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Abstract

Major Depressive Disorder (MDD) is characterized by symptoms such as cognitive dysfunctions, inflammatory changes, and motivational symptoms such as amotivation, fatigue, and anergia. While depressed people are commonly treated by traditional antidepressants such as serotonin reuptake inhibitors (SSRIs), previous studies have reported that SSRI medications do not treat fatigue and anergia symptoms well, and in some cases, can even worsen those symptoms. Subjects treated with dopamine (DA) uptake inhibitors, on the other hand, have been less likely to report symptoms of anergia and fatigue compared to those treated with SSRIs. Common DA uptake inhibitors such as methylphenidate and amphetamines, however, have undesirable side effects, so development of atypical DA uptake inhibitors to combat these side effects is needed. Several highly selective atypical DA uptake inhibitors have recently been developed, which are currently being assessed for their effects on effort-based decision making in rodents to model motivational symptoms seen in humans with MDD. This project is assessing a novel atypical DAT inhibitor, CE-158, for its effects on extracellular DA levels in the nucleus accumbens. Microdialysis and high-performance liquid chromatography with electrochemical detection is being used to measure extracellular DA changes at various time points after administration. Elevated nucleus accumbens dopamine has been linked to increases in effort-based decision making and other aspects of motivation, so findings from this study may reveal whether CE-158 could ultimately be used as a suitable treatment option for effort-related motivational dysfunction in humans suffering from MDD. Through this study it was determined that injections of CE-158 significantly increased extracellular DA levels in the nucleus accumbens.

Keywords: dopamine, depression, nucleus accumbens, microdialysis, fatigue

Introduction

Individuals diagnosed with Major Depressive Disorder (MDD) suffer from a range of symptoms, including cognitive dysfunctions, inflammatory changes, and motivational symptoms. The motivational symptoms often reported in people with MDD are fatigue, anergia, and psychomotor slowing. These conditions of fatigue and anergia are correlated with the general severity of symptoms seen in patients with depression (Stahl, 2002; Gullion & Rush, 1998). Additionally, these symptoms can have long-term functional limitations and debilitating effects on individuals (Stahl, 2002; Demyttenaere et al., 2005; Salamone et al, 2006; Friedman el al., 2007; Treadway & Zald, 2011; Fava et al., 2014; Rothschild et al., 2014; Chong et al., 2015; Salamone et al., 2016a,b,c; Salamone et al., 2017). Fatigue and psychomotor deficits are primary symptoms in depression, but are very difficult to treat. Studies have shown that about half of patients being treated for depression do not report sufficient symptom improvement and commonly experience psychomotor deficits as a residual symptom (Tylee et al., 1999).

The most commonly used antidepressant medications are serotonin selective reuptake inhibitors (SSRIs). However, research shows that SSRIs do not treat the primary symptoms of anergia and fatigue well, and in some cases may even worsen these conditions (Katz et al., 2004; Nutt et al., 2007; Padala et al., 2012; Stenman & Lilja, 2013; Rothschild et al., 2014; Fava et al., 2014; Yohn et al., 2016a,b). Frequently patients treated with SSRIs suffer from residual symptoms, including sleepiness, fatigue, and anergia, even if symptoms associated with mood and anxiety improved (Targum & Fava, 2011; Fava et al. 2014; Cooper et al., 2014; Rothschild et al., 2014; Ferguson et al., 2014). Motivational symptoms can be extremely detrimental to daily life, and can interfere with many basic functions such as physical activity and effort expenditure.

Thus, recent studies have been investigating other possible treatment options to improve the motivational dysfunction seen in depression and related disorders.

Considerable evidence has pointed towards significant involvement of the central dopamine (DA) systems and striatal areas of the brain in motivational functions related to depression (Stahl, 2002; Salamone et al., 2006; Treadway & Zald, 2011). There has been a correlation found between striatal DA neurotransmission and willingness to exert effort for large rewards, even when reward probability is low (Treadway et al., 2012). Additionally, some studies have shown that stimulating this DA neurotransmission can improve motivational symptoms in humans (Stotz et al., 1999; Papakostas et al., 2006). Importantly, when compared to SSRIs, depressed patients who were treated with bupropion, an antidepressant drug that acts on DA and norepinephrine (NE), were less likely to report suffering residual symptoms of sleepiness and fatigue (Cooper et al., 2014). Moreover, drugs that inhibit DA transporters (DAT), including d-amphetamine and methylphenidate, improve motivational function (Stotz et al., 1999). While these psychomotor stimulants that block DAT have benefits to treating motivational symptoms, they also have undesirable effects, including abuse liability and induction of psychotic symptoms (Todtenkopf & Carlezon, 2016; Ostlund et al., 2014; Dong et al., 2017). Due to these effects, studies have been focused on developing drugs that are both highly selective towards DAT and contain atypical neurochemical characteristics to attenuate undesirable side effects.

There has been recent interest in the compound known as modafinil, in terms of its ability to act as a DAT inhibitor with atypical characteristics. Modafinil is a nonamphetamine nootropic, or cognitive enhancing, drug that mimics that action of central nervous system catecholamines (Rang et al., 2016; Katzung & Trevor, 2018; Warner et al., 2018; Dinis-Oliviera, 2014; Dinis-

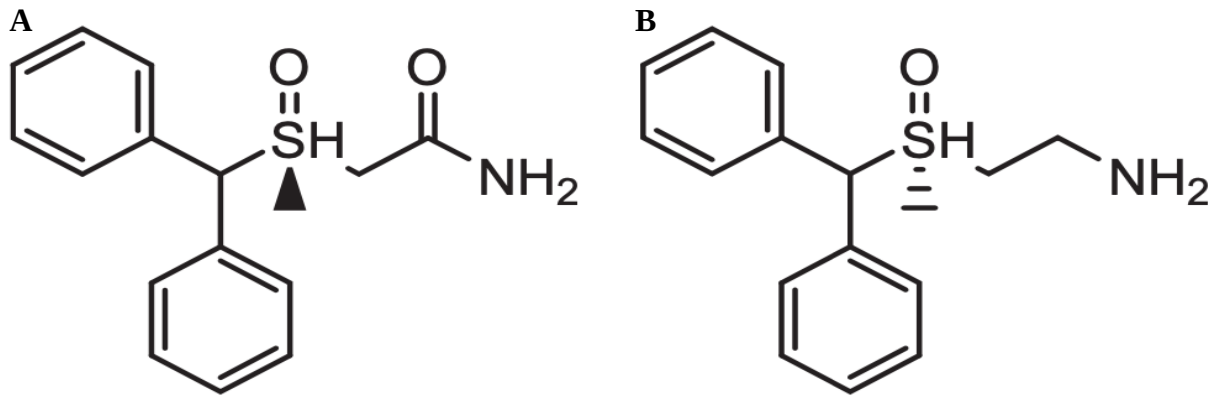

Figure 1. Chemical structure of *R*-Modafinil **(A)** and *S*-Modafinil **(B)**.

Oliviera, 2015; Dinis-Oliviera, 2017; Sousa & Dinis-Oliveira, 2020). **Figure 1** depicts the chemical structures of the *R*- and *S*-enantiomers of modafinil. Modafinil has effects on DA levels within various areas of the brain by binding to DAT, blocking DA reuptake, and causing an increase in DA (Mignot et al., 1994; Ballon & Feifel, 2006; Solinas et al., 2006; Zolkowska et al., 2009; Dell'Osso et al., 2014, Mereu et al., 2017; Sousa & Dinis-Oliveira, 2020). One unique property about modafinil compared to other psychostimulants, such as cocaine, is its pharmacokinetic profile. When binding to DAT, modafinil preferentially binds to a conformation similar to that of an atypical DAT inhibitor, and different from the cocaine-bound conformation (Loland et al., 2012; Reith et al., 2015; Cao et al., 2016; Sousa & Dinis-Oliveira, 2020). Interestingly when compared with other psychostimulants that also increase DA transmission, modafinil stimulates distinct brain areas, specifically in the striatum and cortex, inducing neurological activation more directed towards wakefulness, with reports suggesting contrasting epigenetics and transcriptional consequences leading to the varying clinical effects (Ballon & Feifel, 2006; Ishizuka et al., 2012; Gonzalez et al., 2019). While modafinil is typically used as a wakefulness agent for the treatment of narcolepsy and other disorders, modafinil also has been

employed in psychiatry to help improve symptoms in patients with MDD and bipolar depression (Dell'Osso et al., 2014; Perugi et al., 2017; Barateau & Dauvilliers, 2019; Greenblatt & Adams, 2019; Sousa & Dinis-Oliveira, 2020). Studies are suggesting that modafinil may be a beneficial treatment for depressed individuals suffering from cognitive impairments, specifically sleepiness and fatigue (Perugi et al., 2017; Dell'Osso & Ketter, 2013; Dell'Osso et al., 2013a,b). Furthermore, Teodorini et al. (2020) suggests that patients diagnosed with a psychiatric disorder reported higher perceived benefits of modafinil when used more frequently, while there was no association between increased frequency of use and perceived risks. An additional significant benefit to modafinil includes its seemingly low abuse potential in comparison to other catecholaminergic agents, like amphetamines (McGregor et al., 2008; Schmitt & Reith, 2011; Dackis et al., 2012; Loland et al., 2012; Sangroula et al., 2017; Sousa & Dinis-Oliveira, 2020; Teodorini et al., 2020).

Investigation of modafinil analogs is being done to discover a distinct class of drugs that have pharmacological profiles similar to modafinil, that show possible benefits in terms of their ability to treat motivational symptoms, like fatigue. In order to serve as a promising candidate for clinical use, an important component of the analogs being tested is their ability to significantly inhibit DA-reuptake with high specificity, without causing an efflux of DA, as seen in amphetamines (Kalaba et al., 2017; Sousa & Dinis-Oliveira, 2020). A recent study showed analogs with their amide group replaced with 2-methypyrimidine-4-ol, 2-thiophenyl, and 3-thiophenyl groups had higher DAT inhibition activity than modafinil (Kalaba et al., 2017). For example, a recently synthesized analog of modafinil, known as (*S*)-CE-123, has shown to be highly selective for DAT inhibition. (*S*)-CE-123 has shown a 100-fold selectivity for DAT relative to the NE transporter (NET), while modafinil only has a 25-fold selectivity for DAT

relative to NET (Kalaba et al., 2017; Kristofova et al., 2018). Results from a recent study conducted by Rotolo et al. (2019) determined that systemic injections of (*S*)-CE-123 in rats produced a significant increase in extracellular DA in the nucleus accumbens compared to baseline.

The effect of DA on motivational dysfunction can be modeled through tasks that measure effort-related choice behavior in animals (Salamone et al., 2006; Salamone et al., 2016a,b,c; Salamone et al., 2018). These tasks offer animals a choice between high-effort instrumental actions leading to highly valued reinforcers versus low-effort options leading to low reward options. Effort-related choice impairments are induced through injections of the vesicular monoamine transporter type-2 inhibitor tetrabenazine (TBZ), seen as a reduction in high effort behavior (lever pressing) and an increase in low effort (chow intake) behavior in the fixed ratio 5 (FR5)/chow feeding choice task (Nunes et al., 2013; Randall et al., 2014; Yohn et al., 2015a,b). The administration of (*S*)-CE-123 showed a significant, but partial reversal of the effects of TBZ (Rotolo et al., 2019), signifying a potential to improve motivational function in depressed humans. Several other highly selective atypical DA uptake inhibitors are also being assessed for their effects on effort-based decision making in rodents to model motivational symptoms seen in humans with MDD (Rotolo et al., 2020, manuscript in prep). It is important to continue investigations into this family of compounds to determine which is the most effective at reversing effort-related impairments and increasing extracellular DA without an abuse potential.

In this present study, a recently synthesized novel atypical DAT inhibitor modafinil analog, CE-158, is being investigated for its ability to increase extracellular DA levels within the nucleus accumbens in rats. This experiment will use the process of microdialysis and high-performance liquid chromatography with electrochemical detection to determine the changes in

extracellular DA levels at various time points after systemic administration of CE-158. These findings, in conjunction with behavioral testing being conducted in parallel, will determine whether CE-158 could be used as a suitable treatment option for effort-related motivational dysfunction in humans suffering from MDD.

Methods

Subjects

Adult male, drug-naïve, Sprague Dawley rats (n = 9, weight 279-299 g upon arrival) were housed in a colony that was kept at 23$_{o}$C and 12-hour light/dark cycles (lights on at 07:00 hours). The rats were housed in pairs presurgical procedures, and individually housed postsurgical procedures. Rats were provided standard laboratory chow and water *ad libitum* in their home cages. Animal protocols were approved by the University of Connecticut Institutional Animal Care and Use Committee, and were in accordance with National Institutes of Health guidelines.

Pharmacological agents and selection of doses

CE-158 was obtained from the Lubec Laboratory (University of Vienna, Austria) and dissolved in dimethyl sulfoxide (DMSO), Tween 80, and 0.9% saline. The DMSO/Tween 80/saline solution was administered as the vehicle control. The dose of CE-158 used in the microdialysis experiment was selected based on extensive pilot studies and information about its relative affinity for DAT. Recent behavioral pharmacology experiments revealed that a dose of 8.0 mg/kg CE-158 significantly reversed the effort-related motivational impairments caused by 1.0 mg/kg tetrabenazine on a fixed ratio 5 feeding choice task, and increased lever pressing and decreased chow consumption on a progressive ratio feeding choice task when administered alone (Rotolo et al. 2020, manuscript in prep).

In Vivo Microdialysis

Surgery. Adult male, drug-naïve, Sprague Dawley rats were anesthetized with intraperitoneal (IP) injections of 100 mg/mL ketamine and 10.0 mg/mL xylazine. Rats were placed in a stereotaxic apparatus (incisor bar 5.0 mm above interaural line), and a guide cannula (Bioanalytical Systems) was unilaterally implanted. In accordance with the rat brain atlas of Paxinos and Watson (1998), the tip of the guide cannula was implanted 2.0 mm dorsal to the accumbens core (anterior/posterior: +2.8 mm, medial/lateral ±1.8 mm, dorsal/ventral: -6.8 mm from bregma). The rats were counterbalanced by four implanted with the guide cannula on the left and five implanted on the right. The guide cannulae were secured to the skulls with three stainless steel screws and cement. A stainless-steel stylet was also inserted through the guide cannula to insure integrity. Following surgery, rats were individually housed and allowed a 7-day postsurgical recovery period.

Microdialysis and HPLC. The day before the samples were collected, the cannula implanted rats were habituated in Plexiglass chambers for 8 hours. On the sampling day, dialysis probes (Bioanalytical Systems; 2.0 mm active surface) that were connected to infusion pumps were inserted through the cannulas. An artificial cerebrospinal fluid (aCSF; 147.2 mm NaCl, 2.4 mm $CaCl_2$, 4.0 mm KCl) was pumped through the system at a rate of 2.0 µL/min by a syringe pump, and samples collected every 30 minutes. The samples were collected in microcentrifuge tubes that contained 2.0 µL of ascorbic acid and sodium metabisulfite to prevent oxidation of DA. Samples were collected starting 1 hour after the initial probe insertion. Up to 7 samples were used to establish a stable DA level. The last three of those baseline samples were used as the statistical baseline. The additional samples were collected after rats received an IP injection of either vehicle or 8.0 mg/kg CE-158. Samples were either frozen and analyzed the following

day, or immediately analyzed for DA content using reverse-phase high performance liquid chromatography with electrochemical detection. The electrochemical parameters were: channel 1 = –100 mV, channel 2 = +200 mV, and guard cell = +350 mV. The mobile phase contained 27.5 g sodium phosphate monobasic, 7.0% methanol, 750 μL of 0.1 m EDTA, and 2200 μL of 0.4 m sodium octyl sulfate dissolved in deionized ultrapure H2O with a final pH of 4.5 per liter. The flow rate was 1.0 mL/min. After sampling, the probe was removed and placement was verified through histological analysis.

Histology. After completion of microdialysis experiments, each rat was anesthetized with CO2, perfused intracardially with physiological saline, and then with a 3.7% formaldehyde solution. The brains of the rats were removed and stored in formaldehyde. A vibratome was used to slice 60.0 μm sections that were then mounted on glass microscope slides. Microscopic observations of the cresyl violet stained slides were performed to verify correct placement of the probe. Any rat with an improper placement or significant damage to the injection site was excluded from the analysis.

Statistical Analysis

Changes in extracellular DA levels were calculated as the percent change from baseline, with the mean of the three samples immediately preceding the drug injections serving as the 100% baseline level. A 2 × 7 factorial ANOVA with the treatment (drug vs. vehicle) factor being between groups, and the sample factor (samples collected after drug injection) being repeated measures, was used to test for post-injection differences in extracellular levels of DA. The raw DA levels of the baseline samples were analyzed using t test to verify that the baseline DA levels were not different between conditions. Nonorthogonal planned comparisons were performed

using the error term from the between-subjects analysis to assess differences between the two treatments at each particular sample.

Results

Extracellular DA levels in the nucleus accumbens were significantly increased after administration of CE-158, as seen in **Figure 2.** Factorial ANOVA revealed that there was a significant overall difference between treatment groups (vehicle control vs. CE-158) across the seven samples [$F(1,7)=703.162$, $p<0.001$]. Additionally, the factorial ANOVA with repeated measures on the sample factor revealed a significant overall difference across samples [$F(6,42)=3.339$, $p<0.01$], and a significant sample x treatment interaction [$F(6,42)=3.011$, $p<0.05$]. There also was a significant quadratic trend for the sample x treatment interaction

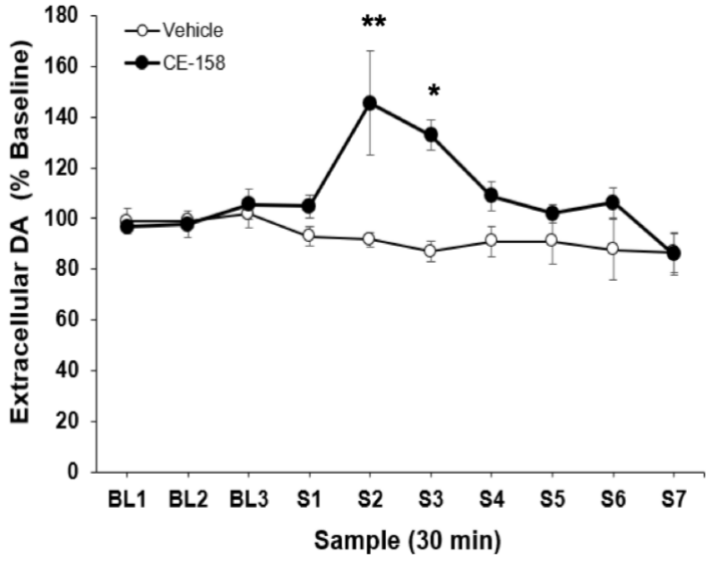

Figure 2. Effect of vehicle or CE-158 on extracellular DA levels in the nucleus accumbens. Mean (± SEM) extracellular DA (expressed percent baseline) measured by microdialysis in 30-minute intervals. Three baseline (BL) samples collected before injection, and seven samples (S1-7) collected post injection of vehicle or 8.0 mg/kg CE-158. **Significant difference from vehicle at S2, $p < 0.01$. *Significant difference from vehicle at S3, $p < 0.05$.

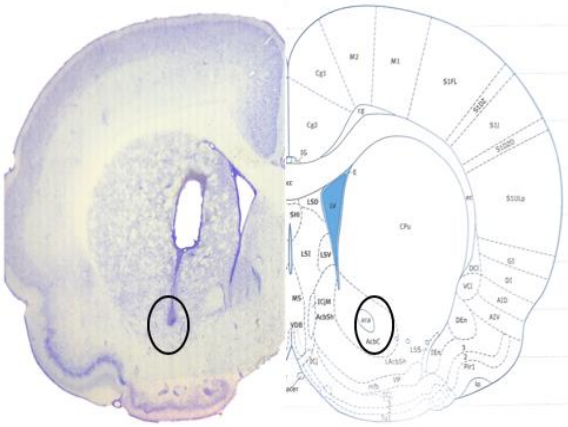

Figure 3. Histology slide (left) displaying the microdialysis probe placement within the nucleus accumbens in comparison to the atlas drawing (right) from Paxinos and Watson (1998).

[F(1,7)=912.422, p<0.01]. Nonorthogonal planned comparisons was used to assess the difference between the vehicle and CE-158 treatments. There revealed to be a significant difference at sample 2 (S2) [F(1,7)=14.376, p<0.01] and sample 3 (S3) [F(1,7)=10.509, p<0.05]. **Figure 3** depicts the histology slide and atlas drawing of a representative microdialysis probe placement in the nucleus accumbens core.

Discussion

This experiment assessed the effects of the novel atypical DAT inhibitor CE-158 on extracellular DA levels in the nucleus accumbens. These studies were undertaken to provide a neurochemical profile of this experimental drug. Injections of CE-158 resulted in a significant increase in the extracellular DA in the nucleus accumbens as measured by microdialysis. In comparison to the vehicle injection, the significant increases occurred in sample 2 collected 30-60 minutes after the injection of CE-158, as well as in the 60-90-minute post-injection sample 3 (**Figure 2**). Specifically, the largest difference between the CE-158 treatment group and the vehicle group was detected at sample 2. Additionally, the treatment group by sample interaction revealed an overall quadratic trend in the sample data. Through analysis of this relationship between the drug treatment group and vehicle group, it can be determined that it is the effects of the drug CE-158 that induces a peak in extracellular DA over a certain time span before returning back to baseline DA levels. These results expand on the information about the pharmacological profile and characteristics of CE-158. The importance of the increase of DA within the nucleus accumbens in the present study is that previous work has discovered this area of the striatal complex that is most critical for regulating effort-based choice, which is a behavioral model that is being used to study human motivational dysfunction (Salamone et al., 2006; Salamone et al., 2016a,b,c; Salamone et al., 2018).

Behavioral experiments on the effects of CE-158 were conducted in conjunction with the current microdialysis study to provide meaningful insight into the presented results. One of the experiments performed by Rotolo et al. (2020, manuscript in prep) was designed to determine the ability of CE-158 to reverse the effort-related effects of TBZ by measuring effort-based choice in an animal model. That experiment trained rats on a FR5/chow feeding choice task that when treated with TBZ shifted the rodents' behaviors from the high-effort option of lever pressing to the low-effort option of chow intake (Rotolo et al., 2020, manuscript in prep), which is consistent with previous studies in modeling motivational symptoms in animal models (Nunes et al., 2013; Randall et al., 2014; Yohn et al., 2015a; Yohn et al., 2016b,c). It was then determined that the co-administration of TBZ with 8.0 mg/kg CE-158 produced a significant reversal of the effect of TBZ, represented by the increased lever pressing and decreased chow intake (Rotolo et al., 2020, manuscript in prep).

In an additional experiment, CE-158 was administered to rats trained on the progressive ratio (PROG)/chow feeding choice task in the absence of TBZ in order to assess the effects of CE-158 on the performance of effort-based choice without any pharmacologically induced impairment (Rotolo et al., 2020, manuscript in prep). The PROG schedule provides a stringent work requirement, because the number of lever presses required for receiving reinforcement gradually increases throughout the session. Through the use of this task, the ability of drugs to enhance the selection of high-effort PROG lever pressing is assessed (Randall et al., 2012; Randall et al., 2015). It was determined that treatment with CE-158 at a 4.0 mg/kg dose and an 8.0 mg/kg dose significantly increased lever presses, as well as significantly decreasing chow intake compared to the vehicle (Rotolo et al., 2020, manuscript in prep). In comparison, the NET inhibitors desipramine and atomoxetine, and the SERT inhibitor fluoxetine do not increase

PROG lever pressing (Yohn et al., 2016d), while all the following DAT inhibitors increase selection of PROG responding: bupropion, lisdexamfetamine, PRX-14040, MRZ-9547, and GBR12909 (Sommer et al., 2014; Randall et al., 2015; Yohn et al., 2016b,c,d). Through evaluation of these additional studies, it can be determined that an effective dose of CE-158 is 8.0 mg/kg, as shown in both the TBZ reversal and PROG/chow feeding choice studies (Rotolo et al., 2020, manuscript in prep). The results of these experiments initiated the investigation of 8.0 mg/kg CE-158 in the current microdialysis study (Rotolo et al., 2020, manuscript in prep).

The current study determined that 8.0 mg/kg CE-158 also has the ability to significantly increase the extracellular DA in the nucleus accumbens. This increase in DA in the present experiment was well aligned with the time course of the CE-158 in the behavioral experiments. In those experiments, the drug was injected 30 minutes prior to run time, thus showing the behavioral TBZ reversal effects during the same 30-60-minute time span as the extracellular DA peak seen in the microdialysis (Rotolo et al., 2020, manuscript in prep). The relationship between the increased extracellular DA and the behavioral studies illustrates that this novel atypical DAT inhibitor modafinil analog has the potential to be a suitable treatment option for motivational dysfunction. Previous studies have shown evidence of some DAT inhibitors having pro-motivational effects in animal models (Nunes et al., 2013; Randall et al., 2015; Sommer et al., 2014; Yohn et al., 2016a,b,c,d). However, there is a large variety of drugs with varying characteristics that fall into this DAT inhibitor category, including cocaine and d-amphetamine. With this heterogeneity among the DAT inhibitor drug class comes limits on which are suitable treatment options for motivational dysfunction. A main concern is that most classical DAT inhibitors, for example cocaine, have a high abuse liability (Todtenkopf & Carlezon, 2006; Ostlund et al., 2014; Dong et al., 2017). Drugs with high abuse liability have limited therapeutic

utility in psychiatry for treating motivational dysfunction, however not all DAT inhibitors have the same pharmacological profile and abuse liability. Recently, there has been investigation into atypical DAT inhibitors, that differ from cocaine, through development of drugs that bind to alternative functional configurations of the DAT.

CE-158 is a modafinil analog that binds to one of the atypical configurations of the DAT (Schmitt & Reith, 2011; Cao et al., 2016). The interest in the development of a modafinil analog comes from its beneficial pharmacological profile. Modafinil is a DAT inhibitor that increases extracellular DA over a long period of time (Mereu et al., 2017), while also having a relatively low abuse liability (Mereu et al., 2013; Müller et al., 2013). In regards to its effects on motivational dysfunction, there has been evidence that modafinil has pro-motivational effects; this drug has been seen to improve fatigue symptoms in patients suffering from depression (Lam et al., 2007). Additionally, modafinil has shown to reverse the TBZ induced low-effort bias in rats (Salamone et al., 2016a; Yohn et al., 2016c). Since CE-158 is an analog of modafinil, there is the possibility that this drug will maintain similar beneficial characteristics, or even to a better capacity.

Results from a recent study on the effects of (*S*)-CE-123 were used as a foundation for the current study, as each compound is being investigated for the potential to serve as a suitable DA uptake inhibitor treatment for motivational dysfunction (Rotolo et al., 2019). (*S*)-CE-123 provides a comparison of modafinil analogs for the current study on CE-158 to be used for further development of atypical DAT inhibitors. In the present study, injection of CE-158 exhibited a significant increase of extracellular DA in the nucleus accumbens from baseline of approximately 145% at sample 2 (30-60 minutes post-injection) and 135% at sample 3 (60-90 minutes post-injection), followed by insignificant variance between the treatment group and

vehicle for the remaining samples (**Figure 2**). (*S*)-CE-123, however, showed a significant increase of extracellular DA from baseline of approximately 190% at sample 2 (20-40 minutes post-injection), 220% at sample 3 (40-60 minutes post-injection), and then hovers near a 180% significant increase from baseline for samples 4-9 (60-180 minutes post-injection) (Rotolo et al., 2019). An initial comparison would reveal that (*S*)-CE-123 shows a larger and more prolonged significant increase in extracellular DA than CE-158. A similarity, though, between the two drugs is that both CE-158 and (*S*)-CE-123 experience maximum DA levels at the 30-60-minute post injection time span. Something to consider, however, is the effective dose determined for (*S*)-CE-123 was 24.0 mg/kg (Rotolo et al., 2019), while CE-158 has a much lower effective dose of 8.0 mg/kg. Although (*S*)-CE-123 had a larger effective dose, CE-158 actually reverses the effects of TBZ in the behavioral experiments to much greater magnitude. (*S*)-CE-123 showed a partial reversal of TBZ-induced changes in performance with an approximate 45% restoration of responding in lever presses (Rotolo et al., 2019), while CE-158 had an approximate 80% restoration of response (Rotolo et al., 2020, manuscript in prep). This indicates that CE-158 has a higher efficacy for reversing the effects of TBZ compared to (*S*)-CE-123. While the microdialysis may not be the same for these two modafinil analogs, the combination of the behavioral and neurochemical results suggests the overall greater potency of CE-158 than (*S*)-CE-123. The relative affinity for DAT of CE-158 will need to be investigated further because affinity is potentially related to these differences in potency. Drugs, including d-amphetamine, methylphenidate, PRX-14040, and GBR12909, that have shown a high potency for reversing TBZ induced effects, have also had a high affinity for DAT (Salamone et al., 2016a; Yohn et al., 2016a,b,c). While in comparison, relatively low affinity to DAT drugs, such as bupropion,

modafinil, and (*S*)-CE-123, have shown a lower magnitude of reversal of the effects of TBZ (Nunes et al., 2013; Salamone et al., 2017; Rotolo et al., 2019).

Conclusion

Additional studies should further develop and examine atypical DAT inhibitors in order to investigate the neurochemical characteristics from a larger group of these compounds. Future studies should compare DAT affinity, selectivity and binding locus, dynamics of effects on extracellular DA, and effort-related behavioral effects of these atypical DAT inhibitors. It is essential for further studies to also explore the abuse liability for modafinil analogs such as CE-158, as well as other atypical DAT blockers, because although classic DAT inhibitors have shown the possibility to improve motivational function in depressed patients (Stotz et al., 1999), many, like amphetamine, have a high abuse liability. The recent interest in atypical DAT inhibitors is critical due to these drugs showing signs of improving motivational function in depressed people (Lam et al., 2007), but with much lower abuse liability. Through the present study discovering the effects CE-158 has on the extracellular DA in the nucleus accumbens, and recent developments on its effort-related behavior effects, it is reasonable to suggest similar novel modafinil analogs may ultimately be used as a suitable treatment option for effort-related motivational dysfunction in humans suffering from MDD.

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