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Rivastigmine does not alter cocaine-induced subjective effects or self-administration

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ABSTRACT

Background: Acetylcholinergic (ACh) neurons interface with the mesolimbic dopamine pathway implicated in addiction, and acetylcholinesterase inhibitors (AChEis) have been shown to reduce the immediate effects of cocaine and amount used. Our study is the first to examine if the safe and low-interaction AChEi rivastigmine (riv) alters the subjective effects produced by cocaine administration.

Methods: Cocaine-dependent subjects were randomized to daily placebo, riv 3 mg, or riv 6 mg, administered inpatient for 10 days. On day 1 (pre-dose) and day 9, subjects received both IV cocaine 40 mg or placebo in a randomized order with subsequent serial assessments of visual analog scale (VAS) subjective effects and pharmacokinetic measurements. On day 10 all participants received one baseline dose of cocaine 20 mg with assessment of subjective effects, and were then able to purchase additional doses at 15 min intervals with study earnings.

Results: 40 subjects were randomized to placebo (n = 16), riv 3 mg (n = 13), or riv 6 mg (n = 12). All subjects completed the study and there were no demographic differences between treatment groups. Pre- and post-treatment, there were no significant pharmacokinetic differences (blood levels of cocaine, BE, EME) following cocaine administration. In a two-way ANOVA, IV cocaine significantly increased positive VAS category ratings compared to placebo, but rivastigmine treatment at either dose had no significant effect on any VAS category ratings. Similarly, there was no significant rivastigmine effect on any category in the day 10 cocaine administration, and no effect on number of subsequent doses participants purchased.

Conclusion: Rivastigmine 3 or 6 mg had no significant effect on the subjective effects of cocaine after 9 days of treatment. This is an important finding as other drugs in the AChEi class (donepezil, Huperzine A) have produced significant results, but differ in their receptor specificity and PK parameters.

1. Introduction

Cocaine is an addictive drug of abuse that is linked to long-term negative consequences including early cognitive decline, mental illness, multi-organ failure, and death (Antai-Otong, 2006; Ersche et al., 2013). In 2017, 0.8% of the U.S. population, about 2.2 million people, had used cocaine in the past month. Half of this group met criteria for cocaine use disorder, characterized by functional decline, risky use, and loss of control over drug use patterns (Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration, 2018). Given cocaine's detrimental effects on mental and physical health, it is of clear interest to evaluate therapies that could reduce its addictive potential. The addictive- and non-addictive learning and reward properties of cocaine arise from the dopaminergic neurons that project from the ventral tegmental area to the nucleus accumbens as well as other limbic targets along the mesolimbic pathway (Pierce and Kumaresan, 2006). Dopaminergic signaling is important in mediating cocaine's subjective effects, and modulation of dopamine pathways can reduce cocaine's salience (Pierce and Kumaresan, 2006). Studies of pharmacotherapeutic agents directly targeting dopamine for treatment of cocaine use disorder have been extensive and promising (Anderson et al., 2009; Kosten et al., 2013; Mooney et al., 2009; Oliveto et al., 2011; Verrico et al., 2013, p. 20). However, some of these treatment options are limited by their own addictive and abuse potential (Mooney et al., 2009), and those with less risk may be less efficacious (Anderson et al., 2009; Mooney et al., 2009).

Although the dopamine system is one potential treatment target, another possibility lies in the upstream cholinergic circuitry that synapses on dopamine neurons within the midbrain (Lester et al., 2010).

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In the reward pathway, dopamine and acetylcholine (ACh) are known to cross-regulate one another in a complicated and at times paradoxical manner that continues to be elucidated (Surmeier and Graybiel, 2012; Williams and Adinoff, 2008; Zhang et al., 2002). Within the striatum, increased cholinergic tone has been widely associated with aversion and dysphoria; for example, increases in ACh levels are observed following stressful or aversive stimuli, and are also observed during withdrawal periods from morphine, alcohol, and benzodiazepines (Mark et al., 1995; Rada et al., 2004; Rada et al., 1991; Rada and Hoebel, 2001). Although some studies have reported increases in striatal ACh following rewarding stimulant exposures (Imperato et al., 1993; Zocchi and Pert, 1994) including dose-dependent increases related to cocaine acquisition (Berlanga et al., 2003; Mark et al., 1999), this cholinergic increase appears to be a downstream consequence of dopaminergic efflux, as increases in striatal ACh were blocked by D1 antagonists (Zocchi and Pert, 1994). Further investigation has confirmed that VTA dopaminergic input is a primary regulator of striatal cholinergic tone, and the structural presence of D1 and D2 receptors located on somata, dendrites, and axons of striatal cholinergic neurons (Williams and Adinoff, 2008). Furthermore, ACh in the striatum appears to then exert local feedback regulation on striatal dopamine levels (Zhang et al., 2002).

In addition to the striatal effects above, past research has associated generalized increases in cholinergic tone with depressive-like behaviors, especially related to behavioral inhibition (Janowsky and Overstreet, 1995; Williams and Adinoff, 2008). Rats administered cholinomimetic agents such as physostigmine consistently exhibit lethargy, hypoactivity, decreases in self-stimulation, behavioral despair in the forced-swim test, and reduced saccharine preference (Janowsky and Overstreet, 1995). This hyper-cholinergic state of reduced motivation has been correlated with reduced drug-seeking behavior and reduced rewarding effects of substances. For example, rhesus monkeys given the acetylcholinesterase-inhibitor (AChEi) physostigmine exhibit decreased cocaine self-administration (de la Garza and Johanson, 1982). In rat lines, Grasing et al. (2011) observed decreased self-administration of cocaine during active treatment with AChEis rivastigmine and donepezil, with persistent decreases noted after discontinuation of donepezil but not rivastigmine. In human subjects, AChEis have been shown to reduce both subjective effects as well as methamphetamine use (De La Garza et al., 2008a; De La Garza et al., 2012; De La Garza et al., 2008b). In cocaine-dependent subjects, the AChEi Huperazine A attenuated the positive subjective effects produced by cocaine administration (De La Garza et al., 2015). Donepezil, another AChEi, also attenuated subjective responses to low-dose cocaine, although it was not effective in high-dose cocaine (Grasing et al., 2010; Winhusen et al., 2005).

Rivastigmine is a pseudo-irreversible, carbamate inhibitor that inhibits both AChE and butyrylcholinesterase and is neuro-selective compared with peripheral tissue (Onor et al., 2007). It is currently FDA-approved for treatment of Alzheimer's dementia, and its low protein-binding characteristics make it an attractive treatment option for substance use, as it is minimally metabolized by the cytochrome P450 system, and is low-risk for interaction with other drugs and substances (Onor et al., 2007). Based on the theoretical involvement of the ACh system in mediating reward, as well as past studies utilizing AChEis and increased cholinergic tone to modulate stimulant effects and reinforcement, we hypothesized that rivastigmine would decrease the positive subjective effects and self-administration of cocaine in a controlled laboratory setting.

2. Methods

Preliminary cognitive outcomes from this study were previously published and all methods are the same as described in that paper (Mahoney et al., 2014).

2.1. Participants

The double-blind, placebo-controlled study was approved by the Baylor College of Medicine (BCM) and Michael E. DeBakey Veterans Affairs Medical Center (MEDVAMC) in Houston, Texas. Participants were recruited through local radio and newspaper advertisements. All participants completed an initial telephone screen and if eligible were invited to complete an in-person screen at the MEDVAMC Research Commons, during which participants were informed of risks, benefits, and purpose of the study before providing informed consent to participate in the study.

Eligibility criteria were persons between 18 and 55 years of age who met DSM-IV criteria for cocaine-dependence, provided a urine drug screen positive for cocaine within 2 weeks prior to study enrollment, and used cocaine via an intravenous or smoked route. Exclusion criteria included serious psychiatric or medical illness including serious neurological or seizure disorders, use of any psychoactive medication, and any substance use disorder excluding cocaine and nicotine. Women were also excluded if they were breastfeeding, pregnant, or could become pregnant during the study. Participants were compensated $40 for completing the screen, and received an additional $550 for completing inpatient procedures.

2.2. Drugs

Dosing was based on prior work by our group utilizing rivastigmine to ameliorate methamphetamine effects. Rivastigmine is currently dosed in a range between 1.5 and 12 mg for pro-cognitive treatment in Alzheimer's dementia, and is therefore known to be safe for human administration within these ranges (Khoury et al., 2018). The plasma half-life of rivastigmine and its primary metabolite are roughly 1 and 2 h; however, cholinesterase inhibition lasts between 8 and 10 h (Feldman and Lane, 2007; Kennedy et al., 1999). Concentrations reach steady-state within a few days (De La Garza et al., 2008a, 2012; Feldman and Lane, 2007; Kennedy et al., 1999; Mahoney et al., 2014). Rivastigmine and matching placebo were encapsulated by Greenpark Pharmacy (Houston, TX). Human use cocaine HCl was provided by Research Triangle Institute International (Research Triangle Park, NC).

2.3. Procedures

All study visits were conducted at the MEDVAMC Research Commons. Subject screens included a medical and drug history, electrocardiogram, and vital signs. If eligible, participants were admitted to the inpatient phase of the study and randomized into 1 of 3 groups: 0, 3, or 6 mg, rivastigmine. A random number generator was utilized to allocate each participant to a treatment group at time of enrollment, with each treatment group having an equal chance of assignment, and therefore the final number allocated to each group was slightly uneven. The placebo group in this study was also used as the placebo comparison group in a companion paper describing 2 additional test groups (Huperazine 0.4 and 0.8 mg) (De La Garza et al., 2015). A protocol timeline is shown in Fig. 1.

2.3.1. Pre-randomization (day 1)

Subjects received double-blind intravenous (IV) infusions of both saline and cocaine (40 mg, IV), in a randomized manner at 10 AM and 2 PM on day 1. The infusions were given by IV push over a 2 minute period by a study physician, and vital signs recorded at −15, 5, 10, 15, 20, 30, 45, 60, 90, 120, and 180 minute time points. To assess subjective effects, participants completed visual analog scales (VAS) at the same time points. VAS ratings were completed for categories of “Any Drug Effect,” “Stimulated,” “Good Effects,” “Like,” “Desire Cocaine,” “High,” “Likely to use if given access,” “Anxious,” “Bad Effects,” and “Depressed.” These scales ranged from 0 (no effect) to 100 (maximal effect).

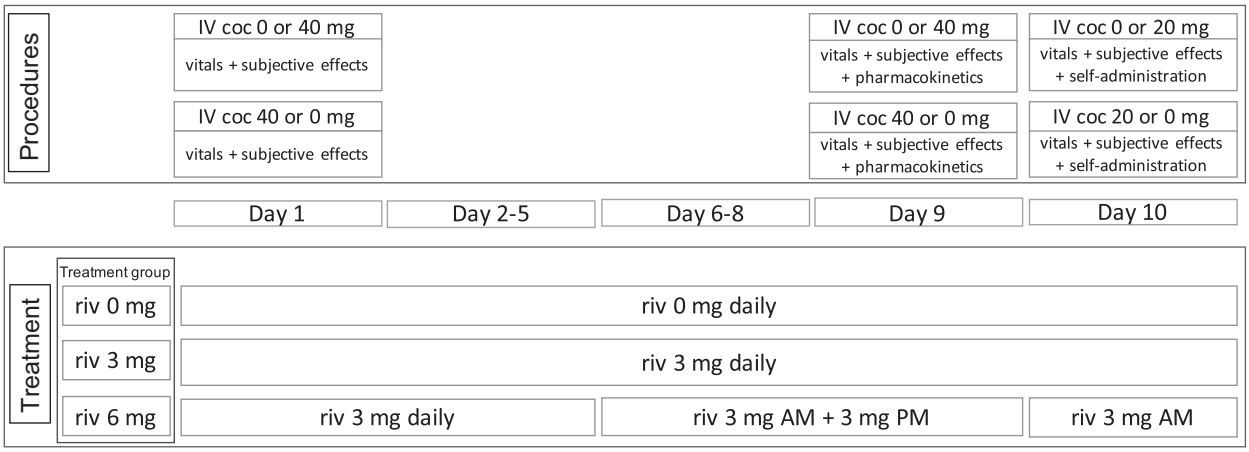

Fig. 1. A timeline of the study protocol.

2.3.2. Study medication randomization and dose escalation

On days 1 (after cocaine/saline infusions) to 10, rivastigmine or placebo was administered once daily. To assure the safety of subjects, a gradual dose escalation was utilized. The placebo group received the same dosage (0 mg) throughout the study. The 3 mg rivastigmine group received 3 mg rivastigmine on days 2–10. The 6 mg rivastigmine group received 3 mg rivastigmine only in the evening of day 1, 3 mg in the morning only on days 2–5, 3 mg twice daily on days 6–9, and 3 mg in the morning of day 10.

2.3.3. Post-randomization (day 9)

The procedures on this day were identical to those on day 1 of the study. The VAS was used to evaluate medication effects. Pharmacokinetic (PK) and subjective effects were measured at −15, 5, 10, 15, 20, 30, 45, 60, 90, and 120 min. Due to barriers that limited the blood draw protocol such as a lack of venous access or health care worker unavailable at the specified time point to complete the draw, PK effects were only collected and analyzed for 4 subjects from each treatment group.

2.3.4. Self-administration session (day 10)

On day 10, subjects participated in a two self-administration sessions; during one session, infusions of saline were available and during the other session, infusions of cocaine were available. During both sessions, all subjects were first administered a non-contingent infusion of saline or cocaine (20 mg, IV) as a sample of the dose that was available for purchase during that session. The baseline administration given to all participants was followed by 5 and 10 minute cardiovascular and subjective effects measures. Participants were then provided $25 from their study earnings and given the opportunity, at 15-minute intervals, to either purchase additional infusions or keep $5. Infusion choices were self-administered by participants via a patient-controlled analgesia (PCA) setup, and choices to keep the money for that interval were indicated verbally to the research coordinator. PCA infusions occurred over 2 min and were limited if the subject's vital signs were outside pre-determined safety parameters.

2.3.5. Study conclusion

Subjects received their final dose of study drug on the morning of day 10. They were subsequently monitored for 24 h and discharged the following morning if medically stable.

2.4. Safety

Adverse events (AEs) were summarized by Medical Dictionary for Drug Regulatory Affairs system organ class, preferred term, and observation period (placebo-only period, placebo-cocaine period, and treatment-cocaine period). AEs were described by incidence, incidence by severity, incidence by relationship to study drug, and incidence by relationship to cocaine.

A physician monitored all vitals and electrocardiogram results during cocaine infusions (days 1, 9 and 10). Safety parameters were set for vital signs (i.e. heart rate > 130 bpm, systolic blood pressure > 165 mmHg, and diastolic blood pressure > 100 mmHg) and cocaine was not administered if readings were outside that range. In our study, there were no instances of cocaine being withheld due to abnormal readings.

2.5. Statistical analyses

All data were analyzed in Microsoft Excel and SPSS Version 25 (IBM SPSS Statistics for Macintosh 2015, n.d.). For all measures, statistical significance was set at $p < 0.05$. Due to the non-independent nature of the subscales (i.e. “High” and “Effect” would have significant correlation) there was no correction made for multiple comparisons, and all findings are presented as raw, unadjusted results. All data are presented as mean ± SEM.

PK data were calculated for pre-randomization (day 1) versus post-randomization (day 9) cocaine infusion (40 mg, IV). Plasma concentration-time profiles were used to obtain estimates of area under the curve (AUC), maximum concentration (Cmax), and time to maximum concentration (Tmax), for cocaine and metabolites. AUC was calculated with the trapezoidal method, utilizing the mean recorded values at each time point and averaging them across that time span. Data were analyzed using two-way ANOVA with day (pre-randomization vs post-randomization) and rivastigmine dose (0, 3, or 6 mg) as factors.

VAS subjective effects ratings on Day 9 and Day 10 were compared in two manners: (1) using *maximal/peak reported effect* across time points, and (2) using the composite *AUC statistic* summating all time points for each category. These outcomes were compared for categories of “Any Drug Effect,” “Stimulated,” “Good Effects,” “Like,” “Desire Cocaine,” “High,” “Likely to use if given access,” “Anxious,” “Bad Effects,” and “Depressed.” Day 9 data were run through a two-way analysis of variance for main and interaction effects of treatment (riv 3, riv 6, or placebo) and cocaine dose (0 vs 40 mg).

For the self-administration portion of the study on Day 10, all subjects received, at a minimum, one baseline dose of cocaine 20 mg, even if they declined to pay for further doses. Ratings collected at the two time points 5 and 10 min after the baseline administration were averaged to simplify calculations and analyzed using one-way analysis of variance for effect of treatment. In addition to the VAS ratings, the number of subsequent paid cocaine self-administrations (out of 5 possible) was an additional outcome variable. If an individual did not self-administer doses of cocaine or saline, their data were excluded from

Table 1
Demographic means (± SD) of study participants.

	Placebo (n = 16)	Rivastigmine 3 (n = 13)	Rivastigmine 6 (n = 14)
Gender			
Male	14	10	11
Female	2	3	3
Race/ethnicity			
African American	11	9	11
Caucasian	2	3	3
Hispanic	0	0	0
Other	3	1	0
Age	40.44 ± 7.91	43.69 ± 5.92	43.93 ± 5.51
Education (years)	12.11 ± 1.11	12.68 ± 1.82	13.14 ± 1.82
Cocaine use			
Years	15.62 ± 7.21	17.07 ± 8.47	16.29 ± 7.60
Past 30 days	16.19 ± 7.49	18.77 ± 6.98	17.21 ± 9.27
Grams/day	1.70 ± 1.01	2.95 ± 3.56	1.85 ± 1.38
Nicotine use			
Years	15.25 ± 9.34	18.69 ± 9.41	19.14 ± 12.00
Cigarettes/day	8.13 ± 6.80	5.67 ± 4.38	11.36 ± 7.06
Alcohol use			
Years	17.94 ± 9.62	21.38 ± 10.69	17.32 ± 9.12
Past 30 days	8.94 ± 8.07	8.62 ± 7.60	8.93 ± 9.06

these analyses.

3. Results

3.1. Participants

Eligible subjects were randomized to placebo (n = 16), riv 3 (n = 13) or riv 6 (n = 14). On average, participants were African American (72%) males (81%) who were approximately 43 (± 7) years of age. On average, participants smoked 2 (± 2) g of cocaine daily, and used cocaine on 17 (± 8) days in the past month. The majority of participants (> 50%) also smoked tobacco (cigarettes) and used alcohol. ANOVA revealed no significant differences for demographic or drug use variables between groups (Table 1).

3.2. PKs

Summary results for PK analysis are shown in Table 2. Comparisons were run for both AUC and max concentration (C_{max}) of bloodstream cocaine, benzoylecgonine (BE), and ecgonine methyl ester (EME) between placebo and rivastigmine treatment groups. Time to maximum concentration (T_{max}) was also compared. Separate ANOVAs were run for day 1 pre-treatment and day 9 post-treatment. There were no significant effects of treatment group on either day. An additional ANOVA was run using day (day 1 vs. day 9) as a predictor variable along with treatment (rivastigmine vs. placebo). Within this model, day was a significant predictor of EME AUC (p = 0.006) but otherwise was a non-significant predictor. After adjustment for multiple comparisons, this finding was non-significant. Treatment remained a non-significant predictor within this model, as expected based on the individual day analyses.

3.3. Post-randomization cardiovascular responses day 9

Fig. 2 shows heart rate, systolic blood pressure, and diastolic blood pressure averages within each treatment group after one week of treatment. For heart rate, ANOVA of AUC revealed no main effect of rivastigmine (F(2,76) = 0.24, p = 0.780), a significant effect for cocaine versus placebo (F (1,76) = 8.187, p = 0.005), but no significant rivastigmine x cocaine interactions (F (2,76) = 0.090, p = 0.914). Exploratory pair-wise comparisons within the 40 mg cocaine dose showed no significant differences between placebo vs rivastigmine 3 mg (p > 0.999) and placebo versus rivastigmine 6 mg (p = 0.883).

For systolic blood pressure, ANOVA of AUC revealed no main effect of rivastigmine (F(2,76) = 0.739, p = 0.481), a significant effect for cocaine versus placebo (F (1,76) = 9.128, p = 0.003), but no significant rivastigmine x cocaine interactions (F (2,76) = 0.194, p = 0.824). Exploratory pair-wise comparisons within the 40 mg cocaine dose showed no significant differences between placebo vs rivastigmine 3 MG (p = 0.964) and placebo versus rivastigmine 6 MG (p = 0.791).

For diastolic blood pressure, ANOVA of AUC revealed no main effect of rivastigmine (F(2,76) = 0.411, p = 0.665), no significant effect for cocaine versus placebo (F (1,76) = 1.662, p = 0.201), and no significant rivastigmine x cocaine interactions (F (2,76) = 0.139, p = 0.871). No exploratory pair-wise comparisons were performed.

Peak physiological effects during administration of cocaine 40 mg were also compared and non-significant between groups for heart rate (F(2,40) = 0.330, p = 0.720)), systolic blood pressure (F(2, 40) = 1.335, p = 0.275), and diastolic blood pressure (F(2, 40) = 0.812, p = 0.451)).

3.4. Post-randomization subjective effects day 9

See Table 3 for full results. Cocaine dose was a significant (p < 0.05) predictor of Peak effect for all VAS categories except for "Depressed," and of a significant predictor of AUC for all VAS categories, except "Bad," "Depressed," and "Anxious" (generally considered negative subjective effects). For all categories, rivastigmine treatment at either dose was not a significant predictor of Peak or AUC ratings in initial or post-hoc pairwise comparisons. There were also no significant effects for rivastigmine treatment x cocaine dose.

Table 2
Pharmacokinetic parameters for cocaine and metabolites following cocaine (40 mg, IV) vs saline on days 1 and 9. All data reflect mean ± SEM, Cmax reflects maximum concentration in ng/ml; Tmax reflects time to maximum concentration in minutes.

		Day 1			Day 9		
		Placebo	Riv 3 MG	Riv 6 MG	Placebo	Riv 3 MG	Riv 6 MG
Cocaine	AUC	14,432.45 ± 1053.32	77,010.00 ± 62,386.73	20,029.17 ± 5645.83	21,875.68 ± 6529.30	48,539.00 ± 33,060.98	21,080.48 ± 6953.47
	C Max	238.15 ± 38.95	5135.95 ± 4824.15	2776.20 ± 2244.32	739.20 ± 298.01	1786.15 ± 1413.86	416.68 ± 163.52
	T Max	9.75 ± 6.79	5.25 ± 3.25	5.25 ± 3.25	15.50 ± 5.72	2.75 ± 0.75	39.00 ± 37.00
BE	AUC	34,384.93 ± 5948.18	41,654.63 ± 10,354.89	28,732.20 ± 4421.30	26,957.17 ± 4138.31	32,905.05 ± 21,017.22	29,481.48 ± 2837.85
	C Max	228.20 ± 34.04	942.25 ± 731.41	198.43 ± 22.06	213.40 ± 33.54	364.60 ± 135.85	185.50 ± 14.25
	T Max	105.00 ± 19.36	78.75 ± 25.53	135.00 ± 19.36	82.50 ± 18.87	60.50 ± 24.09	75.00 ± 25.98
EME	AUC	5158.95 ± 1464.13	5488.68 ± 773.41	4440.80 ± 1179.66	3273.38 ± 414.32	2938.63 ± 436.67	2167.85 ± 295.12
	C Max	33.98 ± 7.91	85.43 ± 52.59	52.95 ± 20.90	29.48 ± 4.51	32.55 ± 14.71	16.20 ± 3.01
	T Max	135.00 ± 8.66	108.75 ± 32.04	98.00 ± 37.07	56.25 ± 23.22	90.50 ± 36.34	150.00 ± 0.00

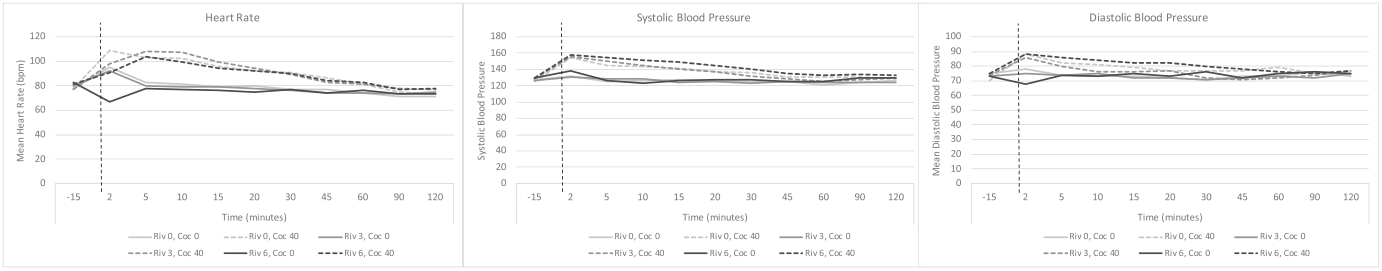

Fig. 2. Longitudinal cardiovascular responses from day 9 cocaine administration (0 and 40 mg) in participants treated with rivastigmine (0, 3, and 6 mg).

Table 3
Mean ratings for day 9 VAS subjective effects. All p-values refer to main effects in a two-way analysis of variance (rivastigmine dose, cocaine dose).

			Total AUC				Max/peak effect			
	Riv dose	Cocaine dose	Mean AUC	St dev	Riv dose p value	Coc dose p- value	Mean max eff	St dev	Riv dose p value	Coc dose p- value
Effect	0 MG	0 MG	100.00	387.30	0.101	< 0.0001	0.00	0.00	0.7891	< 0.0001
		40 MG	3026.56	3299.97			68.13	34.88		
	3 MG	0 MG	0.00	0.00			0.00	0.00		
		40 MG	2326.92	1653.99			60.00	26.46		
	6 MG	0 MG	3.57	13.36			1.43	5.35		
		40 MG	1321.43	751.02			62.86	31.48		
High	0 MG	0 MG	68.75	266.27	0.168	< 0.0001	0.00	0.00	0.9953	< 0.0001
		40 MG	2806.25	3238.58			64.38	33.86		
	3 MG	0 MG	0.00	0.00			0.00	0.00		
		40 MG	1888.46	1701.52			63.85	26.00		
	6 MG	0 MG	0.00	0.00			0.00	0.00		
		40 MG	1328.57	869.34			65.00	32.05		
Good	0 MG	0 MG	250.00	706.22	0.327	< 0.0001	1.88	7.50	0.8314	< 0.0001
		40 MG	2595.31	3083.44			57.50	37.15		
	3 MG	0 MG	0.00	0.00			0.00	0.00		
		40 MG	2269.23	1751.43			64.62	24.36		
	6 MG	0 MG	64.29	240.54			2.86	10.69		
		40 MG	1521.43	1266.77			63.57	32.49		
Bad	0 MG	0 MG	770.31	2341.83	0.112	0.078	8.13	24.00	0.0736	< 0.0001
		40 MG	1501.56	2131.85			37.50	35.68		
	3 MG	0 MG	0.00	0.00			0.00	0.00		
		40 MG	480.77	1284.98			15.39	23.00		
	6 MG	0 MG	4.29	16.04			4.29	16.04		
		40 MG	31.43	30.60			31.43	30.60		
Like	0 MG	0 MG	351.56	1010.82	0.97	< 0.0001	3.75	12.58	0.2721	< 0.0001
		40 MG	2959.38	3438.58			53.13	37.19		
	3 MG	0 MG	319.23	1121.30			6.92	17.02		
		40 MG	2757.69	1848.72			71.54	25.12		
	6 MG	0 MG	710.71	2505.24			7.14	24.00		
		40 MG	2344.64	2287.01			65.71	33.45		
Desire	0 MG	0 MG	1406.25	2356.96	0.165	0.033	20.00	34.83	0.2783	< 0.0001
		40 MG	3448.44	3793.33			57.50	35.31		
	3 MG	0 MG	2175.00	4100.74			22.30	38.98		
		40 MG	3180.77	3348.99			64.62	29.05		
	6 MG	0 MG	571.43	2138.09			5.71	21.38		
		40 MG	1842.86	1905.19			52.86	35.61		
Depressed	0 MG	0 MG	162.50	629.36	0.277	0.121	2.50	10.00	0.2181	0.0746
		40 MG	723.44	1685.02			13.13	25.75		
	3 MG	0 MG	17.31	62.40			2.31	8.32		
		40 MG	107.69	255.64			3.85	7.68		
	6 MG	0 MG	234.29	895.33			7.14	26.73		
		40 MG	562.50	1000.42			17.14	22.00		
Anxious	0 MG	0 MG	345.31	705.63	0.823	0.049	13.13	20.57	0.7313	< 0.0001
		40 MG	1889.06	2702.08			39.38	38.38		
	3 MG	0 MG	1069.23	3173.71			13.85	30.70		
		40 MG	1950.00	3342.37			49.23	34.51		
	6 MG	0 MG	996.43	2244.50			15.00	30.06		
		40 MG	1762.50	1836.14			48.57	26.85		
Stimulated	0 MG	0 MG	226.56	668.57	0.426	< 0.0001	2.50	10.00	0.8969	< 0.0001
		40 MG	2637.50	2697.90			65.63	32.45		
	3 MG	0 MG	15.38	55.47			0.77	2.77		
		40 MG	1840.38	1769.81			61.54	29.68		
	6 MG	0 MG	548.21	1706.79			5.38	19.41		
		40 MG	1450.00	1236.58			60.00	30.28		

3.5. Post-randomization subjective effects day 10

In *t*-test comparisons, cocaine dose (0 vs. 20 mg) was a significant ($p < 0.05$) predictor of all vital signs, and a significant predictor of all subjective effects except "Bad" and "Depressed." After correction for multiple comparisons for the ten subjective effects categories, this significance remained. Following the baseline 20 mg cocaine administration given to all participants, rivastigmine treatment was not a significant predictor of any VAS category rating on day 10 (all p-values > 0.05) and was not a significant predictor of the number of subsequent cocaine doses that were self-administered by participants (all p-values > 0.05), with self-administration for treatment group riv 0 = 2.29 (± 2.09), riv 3 = 1.77 (± 1.74), and riv 6 = 2.14 (± 2.00). Full data are available upon request.

4. Discussion

Our study is the first to examine rivastigmine as a potential therapeutic for cocaine use disorder. We hypothesized that potentiation of ACh with rivastigmine would lead to attenuated subjective effects produced by cocaine, given the results of prior studies that found that increased cholinergic tone reduced drug-seeking behavior, and recent studies indicating the importance of cholinergic modulation of the mesolimbic reward circuit, particularly the bidirectional regulation of ACh and dopamine within the striatum (Williams and Adinoff, 2008).

Our study found no significant change in the PK of cocaine or its metabolites after receiving 3 or 6 mg of rivastigmine for one week. Cocaine's euphoric properties are effected in its unmetabolized state (Ciccarone, 2011), and it is subsequently converted into primary metabolite BE in the liver in a hydrolytic conversion mediated by carboxylesterase (Kolbrich et al., 2006). Rivastigmine would not affect this conversion; however, cocaine's minor metabolite EME is produced via butyrylcholinesterase, a nonspecific cholinesterase enzyme that rivastigmine inhibits (Kolbrich et al., 2006). This is important as altered metabolism could change cocaine levels in the bloodstream, producing more or less intense subjective effects. Our finding this inhibition does not occur in any significant way, helps rule-out alterations in metabolism as a mechanism of rivastigmine's potential action on cocaine use. One major limitation of this analysis was that blood samples could not be collected for every participant at the necessary time points, and therefore only 4 participants were included in the pharmacokinetic analysis.

Our study also found that rivastigmine at 3 or 6 mg does not significantly alter the subjective effects produced by cocaine and did not reduce cocaine self-administration as measured by a pay-for-dose procedure. Testing both subjective effects and self-administration provides a comprehensive view to capture any differences in cocaine effect. Subjective effects are theoretically more sensitive to small changes than self-administration, as the visual-analog scale is continuous and would capture any reported difference between the two conditions, even if the cocaine dosing did not trigger a "break point," or effect at which a user would prefer additional cocaine over money (Eaton et al., 2012). However, since users have been shown to under-report use, the self-administration component provides supplementary objective behavioral data about use that may escape self-report (Clark et al., 2016). There are several explanatory hypotheses for our negative findings.

First, that the rivastigmine did not provide a sufficient level of relevant AChE blockade to have a significant effect. This is especially notable when comparing our negative results to the positive outcomes found in the parallel Huperzine A study conducted by our lab (De La Garza et al., 2015). Huperzine A has better oral bioavailability and blood-brain barrier penetration AChEi when compared to rivastigmine and donepezil (De La Garza et al., 2015). Furthermore, and of notable importance, Huperzine A preferentially inhibits the AChE G4 form, whereas rivastigmine acts on the G1 form (De La Garza et al., 2015; Onor et al., 2007). It has been postulated that part of rivastigmine's procognitive efficacy in Alzheimer's disease is a progressive increase in the G1:G4 ratio with age and in dementia pathology (Onor et al., 2007). However, in our relatively young (mean age = 43 years) sample, the proportion of G1 form that is present is likely negligible. This indicates that AChEis might lack generalizable class-wide efficacy when used in cocaine or other drug use, but instead have efficacy dependent on the individual molecular targets of each agent. It also indicates that rivastigmine may have had significant effects if our sample group had been older. Another possible reason for insufficient blockade is present for the 3 mg group participants, who were dosed once daily rather than BID as in the 6 mg group. Because the AChEi activity of rivastigmine has been measured to last approximately 8.4 h, the once-daily group may have experienced gaps in pharmacologic activity.

Another limitation was our primary outcome of subjective effects produced by IV cocaine in a hospital setting. Because ACh is a neurotransmitter with broad cortical and sub-cortical targets, increasing its tone has other effects on mood, motivation, and behavior independent of the subjective conscious response to cocaine. For example, cholinergic tone has been robustly linked to induced depression, increases in cortisol and stress response, and learned helplessness (Higley and Picciotto, 2014; Janowsky and Overstreet, 1995). In this state, induced anhedonia, feelings of overwhelm, and amotivation could reduce drug-seeking independent of alterations in the rewarding effects of the drug itself; however, our study may have artificially overcome this barrier by presenting subjects with readily available cocaine in the hospital, thereby missing the effect. Generally, cholinergically-induced affective changes are an obvious obstacle to the use of AChEis in a clinical setting, particularly in early cocaine users lacking the chronic cholinergic downregulation hypothesized with long-term stimulant use (De La Garza et al., 2008b). Currently, AChEis are primarily clinically utilized in Alzheimer's disease, where patients have experienced pathologic reductions of the cholinergic system, and therefore increases in cholinergic tone are only used to restore normative cholinergic tone and do not push patients into supraphysiologic levels where affective side effects would be problematic (Frölich, 2002).

The route of use is also notable, as cocaine-related cues (holding smoking paraphernalia, etc.) have been shown to increase striatal dopamine in recreational users independent of the drug itself (Cox et al., 2017). For safety reasons, only subjects with intravenous or smoked use were eligible for our study; however, of the 40 participants, 39 smoked cocaine and only one routinely used intravenously. Because our administration was done through intravenous access in a relatively cue-free setting, the effects experienced from cocaine administration were possibly less robust than in a usual recreational setting, again making it more difficult to detect a difference in treatment effect.

It is also worth noting that our study was conducted in cocaine-dependent participants, who had used cocaine over half the days in the past month (mean = 18/30 days). Several studies have found adaptive downregulations in the ACh system following cocaine administration, including reductions in AChE receptors and in levels of choline synthesis enzyme choline acetyltransferase (ChAT) (Macêdo et al., 2004; Wilson et al., 1994). Post-mortem data on methamphetamine-dependent subjects also has found an overall downregulation of the ACh system in chronic users (De La Garza et al., 2008b). This suggests that the effects of AChEis in chronic cocaine users may differ from the effects in naïve subjects, and indeed, ACEIs have blocked induction of cocaine-induced behavioral sensitization when given prior to cocaine exposure, but did not inhibit the expression of sensitization in already-exposed subjects (Heidbreder and Shippenberg, 1996; Hikida et al., 2003).Therefore, the results of our study are limited to implications in chronic cocaine users, but may have different effects in subjects with early or very intermittent use.

Finally, our sample size was relatively small due to limitations when recruiting participants from a cocaine-dependent population. However, based on the mean VAS effects and standard deviations from the study (e.g. "High" overall mean 64, SD 30; "Effect" overall mean 64, SD 31;

"Desire" overall mean 58, SD 33), our study had a power of 0.8 to pick up a 50% reduction in effect (i.e. a reduction in VAS rating to 30). For VAS categories that represented negative feelings, for example "Bad" (overall mean 29, SD 31) or "Depressed" (overall mean 12, SD 21) the reported effects were less robust, and therefore it would be more difficult to pick up a difference. We also acknowledge that using a single dose of cocaine is a limitation in this study.

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