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Varenicline and GZ-793A Differentially Decrease Methamphetamine Self-Administration under a Multiple Schedule of Reinforcement in Rats

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Abstract

Methamphetamine is a potent psychostimulant with high abuse rates. Currently, there is no FDA-approved pharmacotherapy for methamphetamine addiction. Ideally, a pharmacotherapy should selectively decrease methamphetamine self-administration without affecting responding for other reinforcers. One way to test this is with the use of a multiple schedule of reinforcement, in which drug and food are available in alternating components within a session. The present study evaluated GZ-793A, a vesicular monoamine transporter-2 (VMAT2) inhibitor, and varenicline, a partial agonist at $\alpha 4\beta 2$ and full agonist at $\alpha 7$ nicotinic acetylcholine receptors (nAChRs), for their ability to decrease methamphetamine and food self-administration using a multiple schedule of reinforcement. Male Sprague-Dawley rats self-administered i.v. methamphetamine (0.03 mg/kg/infusion) and food pellets under a multiple schedule of reinforcement. GZ-793A or varenicline was administered prior to multiple schedule sessions. GZ-793A (5 and 20 mg/kg) significantly decreased methamphetamine intake compared to saline, and did not alter food-maintained responding. In contrast, varenicline decreased methamphetamine intake less specific across time. The results suggest that VMAT2 inhibition may be a viable pharmacological target for the treatment of methamphetamine use disorders.

Keywords

rats; methamphetamine; multiple schedule; self-administration; GZ-793A; varenicline; rat

Introduction

Methamphetamine is a potent psychostimulant with high abuse potential. There is severe risk associated with repeated use of methamphetamine, as it can cause cardiovascular

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problems, hyperthermia, convulsions, anxiety, emotional and cognitive problems, psychotic symptoms, addiction, and even death (National Institute on Drug Abuse, 2013). In 2011, recreational methamphetamine use resulted in over 100,000 emergency room visits (National Institute on Drug Abuse, 2013). According to the National Survey on Drug Use and Health (NSDUH) by SAMHSA, in 2015, a large number (~897,000, or 0.4%) of Americans age 12 and older reported using methamphetamine within the past month (Center for Behavioral Health Statistics and Quality, 2015). Unlike heroin and nicotine addiction, there are currently no FDA-approved pharmacological treatments for methamphetamine addiction (Ballester et al., 2017).

Two compounds showing promise as potential pharmacotherapeutic candidates for methamphetamine use disorders include *R-N*-(2,3-dihydroxypropyl)-2, 6-*cis*-di-(4-methoxyphenylethyl) piperidine hydrochloride (GZ-793A; (Meyer et al., 2013)) and varenicline (Verrico et al., 2014). GZ-793A, a lobelane analog and vesicular monoamine transporter-2 (VMAT2) inhibitor, reduces the ability of methamphetamine to release dopamine (DA) from the presynaptic terminal into the extracellular space (Meyer et al., 2013). Typically, VMAT2 functions to actively transport leaked DA back into the vesicle (Eisenhofer et al., 2004a, 2004b). However, with methamphetamine on board, increased DA binding occurs inside the transporter; DA is then moved to the outside of the transporter and released into the cytosol (Horton et al., 2013). Methamphetamine inhibits monoamine oxidase, which prevents metabolism of this cytosolic DA, and reverses the dopamine transporter (DAT), thus releasing cytosolic DA into the extracellular space. Additionally, methamphetamine increases synaptic DA by inhibiting cytosolic DA uptake into the vesicles at VMAT2, and by up-regulating tyrosine hydroxylase (TH) which increases DA synthesis (Sulzer et al., 1995; Sulzer et al., 2005).

GZ-793A inhibits DA uptake at VMAT2 (Meyer et al., 2013) and inhibits methamphetamine-induced DA release at the vesicle through activity at the extravesicular DA uptake and intravesicular DA release sites on VMAT2 (Horton et al., 2013; Nickell et al., 2017), effects which augment end-product inhibition of TH. GZ-793A does not inhibit nicotine- or field-stimulation-evoked DA release, suggesting selectivity for VMAT2 (Nickell et al., 2017). GZ-793A may also indirectly regulate TH activity by reducing the methamphetamine-induced increase in tissue DOPA (Meyer et al., 2013). Previous results show that GZ-793A pretreatment decreases methamphetamine self-administration in rats (Meyer et al., 2013). Further, GZ-793A decreases methamphetamine self-administration following oral pretreatment in rats, the preferred therapeutic route of administration in humans (Wilmouth et al., 2013). Additionally, GZ-793A decreases methamphetamine reinstatement in rats following extinction (Alvers et al., 2012), suggesting therapeutic benefit with respect to reducing methamphetamine seeking and relapse.

Similar to VMAT2, nicotinic acetylcholine receptors (nAChRs) also provide a novel therapeutic target for attenuating methamphetamine self-administration. Research suggests that the nicotinic acetylcholinergic system is involved in the pharmacological effects of methamphetamine (Hiranita et al., 2004, 2006), perhaps specifically at $\alpha 3\beta 4$ (Glick et al., 2008) and $\alpha 7$ nAChRs (Escubedo et al., 2005). Additionally, evidence shows that $\alpha 4\beta 2$ nAChR agonists produce methamphetamine discriminative stimulus-like effects in rats

(Desai & Bergman, 2010, 2014). Unlike GZ-793A, which exhibits minimal activity at nAChRs (Meyer et al., 2013; Nickell et al., 2017), varenicline, an FDA-approved tobacco smoking cessation agent, acts as a partial agonist at $\alpha 4\beta 2$ and full agonist at $\alpha 7$ nAChRs (Mihalak et al., 2006). Varenicline is safe in humans who self-administer methamphetamine (Zorick et al., 2009). It also reduces the positive subjective effects of smoked methamphetamine (Verrico et al., 2014) and improves reaction time in methamphetamine-dependent individuals (Kalechstein et al., 2014). A recent study showed that varenicline produced a small but significant decrease in responding for methamphetamine and saline in female rats (Pittenger et al., 2016), but not male rats (Pittenger et al., 2017), suggesting that varenicline may have potential as a pharmacotherapeutic for methamphetamine. However, these results indicated that varenicline had non-specific effects on responding, and selectivity of varenicline on behavior maintained by an alternative non-drug natural reinforcer was not determined. Because of the paucity of research examining varenicline as a pretreatment to reduce methamphetamine self-administration, in conjunction with research support for the action of nAChRs in the effects of methamphetamine, varenicline was selected as an additional potential pharmacotherapy for the present study.

One step in developing pharmacotherapies for substance use disorders is testing potential candidate compounds using a rodent self-administration paradigm. Such pharmacotherapeutic candidates should decrease specifically drug intake without affecting responding for a non-drug reinforcers, such as food (Grabowski et al., 2004). The inability of a pharmacotherapy to specifically decrease drug intake, while altering behavior maintained by other reinforcers, may indicate adverse side effects that would limit clinical effectiveness (Mello & Negus, 1996). The selectivity of a potential pharmacotherapeutic candidate for methamphetamine use disorders is commonly tested using a between-groups design, with one group of animals self-administering methamphetamine and the other a non-drug reinforcer, such as food or sucrose pellets (Baladi, et al., 2014; Beckmann et al., 2012; Haney & Spealman, 2008; Mello & Negus, 1996; Reichel et al., 2009; Wilmouth et al., 2013). Between-groups designs in self-administration studies allow researchers a straightforward test for pharmacotherapy selectivity that facilitates faster and easier training for animals. However, a within-subjects design to test for selectivity may have greater internal validity and has the added statistical advantage of removing between-subject variability while simultaneously equating behavioral and drug histories in animals tested with the experimental compound.

One way to examine the selectivity of a potential pharmacotherapy in a within-subjects design is to use a multiple schedule of methamphetamine and food reinforcement. Under this schedule, rats alternate between periods of time where methamphetamine infusions or food pellets are available following completion of the schedule requirement. Previous research with monkeys demonstrated that a multiple schedule may be useful in examining the ability of various pretreatments to specifically decrease self-administration of either cocaine or methamphetamine, while not altering behavior maintained by a non-drug reinforcer (Kleven & Woolverton, 1993; Schindler et al., 2011; Woolverton & Virus, 1989). Previous studies using a multiple schedule of drug or sucrose reinforcement in rats found that self-administration of nicotine or cocaine was decreased specifically following pharmacological pretreatments (Caine & Koob, 1994, 1995; Stairs et al., 2010). The value of

using a within-subjects design in preclinical testing of potential pharmacotherapies for methamphetamine addiction is illustrated in preclinical studies examining buprenorphine for opioid use. In studies using alternating food and drug components in monkeys, buprenorphine selectively reduced speedball and heroin intake (Mello & Negus, 1998) and outperformed methadone at selectively decreasing opiate self-administration (Mello et al., 1983). Thus, while an approved medication for stimulant use disorders has not been identified, the use of schedules with alternating food and drug components may have predictive validity in development of clinically useful pharmacotherapies.

While previous studies have shown that GZ-793A is effective in reducing methamphetamine-induced DA release and in decreasing methamphetamine self-administration, and while initial studies with varenicline have been performed in humans using methamphetamine, experiments have not investigated the selectivity of GZ-793A and varenicline to decrease methamphetamine self-administration using a within-subjects design. The current study determined the selectivity of GZ-793A and varenicline in decreasing methamphetamine self-administration using a multiple schedule of methamphetamine and food self-administration.

Methods

Subjects

Sixteen male Sprague-Dawley rats (Harlan Industries; aka Envigo Inc., Indianapolis, IN, USA) were received on post-natal day (PND) 51 and were acclimated to their housing conditions for 7 days with free access to food and water. Rats were housed in clear plastic shoebox cages with shredded aspen bedding for the duration of the experiment and were maintained on a 12/12 hour light/dark cycle with lights on from 07:00 to 19:00 h. All protocols were approved by the Creighton University Animal Care and Use Committee and conformed to the NIH Guide for the Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources (U.S.), 2011).

Apparatus

Standard operant conditioning chambers (28 × 21 × 21 cm; ENV-001; MED Associates, St. Albans, VT) with alternating aluminum and Plexiglas walls including a metal rod floor were located inside sound-attenuating chambers (ENV-018M; MED Associates, St. Albans, VT). A recessed food tray (5 × 4.2 cm) was located 2 cm above the floor in the center of one of the aluminum walls, and a response lever was located 6 cm above the floor on each side of the food tray. A white stimulus light (28 v, 3 cm diameter) was located 6 cm above each lever. The standard operant chambers were retrofitted with a counterbalance arm, swivel, and leash (PHM-110, PHM-115I and PHM-120A, respectively; MED Associates, St. Albans, VT). Drug infusions were delivered via a MED Associates infusion pump located outside of the sound-attenuating chamber (PHM-100; MED Associates, St Albans, VT). Responses were recorded and programmed consequences were controlled by a computer in an adjacent room equipped with Med-PC software (Med Associates, St. Albans, VT).

Procedure

Food training—Following PND 58, the animals were weighed to determine their free-feeding body weights. Each rat's weight was then multiplied by .85 to determine the animal's 85% free-feeding weight. The animals then underwent 7 days of food restriction (given ~15–18 g per day) to bring their body weights down to 85% of their free-feeding weights. After restriction, the mean body weight of the rats was 232.5 g ($\pm$ 17.64). On the day prior to the start of food training, rats were exposed to 5 g of food pellets (45 mg pellets; BioServ, Frenchtown, NJ) to alleviate neophobia during training on the following day. During food training rats were placed in the operant chambers for daily one-hour sessions, with the houselight on and with only one of the two levers present in the chamber. The initially available lever was designated as the food lever, and its placement was counterbalanced across subjects. Responses on the food lever resulted in delivery of a food reinforcer. Reinforcement schedules were as follows: Fixed ratio (FR) 1 for 3 days, FR3 for 2 days, FR5 for 3 days, and FR5 with a 60-s timeout following food pellet delivery (FR5 + 60s TO) for 3 days. Following food training, rats were given free access to food for 7 days to regain body weights prior to surgery.

Catheterization Surgery—Following return to free-feeding body weight, all rats were implanted with an indwelling catheter in the jugular vein. Rats were anesthetized with ketamine (100 mg/kg, i.p.) and midazolam (5 mg/kg, i.p.). The silastic catheter (0.2 mm i.d.; Fisher Scientific) was threaded subcutaneously to exit from a piece of stainless-steel hypodermic tubing (22 ga) embedded in a dental acrylic head cap mounted with 4 stainless-steel jeweler's screws to the top of the skull. For 7 days following surgery, catheters were flushed daily with 0.1 mg/ml heparinized saline (0.25 ml/day) to maintain patency.

Methamphetamine Self-Administration—Following the 7-day recovery period after surgery, rats were fed ~18 g of rat chow per day to reduce weight and maintain 85% free-feed body weight for the remainder of the experiment. For acquisition of methamphetamine self-administration, rats were placed in the operant conditioning chambers with only the lever (drug lever) opposite the previously established food lever extended into the chamber for one-hour daily sessions. Responses on the drug lever resulted in a 0.03 mg/kg/infusion of methamphetamine delivered over 5.6 seconds. Cue lights were illuminated above the lever to signal the delivery of the methamphetamine infusion and the start of a 60-s timeout. The houselight was off during the drug-alone sessions. Regardless of the number of methamphetamine infusions earned, the reinforcement schedule increased in the following sequence: FR 1 for 3 days, FR3 for 2 days, FR5 for 3 days, and FR5 + 60s TO. This set schedule of increasing FR values was used to speed training, given concern for maintaining catheter patency through the duration of the entire experiment.

Multiple Schedule—After acquiring stable methamphetamine self-administration on the drug-alone sessions, which was defined as responding on the drug lever with no more than 20% variability across four consecutive sessions, the multiple schedule of reinforcement, in which both levers were present, was initiated. Multiple schedule sessions were 75 minutes in length with four 15-minute components, and a 5-minute timeout period between each component. Timeouts were signaled via continuous illumination of both cue lights, with

both levers remaining extended in the chamber. During components 1 and 3, the houselight was off and completion of the FR requirement on the drug lever yielded delivery of methamphetamine (0.03 mg/kg/infusion). The FR requirement remained at FR5 + 60s TO for both food and methamphetamine for the remainder of the experiment. Responses on the food lever were recorded, but had no programmed consequence. In components 2 and 4, the houselight was on and responses and completion of the FR requirement on the food lever resulted in delivery of a food pellet. Responses on the drug lever were recorded, but had no programmed consequence. During all components, a signaled (onset of cue lights above levers) 60-s timeout occurred following the delivery of either reinforcer. Responses on both levers during timeouts were recorded, but had no programmed consequence. Stable responding on the multiple schedule was defined using two criteria during the drug components (components 1 & 3): rats were required to self-administer at least 10 infusions of methamphetamine in the drug components for four consecutive sessions, and demonstration of lever discrimination during the drug component was required of at least a 2:1 ratio on the drug lever compared to the food lever. Individual subjects had to meet these stability criteria prior to initiating drug pretreatments.

GZ-793A and Varenicline Pretreatment—Once the above stability criteria were met, rats entered the pretreatment phase. In this phase, rats received twice-weekly pretreatments 15-minutes prior to the start of the daily multiple schedule session. Pretreatment times were selected to be comparable with those of previously published studies using rats (Beckmann et al., 2012; Alvers et al., 2011; Desai & Bergman 2010) and squirrel monkeys (Desai & Bergman, 2014). Pretreatments of varenicline (0, 0.3, 1, or 2 mg/kg s.c.; (Desai & Bergman, 2010; Steensland et al., 2007) and GZ-793A (0, 10, 15, or 30 mg/kg s.c.; (Beckmann et al., 2012)) were administered according to a Latin square design. The order of drug treatments was counterbalanced across subjects, such that half of the animals received GZ-793A first followed by varenicline, and the other half received varenicline first followed by GZ-793A. Two maintenance sessions occurred between pretreatment days to maintain stable responding for methamphetamine.

Drugs

Methamphetamine and varenicline were purchased from Sigma/RBI (St. Louis, MO) and dissolved in 0.9% w/v NaCl (saline) in a volume of 1 ml/kg. Methamphetamine was self-administered i.v., while varenicline was administered subcutaneously (s.c). GZ-793A was synthesized as previously described (Horton et al., 2011), dissolved in saline at 2 ml/kg volume, and administered s.c.

Statistical Analyses

Five animals lost catheter patency before completion of the study; their data were excluded from analyses. Given the differences in rates of responding maintained by methamphetamine and food reinforcers, data were analyzed as number of reinforcers as a percent change from the previous day (control session). To determine that order of drug pretreatment (i.e. GZ-793A administered first versus varenicline administered first) did not have an effect on how the drugs affected responding, repeated measures analyses of variance (ANOVAs) were performed on the percent change in the number of reinforcers earned according to whether

GZ-793A or varenicline was given first. These analyses revealed there was no effect of or interaction with pretreatment order, so the data were collapsed, ignoring pretreatment order, in subsequent statistical analyses. Given our a priori interest in the effects of the individual compounds on responding under the multiple schedule of methamphetamine and food, separate repeated-measures ANOVAs were conducted on both the percent change in the number of reinforcers earned and the overall response rates (resp/sec) for the effects of both GZ-793A and varenicline, with pretreatment dose, reinforcer type, and lever as within-subjects factors for both analyses. Post hoc comparisons were conducted using a Tukey's HSD test. For tests of significance, an alpha of 0.05 was used.

Results

Multiple Schedule Methamphetamine Acquisition

Of the eleven animals with patent catheters throughout the duration of the experiment, six acquired methamphetamine self-administration under a multiple schedule of reinforcement using our stability criteria. The average number of sessions on the multiple schedule required to reach stable methamphetamine self-administration was 21.7 ($\pm$ 5.74) sessions. A repeated-measures ANOVA on the level of responding during the session prior to the start of pretreatment revealed a significant interaction of reinforcer type $\times$ lever [$F(1,6) = 20.973$, $p < 0.001$]. Post hoc analysis revealed that the animals exhibited discrimination, in that, when methamphetamine was available, responding was significantly higher on the active methamphetamine lever than the inactive food lever. Also, responses on the active methamphetamine lever during the methamphetamine components were significantly higher than responses on the methamphetamine lever during the food components (Figure 1).

GZ-793A

A repeated-measures ANOVA of the effect for GZ-793A on the percent change in the number of reinforcers from control sessions revealed a significant reinforcer $\times$ dose interaction [$F(3,15) = 3.49$, $p < 0.05$]. Post hoc comparisons revealed that across all three doses tested, GZ-793A decreased methamphetamine responding compared to food-maintained responding. Additionally, the 5 and 20 mg/kg doses of GZ-793A significantly decreased methamphetamine intake compared to saline control injections (Figure 2A).

To evaluate GZ-793A effects across the session, the session was divided into the first and second methamphetamine and food components. A repeated-measures ANOVA on the first methamphetamine and food components revealed only a significant main effect of reinforcer [$F(1,5) = 17.27$, $p < 0.01$]. Post hoc analysis revealed that the 10 and 20 mg/kg dose of GZ-793A decreased methamphetamine intake compared to food intake (Figure 2B). A repeated-measures ANOVA on the second methamphetamine and food components revealed a significant dose $\times$ reinforcer interaction [$F(3,15) = 5.09$, $p < 0.05$]. Post hoc comparisons indicated that all three active doses of GZ-793A decreased methamphetamine intake compared to food intake and decreased methamphetamine intake relative to the saline control injection (Figure 2C). A repeated-measures ANOVA on the effects of GZ-793A on inactive lever responding across the entire session revealed no significant effects in either the methamphetamine or food components (Figure 2D).

Given that the transformed data displayed in Figures 2 can lead to difficulty in interpretation, the effects of GZ-793A on overall response rates are displayed in Figures 3A & B. A repeated-measures ANOVA found a significant GZ-793A dose $\times$ reinforcer interaction [$F(4, 20) = 3.81, p = 0.01$]. Post hoc comparisons revealed that 5 and 20 mg/kg doses significantly decreased responding compared to saline control injections and there was no effect of rates of responding maintained by food.

Varenicline

A repeated-measures ANOVA of the effect of varenicline over the total session revealed only significant main effects of dose [$F(3,18) = 3.29, p = 0.05$] and reinforcer [$F(3,6) = 7.75, p = 0.05$] but no significant interactions. The post hoc tests of dose revealed that the highest dose of varenicline had a decreasing effect compared to the 0.3 mg/kg dose but was not significantly different from saline control injections. A repeated-measures ANOVA on the first methamphetamine and food components revealed a main effect of dose [$F(3,18) = 4.24, p = 0.01$]; this appears to be attributed to only the 2.0 mg/kg dose decreasing methamphetamine intake compared to saline, although a Tukey's post hoc test did not indicate a significant difference between the 2.0 mg/kg dose and saline (Figure 4B). A repeated-measures ANOVA on the second methamphetamine and food components revealed a significant main effect of reinforcer [$F(1,5) = 26.74, p < 0.01$]. Post hoc comparisons indicated that the highest dose of varenicline (2.0 mg/kg) decreased methamphetamine intake compared to food intake. Also, the 2.0 mg/kg dose of varenicline significantly decreased methamphetamine intake relative to the saline control injection (Figure 4C). A repeated-measures ANOVA on the effects of varenicline on inactive lever responding across the entire session revealed no significant effects in either the methamphetamine or food components (Figure 4D).

A repeated-measures ANOVA on the effects of varenicline on overall response rates revealed significant main effects of dose [$F(4, 20) = 5.29, p = 0.01$] and reinforcer [$F(1, 5) = 5551.8, p = 0.001$] but no significant interaction. Post hoc comparisons revealed that the 2.0 mg/kg dose of varenicline significantly decreased response rates compared to control and saline injections in the food components (Figure 5A&B).

Discussion

The present results demonstrate that methamphetamine self-administration was maintained using the current multiple schedule of i.v. methamphetamine and food reinforcement. Once responding for methamphetamine or food was stable, pretreatment with the VMAT2 inhibitor GZ-793A specifically decreased methamphetamine intake without affecting food-maintained behavior. The efficacy of GZ-793A to decrease responding for methamphetamine was consistent across both the first and second methamphetamine components of the schedule for both the percent change dose effect curves and the overall response rate curves (data not shown). In contrast, the effect of varenicline on methamphetamine intake was not as selective for methamphetamine or consistent across time: there was a significant main effect of varenicline dose but no doses significantly decreased methamphetamine intake compared to saline across the entire session for both the

percent change and overall response rate measures. The highest varenicline dose (2.0 mg/kg) disrupted food-maintained behavior, particularly in the first food component and in the overall response rate measure for the entire session, which may indicate a lack of selectivity for methamphetamine at that dose. Finally, the highest dose of varenicline significantly decreased methamphetamine intake during the second methamphetamine component of the session using the percent change measure, although this effect was not replicated in the overall response rate data (data not shown).

The current study, demonstrating methamphetamine self-administration under a multiple schedule of reinforcement, extends previous findings that nicotine and cocaine maintain responding under the identical multiple schedule of reinforcement (Stairs et al., 2010). Furthermore, GZ-793A decreased responding for methamphetamine without altering responding for food, consistent with previous literature, which shows that with a similar pretreatment time to the current study, moderate to high doses (10–30 mg/kg; s.c., 20 min) of GZ-793A effectively reduced methamphetamine self-administration (Beckmann et al., 2012). Although the current study did not find a significant effect across the entire session of GZ-793A at 10 mg/kg, the 5 and 20 mg/kg doses decreased methamphetamine intake. Similarly, oral administration of GZ-793A decreased methamphetamine self-administration, demonstrating that the compound has oral bioavailability (Wilmouth et al., 2013). Interestingly, previous studies have found that GZ-793A either did not affect (Wilmouth et al., 2013) or, after consecutive sessions, even increased (Beckmann et al., 2012) food self-administration. However, both Wilmouth et al. (2013) and Beckmann et al. (2012) used between-groups designs, and thus, the present study extends those findings by using a within-groups design. Taken together with the previous results, the current results indicate that GZ-793A is effective at specifically decreasing methamphetamine self-administration without simultaneously altering non-drug reinforced behavior.

Recently, varenicline has been reported to decrease the positive subjective effects of methamphetamine in human subjects (Verrico et al., 2014), consistent with the current preclinical findings. In a preclinical study varenicline has been shown to produce about a 10% decrease in responding for methamphetamine; however, responding maintained by saline was also decreased, indicating a nonspecific effect (Pittenger et al., 2016). Pittenger and colleagues later found no effect of varenicline on responding for methamphetamine in male rats, and that methamphetamine-primed reinstatement responding increased with 0.3 and 1.0 mg/kg doses of varenicline (Pittenger et al., 2017). In the current study, the high dose (2.0 mg/kg) of varenicline resulted in a ~50% decrease in methamphetamine intake and a ~20% decrease in responding for food across the entire session. However, neither of these effects was statistically significant compared to saline. Varenicline (0.03–3.0 mg/kg) did not attenuate the discriminative stimulus effects of methamphetamine in methamphetamine-trained rats; however, at doses ranging from 0.01–3.0 mg/kg, varenicline partially substituted for methamphetamine when administered 20 minutes before the start of the session (Desai & Bergman, 2010). Despite knowledge of the specific site of action of varenicline, it is difficult to speculate on the mechanism by which varenicline alters the effects of methamphetamine.

Findings from the literature indicate that nAChRs mediate part of the pharmacological effects of methamphetamine (Desai & Bergman, 2010; Glick et al., 2008; Hiranita et al.,

2004). Although varenicline has a low abuse liability, its actions as a partial agonist at $\alpha 4\beta 2$ and as a full agonist at $\alpha 7$ nAChRs on dopaminergic, glutamatergic and GABAergic neurons in the ventral tegmental area lead to DA release in nucleus accumbens (Polosa & Benowitz, 2011). In humans, varenicline has demonstrated utility as a pharmacotherapy for nicotine use disorders (Gonzales et al., 2006; Mihalak et al., 2006) and has been evaluated with mixed results as a treatment for alcohol use disorders (Erwin & Slaton, 2014; McKee et al., 2009; Plebani et al., 2013). Varenicline has also been tested for as a potential treatment for cocaine use disorders in humans, with some positive effects (Plebani et al., 2012). These clinical results are congruent with some of the preclinical animal models testing the effects of varenicline on cocaine-related behaviors (Gould et al., 2011; Guillem & Peoples, 2010; Mello et al., 2014). While varenicline appears promising as a pharmacotherapeutic approach for cocaine use, it appears that varenicline may not be an effective pharmacotherapy for methamphetamine use given its limited effectiveness in preclinical animal models of methamphetamine-related behaviors (Pittenger et al., 2016, 2017; current results).

A promising mechanistic approach to the discovery of an efficacious pharmacotherapy for methamphetamine use disorders may be by direct alteration of dopaminergic presynaptic function. Methamphetamine increases extracellular DA via multiple mechanisms, including vesicular release, VMAT2 inhibition, TH upregulation, and reversal of DAT function (Meyer et al., 2013). GZ-793A inhibits methamphetamine-induced DA release from synaptic vesicles and DA uptake at VMAT2, and alone increases levels of cytosolic DA, increasing end-product inhibition of TH, all of which results in decreased extracellular DA concentrations (Dwoskin & Crooks, 2002; Horton et al., 2011, 2013; Meyer et al., 2013). VMAT2 inhibitors such as GZ-793A appear to have a low abuse liability as rats do not self-administer GZ-793A alone, nor does GZ-793A produce conditioned place preference (Beckmann et al., 2012). Given the low abuse liability and the current results showing the ability of GZ-793A to specifically decrease methamphetamine self-administration, VMAT2 inhibition may be a promising mechanism for the discovery of a methamphetamine pharmacotherapy.

While the current results suggest that VMAT2 may be a promising pharmacological target for methamphetamine use disorders, these results have limitations. First, five animals did not meet criteria for stable methamphetamine self-administration using the multiple schedule. This was usually due to the animals failing to self-administer a minimum of 10 infusions consistently across 4 consecutive sessions. These criteria were established on the basis of a previous study that used similar criterion for rats self-administering the same dose of methamphetamine during 60 min daily sessions (Beckmann, et al., 2012). In retrospect, these criteria may have been too conservative given that, in the current study, the rats only had access to methamphetamine for 30 minutes during the multiple schedule. Increasing the amount of time drug was available during the daily sessions would have likely increased the number of rats that meet our stability criteria.

A second limitation was that food-maintained responding was at a higher rate than methamphetamine-maintained responding, as previously reported using multiple schedules of drug and food reinforcement (Caine & Koob, 1994; Stairs et al., 2010). Given the differences in baseline rates of responding maintained by food and methamphetamine, it

could be argued that changes in behavior following varenicline and GZ-793A could be due to rate-dependent effects (Dews, 1958; McMillan, 1969; D.E. McMillan & Leander, 1976). However, a rate-dependent interpretation of the current data seems unlikely given that the low rates of responding maintained by both methamphetamine and the inactive lever did not increase following pretreatment of either GZ-793A or varenicline. The typical rate-dependent effect seen following drug pretreatments is an increase in low rates of behavior and decreases in high rates of behavior (Dews & Wenger, 1977; Perkins, 1999; Snider et al., 2016).

While the current data likely can rule out a rate-dependent effect of GZ-793A and varenicline, it is more difficult to rule out a behavioral economic explanation. Given the much lower rates of behavior and intake maintained by methamphetamine relative to food in the current multiple schedule, it is possible that responding maintained by methamphetamine is more easily disrupted (i.e. decreased) because methamphetamine has weaker essential value relative to food. Essential value of a reinforcer can be seen as a measure of the reinforcer's elasticity of demand (Christensen et al., 2008; Hursh & Silberberg, 2008); as the price of a reinforcer increases (i.e. increases in response requirements), consumption of the reinforcer decreases (Galuska et al., 2011; Hursh & Silberberg, 2008). Previous studies examining the essential value of stimulant drugs as reinforcers have found that cocaine and methamphetamine have lower essential value relative to food reinforcers, particularly when tested using a multiple schedule of reinforcement (Christensen et al., 2008; Galuska et al., 2011). Given that in the current study the ratio value (FR5) of the methamphetamine and food reinforcers were the same but food maintained a 3-times higher rate of response and reinforcers earned, it is likely that methamphetamine had a lower essential value than food. It is possible that GZ-793A and varenicline tended to decrease methamphetamine intake while not effecting food intake because methamphetamine has a lower essential value as a reinforcer and maintained a much lower response rate that is more easily disrupted by drug pretreatments, which would also fit with a behavioral momentum explanation of the data (Nevin & Grace, 2000). Future studies using the current multiple schedule may be able to better control for differences in reinforcer density by increasing the ratio value for the food or potentially by shortening the length of the components. Previous research by Ginsburg and Lamb (2014a,b) using a multiple schedule of oral ethanol and food found that reinforcer density between the two reinforcers could be equated by shortening the components to 5 minutes.

Finally, another limitation in the current study may be that use of a multiple schedule may overestimate the selectivity of a potential pharmacotherapy. Research by Ginsburg and Lamb (2014a,b) found that varenicline and other drugs showed selectivity in decreasing ethanol intake under a multiple schedule of ethanol and food, but this selectivity was decreased or reversed when ethanol and food were available concurrently. It is possible that the selectivity seen in GZ-793A may be due to the use of a multiple schedule, although previous research using group designs also showed selectivity. An alternative approach would be to use a concurrent schedule, which has been used successfully in rhesus monkeys (Banks, 2017; Banks & Blough, 2015; John et al., 2015) and in rats (Ping & Kruzich, 2008). However, caution should be exercised when using a concurrent schedule of methamphetamine and food reinforcement with rats, as previous research has found that when food and

methamphetamine were available concurrently, rats self-administered very low levels of methamphetamine and showed a strong preference for food reinforcers (Caprioli et al., 2015; Ping & Kruzich, 2008). A reduction in methamphetamine intake when food is concurrently available may be problematic when examining pharmacotherapies intended to decrease methamphetamine self-administration.

In summary, consistent with previous research, GZ-793A attenuated methamphetamine self-administration, without altering food-maintained responding, under a multiple schedule of reinforcement. Further, GZ-793A appears to be superior to varenicline in this regard. Overall, these results suggest that VMAT2 rather than nAChRs may be a more promising pharmacological target for the treatment of methamphetamine use disorders. Finally, future studies should attempt to better equate reinforcement densities of food and methamphetamine and/or use a reinforcement schedule in which methamphetamine and food are concurrently available.

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Figure 1.

Responses on methamphetamine and food levers in methamphetamine and food components of the multiple schedule sessions. Data are expressed as mean (+ SEM; N = 6) number of total responses on methamphetamine and food levers during each of the methamphetamine and food components. The numerical values above the bars are the average overall response rates (resp/sec) for the rats during those components. *Significant difference from food lever in methamphetamine components, $p < 0.05$. # Significant difference from methamphetamine lever in food components, $p < 0.05$.

Figure 2.

Effects of GZ-793A during the entire multiple schedule session (Panel A), the first methamphetamine and food components (Panel B), and the second methamphetamine and food components (Panel C). Panel D shows the effects of GZ-793A on responding on the inactive lever: note the change in scale relative to Panels A–C. Data are expressed as mean ($\pm$ SEM, N = 6) number of reinforcers (A–C) or inactive lever presses (D) as a percentage of performance during the control session prior to injection. * Significant difference from food-maintained responses. & Significant difference from saline.

Figure 3.

The effects of GZ-793A on the overall response rates (resp/sec) during methamphetamine components (Panel A) and during food components (Panel B). Data are means ($\pm$ SEM, $N = 6$). C = control session prior to pretreatments. S = Saline pretreatments. & indicates a significant difference from saline.

Figure 4.

Effects of varenicline during the entire session (Panel A), the first methamphetamine and food components (Panel B) and the second methamphetamine and food components (Panel C). Panel D shows the effects of varenicline on responding on the inactive lever: note the change in scale relative to Panels (A–C). Data are expressed as mean ($\pm$ SEM, $N = 6$) number of reinforcers (A–C) or inactive lever presses (D) as a percentage of performance during the control session prior to injection. * Significant difference from food-maintained responses. & Significant difference from saline.

Figure 5. The effects of varenicline on the overall response rates (resp/sec) during methamphetamine components (Panel A) and during food components (Panel B). Data are mean ($\pm$ SEM, $N = 6$). C = control session prior to pretreatments. S = Saline pretreatments. & indicates a significant difference from saline.