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RESEARCH PAPER

Drug effects on multiple and concurrent schedules of ethanol- and food-maintained behaviour: context-dependent selectivity

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BACKGROUND AND PURPOSE

Drugs that more potently or effectively reduce ethanol-maintained behaviour versus an alternative are considered selective and are considered promising pharmacotherapies for alcoholism. Such results are often obtained using separate groups or multiple schedules where ethanol and the alternative are available alone or sequentially. Recently, we observed that when ethanol and food were available sequentially under a multiple schedule, fluvoxamine and varenicline were selective; yet this selectivity disappeared when ethanol and food were concurrently available.

EXPERIMENTAL APPROACH

We examined the generality of these findings by comparing doses of several drugs required to decrease ethanol- and food-maintained responding under a multiple schedule and under a concurrent schedule. Effects were determined for chlordiazepoxide, 2,5-dimethoxy-4-iodoamphetamine (DOI), meta-chlorophenylpiperazine (mCPP), morphine, naltrexone and d-amphetamine.

KEY RESULTS

Under the multiple schedule, ED_{50} values for decreases in ethanol-maintained responding were significantly different and lower than ED_{50}s for decreases in food-maintained responding (demonstrating selectivity) for each drug except for chlordiazepoxide (which was equipotent) and naltrexone (which did not affect responding). However, this selectivity vanished or even inverted under the concurrent schedule, such that ED_{50} values for decreasing ethanol- and food-maintained responding were not different (or, following DOI, the ED_{50} for food-maintained responding was lower than for ethanol-maintained responding).

CONCLUSIONS AND IMPLICATIONS

Results are consistent with those seen following fluvoxamine and varenicline administration, and suggest that selectivity is assay-dependent. These results indicate the need for careful interpretation of selective drug effects, especially when obtained in situations where ethanol or the alternative is the only programmed reinforcement available.

Abbreviations

5-HT, 5-hydroxytryptamine; DOI, 2,5-dimethoxy-4-iodoamphetamine; IQR, interquartile range; mCPP, meta-chlorophenylpiperazine

Introduction

Agents that reduce ethanol-maintained behaviour more or at lower doses than behaviour maintained by another event are thought to hold promise as pharmacotherapies for alcoholism (Koob and Weiss, 1990). The underlying concept for these types of studies is that drugs that reduce behaviour maintained by ethanol at lower doses or to a greater extent than behaviour maintained by another event (e.g. food) are potentially useful as therapeutics and act at neurobiological targets preferentially involved in ethanol reinforcement. For example, both fluvoxamine and varenicline decrease ethanol-maintained behaviour in one group of rats at lower doses than behaviour maintained by food or sucrose solution in another group of rats (Lamb and Järbe, 2001; Steensland *et al.*, 2007). These and other similar findings have led researchers to propose that the 5-hydroxytryptaminergic and nicotinic systems are preferentially involved in ethanol reinforcement, and that fluvoxamine and varenicline could be effective treatments for problematic drinking (Naranjo and Knoke, 2001; Lajtha and Sershen, 2010). Recent clinical studies have revealed that fluvoxamine has very limited effectiveness as a therapeutic while varenicline has been effective in clinical trials to date (Chick *et al.*, 2004; Litten *et al.*, 2013). Thus, the utility of this approach to identify effective therapies for problem drinking is unclear. This may be due to the fact that selective drug effects can be determined by many things, and therefore, isolating whether the selectivity is due to preferential effects on ethanol reinforcement is challenging.

There are a number of factors beyond preferential effects on ethanol reinforcement that could result in drugs selectively reducing behaviour maintained by ethanol versus an alternative. These include differences in ethanol exposure history, baseline response rates (e.g. Kelleher and Morse, 1968), reinforcement amounts (e.g. Cohen, 1986), or the context of the availability of ethanol and the alternative (whether they are available separately or concurrently).

We have previously undertaken studies to address each of these issues. We found that the selective effects on ethanol versus food-maintained behaviour we (and others) have repeatedly observed with separate groups are unlikely to be due to differences in ethanol exposure history, response rate or reinforcement amount. However, selective effects observed when ethanol is available alone appear to vanish or even invert when ethanol and food are concurrently available.

Previously, we matched ethanol exposure history, response rates and number of reinforcement deliveries for ethanol and food using a multiple schedule comprised of separate 5 min components where food and ethanol were each available after five correct responses (FR5). The selective effects of fluvoxamine and varenicline observed in separate groups were also apparent under this multiple schedule (Ginsburg *et al.*, 2005; Ginsburg and Lamb, 2014). Further, we found little evidence of rate-dependent effects of fluvoxamine (Ginsburg and Lamb, 2008; Lamb and Ginsburg, 2008). We also found little evidence that fluvoxamine effects depended on reinforcement amount of either food or ethanol (Lamb and Järbe, 2001; Ginsburg *et al.*, 2005; Ginsburg and Lamb, 2008; Lamb and Ginsburg, 2008). Thus, the selective effects of fluvoxamine are unlikely to be due to differences in ethanol exposure history, response rates or reinforcement amounts.

However, when we examined the effects of fluvoxamine and varenicline in rats responding under conditions of concurrent access to ethanol and food, we found no selective effect on ethanol-maintained responding (Ginsburg and Lamb, 2006b; 2013). In these studies, we used parameters that resulted in matched numbers of ethanol and food deliveries across a 30 min session, although response rates were significantly greater for food. We also used a concurrent VI schedule of ethanol and food delivery to match response rates across the session, and then varied the VI schedule to increase or remove food availability, and found that the potency of fluvoxamine to decrease ethanol-maintained responding depended only on whether food was concurrently available or not, rather than the density of food reinforcement (Ginsburg *et al.*, 2012). These studies provided further evidence that the selective effects seen in separate groups or under the multiple schedule were not due to differences in response rates or reinforcement amounts. However, these studies also showed that selective effects are more likely to be seen when ethanol and food are available separately than when they are concurrently available.

Here we investigated the generality of this finding by examining the effects of several drugs on responding maintained by ethanol or food. One group of rats was trained to respond on the multiple schedule we have previously described (Ginsburg *et al.*, 2005; Ginsburg and Lamb, 2006a), in which ethanol and food are available in separate components of the same session. Another group was trained under a concurrent schedule of food and ethanol reinforcement we have also previously described (Ginsburg and Lamb, 2006b; 2013). The relative potency of each drug to decrease ethanol- versus food-maintained responding was determined in each group in order to evaluate the generality of the inversion in selectivity we have seen for fluvoxamine and varenicline that depend on the concurrent presence or absence of food. If assay-dependent selectivity generalizes across many different drugs, it would suggest that the assay used must be considered when interpreting selective effects of drugs on ethanol-maintained behaviour as reflecting preferential effects on ethanol reinforcement.

The drugs selected for this study fall into four broad categories: chlordiazepoxide is a benzodiazepine, 2,5-dimethoxy-4-iodoamphetamine (DOI) and meta-chlorophenylpiperazine (mCPP) are 5-HT agonists, morphine and naltrexone are an opioid agonist and antagonist, and amphetamine is a stimulant that works by releasing monoamines and inhibiting uptake. Chlordiazepoxide is a first-line agent used to reduce symptoms of ethanol withdrawal, and might act as a substitution therapy, based on discriminative stimulus effects it shares with ethanol (De Vry and Slangen, 1986; Kumar *et al.*, 2009; Manasco *et al.*, 2012). However, a recent study using a multiple schedule of ethanol and food-maintained responding in rats failed to show robust selective effects (Amato *et al.*, 2012), consistent with effects of other benzodizepines in a similar study (Shelton and Balster, 1997). The 5-hydroxytryptaminergic system has been implicated in alcoholism and in the reinforcing effects of ethanol (Sari *et al.*, 2011). Both mCPP and DOI are 5-HT receptor agonists. mCPP can produce alcohol-like effects and reduce craving for

alcohol among alcoholics (Buydens-Branchey *et al.*, 1997; Gatch, 2005). This effect may depend on the particular alcoholic subtype, and this subtype specificity has led to the idea that 5-HT_2 receptors (for nomenclature see Alexander *et al.*, 2013) may be disregulated in some alcoholics (Krystal *et al.*, 1996; George *et al.*, 1997). DOI is a 5-HT_2 receptor agonist, and both DOI and mCPP have been shown to reduce ethanol-maintained responding, but not water-maintained responding when both ethanol and water were available and responding was predominately on the ethanol-associated lever (Maurel *et al.*, 1999). Similarly, the opioidergic system has also been implicated in the reinforcing effects of ethanol, and naltrexone, a μ opioid receptor antagonist, is one of only three medications approved for the treatment of alcoholism in the United States (Volpicelli *et al.*, 1992; Carmen *et al.*, 2004; Méndez and Morales-Mulia, 2008). Because selective effects on ethanol self-administration versus an alternative are thought to predict clinical effectiveness, we would expect naltrexone to selectively reduce responding maintained by ethanol. Finally, amphetamine is an indirect dopamine, noradrenaline and 5-HT agonist. While some have suggested the use of similar agents to reduce withdrawal effects among those in recovery from alcoholism (Rothman *et al.*, 2007), local administration of amphetamine into the striatum increases the duration of a drinking bout (Samson *et al.*, 1992; 1993), and would not be expected to selectively reduce ethanol- versus food-maintained behaviour.

Methods

Test systems used

Apparatus. Eight operant conditioning chambers were used (MedAssociates, Georgia, VT, USA), each equipped with an overhead house light, a rear stimulus light, two response levers, two lever lights (one above each lever), a dipper mechanism capable of delivering 0.1 mL of ethanol solution, and a pellet magazine capable of delivering 45 mg food pellets. Dipper presentation and food delivery occurred in a bin between the two levers. Each chamber was housed in a light- and sound-proof cubicle (MedAssociates). Chambers were interfaced with a computer. Commercially available software was programmed to coordinate light presentations, deliver reinforcement, and record lever responses (Med-PC, MedAssociates).

Drugs

Chlordiazepoxide hydrochloride, d-amphetamine sulphate and mCPP hydrochloride, were purchased from Sigma, Inc. (St. Louis, MO, USA). Morphine sulphate, naltrexone hydrochloride and DOI hydrochloride were provided by the National Institute on Drug Abuse (Bethesda, MD, USA). Each drug was dissolved in saline and administered i.p. at a volume of 1 $mL \cdot kg^{-1}$. Pretreatment times were as follows: amphetamine – 10 min; chlordiazepoxide, morphine, mCPP, and DOI – 15 min; and naltrexone – 20 min. Concentrations are expressed by weight of the salt.

Experimental design

Subjects. Two separate groups of male Lewis rats (Harlan, Inc., St. Louis, MO, USA) served as subjects. Rats arrived at our facility at approximately 6-weeks-old and were provided with food and water *ad libitum* for at least 1 week before the initiation of training. Subsequently, food was restricted and provided after operant sessions in order to maintain weights of 300–330 g for the duration of the study. Rats were housed under a 14/10 h light/dark cycle. All procedures were approved by the Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). All studies involving animals are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (Kilkenny *et al.*, 2010; McGrath *et al.*, 2010). Subject numbers varied for each treatment: for chlordiazepoxide, n = 6 rats per group were tested; for mCPP and DOI, n = 6 rats per group were tested, except for mCPP under the concurrent schedule, which was tested in n = 5 rats; morphine and naltrexone effects were tested in n = 8 rats in the multiple schedule (distinct from those used to test the other drugs under this schedule) and n = 6 rats in the concurrent schedule; and amphetamine effects were tested in n = 3 rats under the multiple schedule and n = 4 rats under the concurrent schedule.

Treatments. Doses of each drug or vehicle were administered on Tuesdays and Fridays. Vehicle was also administered on Thursday to confirm that behaviour remained stable across the weeks. Each dose was administered twice and effects were averaged for each subject. Doses of each drug were not administered in any systematic order across subjects. Generally, a dose range that encompassed doses with no effect to a dose that reduced responding by at least 50% was used. Doses of DOI that were without effect were mistakenly omitted from testing under the concurrent procedure. In the case of naltrexone, doses were tested up to a dose that blocks over 99% of available μ receptors (Walker *et al.*, 1994). The order of drug administration for each schedule is shown in Table 1.

Training. Training was the same for both groups up to the point where the final schedules were introduced. Rats were trained to press the ethanol-associated lever for a sucrose solution then sucrose was gradually faded out of and ethanol gradually faded into the solution, and the response requirement was increased (Samson *et al.*, 1988). Eventually, rats

Table 1

Order of drug testing under each schedule

Multiple schedule	Concurrent schedule
Naltrexone[a]	Naltrexone
Morphine[a]	Morphine
DOI	CDP
mCPP	Amphetamine
CDP	DOI
Amphetamine	mCPP

[a]Indicates tests were performed in a separate group from those below.

responded for 8% (w v^{-1}) ethanol in filtered water on a FR5 schedule of reinforcement during a 30 min session. Illumination of the light above the left lever indicated ethanol availability and completion of the response requirement on the indicated lever turned off the stimulus light, illuminated the rear stimulus light, and provided access to 0.1 mL of solution for 30 s. Once this behaviour stabilized, a second 30 min session was introduced immediately after ethanol self-administration in which rats were trained to press the other lever for food pellets (45 mg, Bio-Serv, New Brunswick, NJ, USA). Under this condition, the light above the right lever was illuminated and indicated food availability, and completion of the ratio requirement turned off the lever light and illuminated the overhead house light for 30 s, and released two food pellets into the hopper. Training proceeded until rats were performing stably on a FR5 schedule during each of two consecutive 30 min sessions (ethanol then food). At this point, rats moved to the appropriate final schedule.

Multiple schedule. The multiple schedule consisted of a 15 min session comprised of three separate 5 min components in which food, ethanol, and then food were available in succession. During the first component, the right lever light was illuminated, and five responses (FR5) on the right lever produced food. During the second component, the left lever light was illuminated and five responses (FR5) produced ethanol solution. Finally, during the third component, the right lever light was again illuminated and five responses (FR5) on the right lever again produced food. Completion of each fixed ratio was followed by a 30 s timeout signalled by the house light during which responses had no programmed consequence. 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. These parameters were selected because they result in similar numbers of responses for and deliveries of ethanol and food during each component. Training rats to respond for ethanol and food in separate sessions required 56–58 [range, median = 51, interquartile range (IQR) = (51–51)] sessions. Stability under the multiple schedule was achieved over the next 24 sessions in all rats. Testing occurred over the next 114–234 (range, median = 216, IQR = 199–233) sessions. Opioids were tested in a separate group, these rats each required 85 sessions to acquire stable responding for ethanol and food in separate sessions, and stability on the multiple schedule was achieved over the next 52 sessions for all rats. Testing occurred during a subsequent period of 36–38 (range, median = 37, IQR = 36–38) sessions.

Concurrent schedule. The concurrent schedule consisted of a single 30 min session in which both lever lights were illuminated to signal concurrent availability of food and ethanol. Completion of the ratio requirement on the left lever turned both lever lights off, illuminated the rear stimulus light, and provided ethanol solution access (0.1 mL) for 30 s. Completion of the ratio requirement on the right lever turned both lever lights off, illuminated the overhead house light, and provided two food pellets. Following completion of a fixed ratio for food or ethanol, a 30 s post-reinforcement timeout was present during which lever lights were turned off, and responses had no programmed consequences. There was no penalty for switching levers before the completion of a ratio, and completion of a ratio on one lever did not reset the ratio on the alternative lever. Initially, the response requirement was FR5 for both food and ethanol. Subsequently, FR requirements for food were increased for each rat so that the difference between the number of food and ethanol deliveries was less than 20% of the total number of deliveries earned. This resulted in three rats responding under FR35 for food, one responding under FR25 for food and two responding under FR20 for food. Ethanol was available under FR5 for all rats. Once the criterion was consistently met in a subject for 5 consecutive days, testing began. Training rats to respond for ethanol and food in separate sessions required 30–67 (range, median = 35, IQR = 35–36) sessions. Stability under the concurrent schedule was achieved over the next 46–76 (range, median = 66, IQR = 64–69) sessions in all rats. Testing occurred over the next 289–386 (range, median = 361, IQR = 331–369) sessions.

Measurements made and data analysis

Analysis. ED_{50} values for reductions in responding on each lever were calculated and compared for each schedule. ED_{50} values for food-maintained responding that fell outside of the 95% confidence limits for the ED_{50} for ethanol-maintained responding were considered significant ($P < 0.05$). Similarly, ED_{50} values for effects in the second food component (Food 2) that fall outside the confidence limits for the first food component (Food 1) were considered significantly different. ED_{50} values were calculated by expressing the number of responses on each lever following each active dose as a percentage of control responding following vehicle. A linear regression on the descending limb of the dose–effect curve was performed for each subject, and from this model, the dose at which a 50% reduction occurred was determined. These ED_{50} values were averaged to arrive at the group ED_{50}, and confidence limits were calculated by multiplying the SEM by the critical value of t for $P < 0.05$. In the case of DOI under the concurrent schedule, no dose tested reduced responding for the group by less than 50%, and ED_{50} values were not calculated. In the case of responding in the Food components (Food 1 and Food 2) of the multiple schedule following amphetamine treatment, no dose tested decreased responding by more than 50% for the group. In this case, the ED_{50} value was extrapolated.

Ethanol consumption. We have previously reported estimated blood ethanol levels that demonstrate that rats consumed ethanol earned in the multiple schedule (Ginsburg *et al.*, 2005). Here, we measured estimated blood ethanol levels in the rats responding under the concurrent schedule following a session when no drug or vehicle was administered, using a method we have previously described (Javors *et al.*, 2005). In this session, rats earned 10.2 ± 0.6 ethanol deliveries, or approximately 0.24 $g \cdot kg^{-1}$. Immediately after this session, breath ethanol levels were assessed in each rat and from these, blood levels were estimated at 0.58 ± 0.10 $g \cdot L^{-1}$. This demonstrates that rats were consuming earned ethanol.

Results

Baseline behaviour

Rats responding under the multiple schedule earned 9.1 ± 0.4 and 9.5 ± 0.5 food deliveries following vehicle in Food 1 and Food 2 components respectively. Rats earned 8.9 ± 0.3 ethanol deliveries, or 0.21 g·kg^{-1} ethanol per session. Following vehicle treatment in the concurrent schedule, rats earned 12.1 ± 1.9 food deliveries and 10.3 ± 0.9 ethanol deliveries, corresponding to 0.25 g·kg^{-1} per session.

Chlordiazepoxide

Under the multiple schedule, chlordiazepoxide reduced responding similarly in all three components (Figure 1A). As shown in Table 2, ED_{50} values were not different among the three components. Under the concurrent schedule, chlordiazepoxide also similarly reduced responding maintained by food and ethanol (Figure 1B). Table 2 shows that the ED_{50}s for reduction of ethanol- and food-maintained responding did not differ under the concurrent schedule.

mCPP

Under the multiple schedule, mCPP reduced ethanol-maintained responding more potently than food-maintained responding (Figure 2A). Table 2 shows that the ED_{50} during the ethanol component significantly differed from the ED_{50} in either food component, but ED_{50}s for the food components did not differ significantly. As shown in Figure 2B and in Table 2, under the concurrent schedule, ethanol-maintained responding tended to be decreased at lower doses than food-maintained responding. However, this effect was not significant.

DOI

Generally, DOI was more potent at reducing ethanol-maintained responding than food-maintained responding under the multiple schedule (Figure 2C). The difference in ED_{50} during Food 1 and ethanol components was significantly different; however, the potency difference between ethanol and Food2 components was not (Table 2). Likewise, the difference between the ED_{50} for Food 1 and Food 2 was significantly different. Unfortunately, DOI was more potent at decreasing behaviour under the concurrent schedule, and we failed to test ineffective doses. As noted in Table 2, the ED_{50} for both ethanol and food-maintained responding was less than 0.32 mg·kg^{-1}, but because of the truncated dose–effect function comparision of DOI effects was not possible.

Morphine

Under the multiple schedule, morphine was more potent at reducing ethanol- versus food-maintained responding (Figure 3A). As shown in Table 2, this potency difference was significant for comparisons with both Food 1 and Food 2. Food 1 and Food 2 ED_{50}s did not differ significantly from each other. Under the concurrent schedule (Figure 3B), for morphine the selectivity inverted such that the potency to reduce ethanol-maintained responding was lower than the potency to reduce food-maintained responding (Table 2).

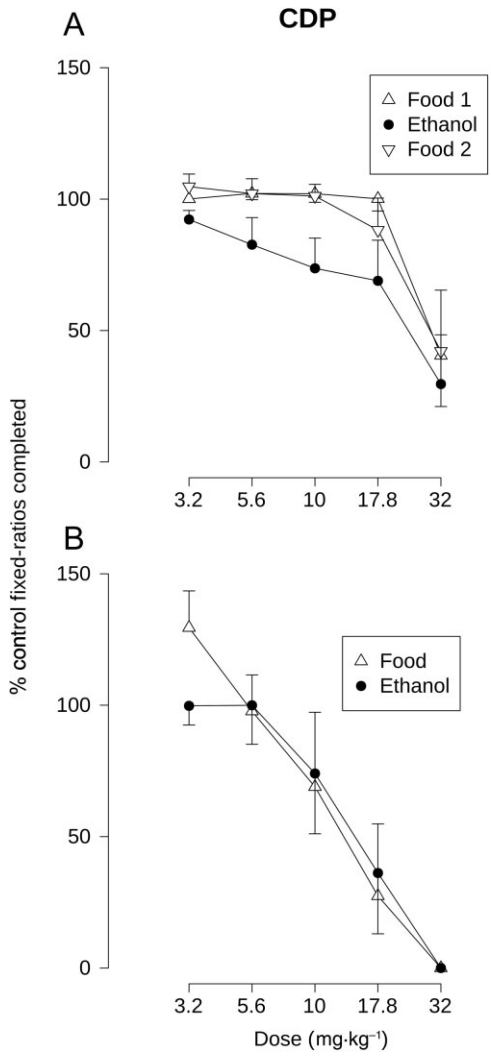

Figure 1

Effects of chlordiazepoxide on responding maintained by food or ethanol under the multiple schedule (A) and concurrent schedule (B). Points represent the mean ± SEM for $n = 6$ rats for each schedule, expressed as a % of responding following vehicle for each rat. Plots illustrate the number of fixed ratios completed as a function of the dose of chlordizaepoxide administered before the session. Filled symbols represent ethanol-maintained responding and open symbols represent food-maintained responding. Doses are plotted on a log scale.

Naltrexone

Naltrexone did not affect ethanol- or food-maintained responding in any component of the multiple schedule at any dose tested (Figure 3C). Because naltrexone appeared to have an effect in the concurrent schedule, but the effect did not allow ED_{50} calculation, we analysed the data using a repeated measures ANOVA with dose and maintaining event as within-subject factors. Under the concurrent schedule, naltrexone effects on ethanol- versus food-maintained responding were significantly different [$F(1,4) = 79.21$, $P < 0.001$]. Subsequent one-sample *t*-tests (comparing effects at each dose with 100) revealed that no dose of naltrexone resulted in

Table 2

ED_{50} values for each drug under each schedule

Multiple schedule			Concurrent schedule	
CDP	Food 1	17.56 (8.08–38.17)		
	Food 2	15.23 (6.44–36.02)	Food	18.43 (7.60–29.26)
	Ethanol	17.14 (12.00–24.49)	Ethanol	15.15 (7.57–22.73)
mCPP	Food 1	1.02 (0.57–1.84)		
	Food 2	1.40 (1.07–1.83)	Food	0.42 (0.23–0.79)
	Ethanol	**0.59 (0.36–0.95)**	Ethanol	0.24 (0.09–0.60)
DOI	Food 1	1.25 (0.80–1.95)		
	Food 2	0.74 (0.37–1.52)	Food	<0.32 mgkg^{-1}
	Ethanol	***0.37(0.17–0.80)***	Ethanol	<0.32 mgkg^{-1}
Morphine	Food 1	2.55 (2.03–3.18)		
	Food 2	2.60 (2.01–3.38)	Food	1.10 (0.27–4.48)
	Ethanol	**0.56 (0.13–2.51)**	*Ethanol*	*3.64 (2.88–4.60)*
Amphetamine	Food 1	8.80 (1.60–49.00)		
	Food 2	7.70 (3.10–19.40)	Food	0.76 (0.51–1.13)
	Ethanol	**0.70 (0.50–1.00)**	Ethanol	0.81 (0.43–1.54)

Bold, ethanol ED_{50} <food ED_{50} ($P < 0.05$); bold italic, ethanol ED_{50} < food ED_{50} (Food 1 only; $P < 0.05$); italic, food ED_{50} < ethanol ED_{50}.

significant changes in food-maintained responding compared with saline. However, 0.3 and 1.0 mg·kg^{-1} produced significant effects, decreasing ethanol-maintained responding ($P < 0.05$ after Benjamini and Hochberg, 1995 correction for multiple comparisons). While this effect is consistent with reports of the effectiveness of naltrexone at reducing problematic drinking (Volpicelli *et al.*, 1992), the lack of clear dose-dependent effects complicate the interpretation of this effect.

In order to ensure that naltrexone was antagonizing μ opioid receptors as expected, 3.2 mg·kg^{-1} naltrexone was administered 5 min before 10 mg·kg^{-1} morphine and behavioural sessions began 15 min later. As shown in Figure 3A and B (grey points), 3.2 mg·kg^{-1} naltrexone antagonized the effects of 10 mg·kg^{-1} morphine. This antagonism was similar for ethanol- and food-maintained responding under both the multiple and concurrent schedules.

Amphetamine

Under the multiple schedule, amphetamine was more potent at reducing ethanol- versus food-maintained responding in both food components (Figure 4A). As shown in Table 2, the ED_{50} during the ethanol component was significantly different from the ED_{50} in either food component, although ED_{50}s for the two food components did not differ. Under the concurrent schedule, amphetamine reduced ethanol- and food-maintained responding equipotently (Figure 4B; Table 2).

Discussion

Here we show that selective effects on ethanol- versus food-maintained responding observed under a multiple schedule are eliminated or inverted when ethanol and food are concurrently available. This result is consistent with our previous work showing similar results following fluvoxamine or varenicline treatment. These results indicate that this pattern generalizes to several drugs with different pharmacological targets. These results indicate that caution should be taken in interpreting reports of selective effects on behaviours maintained by different reinforcing events determined when each event is available separately.

Chlordiazepoxide is a benzodiazapine and is a first-line agent used to reduce symptoms of ethanol withdrawal and can produce ethanol-appropriate responding in ethanol drug discrimination procedures (De Vry and Slangen, 1986; Kumar *et al.*, 2009; Manasco *et al.*, 2012). However, pretreatment with chlordiazepoxide or the closely related drug diazepam does not differentially reduce responding maintained by ethanol versus saccharin (Shelton and Balster, 1997) or food (Amato *et al.*, 2012) under multiple schedules. This is consistent with results from the present study in which chlordiazepoxide reduced ethanol- and food-maintained responding with similar potency under the multiple schedule. However, the consistency with another report comparing effects of chlordiazeposixde on behaviour maintained by ethanol versus water or sucrose under concurrent schedules is less clear. Samson and Grant (1985) found evidence that chlordiazepoxide reduced responding maintained by ethanol at lower doses than responding maintained by either water or sucrose solution under conditions of concurrent access. While we did not find evidence of a selective effect of chlordiazepoxide under the concurrent schedule, their results are consistent with effects we saw with other drugs.

In the study by Samson and Grant (1985), responding for water was at relatively low levels, and responding occurred predominately on the ethanol-associated lever (75.5 ± 4.7%, mean ± SEM). When sucrose was concurrently available,

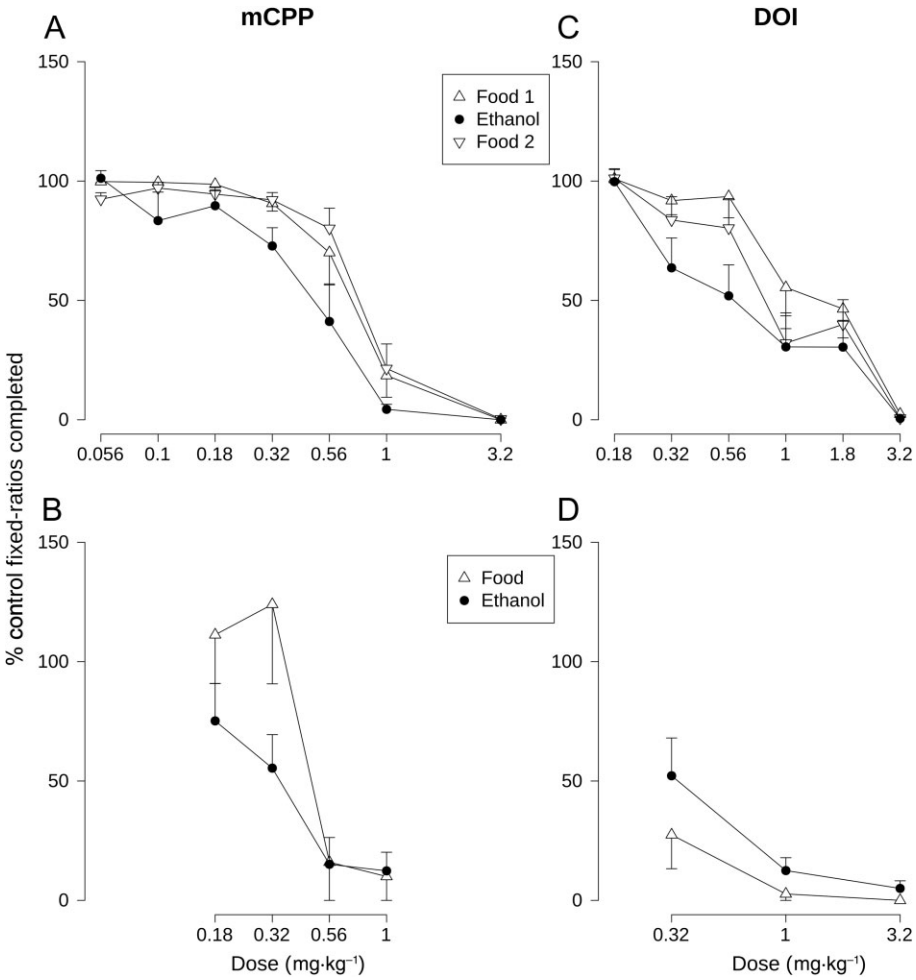

Figure 2

Effects of mCPP (A and B) and DOI (C and D) on responding maintained by food or ethanol under the multiple (A and C) and concurrent (B and D) schedules. Points represent the mean ± SEM for $n = 6$ rats for each schedule (except for mCPP effects under the concurrent schedule, which had $n = 5$ rats) expressed as a % of responding following vehicle for each rat. Plots illustrate the number of fixed ratios completed as a function of the dose of mCPP or DOI administered before the session. Filled symbols represent ethanol-maintained responding and open symbols represent food-maintained responding. Doses are plotted on a log scale.

responding was more evenly distributed between both levers (44.6 ± 4.2%). Thus, in that study, rats chose ethanol over water, while the choice of ethanol versus sucrose was similar, and the potency of chlordiazepoxide to reduce responding maintained by ethanol was greater when water was concurrently available than when sucrose was concurrently available. Thus, the potency of chlordiazepoxide to reduce responding for ethanol depended on the relative choice of ethanol versus the available alternative. We have previously demonstrated that the potency of fluvoxamine to reduce ethanol-maintained responding can depend on the presence or absence of concurrently available food (Ginsburg *et al.*, 2012). Thus, the potency of drugs to reduce ethanol-maintained responding may depend on the relative choice between ethanol versus the alternative that is available. Whether this might explain assay-dependent selective effects is unclear, but certainly plausible.

The 5-hydroxytryptaminergic system has been implicated in the aetiology of alcoholism (Sari *et al.*, 2011). Previously, several groups have shown that selective 5-HT reuptake inhibitors, which act as indirect 5-HT agonists, can selectively reduce ethanol-maintained responding in rats when no alternative is concurrently available, although this selective effect is abolished or inverted when food is concurrently available. Both of the direct 5-HT agonists DOI and mCPP have been shown to reduce ethanol-maintained responding in rats (Maurel *et al.*, 1999). When compared with concurrent responding reinforced by water delivery, these effects appeared to be selective. However, responding maintained by water occurred at very low rates, and was chosen less than ethanol. Others have shown similar results using free access to ethanol and water, but again water intake was substantially lower than ethanol intake (Buczek *et al.*, 1994). In the present study, both DOI and mCPP appeared to selectively reduce ethanol- versus food-maintained responding under the multiple schedule. When ethanol and food were concurrently available, this selectivity was not maintained for mCPP, and appeared to not be maintained for DOI, although as

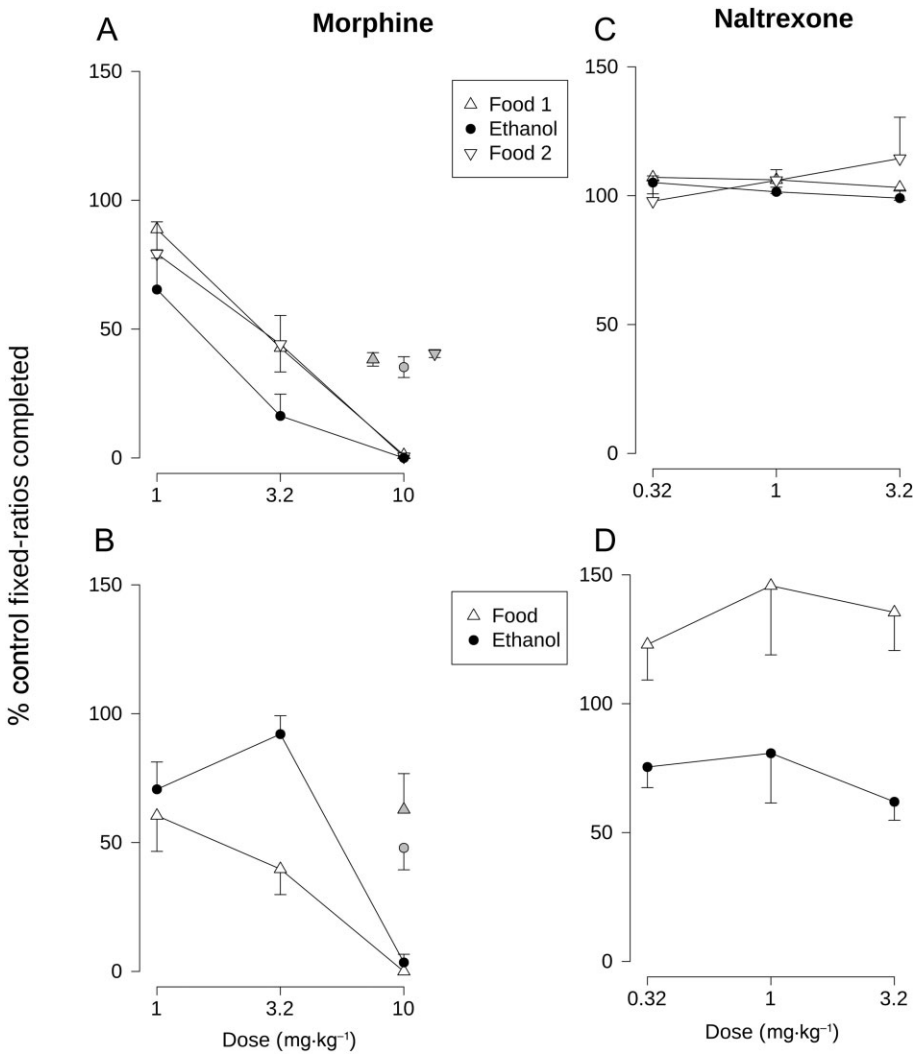

Figure 3

Effects of morphine (A and B) and naltrexone (C and D) on responding maintained by food or ethanol under the multiple (A and C) and concurrent (B and D) schedules. Grey points in the left panels represent effects following naltrexone pretreatment followed 5 min later by morphine treatment see text for details. Points represent the mean ± SEM for $n = 8$ rats in the multiple schedule and $n = 6$ rats in the concurrent schedule, expressed as a % of responding following vehicle for each rat. Plots illustrate the number of fixed ratios completed as a function of the dose of morphine, naltrexone or the combination of both administered before the session. Filled symbols represent ethanol-maintained responding and open symbols represent food-maintained responding. Doses are plotted on a log scale.

indicated, this interpretation is hindered by the limited doses of DOI tested in the concurrent schedule.

Morphine has been shown to reduce responding for ethanol in rats and monkeys. In rats, lower doses of morphine increased ethanol-maintained responding while higher doses decreased it (Schwarz-Stevens *et al.*, 1992; Hodge *et al.*, 1995). When compared with effects on responding reinforced with concurrently available water, these effects appeared selective. Very similar results have been reported in rhesus monkeys using an operant task in which ethanol and water were concurrently available (Williams *et al.*, 2001). However, responding for water in each of these studies was extremely low and was chosen less than ethanol. Thus, these results seem consistent with ours, where morphine exerted selective effects on ethanol-maintained behaviour under the multiple schedule, but non-selectively reduced ethanol- and food-maintained responding under the concurrent schedule.

We found that under the multiple schedule, naltrexone did not affect responding maintained by ethanol or food across the dose range tested, similar to results reported by Bienkowski *et al.* (1999). However, under the concurrent schedule, naltrexone modestly reduced ethanol-maintained responding, but did not affect food-maintained responding, although this effect was not dose-dependent across the range of doses studied. Other groups have also demonstrated that naltrexone can reduce ethanol-maintained responding (e.g. Ulm *et al.*, 1995). Still others have observed non-selective and similar reductions in responding maintained by ethanol versus a sweetened solution in separate groups of rats and in a multiple schedule in monkeys (Shelton and Grant, 2001;

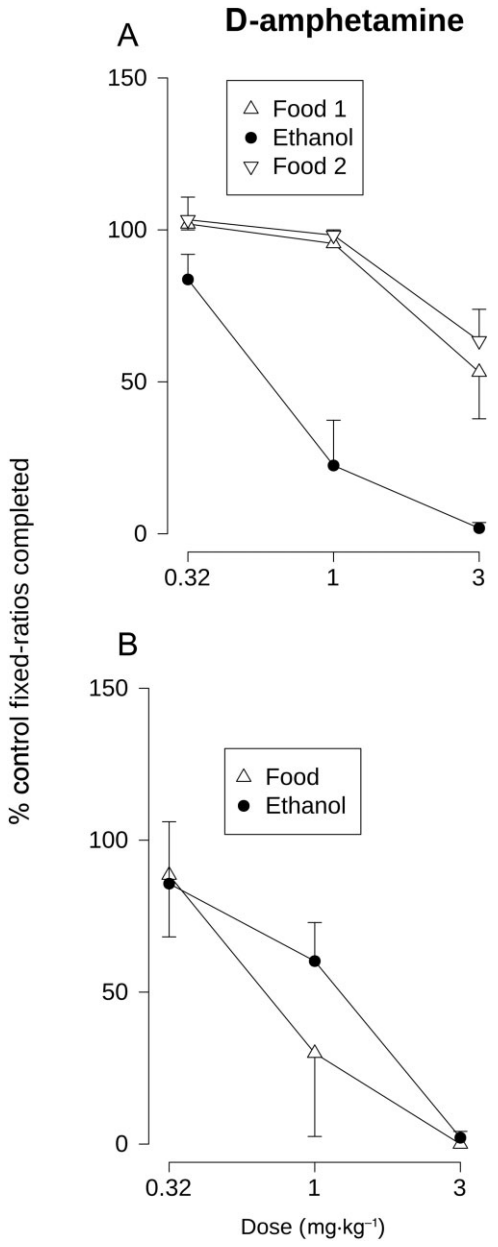

Figure 4

Effects of amphetamine on responding maintained by food or ethanol under the multiple (A) and concurrent (B) schedules. Points represent the mean ± SEM for $n = 3$ rats in the multiple schedule and $n = 4$ rats in the concurrent schedule, expressed as a % of responding following vehicle for each rat. Plots illustrate the number of fixed ratios completed as a function of the dose of amphetamine administered before the session. Filled symbols represent ethanol-maintained responding and open symbols represent food-maintained responding. Doses are plotted on a log scale.

Steensland *et al.*, 2007). The reason for these disparate results remains unclear, although there are several possibilities. Ethanol concentration, naltrexone dose and pharmacokinetics, and species have been suggested (Boyle *et al.*, 1998).

Other factors, including the route of administration, daily food ration and feeding time relative to the experimental session could have blunted the potency of naltrexone to reduce ethanol- and food-maintained behaviour. There is evidence that the route of administration can affect the potency of naltrexone to reduce ethanol self-administration, with i.p. administration (as used in the present study) being less potent than s.c. administration (Williams and Broadbridge, 2009). The acute effects of naltrexone may also be blunted in food-restricted rats (as were used in the present study) compared with *ad libitum* fed rats (Williams, 2007). Recently, data consistent with this result were reported by Paronis (Paronis, 2013), where naltrexone (up to 10 mg·kg^{-1}) had very modest effects on behaviour maintained by concurrent availability of milk and milk + ethanol in food-restricted rats. Further, the ability of naltrexone to reduce ethanol- and food-maintained responding diminishes as the availability of food or ethanol outside of the experimental session increases (Nestby *et al.*, 1999; Carroll *et al.*, 2000). In the present study, food was available after the session, while ethanol was not. Thus, these results suggest that the availability of food after the session could affect the ability of naltrexone to decrease responding maintained by food as well as by ethanol when both are available in the same session. Together, these factors may have combined to substantially blunt the effect of naltrexone in the present study.

While a difference in the availability of food versus ethanol outside the session might explain the lack of naltrexone effects in the multiple schedule, it does not explain the change in selectivity of drugs between the multiple and concurrent schedules, as the availability of food and ethanol outside the session was the same for both studies. However, it does highlight a potentially important factor in drug effects on behaviour maintained by two different outcomes. The extent to which the availability of either reinforcer outside the session influences drug effects has received very limited attention from behavioural pharmacologists.

In the present study, rats earned 0.21 g·kg^{-1}5 min^{-1} (multiple schedule) or 0.24 g·kg^{-1}30 min^{-1} (concurrent schedule) ethanol. Here, rats responding under the concurrent schedule achieved an average blood ethanol concentration of 0.58 g·L^{-1}, which is similar to levels achieved by adult male P-rats (0.50–0.62 g·L^{-1}) during unscheduled access in the dark phase, when consumption peaked (Murphy *et al.*, 1986). However, scaled for time, the amount consumed by rats in the present study is lower than the amount of ethanol earned by rats considered to consume high levels of ethanol, such as high-alcohol drinking (HAD) 1 and HAD2, alcohol preferring lines of rats, which can earn as much as 0.71–0.89 g·kg^{-1}30 min^{-1} (Rodd *et al.*, 2003; Bell *et al.*, 2006; Oster *et al.*, 2006). Recently, far higher blood ethanol levels have been achieved in these alcholol-preferring rats (Bell *et al.*, 2013). Longer periods of greater ethanol consumption could affect the results reported here. Thus, it remains unclear whether (but likely that) the assay-dependent difference in drug effects on ethanol- versus food-maintained responding observed here would remain in rats that consumed even higher amounts of ethanol, particularly during relatively short access periods.

Taken together, these data demonstrate that the relative potency of a treatment to reduce ethanol- versus food-maintained responding can depend on the schedule arrangement. In general, a selective effect on ethanol-maintained

responding was more likely to be observed under the multiple schedule, where programmed, alternative reinforcement was not concurrently available. Selectivity appears to decrease as the choice of ethanol versus an alternative becomes more similar. This is true for drugs that are thought to act at sites involved in ethanol reinforcement (DOI, mCPP), as well as drugs that would *not* be expected to be effective therapies (morphine, amphetamine). However, the effect was not present for a drug with substantially similar targets and discriminative stimulus effects to ethanol (chlordiazepoxide). While not every drug tested showed this selective effect under the multiple schedule, for those that did, the selectivity was abolished or even inverted under the concurrent schedule. These results indicate that reports of potential medications or other pharmacological tools exerting selective effects on behaviour maintained by ethanol versus an alternative should be interpreted with caution, especially if ethanol and the alternative were not concurrently available. Further work is necessary to understand why the schedule arrangement can affect relative potency in this manner, and more importantly, how potency differences under different behavioural procedures should be interpreted.

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Conflict of interest

The authors have no conflict of interest.

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