA inhibition. While (S)-CE-123 has demonstrated pro-cognitive effects and preclinical effectiveness without adverse side effects, (*S, S*)-CE-158 and a range of modafinil-related drugs have been included in the investigation to identify the most promising candidate to treat motivational dysfunction (Nikiforuk et al. 2017; Kalaba et al. 2017; Kristofova et al. 2018; Camats-Perna et al. 2019). In the Rotolo et al. 2020 study, rats were administered five treatment options: TBZ vehicle + (*S, S*)-CE-158 vehicle; 1.0 mg/kg TBZ + (*S, S*)-CE-158 vehicle; 1.0 mg/kg TBZ + 2.0 mg/kg (*S, S*)-CE-158; 1.0 mg/kg TBZ + 4.0 mg/kg (*S, S*)-CE-158; 1.0 mg/kg TBZ + 8.0 mg/kg (*S, S*)-CE-158. The co-administration of 8.0 mg/kg (*S, S*)-CE-158 plus TBZ yielded a significant increase in lever pressing and decrease in chow intake compared to TBZ plus vehicle ($P<0.001$). Repeated measures ANOVA identified a significant increase in lever pressing and high-effort responding on the concurrent PROG/chow feeding choice procedure at a co-administered minimum dose of 4.0 mg/kg (*S, S*)-CE-158 plus TBZ compared to TBZ plus vehicle ($P<0.01$).

Methylphenidate

Methylphenidate, or MET, is a stimulant drug with DA reversal properties that has been commonly tested in human clinical populations. In the Yohn et al. 2016 PREXA study, the co-administration of MET with TBZ in rats successfully attenuated the effects of TBZ alone, but it resulted in similar comparisons as modafinil. Compared to the co-administration of PRX and

BUP, MET yielded significantly lower lever presses on the FR5 procedure. Although methylphenidate improves motivational deficits by inhibiting the dopamine transporter (DAT), its stimulant nature also results in a number of undesirable side effects such as strong abuse liability and psychotic symptoms (Rotolo et al., 2019).

DAT Blocker	Affinity Value	Citation
Bupropion	Ki = 630nM	(Meyer et al., 2002; Richelson and Pfenning 1984; Tatsumi et al. 1997; Schmitt et al., 2008)
GBR12909	Kapp = 121∓ 21nM	(Schmitt et al., 2008; Vladimir et al., 2008)
LDX	Km = 200nM	(Yohn et al., 2016)
PRX-14040	Ki = 9.43nM	(Yohn et al., 2016; Gu et al., 1994; Giros and Caron, 1993; Galli et al., 1995; Shearman et al., 1998)
Modafinil	Km = 3050 ∓ 258nM	(Salamone et al., 2016; Zhang et al. 2017)
(*S*)-CE-123	IC50 = 4.6 μM	(Sagheddu et al., 2020)
(*S, S*)-CE-158	IC50 = 0.2271μM	(Rotolo et al., 2020)
Methylphenidate	IC50 = 224 ∓ 19nM	(Salamone et al., 2016; Wayment et al., 1999)

Table 1. List of dopamine transport inhibitors from selective literature and corresponding affinity values (nM).

	FR5 (mg/kg)	DAT (nM)	PROG (mg/kg)
BUP	**10**	**630**	**20**
GBR12909	**2.5**	**121**	**5**
LDX	**0.1875**	**200**	**0.75**
PRX-14040	**2.5**	**9.43**	**20**
MOD	**7.5**	**3050**	
(*S*)-CE-123	**24**	**4606**	**24**
(*S*, *S*)-CE-158	**8**	**227**	**4**
METH	**0.5**	**224**	**4**

BUP = bupropion; LDX = lisdexamfetamine; MOD = modafinil; METH = methylphenidate

Table 2. List of lowest effective doses (mg/kg) calculated from FR5 and PROG dose-response curves and corresponding DAT affinities (nM).

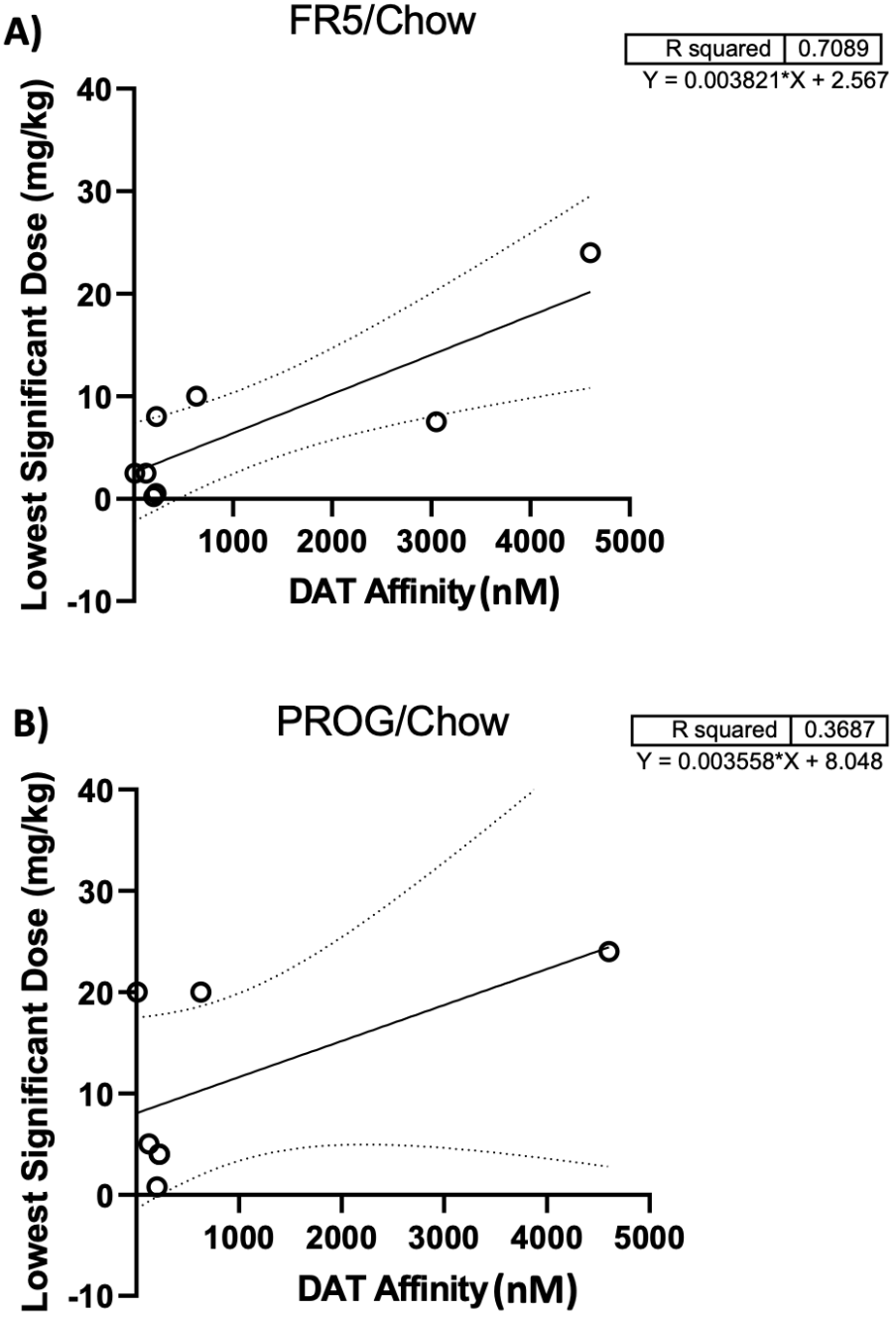

Figure 1. A) Scatterplot with regression line (solid line) and 95% confidence intervals (dotted curves) showing the relation between affinity of DA transport blocker vs. its minimum effective dose on concurrent FR5/Chow-choice Procedure. $R^2 = 0.7089$ and the graph has a 95% confidence interval of (0.3376, 0.9707) with a standard error of estimate (sYX) of 4.599. B)

Scatterplot with regression line (solid line) and 95% confidence intervals (dotted curves) showing the relation between affinity of dopamine transport blocker against its minimum effective dose on PROG/Chow Feeding Choice Task. $R^2 = 0.3687$ and the graph has a 95% confidence interval of (-0.2687, 0.9334) with a sYX of 8.483.

Discussion

In determining the "goodness of fit" on a correlational analysis, the coefficient of determination (R^2) and standard error of estimate (sYX) are common indicators. Variance, or the scatter around the line of best fit, refers in this study to the minimum dose values (mg/kg) that can be predicted by DAT affinity values (nM). The R^2 calculations of both graphs indicate that 71% of the variance in data can be explained in the concurrent FR5 procedure (**Figure 1A**), yet only 37% can be explained in the concurrent PROG procedure (**Figure 1B**). The 95% confidence bands also demonstrate a larger gap in variance on the PROG interval of (-0.2687, 0.9334) compared to the FR5 interval of (0.3376, 0.9707). The standard error of estimate, or sYX, measures both the variability of the observed scatter and reliability of the estimating equation (Y=mx+b). The predicted sYX value for DAT vs FR5 is 4.599, nearly half the DAT vs PROG value of 8.483. The strength of the correlation between affinity and behavioral potency can be characterized with significantly less dispersion on the FR5 procedure relative to the PROG procedure.

The Pearson product-moment correlation coefficient (r) captures linear relationships on a range of -1 to +1, corresponding to negative and positive slopes respectively. An absence of a linear pattern falls closer to a value of 0. GraphPad Prism 8.4.1 computed r = 0.8420 for **Figure 1A** and r = 0.6072 for **Figure 1B**, indicating stronger linearity on the FR5 procedure relative to the

PROG procedure. A computational two-tailed hypothesis test ($\alpha = 0.05$) yielded a p-value of 0.0087 for DAT vs FR5 and a p-value of 0.1482 for DAT vs PROG. For the regression graph of FR5, $P<0.05$ and the null hypothesis can be rejected, concluding a strong linear correlation between the variables. The PROG data, however, fails to reject H_o in favor of H_a ($P>0.05$) and lacks substantial evidence to conclude a linear relationship. The correlation between the dose at which a DAT blocker has its effect and its unique binding affinity can be summarized as statistically significant for the FR5/chow choice task, but not conclusive for the PROG/chow choice task.

The increased variability in PROG dispersion compared to that of the FR5 procedure could be partially attributed to its exclusion of modafinil, reducing its total drug count to $n = 7$. The drugs selected for this study consist of both typical and atypical DA transport blockers. Atypical analogs of modafinil such as (*S*)-CE-123 and (*S, S*)-CE-158 indicate a better profile for reversing depression-like symptoms while decreasing potential side effects. In addition to DAT affinity, the selectivity and binding locus of a DAT blocker have profound effects on DAT trafficking and the ability to reduce abuse liability and psychotic symptoms (Rotolo et al., 2019). The correlation in this study is strictly between drug affinity and potency; there is no noticeable distinction based on binding selectivity.

In summary, there is a strong relationship between the concentration at which these various DAT inhibitors affect DA transport *in vitro* and the doses at which the same compounds reverse the effects of TBZ in *vivo* in rats responding on the FR5/chow feeding choice task. However, the results regarding the PROG task were inconclusive. Future studies may consider drafting more candidates for the regression analysis on the PROG procedure and investigating differences between atypical or typical binding in relation to potency. Ongoing research on this topic could

provide insight on dosage in the therapeutic administration of pharmacological agents used to treat motivational dysfunction and psychomotor challenges.

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