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Relative potency of varenicline or fluvoxamine to reduce responding for ethanol versus food depends on the presence or absence of concurrently earned food

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Abstract

Background—Varenicline, a nicotinic partial agonist, selectively reduces ethanol- versus sucrose-maintained behavior when tested in separate groups, yet like the indirect serotonin agonist fluvoxamine, this selectivity inverts when ethanol and food are concurrently available.

Materials and Methods—Here we extend these findings by examining varenicline and fluvoxamine effects under a multiple concurrent schedule where food and ethanol are concurrently available in different components: Component1 where the food fixed-ratio was 25 and Component2 where the food fixed-ratio was 75. The ethanol fixed-ratio was always 5, and food-maintained responding predominated in Component1 while ethanol-maintained responding predominated in Component2. In a second experiment, varenicline effects were assessed under a multiple schedule where food, then ethanol, then food was available in separate 5-min components with fixed-ratios of 5 for each reinforcement.

Results—In the multiple concurrent schedule, varenicline was more potent at reducing food- versus ethanol-maintained responding in both components, and reduced ethanol-maintained responding more potently during Component1 (when food was almost never earned) than in Component2 (where food was often earned). Fluvoxamine was similarly potent at reducing food- and ethanol-maintained responding. Under the multiple schedule, varenicline, like fluvoxamine, more potently decreases ethanol- versus food-maintained responding when only food or ethanol are available in separate components.

Conclusions—These results demonstrate that selective effects on drug- versus alternative-maintained behavior depend on the schedule arrangement, and assays in which ethanol or an alternative is the only programmed reinforcement may overestimate the selectivity of treatments to decrease ethanol self-administration. Thus, selective effects obtained under one assay may not generalize to another. Better understanding the behavioral mechanisms responsible for these results may help to guide pharmacotherapeutic development for substance use disorders.

Keywords

operant; alcohol; selectivity; pharmacotherapy

Introduction

Drugs that reduce ethanol-maintained behavior more potently than an alternative behavior are considered selective and thought to hold promise as therapeutic agents for alcoholism because such selectivity presumably reflects a greater potency to decrease drinking versus other, more desirable behavior (see Shoblock et al., 2011 for a recent example). However, such selectivity appears to be assay-dependent (Ginsburg et al., 2005; Ginsburg and Lamb, 2006, 2013). Further, many drugs that selectively reduce ethanol-maintained behavior in common preclinical assays fail or show limited effectiveness in the clinic. Understanding why selective effects on ethanol-self-administration are assay-dependent could help improve preclinical models of alcoholism and make the search for novel treatments more efficient.

Many drugs decrease ethanol-maintained behavior more potently or to a greater extent than behavior maintained by a different reinforcer than ethanol when either ethanol or the non-ethanol reinforcer is the only programmed reinforcement. For example, fluvoxamine, a selective serotonin reuptake inhibitor, more potently reduced ethanol- than food-maintained responding in separate groups of rats (Lamb and Järbe, 2001). This selectivity was also present in a subsequent study in rats responding under a multiple schedule where food then ethanol then food were available in separate components of the same session, eliminating differences in drug or behavioral histories or an interaction between fluvoxamine and ethanol as explanations for the effect (Ginsburg et al., 2005).

In contrast, in rats responding under a concurrent schedule of food and ethanol reinforcement, fluvoxamine less potently reduced ethanol-maintained behavior than food-maintained behavior (Ginsburg and Lamb, 2006). This inversion of fluvoxamine selectivity appears to result from the presence of concurrently available food (Ginsburg et al., 2012).

These preclinical results parallel clinical studies demonstrating that fluvoxamine is only modestly effective and even then only in particular subgroups of patients (Pettinati, 2001; Chick et al., 2004). In other subgroups of patients, fluvoxamine was less effective than placebo (Chick et al., 2004). Thus, selective effects in separate groups of rats or in a multiple schedule may predict clinical effectiveness in certain subgroups of patients, but do not predict broad effectiveness, while the opposite selectivity seen in a concurrent procedure parallels treatment failures in certain patients.

A similar pattern of results is emerging for varenicline; a drug used to promote smoking cessation with low efficacy at nicotinic receptors *in vitro* (Cahill et al., 1996). In preclinical studies, varenicline reduces ethanol-maintained behavior at doses that do not affect behavior maintained by sucrose in separate groups of rats (Steensland et al., 2007; Wouda et al., 2011). Similarly, varenicline reduced ethanol, but not saccharin consumption in separate two-bottle choice procedures in mice (Kamens et al., 2010a). Recently, however, we found that varenicline selectivity was inverted in rats responding under a concurrent schedule of ethanol and food reinforcement (where both are available simultaneously) such that food-maintained responding was more sensitive to varenicline than ethanol-maintained responding (Ginsburg and Lamb, 2013). While early clinical studies have shown promising

results for varenicline, these studies were conducted in non-treatment seeking subjects whose drinking was substantially lower than is typically seen in treatment-seeking patients (McKee et al., 2009; Mitchell et al., 2012); reduction of drinking in similar populations was seen in early results of clinical studies with fluvoxamine (Angelone et al., 1998). Thus, the broad effectiveness of varenicline remains unknown; though a recent placebo-controlled, double blinded study showed varenicline treatment produced modest, but significant improvement in drinking outcome measures compared to placebo (Litten et al., 2013).

Previously we demonstrated that the potency of fluvoxamine to reduce ethanol-maintained responding depends on the presence or absence of an alternative (Ginsburg et al., 2012). This was done in a within-subjects, but between sessions design. Here we extend those results by examining whether the frequency at which food is concurrently earned affects the selectivity of fluvoxamine and varenicline to decrease ethanol- versus food maintained responding in a within-subject, within-session design (using a multiple concurrent schedule). We compare these results to those obtained using a multiple schedule we have previously described (Ginsburg et al., 2005). The results indicate that the presence of a concurrently available alternative can affect the relative potency of a treatment to reduce ethanol- versus food-maintained behavior. Further, the multiple concurrent procedure provides additional information about treatment effects on the allocation of choice between ethanol and the alternative. These results indicate that selectivity is assay-dependent.

Methods

Subjects

Twelve male Lewis rats (Harlan, Frederick, MD) were trained as described below. Rats weighed ~275g upon arrival, and were allowed at least 1 week to habituate to our facility. Subsequently, daily feeding occurred after the operant session and was limited to maintain their weights at 300–330g throughout the study. Water was always available in rats' home cages.

Apparatus

Experiments occurred in commercially available operant chambers (ENV-008, Med-Associates, Georgia, VT.) with two levers arranged on either side of one wall with a light above each and a houselight above the chamber on the opposite wall. A trough was positioned between the levers where rats could access food pellets (45 mg chow flavored, BioServ, Frenchtown, NJ) and solutions (via a 0.1 mL dipper mechanism) when they were delivered. Chambers were also equipped with a speaker connected to a tone generator (ANL-926, Med-Associates, Georgia, VT). Stimuli presentation, reinforcement delivery, and response recording were controlled using a custom program written and executed with commercially available software (Med-PCIV, Med-Associates, Georgia, VT). Operant chambers were housed in ventilated, light and sound attenuating cubicles (ENV-022V, Med-Associates, Georgia, VT). Pink noise was generated in the room housing operant equipment to further isolate tones within the operant chamber.

Multiple Concurrent Schedule

In this procedure, ethanol and food are always concurrently available, but contingencies and associated stimuli are arranged such that in one component, rats rarely earn food and most responding occurs on the ethanol-associated lever. In the other component, rats earn both food and ethanol, though responding occurs predominately on the food-associated lever. These two types of components alternate (in random order) and an equal number of each are presented throughout the session.

Training—Six rats were trained to respond for an 8% sucrose solution (w/v) during 30-min sessions. During these sessions, presentation of a 16 kHz tone indicated that the solution was available. A single response on the right lever silenced the tone and raised the dipper into an accessible position for 15-sec. Subsequently, the dipper was withdrawn, the 16 kHz tone was again presented, and a response on the left lever again resulted in solution presentation and associated stimulus changes. Once rats consistently responded for sucrose, the response requirement was increased to 5. Ethanol was then added to the solution (10% w/v). The sucrose in the solution was then gradually removed until rats were responding for a 10% ethanol solution in drinking water alone.

Rats were trained to respond for food in a subsequent 30-min session. In this session, an 8 kHz tone was presented, and a single response on the right lever silenced the tone and produced delivery of one food pellet. After 15-sec, the 8 kHz tone was again presented and the lever was again active. Once rats earned at least 80 pellets in a session, the response requirement was increased to 25. Rats then began training under the multiple concurrent schedule.

Under the multiple concurrent schedule, stimulus conditions during each component were presented in random order (by sampling without replacement from the two stimulus conditions) during a 30-min session. In the 16 kHz condition, ethanol solution was available after 5 responses on the left lever and food was available after 75 responses (fixed-ratio 75; FR75) on the right lever. In the 8 kHz condition, ethanol solution remained available after 5 responses, while food was available after 25 responses (fixed-ratio 25, FR25) on the appropriate lever (Figure 1, upper panel). Responses on one lever did not reset the response counter for the other lever. Rats were exposed to one multiple concurrent schedule session each weekday until responding on the ethanol lever in the presence of the 16 kHz tone was >50% and responding on the ethanol lever in the presence of the 8 kHz tone was <50%. Once rats met this criteria, testing commenced. Following training, multiple concurrent schedule studies were completed in 27 experimental sessions, conducted over the course of 4 months. During this time, subjects continued to train under the multiple schedule each weekday, holidays excluded.

Multiple Schedule

In this procedure, rats can respond for food, ethanol, and then food again in sequential 5-min components, so food and ethanol are never concurrently available. Parameters are arranged to produce similar numbers of responses for and deliveries of ethanol and food across the three components. Previously, we found that fluvoxamine was more potent at reducing

ethanol-maintained responding compared with food-maintained responding under this same schedule (Ginsburg et al., 2005).

Training—Six rats were trained to respond for an 8% sucrose solution. During these sessions a light above the right lever indicated a response provided access to 0.1 ml of the solution. A response also turned off the light above the lever and turned on the house light indicating that the solution was available and responses had no programmed consequence. After 30-sec, the dipper was returned to an inaccessible location, the light above the right lever was again illuminated, and a response again provided access to the solution. Once rats reliably responded for the sucrose solution the response requirement was raised to 5, then ethanol (8% w/v) was introduced into the solution, and finally sucrose was gradually removed from the solution.

Once rats were responding for 8% ethanol alone, they were trained to respond for food in a separate 1-hour session. During these sessions a light above the left lever indicated that responses on the left lever produced food delivery. Completion of the response requirement turned off the stimulus light and turned on the house light and delivered a food pellet into the accessible tray. After 30-sec, the house light was turned off, the stimulus light was turned on, and responses again produced food.

Finally rats were introduced to the multiple schedule. During the first component, the left lever light was illuminated, and 5 responses (fixed-ratio 5; FR5) on the left lever produced food. During the second component, the right lever light was illuminated and 5 responses (FR5) produced ethanol solution. Finally, during the third component, the left lever light was again illuminated and 5 responses (FR5) on the left lever again produced food. In each component, responses on the alternative lever were recorded but had no programmed consequence. Initially, components were 30-min long, but component length was reduced over the first several sessions until the terminal parameters were attained: each of the three components was 5-min long (Figure 1, lower panel). Once responding in each of the three components was stable under the terminal parameters, the effects of varenicline and fluvoxamine were assessed. Multiple schedule studies were completed in 35 experimental sessions, conducted over the course of 11 months. During this time, subjects continued to train under the multiple schedule each weekday, holidays excluded.

Testing—For both procedures, effects of fluvoxamine maleate (3.2, 5.6, 10, & 17.8 mg/kg -multiple concurrent or 10 mg/kg multiple schedule, i.p., 30-min pretreatment, Solvay, Weesp, The Netherlands) or varenicline (0.32, 0.56, 1.0, 1.78, & 3.2 mg/kg (multiple concurrent schedule) and additionally 5.6 & 10 mg/kg (multiple schedule), i.p., 15-min pretreatment, Pfizer, Inc, Groton, CT) were determined on Tuesdays and Fridays. Both drugs were dissolved in 0.9% saline to provide 1 ml/kg solutions for each dose tested. Vehicle was administered each Thursday. Vehicle was also administered on Tuesdays and Fridays as part of the dose sequence and served as controls for ED50 calculations. Doses (including vehicle) were administered in mixed order. After each dose had been tested, the order was reversed for each subject and tested again, generating two dose effect curves. Because an ANOVA revealed no order effects in either group, effects of each dose were averaged for each subject for analyses.

Analysis

Statistical significance was established by $p < 0.05$ for all tests. All analyses were performed using R (R Development Core Team, 2012). Data were analyzed in two different ways: ED50 and ANOVA on number of fixed-ratios completed.

ED50 for decreases in fixed-ratio completions on each lever during each component were determined. First, the number of fixed-ratios completed were calculated and normalized to control values (following vehicle administration on Tuesdays and Fridays). The range of doses used in the analysis included monotonic decreasing values for the group (i.e. the descending limb of the dose-response curve). In the case of food-associated responding during food FR75 components in the multiple concurrent schedule and ethanol-associated responding during Food components of the multiple schedule, analyses were not performed as little responding ever occurred in these cases. From these doses and effects, a linear regression was performed for each subject and the dose that results in 50% reduction (ED50) in responding was calculated from the resulting regression equation. Mean ED50 values for the group and associated 95% confidence limits were then calculated for each condition.

In addition, ANOVAs were performed on fixed-ratios completed with the dose of each drug, lever (food or ethanol associated), and component type (food FR25 or food FR75 for multiple concurrent, Food 1, Ethanol and Food 2 for the multiple schedule) as factors. For these ANOVAs, Greenhouse-Geisser corrections for deviation from sphericity were used. When effects of dose were present, dose effects were analyzed using multiple paired t-tests, corrected for multiple comparisons using the method of Benjamini and Hochberg (1995).

The proportion of ethanol-associated responses (ethanol/total (ethanol + food) responses) was calculated for each component of the multiple concurrent schedule following each dose of varenicline and fluvoxamine. During the food FR25 component, responding on the food-associated lever predominated following vehicle treatment, resulting in a low proportion of ethanol responding. Conversely, during the food FR75 component, responding on the ethanol-associated lever predominated, resulting in a relatively high ethanol response proportion. An overall within-subject ANOVA on proportion with dose and component as factors was performed. In the case of varenicline, an interaction was present so pairwise comparisons of dose effects against vehicle data were performed for each component, and corrected using the method of Benjamini and Hochberg (1995). Decreases in the proportion reflect conditions where responding maintained by food was more affected by treatment than responding maintained by ethanol while increases in the proportion reflect conditions where responding maintained by ethanol was more affected by treatment than responding maintained by food.

Fluvoxamine effects on multiple schedule—Confirmation that fluvoxamine exerted greater effect on ethanol- versus food-maintained responding under the multiple schedule was accomplished using two paired t-tests on percent control responding following 10 mg/kg fluvoxamine.

Results

Multiple Concurrent Schedule

Baseline behavior—Once rats were trained, responding was guided by stimuli and contingencies present during the session. During components where the 8 kHz tone was present and Food FR25, responding was primarily on the food-associated lever: rats completed 22.2 ± 6.3 (mean $\pm$ S.E.M.) food fixed-ratios and 6.0 ± 1.8 ethanol fixed-ratios. In contrast, during components where the 16 kHz tone was present and Food FR75, responding was primarily maintained by ethanol delivery. During Tuesday/Friday vehicle sessions, rats completed 0.6 ± 0.8 food fixed-ratios and 27.5 ± 2.7 ethanol fixed-ratios. During these components, three of the subjects never made a response on the food lever following vehicle, one subject completed one fixed-ratio following varenicline vehicle and one fixed-ratio following fluvoxamine vehicle, one subject completed three fixed-ratios following varenicline vehicle and one fixed-ratio following fluvoxamine vehicle, and one subject completed three fixed-ratios following varenicline vehicle but made no responses on the food-associated lever following fluvoxamine vehicle. Thus, food was almost never earned during the food FR75 components. Across the entire session, rats earned 22.8 ± 2.7 pellets and 33.5 ± 4.0 ethanol deliveries. This equates to approximately 1.1 ± 0.1 g/kg ethanol during each session.

Varenicline effects—Varenicline dose-dependently decreased the number of fixed-ratios completed on the ethanol-associated lever in food FR75 components and on the food-associated lever in food FR25 components (Figure 2, upper left). In contrast, in the food FR25 components, varenicline exhibited a biphasic effect on the number of ethanol fixed-ratios completed: lower doses increased and higher doses decreased the number of ethanol deliveries earned. This led to a significant increase in the proportion of ethanol versus food responses made during food FR25 components (Figure 2, lower left). Fixed-ratios for food were rarely completed during food FR75 components across vehicle and active dose conditions. Similar effects on response rates were seen (Table 1).

As shown in Table 2, varenicline more potently reduced responding maintained by ethanol during the Food FR75 component (when food was almost never earned) versus during the Food FR25 component (when food was frequently earned). It is important to note that the ED50 value for ethanol effects during the Food FR25 component is extrapolated as no dose tested reduced responding for the group by more than 50%. Still, the ED50 is at least 3.2 mg/kg if not greater. The ED50 for decreased ethanol-maintained responding was greater in both components than the ED50 for food-maintained responding (during the Food FR25 component). This represents an inversion of the selectivity considered desirable in a potential pharmacotherapy for alcoholism.

Consistent with these results, a repeated-measures ANOVA on number of fixed-ratios completed showed an interaction among varenicline dose, component type, and lever (food or ethanol) $F[5, 25]=9.1$, $p<0.05$). Interactions between dose and lever ($F[5, 25] = 20.8$, $p<0.05$) and component type and lever ($F[1,5] = 39.9$, $p<0.05$) but not between dose and component type ($F[5, 25] = 1.1$) were present. Main effects of dose ($F[5, 25]=18.9$, $p<0.05$), component type ($F[1, 5] = 6.8$, $p<0.05$), and lever ($F[1,5] = 55.4$, $p<0.05$) were also present.

Based on the three-way interaction, post-hoc analyses were performed on varenicline dose-effects (compared to vehicle) on response rate on each lever under each component type. As shown in Figure 2 (upper left panel), lower doses of varenicline (0.32 – 1.0 mg/kg) significantly affected responding during the food FR25 components; increasing the number of ethanol deliveries earned while simultaneously decreasing the number of food deliveries earned. Responding on both levers was decreased following 3.2 mg/kg varenicline. During Food FR75 component, varenicline dose-dependently decreased ethanol-maintained responding, and this occurred at slightly lower doses than those needed to decrease ethanol-maintained responding in the Food FR25 component.

Fluvoxamine effects—Fluvoxamine dose-dependently decreased the number of fixed-ratios completed on both levers in the food FR25 components and on the ethanol lever during food FR75 components (Figure 2, upper right). Responding on the food-associated lever during food FR75 components was not significantly affected, however fixed-ratios for food were rarely completed in these components, even following vehicle. In contrast with varenicline effects, no effect on the proportion of ethanol versus food responses was present in either component (Figure 2, lower right). As shown in Table 2, fluvoxamine equipotently reduced ethanol-maintained responding during food FR25 and food FR75 components. The potency of fluvoxamine to reduce food-maintained responding in the Food FR25 component was also not different than the potency to reduce ethanol-maintained responding in either component.

An ANOVA on fixed-ratios completed revealed an interaction among dose, component type, and lever ($F[4,20]=7.3$, $p<0.05$). Significant interactions were present between dose and lever ($F[4,20]=21.6$, $p<0.05$), and between component and lever ($F[1,5] = 17.1$, $p<0.05$), but not between dose and component type ($F[4, 20]= 0.7$). Main effects were significant for dose ($F[4, 20]=31.6$, $p<0.05$), lever ($F[1, 5] = 59.0$, $p<0.05$), but not component ($F[1,5] = 0.8$). Based on the interaction among component, dose and lever, post-hoc analyses were performed and showed that fluvoxamine decreased responding on both levers during food FR25 components, and on the ethanol-associated lever during food FR75 components.

Multiple Schedule

We extended this study to include varenicline effects using the multiple schedule we have previously described (Ginsburg et al., 2005). In this schedule, treatment effects are assessed in the same subject responding for either food or ethanol in separate components of the same session. First, varenicline effects were assessed to confirm that the selective effect seen in separate groups of animals responding for ethanol or food was observed (Steensland et al., 2007; Wouda et al., 2011). Then, to ensure that previously reported fluvoxamine effects were replicated, we tested a single dose of fluvoxamine (10 mg/kg) to confirm that fluvoxamine exerted the expected selective effects in these rats.

Baseline behavior

Once rats were trained, responses were controlled by the contingencies and associated stimuli presented during each session. During Food components, rats responded primarily on

the food-associated lever: rats earned 7.6 ± 1.7 and 8.1 ± 1.5 food deliveries. During the Ethanol component, rats earned 6 ethanol deliveries or approximately 0.2 g/kg.

Varenicline effects

As shown in Figure 3 (upper panel), varenicline exerted biphasic effects on the number of fixed ratios completed in the Ethanol and Food 2 components (increased at 0.32 for Ethanol and Food 2 components and at 1.0 mg/kg for Food 2 component and decreased at 3.2, 5.6, and 10 mg/kg), and monotonically decreased the number of fixed-ratios completed in the Food 1 component. Responding on the ethanol-associated lever during the two Food components was very low and unaffected by any dose of varenicline. During the Ethanol component, responding on the food-associated lever was increased at the same doses that increased ethanol-associated responding, while higher doses similarly decreased responding on both levers. Note that although responses on the non-reinforced lever are expressed in terms of fixed-ratio completions (FR5 for both levers when active), completion of 5 responses on the non-reinforced lever had no programmed consequence. Effects on response rate (Table 1) were similar to those on fixed-ratios completed.

Table 2 shows that the potency of varenicline to decrease ethanol-maintained responding was greater than its potency to disrupt food-maintained responding in either Food component. These results are shown in Figure 3 (upper panel), where decreases in response rate during the Ethanol component occurred at lower doses than decreases in response rates during the Food components.

An overall ANOVA revealed an interaction among dose, component and lever ($F[10, 50] = 19.0$, $p < 0.05$). Interactions were also present between dose and lever ($F[5, 25] = 23.7$, $p < 0.05$), lever and component ($F[2, 10] = 80.8$, $p < 0.05$), and dose and component ($F[10, 50] = 2.2$, $p < 0.05$). Main effects of dose ($F[5, 25] = 68.1$, $p < 0.05$) and lever ($F[1, 5] = 392.9$, $p < 0.05$), but not component ($F[2, 10] = 0.4$, $p < 0.05$) were also present. Based on the interactions observed, post-hoc analyses were performed to determine effects of each dose compared to vehicle (Figure 3). Responding on the food-associated lever during the Food 2 component following 0.32 mg/kg varenicline was significantly different and higher than the response rate following vehicle. Responding on the food-associated lever during the Ethanol component following 1.0 mg/kg varenicline was also significantly different and higher than the rate following vehicle. Higher doses of varenicline (3.2–10 mg/kg) decreased responding under all conditions.

Fluvoxamine effects

To ensure that we also observed the expected selective effect of fluvoxamine (Ginsburg et al., 2005) on ethanol-maintained versus food-maintained responding in these same rats, we also tested the effects of 10 mg/kg fluvoxamine. Indeed, this dose of fluvoxamine had a greater effect on responding maintained by ethanol versus food. Expressed as a percentage of the Thursday control response rate for that week in each component, 10 mg/kg fluvoxamine reduced responding in the ethanol component (38.2 ± 19.5 %; mean $\pm$ S.E.M.) more than responding in the food components (Food 1: 74.8 ± 16.6 %; Food 2: $89.7 \pm$

4.3%). These differences were significant ($t=2.6$ and 3.1 , $p<0.05$ for ethanol versus Food1 and Food2, respectively).

Discussion

Here, we demonstrate that the relative potency of varenicline to decrease ethanol- versus food-maintained responding is influenced by context in which the behavior occurs.

Generally, these results are consistent with previous findings that concurrent presence or absence of food can affect the relative potency of fluvoxamine to reduce ethanol- versus food-maintained responding. These results have implications for the interpretation of commonly used preclinical procedures for identifying effective pharmacotherapies for alcoholism.

Previously, we demonstrated that at doses that do not abolish operant responding, the selective effect of fluvoxamine (where potency to decrease ethanol-maintained responding is greater than food-maintained responding) seen in separate groups (Lamb and Järbe, 2001) or in the same subjects under a multiple schedule (Ginsburg et al., 2005) disappear or inverts when food is concurrently available (Ginsburg and Lamb, 2006; Ginsburg et al., 2012). Here, we extend these results to show that varenicline selectively reduces ethanol- versus food-maintained responding under a multiple schedule, consistent with previous results in separate groups (Steensland et al., 2007; Wouda et al., 2011). However, this selectivity inverts when food and ethanol are concurrently available (Ginsburg and Lamb, 2013).

The present results show this effect in the same subjects in the same session by using a multiple concurrent schedule: the potency to reduce ethanol maintained responding was greater in food FR75 components (when food was almost never earned) compared to food FR25 components (when both were earned). Together, these results demonstrate that reports of selective effects of novel anti-alcoholism treatments likely depend on the assay, and may not generalize to other assay conditions. These results present a challenge to understanding circumstances where selectivity predicts clinical effectiveness, if it does at all.

It is possible that the increase in ethanol-maintained responding is due to an interaction between varenicline and consumed ethanol. Indeed, there was evidence of an interaction between ethanol and varenicline in the multiple schedule, where the ED50 was lower in the second food component (after ethanol had been consumed) versus the first. Differences between varenicline effects in the first food components (before ethanol is made available) and the second food component (after ethanol has been consumed) likely reflect interactions between varenicline and ethanol. Others have reported that varenicline interacts with ethanol to enhance the sedative and ataxic effects of ethanol (Kamens et al., 2010b), here we see possible evidence of a direct interaction between varenicline and ethanol at lower doses of ethanol.

It is also possible that the effects of varenicline observed here are simply rate-dependent, where lower rates of responding are increased at the same doses that decrease or have no effect on higher rate behavior (Kelleher and Morse, 1968). Varenicline increased responding on the ethanol-associated lever during food FR75 components under the multiple concurrent

schedule, and on the food-associated lever during the Ethanol component of the multiple schedule while simultaneously not affecting or decreasing responding on the alternative lever (which occurred at a higher rate in both cases).

Another explanation for the increases in off-target responding seen following varenicline may be that varenicline increased responding that is normally maintained at low rates due to prevailing conditions. Such effects have been reported for varenicline, but not for fluvoxamine, using serial reaction time tasks (Tsutsui-Kimura et al., 2009; Wouda et al., 2011). This effect of varenicline is biphasic, and occurs at lower doses than those that disrupt responding, consistent with the present results. Increased perseverative responding is unlikely to explain these effects, as increases in perseverative responding have not been reported for nicotine, varenicline, or fluvoxamine (Tsutsui-Kimura et al., 2009; Wouda et al., 2011).

Yet another possible explanation is that the potency of disruptive effects depend on the magnitude or frequency of reinforcement, consistent with concepts described by behavioral momentum (Cohen, 1998; Harper, 1999; Nevin and Grace, 2000). However, we have demonstrated that fluvoxamine effects are unlikely to depend on the magnitude of reinforcement (Lamb and Ginsburg, 2005, 2008; Ginsburg and Lamb, 2008). The extent to which varenicline effects could be influenced by the relative frequencies of reinforcement remains unknown.

Results of studies with fluvoxamine are also consistent with assay-dependent selectivity. Fluvoxamine (10 mg/kg) reduced ethanol-maintained responding more than food-maintained responding under a multiple schedule, replicating our previous results (Ginsburg et al., 2005). In the multiple concurrent schedule, the potency of fluvoxamine to reduce ethanol- and food-maintained responding was similar in both components. This result is also consistent with previous results from our lab where the potency of fluvoxamine to reduce ethanol-maintained responding depended on whether food was concurrently available or not (Ginsburg et al., 2012). Thus, a consistent pattern of greater potency to decrease ethanol- versus food-maintained behavior when each is available alone is clear in separate groups or under the multiple schedule (Lamb and Järbe, 2001; Ginsburg et al., 2005) and this potency difference is not present or inverts in rats responding under a schedule with concurrent access to food and ethanol (Ginsburg and Lamb, 2006; Ginsburg et al., 2012).

In this study, low doses of varenicline generally increased ethanol-maintained responding, which would seem to be an undesirable clinical characteristic. Higher doses of varenicline reduce ethanol and food maintained responding, and, in situations where both food and ethanol are concurrently available, decrease responding maintained by food at lower doses than responding maintained by ethanol. This too would seem to be an undesirable clinical characteristic. Only in situations where ethanol or food is the only reinforcement encountered (under the multiple schedule) did varenicline (at higher doses) decrease responding maintained by ethanol, but not by food. This could be interpreted to suggest that varenicline has clinical utility in situations where ethanol maintains behaviors to the exclusion of most every other activity, however in situations where ethanol-maintained

behavior co-occurs with other activities, the other activities could be more affected by treatment than ethanol use.

It is possible that the increase in ethanol-maintained responding following lower doses of varenicline reflect partial agonist activity at nicotinic acetylcholine receptors (nAChR) comprised of $\alpha 4\beta 2$ subunits, as varenicline has a relatively high affinity for (but low efficacy at) this subtype compared to nAChRs comprised of $\alpha 3\beta 4$ or $\alpha 7$ subunits (Mihalak et al, 2006). If true, a selective full agonist at $\alpha 4\beta 2$ nAChR should increase ethanol-maintained responding even further. Higher doses of varenicline decreased ethanol- and food-maintained responding. This descending limb could reflect recruitment of $\alpha 3\beta 4$ or $\alpha 7$ nAChRs, and other drugs with high efficacy at these subtypes would be predicted to share the context-dependent selective effects on ethanol- versus food-maintained responding reported here. In contrast, drugs that do not target $\alpha 3\beta 4$ or $\alpha 7$ nAChRs may only decrease operant responding maintained by food and ethanol at even higher doses via action at other non-nicotinic sites. While it might be enticing to examine effects of drugs that differ in affinity or efficacy between the $\alpha 3\beta 4$ or $\alpha 7$ subtypes to decrease ethanol- versus food-maintained responding, the relative potency of these actions would also be expected to depend on the schedule arrangement. Further, it remains unclear how a particular systemically administered dose corresponds with stimulation of specific nAChR subtypes, and the range of effective doses in the present study was rather small, constituting just over 1/2 log unit (1.3 – 6 mg/kg) while the difference in affinity for $\alpha 2\beta 4$ versus $\alpha 7$ is closer to a full log unit (2.3 – 18 μ M). Thus, the behavioral effects we observed are unlikely to cleanly distinguish between effects at a particular nAChR subtype.

It should be noted that the ethanol concentrations used in the two schedules here differed (10% w/v in the multiple concurrent versus 8 % w/v in the multiple schedule). Others have shown that the difference in behavior maintained by these two concentrations is modest (Beardsley et al., 1993). Moreover, in an earlier study (Ginsburg et al., 2005), the selective effect of fluvoxamine observed under the multiple schedule maintained by 8% (w/v) ethanol was also seen when the concentration was increased to 16% (see also Lamb and Järbe, 2001). Thus, one would expect that the selective effects of varenicline and fluvoxamine would also have been present had responding under the multiple schedule been maintained by 10% (w/v) ethanol.

This study provides further evidence that selective effects observed in commonly used preclinical models of alcoholism may not generalize to other procedures. Coupled with the fact that many drugs that exhibit selective effects on ethanol-maintained behavior in such models fail or show limited effectiveness in clinical use, these results should challenge preclinical researchers to develop models with better predictive validity. Indeed, it may be that different procedures model different patterns of pathological alcohol use. While few, if any agents have been identified with broad effectiveness for alcoholism, developing assays that can identify effective treatments in particular subtypes of alcoholism could improve targeted pharmacotherapy and benefit particular individuals.

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Multiple Concurrent Schedule

Multiple Schedule

Figure 1.

Illustration of schedules of reinforcement used. Top panel diagrams the multiple concurrent schedule. S1 and S2 represent presentation of the 8kHz and 16kHz tones, respectively. R1 and R2 represent a response on the food- or ethanol-associated lever, respectively. Numeric prefixes show the number of responses on the lever required to produce reinforcement in each component. Completion of the response requirement results in presentation of food or ethanol and the associated stimulus (SR1 or SR2), and prevents completion of the response requirement on the other lever (double-headed arrows). Component order proceeds with a probability of 0.5 for a given component being selected ($p=0.5$ on each branch). The session continues for 30-min (looped arrow). Lower panel diagrams the multiple schedule. This schedule is comprised of three sequential 5-min components. S1 represents illumination of the light above the food-associated lever and S2 represents illumination of the light above the ethanol-associated lever. Other elements are the same as described above. This notation was adopted from Mechner (1959).

Figure 2.

Top Panel: Effects of varenicline (left) and fluvoxamine (right) on the number of responses (expressed as number of fixed-ratios completed) on each lever in each type of component in the multiple concurrent schedule. Note that filled symbols represent the predominant response in each type of component. Bottom Panel: Effects of varenicline (left) and fluvoxamine (right) on ethanol-associated responses as a proportion of total responses for each component of the multiple concurrent schedule. Higher proportions reflect conditions where ethanol-associated responses predominate over food-associated responses. Thus, in

the food FR75 component (filled symbols), almost all responding was on the ethanol-associated lever following every dose of varenicline that did not abolish responding. However, in the food FR25 component (open symbols), food-associated responses predominated following vehicle or fluvoxamine, but varenicline produced a dose-dependent increase in the amount of ethanol- versus food-associated responses. Points represent mean $\pm$ S.E.M. for n=6 rats. * indicates significant difference from vehicle after correction for multiple comparisons.

Figure 3.

Top panel: Effects of varenicline on the number of responses (expressed as number of fixed-ratios completed) on each lever in each of the three components in the multiple schedule. Note that filled symbols represent the predominant response in each type of component.

Bottom Panel: Effects of varenicline on ethanol-associated responses as a proportion of total responses for each component of the multiple concurrent schedule. Note that in contrast with effects in the multiple concurrent schedule, varenicline has no effects on response proportion in Food components where food-associated responding typically predominates (open

symbols), but decreases the proportion of ethanol-associated responses in the Ethanol component where ethanol-associated responding typically predominates (filled symbols), consistent with a selective effect on ethanol-maintained behavior. Points represent mean $\pm$ S.E.M. for n=6 rats. * indicates significant difference from vehicle after correction for multiple comparisons.

Table 1
Response rates (resp/sec) on each lever in each component following doses of varenicline and fluvoxamine

Multiple Concurrent Schedule										
Component		Food FR75			Food FR25					
Lever	Dose	Ethanol	mean	(S.D.)	Food	mean	(S.D.)	Ethanol	mean	(S.D.)
Varenicline		0.00	0.71	(0.26)	0.19	(0.07)	0.25	(0.23)	1.40	(0.52)
		0.32	0.92	(0.24)	0.15	(0.07)	0.57	(0.37)	0.89	(0.25)
		0.56	0.97	(0.27)	0.13	(0.06)	0.56	(0.30)	0.94	(0.44)
		1.00	0.90	(0.28)	0.10	(0.04)	0.48	(0.27)	0.81	(0.46)
		1.80	0.61	(0.43)	0.08	(0.06)	0.53	(0.32)	0.41	(0.37)
		3.20	0.45	(0.23)	0.03	(0.03)	0.57	(0.21)	0.25	(0.30)
Component		Food FR75			Food FR25					
Lever	Dose	Ethanol	mean	(S.D.)	Food	mean	(S.D.)	Ethanol	mean	(S.D.)
Fluvoxamine		0.00	0.76	(0.29)	0.20	(0.10)	0.28	(0.22)	1.34	(0.66)
		3.00	0.65	(0.21)	0.21	(0.16)	0.20	(0.18)	1.32	(0.59)
		5.60	0.45	(0.15)	0.20	(0.05)	0.10	(0.09)	1.24	(0.56)
		10.00	0.53	(0.37)	0.20	(0.18)	0.23	(0.21)	0.94	(0.73)
		17.80	0.08	(0.12)	0.06	(0.12)	0.02	(0.05)	0.38	(0.61)
Multiple Schedule										
Component		Food 1			Food 2					
Lever	Dose	Food	mean	(S.D.)	Ethanol	mean	(S.D.)	Food	mean	(S.D.)
Varenicline		0.00	0.90	(0.34)	0.03	(0.04)	0.10	(0.04)	0.40	(0.21)
		0.32	1.00	(0.51)	0.03	(0.08)	0.27	(0.23)	0.51	(0.26)
		1.00	0.86	(0.30)	0.03	(0.05)	0.20	(0.08)	0.40	(0.26)
		1.78	0.64	(0.29)	0.01	(0.02)	0.13	(0.09)	0.15	(0.14)
		3.20	0.43	(0.23)	0.00	(0.00)	0.08	(0.04)	0.07	(0.07)
		5.60	0.08	(0.14)	0.00	(0.00)	0.01	(0.02)	0.00	(0.00)
Fluvoxamine		10.00	0.01	(0.01)	0.00	(0.00)	0.00	(0.00)	0.00	(0.00)

Table 1

ED50 values for varenicline and fluvoxamine effects on ethanol- versus food-maintained responding under during each type of component.

Varenicline	Schedule	Component	ED50 (mg/kg)	[95% confidence limits]
	Multiple schedule			
		Food 1	2.9	[2.7 – 3.2]
		Food 2	2.7	[2.6 – 2.9]
		Ethanol	2.0	[1.8 – 2.2]
	Multiple Concurrent schedule			
		Food	1.2	[1.0 – 1.4]
		Ethanol	4.3	[3.2 – 5.7]
Fluvoxamine				
	Multiple Concurrent schedule			
		Food	12.6	[10.2 – 15.5]
		Ethanol	9.3	[8.3 – 10.5]