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Effects of disulfiram on choice behavior in a rodent gambling task: association with catecholamine levels

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Abstract

Rationale—Gambling disorder is a growing societal concern, as recognized by its recent classification as an addictive disorder in the DSM-5. Case reports have shown that disulfiram reduces gambling-related behavior in humans.

Objectives—The purpose of the present study was to determine whether disulfiram affects performance on a rat gambling task, a rodent version of the Iowa gambling task in humans, and whether any changes were associated with alterations in dopamine and/or norepinephrine levels.

Methods—Rats were administered disulfiram prior to testing on the rat gambling task or prior to analysis of dopamine or nor-epinephrine levels in brain homogenates. Rats in the behavioral task were divided into two subgroups (optimal vs suboptimal) based on their baseline levels of performance in the rat gambling task. Rats in the optimal group chose the advantageous strategy more, and rats in the suboptimal group (a parallel to problem gambling) chose the disadvantageous

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Conflict of interest The authors declare that they have no conflicts of interest.

strategy more. Rats were not divided into optimal or suboptimal groups prior to neurochemical analysis.

Results—Disulfiram administered 2 h, but not 30 min, before the task dose-dependently improved choice behavior in the rats with an initial disadvantageous “gambling-like” strategy, while having no effect on the rats employing an advantageous strategy. The behavioral effects of disulfiram were associated with increased striatal dopamine and decreased striatal norepinephrine. *Conclusions* These findings suggest that combined actions on dopamine and norepinephrine may be a useful treatment for gambling disorders.

Keywords

Norepinephrine; Dopamine; Gambling; Antabuse

Introduction

At some point in their lives, 1.6% of adults in the USA and Canada meet the DSM criteria for gambling disorder (GD) (American Psychiatric Association 2013). Underscoring its prevalence and cost to society, GD has been re-classed as an addictive disorder in the recently released DSM-5. Despite this, there are no pharmacological treatments for GD that are approved by the FDA. Clinical trials of antidepressants, mood stabilizers, antipsychotics, glutamatergic agents, and opioid antagonists have revealed promise only for the use of opioid antagonists (Grant et al. 2014). However, the paucity of pharmacological approaches available suggests that a greater understanding of the neurobiological mechanisms that control gambling is needed to better inform the development of more effective treatments.

Disulfiram (also known as Antabuse®), discovered in the 1920s, has been used as a treatment for alcoholism (Suh et al. 2006). Recently, disulfiram has been shown to be effective as a potential therapy for other addictions, namely, cocaine abuse (Carroll et al. 2004; George et al. 2000; Petrakis et al. 2000) (see Sofuoglu and Kosten (2006) for review). Based on these observations, it was suggested that disulfiram may be an effective treatment for GD (Mutschler et al. 2010a). Indeed, a case report by Muller et al. (2011) reported that, even though no changes in relapse to gambling were observed, cravings for gambling were reduced by disulfiram. Indeed, disulfiram has shown promise in reducing gambling in one case report (Mutschler et al. 2010b) but not another (Muller et al. 2011).

Disulfiram is effective as a treatment for alcohol use disorder because it inhibits aldehyde dehydrogenase, resulting in acetaldehyde accumulation and “hangover”-like effects that deter further use. Interestingly, the effects of disulfiram on cocaine use have been noticed in humans (Kosten et al. 2013; Sofuoglu and Kosten 2006) and rats (Schroeder et al. 2010) even without alcohol consumption. Thus, the mechanism of action may not be similar to that underlying its efficacy for the treatment of alcoholism. Instead, the effects of disulfiram on cocaine abuse and gambling disorder may be explained by its actions as an inhibitor of the catecholamine biosynthetic enzyme dopamine β -hydroxylase (DBH), and its subsequent actions on dopamine (DA) and norepinephrine (NE). Indeed, administration of disulfiram or the selective DBH inhibitor nepicastat depletes NE and increases DA concentrations in the

rat (Goldstein and Nakajima 1967; Karamanakos et al. 2001; Musacchio et al. 1966; Schroeder et al. 2010) and mouse (Bourdlat-Parks et al. 2005) brain.

Pioneering reports have shown that peripheral measures of DA are elevated when both healthy and problem gamblers gamble (Meyer et al. 2004; Shinohara et al. 1999), while exposure to DA agonists in Parkinson's disease patients also increases the risk of developing impulse control disorders (Seedat et al. 2000; Weintraub et al. 2006). Interestingly, recent results indicate that modulating dopaminergic transmission modulates gambling activities in rodents (Cocker et al. 2011; Cocker et al. 2013; Zeeb et al. 2009). With respect to NE, interest in its role in addiction is re-emerging following earlier beliefs that it was not involved in drug seeking (Weinshenker and Schroeder 2007). For example, engaging in casino gambling elevates activity of the hypothalamic-pituitary axis in gamblers, as indicated by increased plasma levels of NE and cortisol and increased heart rate (Meyer et al. 2004), while higher levels of NE in pathologic gamblers have been found (Roy et al. 1988), as have deficits in NE receptor sensitivity in pathological gamblers (Pallanti et al. 2010). In animal models, DBH inhibitors and NE antagonists reduce cocaine seeking (Gaval-Cruz and Weinshenker 2009; Schroeder et al. 2010; Schroeder et al. 2013; Wee et al. 2008; Zhang and Kosten 2005), alcohol intake (Colombo et al. 2014; Walker et al. 2008), heroin self-administration (Greenwell et al. 2009), nicotine place preference (Forget et al. 2009), and nicotine self-administration (Forget et al. 2010). In humans, findings from PET imaging have revealed correlations between impulsivity in gamblers and binding potential of either [¹¹C]-raclopride (Clark et al. 2012), a D2/D3 antagonist, or [¹¹C]-(+)-PHNO (Boileau et al. 2013), a D2/D3 agonist. Interestingly, the correlation between impulsivity and binding potential in the striatum was negative for [¹¹C]-raclopride, but positive, for [¹¹C]-(+)-PHNO in the substantia nigra, indicating different roles of D2 (striatum) and D3 (substantia nigra) receptors in this disorder (for discussion of differentiation between D2 and D3 receptors, see Le Foll et al. (2014)).

The purpose of the present study was to evaluate the effects of disulfiram in a rat gambling task, and on DA and NE levels in the brain. The rat gambling task (rGT) used in this report is based on the Iowa gambling task (IGT) (Bechara et al. 1994) and, like the IGT, animals must learn to select among various options such as to maximize the total amount of rewards earned over numerous trials. In this task, animals must learn to inhibit “tempting” options with high rewards, as these high rewards are also associated with higher probabilities and durations of a punishing time-out. Smaller rewards are associated with lower probabilities and durations of time-out leading to more overall rewards in a given session. In this study, rats were divided into two groups, those that chose the advantageous option more (optimal group) and those that chose the disadvantageous option more (suboptimal group), to reflect the fact that gamblers choose the disadvantageous option more (Linnet et al. 2006; Petry 2001). For this study, the frontal cortex and striatum were selected for study due to their demonstrated role in the “exploitation” phase of the Iowa gambling task (de Visser et al. 2011). It is hypothesized that disulfiram's ability to inhibit DBH, and thereby decrease NE and increase DA, would reduce gambling-like behavior by decreasing the proportion of disadvantageous choices and increasing the proportion of advantageous choices, especially in the suboptimal group. This investigation will shed some light on a novel mechanism (DBH inhibition) mediating gambling, in the absence of any more selective DBH inhibitors

(nepicastat is no longer available commercially). Different pre-treatment times were chosen such that behaviorally relevant changes in neurochemical findings could be distinguished from those that have no behavioral sequelae in this task.

Methods

Subjects

Subjects were 66 male Long-Evans rats (Charles River, St. Constant, QC) weighing 350–550 g at the time of the study (the different weights represent the different ages of the various cohorts of rats). All animals were single-housed in a climate-controlled environment on a 12-h reverse light/dark cycle (lights off 8.00 am–8.00 pm) so that behavioral testing occurred during the active phase of the animal's circadian rhythm. During behavioral testing, rats were maintained on 18–20 g of rat chow per day, given after their experimental session. This amount of food is less than the free feeding amount but still enough to promote health and weight gain over time.

The rat gambling task

Behavioral testing occurred in five-hole operant conditioning chambers (Med Associates, Roanoke, VA) as previously described (Pushparaj et al. 2015). In the rGT, animals learn about which of four response options has which size of reward and probability/duration of a time-out punishment (see Table 1).

In the rGT, animals initiate each trial by making a nosepoke response at the food tray (with a traylight). This triggers the start of a 5-s inter-trial interval (ITI) before the stimulus lights are turned on in all of the four active holes. A response at one of the illuminated holes results in the offset of all the stimulus lights and either delivery of the pre-set amount of reward for that hole option or the start of the time-out “punishment” period. The following is a summary of possible outcomes:

1. **Reward:** If the animal is rewarded on any trial, food delivery is signaled by onset of the traylight which remains illuminated until the animal collects the reward. Responding at the food tray also initiates the start of the next trial. The size of reward and punishment probability/duration is given in Table 1.
2. **Punishment:** If the animal is punished, the traylight remains off until the end of the time-out period, whereupon this light is turned on to signal that the animal can initiate the next trial. During these time-out periods, the stimulus light within the hole chosen on that trial flashes at a frequency of 0.5 Hz. The size of reward and punishment probability/duration is given in Table 1.
3. **Omission:** If the animal fails to make a response within 10 s after the four stimulus lights are turned on, the stimulus lights are turned off, the trial is scored as an omission, and the traylight is illuminated to signal the beginning of the next trial. Animals are not punished for omitting trials.
4. **Premature responses:** Premature responses made at the array during the ITI are punished by a 5-s time-out period during which no further trials can be initiated.

The duration of the time-out is signaled by illumination of the houselight and terminated by onset of the traylight so that animals can begin another trial.

5. Perseverative responding: Perseverative responding is defined as continued responding in the response hole after making a choice in that hole. It is not associated with any consequences, but recorded.

Training continued for 30 sessions. By this time, responding for the various options had reached a plateau. Animals were randomly assigned to one of two groups (A or B), receiving a different configuration of response outcomes in the holes. These are given in Table 1.

After training, rats were administered disulfiram as described below. Each disulfiram test day was separated by at least 2 days of responding on the rGT. Some rats had to be retested under some doses due to equipment malfunction. Each session was 30 min in length and testing was 5–7 days a week throughout the experiment.

Drug

Disulfiram (Sigma-Aldrich, Oakville, ONT, Canada) was administered acutely at doses of 0, 10, 25, 50, 75, or 100 mg/kg, i.p., and given in a counterbalanced order. Disulfiram was sonicated in sterile saline and injected as a suspension. Doses of disulfiram were chosen based on previous studies with cocaine in rats in which effects on brain catecholamine levels and cocaine-seeking behavior were found at 100 mg/kg but not 10 mg/kg (Schroeder et al. 2010). Pre-treatment times of either 30 or 120 min were chosen based on evidence that disulfiram distribution after i.p. administration is maximal after 0.5–1 h (Faiman et al. 1980; Goldstein and Nakajima 1967); (Schroeder et al. 2010). By selecting pre-treatment times that are expected to have different neurochemical correlates, some insight into the mechanism of action of any effects on the rGT can be gained.

Neurochemical study

Rats were injected with disulfiram (0, 10, or 50 mg/kg, i.p.) either 30 or 120 min prior to euthanasia with carbon dioxide. A separate group of rats was used for each dose at each pre-treatment interval, for a total of six groups. DA and NE levels were measured from the same dose/pre-treatment interval. Brains were then quickly removed and the whole dorsal striatum and whole frontal cortex were dissected over ice. For dissection of the frontal cortex, the olfactory bulb was removed and then a single coronal cut at approximately bregma + 1.5 mm was made, just anterior to the forceps major corpus callosum and striatum. For the striatum, after removal of the hippocampus and thalamus, the striatum was visually identified and removed from the underlying corpus callosum and external capsule with a spatula from an area approximately described by the following coordinates: DV, + 3 to + 8; ML, + 1.5 to + 5; AP, + 2 to –1.5 mm (Paxinos and Watson 1986). Brain tissue was placed in individual eppendorfs and these were placed immediately on dry ice. The 10-mg/kg dose was selected as a control because it produced no significant effects in the behavioral test. The 50-mg/kg dose was selected because it resulted in the peak of the dose-response curve observed in the behavioral test. The effect of higher doses on DA and NE was previously examined (Schroeder et al. 2010).

Tissue samples were stored at -80°C until analysis. Frozen tissue samples were initially prepared by sonication in 20 volumes of ice-cold 0.1 N perchloric acid, followed by centrifugation at $16,100g$ for 30 min at 4°C . A 200- μl aliquot of the resulting supernatant was then filtered through a 0.45- μm PVDF membrane (Sun-SRi, Rockwood, TN, USA) at $4000g$ for 10 min at 4°C and then transferred to 11-mm autosampler vials. Levels of DA and NE were quantified using high-performance liquid chromatography coupled with electrochemical detection using procedures similar to those described previously (Song et al. 2012). Briefly, the system consisted of an ESA MD-150 $\times$ 3.2 mm column, an ESA 5020 guard cell, and an ESA 5600A Coularray detector with an ESA 6210 detector cell (ESA, Chelmsford, MA, USA). The guard cell potential was 475 mV, and the analytic cell potentials were set at -175 , 100, 350, and 425 mV. Samples were eluted at a flow rate of 0.4 ml/min with a mobile phase consisting of 1.7 mM 1-octanesulfonic acid sodium, 75 mM NaH_2PO_4 , 0.25% triethylamine, and 8% acetonitrile at pH 2.9. CoulArray Data Station software (version 3.10, ESA) was used to generate chromatograms and calculate area under the curve for norepinephrine and dopamine peaks derived from experimental samples and experimenter-prepared standards from which unknown concentrations could be interpolated.

Rats and treatments

See Table 2 for a summary. This study consisted of two replications of the gambling study to increase the power and also to provide a counterbalanced order of the pre-treatment times of disulfiram. In the first study, 18 rats received counterbalanced doses of disulfiram 30 min prior to testing on the rGT. After completion of the dose-response, they then received all doses 120 min prior to testing on the rGT. In the replication, 23 rats received all of the disulfiram doses at 120 min prior to testing on the rGT. After completion of this dose-response analysis, they received all doses of disulfiram 30 min prior to testing on the rGT. These 23 rats in the replication were previously used in a gambling study in which they received naltrexone (Di Ciano and Le Foll 2016), and a subset of these rats also received some dopamine ligands as a pilot study (Di Ciano et al. 2015). These rats were then used in the neurochemical study; they received either 50 or 10 mg/kg doses. Additional rats ($n = 25$) were also used in the neurochemical study and were previously administered opiate ligands (Gueye et al. 2016); they received either 10 or 0 mg/kg doses. For the first replication (in which the 30-min pre-treatment time preceded the 120-min pre-treatment time), disulfiram administration began after the initial 30-day training period. For the replication (in which the 120-min pre-treatment time preceded the 30-min pre-treatment time), initial drug treatments started after 30 days of training, but disulfiram administration began after 84 rGT sessions.

Data analyses

The measures chosen are based on Zeeb et al. (2009), which are predicated by studies of the five-choice serial reaction time task. For a discussion of the interpretation of these measures, see Robbins (2002). The measures collected are given below:

1. The percentage of trials on which an animal chose a particular option. This was calculated according to the following formula: number of choices of a particular option/number of trials (including omissions) $\times$ 100
2. The total number of trials initiated

3. The total number of omissions made expressed as a percent ($\#omissions/\#trials$ including omissions $\times 100$)
4. The percentage of premature responses was calculated as the number of premature responses/total number of trials initiated $\times 100$
5. The latency to make a choice after initiation of a trial
6. The latency to collect reward after making a response choice
7. The perseverative responses on punished trials was calculated as the fraction of the total punishment duration
8. Perseverative responses on rewarded trials was calculated as the fraction of the total number of trials rewarded

In the rGT, rats must learn the optimal strategy to obtain the most number of reinforcements per session as possible. To achieve this, rats must inhibit the choices that produce large rewards because this also results in the greatest punishment; choices with smaller rewards produce more pellets per session. In the rGT, there are four choice options (P1, P2, P3, and P4) that produce either 1, 2, 3, or 4 pellets, respectively. If a rat chooses exclusively one option, the number of pellets possible would be greatest with P2 (411), then with P1 (295), then P3 (135), with P4 (99) producing the least number of pellets (see Table 1) (Zeeb et al. 2009). Thus, P1 and P2 are advantageous strategies, while P3 and P4 are disadvantageous “tempting” strategies. In the Iowa gambling task, it is known that pathological gamblers choose these “tempting” options (Linnet et al. 2006; Petry 2001). Thus, the data in the present study were analyzed separately for rats that made the P1 and P2 options combined more than the combination of P3 and P4. To accomplish this, the percent choice of P1 was added to the percent choice of P2 to comprise the advantageous option, while P3 and P4 percent choices were summed to produce the disadvantageous option. Rats that made more advantageous choices under vehicle were analyzed as the optimal group, while those that made more disadvantageous than advantageous choices under vehicle were the suboptimal group. Since the choice of advantageous and disadvantageous strategies may model a form of GD and this may change over time with experience, optimal and suboptimal groups were created separately for each of the 30- and 120-min pre-treatment times. This resulted in some “borderline” rats switching from optimal to suboptimal (4) or suboptimal to optimal (2) groups.

To demonstrate stability of responding across days, the percent of responding for the advantageous choice on the day prior to the first disulfiram injection was compared to choice on the day prior to the last disulfiram injection with paired samples *t* tests. This was done separately for each of the 120- and 30-min pre-treatment intervals.

For the effects of disulfiram on behavior, advantageous responses were analyzed with three-way repeated measure group (optimal vs suboptimal) $\times$ dose (six levels) $\times$ replication (two levels) ANOVAs with group and replication as the between-subject factors, followed by simple effects comparing vehicle to each dose. Since advantageous choice and disadvantageous choice are not independent, only the advantageous choice was analyzed. For analysis of choice behavior, data were analyzed with a dose (six levels) $\times$ group (two

levels) $\times$ choice (four levels; P1, P2, P3, P4) $\times$ replication (two levels) ANOVA. All statistical analyses were conducted using SPSS.

To determine whether there were time-dependent changes in percent of advantageous responses across the session, data were divided into time bins. The percent of advantageous choices during the first, second, third, and fourth quarter of the session were analyzed with a bin (four levels) $\times$ dose (six levels) $\times$ group (two levels) ANOVA. This was conducted separately for the 30- and 120-min pre-treatment intervals.

Neurochemical data were analyzed with two-way dose (three levels) $\times$ replication (two levels) between-subject ANOVAs separately for each brain area (frontal cortex or striatum), neurotransmitter (DA or NE), and pre-treatment interval (30 or 120 min). Significant one-way ANOVAs were followed by Bonferroni-corrected *t* tests between vehicle and each dose to determine whether differences existed between vehicle and either the 10- or 50-mg/kg doses.

Results

Two rats were excluded from behavioral analyses due to problems with the behavioral testing equipment, resulting in a final sample size of 39. All rats received all treatments. The final sample size was $n = 29$ for the optimal group and $n = 10$ for the suboptimal group for the 120-min pre-treatment and $n = 27$ for the optimal group and $n = 12$ for the suboptimal group for the 30-min pre-treatment.

Stability of responding over days

On the day prior to the first disulfiram injection administered 30 min prior to testing, the percent responding on the advantageous lever was 70 ± 4.9 (mean $\pm$ SEM), and it was 63 ± 4.7 on the day prior to the last injection. Comparison of responding on these 2 days with paired samples *t* test did not reveal a significant difference. On the day prior to the first disulfiram injection administered 120 min prior to testing, rats made on average ($\pm$ SEM) $68 \pm 4.3\%$ of their responses on the advantageous lever. On the day prior to the last injection of disulfiram at the 120-min pre-treatment time, rats made on average $67 \pm 4.3\%$ of their responses on the advantageous lever. The difference between these days was not significantly different. Data were not shown.

Advantageous responses

Following disulfiram administration at the 120-min pre-treatment interval (Fig. 1), a dose (six levels) $\times$ group (suboptimal, $n = 10$; optimal, $n = 29$) $\times$ replication ANOVA revealed a significant dose $\times$ group interaction ($F(5, 175) = 4.038$, $p_{GG} = 0.007$), suggesting that the effects of dose were different in each of the optimal or suboptimal groups. Simple effects of vehicle to each dose revealed significant differences between vehicle and the 25-mg/kg ($F(1, 9) = 5.501$, $p = 0.044$) and 50-mg/kg doses ($F(1, 9) = 7.352$, $p = 0.024$) for the suboptimal group. The difference between vehicle and the 75-mg/kg dose approached significance ($p = 0.089$). No comparisons were significant for the optimal group. A dose $\times$ group $\times$ replication ANOVA revealed no effects for the 30-min pre-treatment interval (Fig. 1; optimal, $n = 27$; suboptimal, $n = 12$).

Choice behavior

Following disulfiram administration at the 120-min pre-treatment interval, a dose $\times$ choice $\times$ group $\times$ replication ANOVA revealed a choice $\times$ dose $\times$ group interaction (Fig. 2; $F(15, 525) = 2943$, $p = 0.015$). Choice $\times$ dose ANOVAs separately for each group revealed only an effect of choice, but no interaction, for the optimal group ($F(3, 84) = 118.330$, $p < 0.001$) and suboptimal group ($F(3, 27) = 12.036$, $p < 0.001$), suggesting that rats made different numbers of responses for each of the choices, with no effect of disulfiram. Following the 30-min pre-treatment interval, an ANOVA revealed a choice $\times$ dose $\times$ group interaction ($F(15, 525) = 1.795$, $p = 0.032$). Further analysis with choice $\times$ dose ANOVAs for each group revealed a significant interaction for the optimal group ($F(15, 375) = 2.370$, $p_{GG} = 0.044$). Follow-up analysis on the effect of dose for each choice revealed no significant effects of dose for any choice.

Time bins

Analyses of the time bin data with bin $\times$ dose $\times$ group ANOVAs revealed no overall interactions for either the 30-or 120-min pre-treatment interval. This suggests that responding was stable across the session. For the 120-min pre-treatment interval, a dose $\times$ group interaction was revealed ($F(5, 185) = 2.762$, $p = 0.020$). This is consistent with the findings reported above and was not further analyzed.

Other measures

One hundred twenty-minute pre-treatment—For the 120-min pre-treatment condition, group $\times$ dose $\times$ replication ANOVAs revealed no interactions but a main effect of group only for the number of trials ($F(1, 35) = 15.058$, $p < 0.001$) and the number of premature responses ($F(1, 35) = 5.305$, $p = 0.027$), indicating that the optimal group initiated more trials while the suboptimal group made more premature responses.

Thirty-minute pre-treatment—For the 30-min pre-treatment condition, group $\times$ dose $\times$ replication ANOVAs revealed no interactions but an effect of group only for the number of trials ($F(1, 35) = 22.105$, $p < 0.001$) and the number of premature responses ($F(1, 35) = 6.679$, $p = 0.014$), indicating that the optimal group initiated more trials while the suboptimal group made more premature responses. For omissions, a dose $\times$ group $\times$ replication interaction was found ($F(5, 175) = 3.070$, $p = 0.011$), which was due to a significant dose $\times$ group interaction in only one replication ($F(5, 105) = 3.798$, $p = 0.003$). However, follow-up analyses did not reveal any significant effects of dose for either the optimal or suboptimal group in that replication. Therefore, the two replications do not yield different interpretations of the data. Data are summarized in Table 3.

Neurochemical analyses

The sample size was $n = 7$ –8 for all doses and pre-treatment times.

Dopamine

One hundred twenty-minute pre-treatment interval: Administration of disulfiram to rats increased DA in the striatum at the 120-min pre-treatment interval (Fig. 3). Two-way dose $\times$

replication ANOVAs revealed a significant effect of dose in the striatum ($F(2, 20) = 5.213$, $p = 0.015$) but not the frontal cortex. Post hoc analyses for the striatum revealed that both the 10 and 50 mg/kg doses were different from vehicle ($p < 0.05$). No significant interactions were found.

Thirty-minute pre-treatment interval: For the 30-min pre-treatment interval, no significant interactions or main effects were revealed by dose $\times$ replication ANOVAs for either the frontal cortex or striatum.

Norepinephrine

One hundred twenty-minute pre-treatment interval: At the 120-min pre-treatment interval, replication $\times$ dose ANOVAs for each of the frontal cortex and striatum revealed a significant effect of dose in the striatum ($F(2, 20) = 5.551$, $p = 0.012$) but not in the frontal cortex (Fig. 4). Post hoc analyses revealed that striatal NE levels were significantly lower than vehicle after the 50-mg/kg dose ($p < 0.05$). An effect of replication was found in the frontal cortex ($F(1, 19) = 8.324$, $p = 0.009$), but in the absence of an effect of dose, this suggests that NE levels were lower in one replication, but had no impact on the effect of disulfiram.

Thirty-minute pre-treatment interval: At the 30-min pre-treatment interval, dose $\times$ replication ANOVAs for the frontal cortex and striatum revealed only an effect of dose in the striatum ($F(2, 20) = 6.094$, $p = 0.009$); post hoc analyses revealed that the 10-mg/kg dose was different from vehicle ($p < 0.05$), while the difference between the 50-mg/kg dose and vehicle approached significance ($p = 0.068$). In the frontal cortex, an effect of replication was found ($F(1, 20) = 5.112$, $p = 0.035$) with no effect on dose, indicating that NE levels were lower in one replication, but this had no impact on the effect of disulfiram.

Discussion

The purpose of the present study was to determine the effects of disulfiram in an animal model of gambling and to determine whether any effects were associated with altered DA and/or NE levels. Administration of disulfiram at 120 min, but not 30 min, prior to the task dose-dependently improved strategy in rats that initially had a suboptimal strategy, but had no effect in rats that started with an optimal strategy. Decreases in NE were observed in the striatum at both pre-treatment times, with significant increases in DA being observed in the striatum after the 120-min pre-treatment interval, but not the 30-min pre-treatment interval.

It is known that, in the IGT, pathological gamblers tend to select the disadvantageous options with the higher rewarded outcomes (Linnet et al. 2006; Petry 2001); thus, the suboptimal group represents a phenotype that is similar to those with pathological gambling. Disulfiram has been reported to be efficacious for pathological gambling in case reports (Muller et al. 2011; Mutschler et al. 2010b), and the present results are consistent with these findings. Further, given that disulfiram only affected disadvantageous choices, it may be that disulfiram is uniquely beneficial to those with aberrant choices that lead to problem gambling. The decision-making process that underlies the Iowa gambling task has also been shown to be disrupted in a number of other pathologies such as, for example, substance use

disorder (Biernacki et al. 2016; Stephan et al. 2017), Parkinson's disease (Evens et al. 2016), obsessive-compulsive disorder (Kodaira et al. 2012), and eating disorders (Garrido and Subira 2013), which poses the question as to whether the present findings with disulfiram are specific to gambling or some decision-making process that would impact other pathologies. Although compelling, conclusive evidence cannot be obtained from a single study or animal model, and thus, future studies would need to compile converging data from other animal models of gambling (Cocker et al. 2013). Nevertheless, the rGT has been shown to have predictive validity and the present results are therefore noteworthy (Di Ciano and Le Foll 2016).

The effects observed in the suboptimal group were evident at doses that were somewhat similar to the effective doses in a previous study examining cocaine seeking, using the same pre-treatment regimen (Schroeder et al. 2010). Changes in behavior in the present study were associated with doses and a pre-treatment regimen that produced significant alterations in DA and NE. Indeed, these neurochemical changes were different at the 30-min pre-treatment interval, suggesting that the emergence of behavioral changes may be related to the attainment of a certain change in DA and/or NE levels. Indeed, changes in DA were more pronounced in the striatum (DA) after the 120-min pre-treatment time.

The suppression of NE synthesis and subsequent reduction of adrenergic receptor activation may underlie the behavioral effects of disulfiram in the rGT because adrenergic receptor antagonists attenuate other addiction-related behaviors, such as drug seeking, in rats. For example, blockade of α 1-adrenergic receptors with prazosin reduced intake of cocaine (Wee et al. 2008), heroin (Greenwell et al. 2009), and ethanol (Walker et al. 2008) in "long access" paradigms that more closely model drug dependence and escalated intake than conventional "limited access" protocols. Prazosin also attenuates cocaine-primed reinstatement of cocaine seeking (a model of relapse) in rats (Zhang and Kosten 2005), as well as nicotine taking and reinstatement (Forget et al. 2010). Thus, decreases in NE may contribute to the behavioral effects observed in the present study, either alone or in combination with DA agonism. It should be noted that, although levels of DBH are sparse in the striatum (Berridge et al. 1997; Delfs et al. 1998; Swanson and Hartman 1975), norepinephrine does exist in the striatum and is functionally relevant. For example, administration of reboxetine, a norepinephrine reuptake inhibitor, increased norepinephrine levels in the striatum (Gobert et al. 2004). Infusion of propranolol into the striatum blocked L-DOPA-induced dyskinesia (Lindenbach et al. 2011), while loss of norepinephrine innervation in the striatum reduced abnormal involuntary movements (Barnum et al. 2012).

Previous reports using the rGT have also found effects of DA manipulations on this task. The D2 receptor antagonist eticlopride improved performance in the rGT (Zeeb et al. 2009), while quinpirole, a D2 agonist, impaired performance on a rodent slot machine task (Winstanley et al. 2011). Further, the indirect DA agonist amphetamine decreased optimal P2 choices, while increasing suboptimal P3 choices in the rGT (Zeeb et al. 2009). Although these findings suggest a role of DA in treating gambling, the direction of effect is opposite from our present finding that *increases* in DA were associated with improvements in behavior. It should be noted that in these previous reports, the data were aggregated across

all animals, while in the present study, the data were separated based on baseline choice of the advantageous and disadvantageous strategy.

It is somewhat surprising that we observed significant increases in striatal DA following disulfiram treatment. Another study that also used the 50-mg/kg dose and 120-min pre-treatment time found increases in cortical, but not striatal, DA levels (Devoto et al. 2012). This is presumably because DBH inhibition increases DA production in NE neurons, and noradrenergic innervation of the cortex is dense. By contrast, noradrenergic innervation of the striatum is sparse, and striatal DA levels are already extremely high due to massive innervation from dopaminergic neurons, which do not produce more DA following disulfiram administration. Nevertheless, we found augmentation of striatal DA tissue levels at both disulfiram doses, so we are confident in the veracity of the results. These increases in DA were more pronounced after the 120-min pre-treatment time in the striatum and this may explain the effects observed here, in combination with changes in the NE in the frontal cortex, as mentioned above. It should be considered that the effects of disulfiram on DA were due to metabolites of disulfiram. That is, the metabolites of disulfiram are found in the brain (Winefield et al. 2015). Although they are known to affect alcohol dehydrogenase systemically (Hart and Faiman 1994; Lipsky et al. 2001), the locus of disulfiram's effectiveness in treating alcohol use disorder and the impact of metabolites in the brain are largely unexplored. One study measured dopamine levels in the nucleus accumbens following administration of disulfiram or its metabolites and found them in plasma at 120 min, with some presence also at 30 min. Carbamathione, an active metabolite of disulfiram, increased DA levels in the nucleus accumbens, as did disulfiram. However, when the metabolism of disulfiram was blocked, there was no effect on DA (Faiman et al. 2013). Thus, it seems likely that at least some of the actions of disulfiram on DA are due to active metabolites, and this warrants further investigation.

Alternatively, disulfiram's effects may be explained by combined effects on both the NE and DA systems. Indeed, we found that no single neurochemical change was uniquely associated with the dose (50 mg/kg) and pre-treatment time (120 min) required for the beneficial effects of disulfiram in the rGT, but only that regimen produced a combination of decreased striatal NE and increased striatal DA at 120 min. It has been reported in a recent study that administration of DA or NE reuptake inhibitors had no effect on the rGT, but co-administration of both classes of drugs decreased optimal choice and increased disadvantageous choice (Baarendse et al. 2013). Thus, only simultaneously increasing both DA and NE appears to impair behavior in this task. It is also important to note that because disulfiram increases DA production in noradrenergic neurons, not in dopaminergic neurons, facilitated DA transmission following DBH inhibition is likely to be anatomically and functionally distinct from that produced by reuptake inhibitors.

Limitations

The interpretations in this paper must be tempered by a number of possible limitations. First, the lack of effect in the optimal group may be explained by a ceiling effect in advantageous responses. Although this cannot be entirely dismissed, advantageous responses in this analysis is a combination of the P1 and P2 responses and thus it is possible that increases in

advantageous responses would have been detected in either one of these response options. As can be seen from the present data, P1 and P2 choices were not altered, and thus, it is not likely that the lack of effect on the optimal group is due to a ceiling effect. Second, a further limitation of this study is the lack of correlation between neurochemical measures and behavior. This analysis would have strengthened the present conclusions, but the neurochemical study was conducted post hoc after promising preliminary behavioral findings. Nonetheless, this correlation would have entailed a between-subject dose-response analysis which would have decreased the power in the present study. Related to this, the behavioral data were assessed in optimal vs suboptimal rats, whereas for the neurochemistry data, this distinction was not made. Thus, firm conclusions linking the behavioral and neurochemical data cannot be made and conclusions must be tempered. Future studies will need to further investigate the relationship between combinations of changes in DA and NE and the rGT. In particular, more involved studies should look at whether neurochemical differences exist between optimal and suboptimal rats. Studies investigating the effects of intracerebral infusion of DA or NE agents would be informative, as would administration of systemic agents to block the effects of disulfiram. A third limitation concerns the use of animals from previous studies and some effects of replication. For the neurochemical study, the vehicle and 50-mg/kg doses were in different subsets of rats, yet an orderly dose-response function was still seen (half of the rats in the 10-mg/kg group were from different subsets). An effect of replication was seen when the data for NE in the FC were analyzed. It should be noted, however, that no effects of dose were found in these areas. Thus, the effect of replication was such that one replication had overall lower values than the other, with no impact on the effects of disulfiram. As for the behavioral findings, an effect of replication was seen in the analysis of omissions in that one replication revealed a dose $\times$ group effect. However, given that the main effects of dose were not significant, the effect of this replication on the interpretation of the effects of disulfiram is not affected.

Conclusions

In a rodent model of gambling, the present study shows that disulfiram improved performance in rats that had disadvantageous strategies, similar to humans with GD, with no effect in rats that engaged in predominantly advantageous strategies. After administration of disulfiram, decreases in norepinephrine (in the striatum) and increases in dopamine (in the striatum) were observed. Together, these results suggest that combined actions on DA and NE may represent a novel treatment strategy for gambling disorder.

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Fig. 1.

Disulfiram dose- and time-dependently increased optimal choices exclusively in rats displaying predominantly suboptimal strategies. Top panel: mean $\pm$ SEM percent optimal choices 120 min after pre-treatment with vehicle or disulfiram (10, 25, 50, 75, or 100 mg/kg, i.p.) for the optimal group ($n = 29$; light gray bars) and suboptimal group ($n = 10$; dark gray bars). $*p < 0.05$ compared to vehicle for the suboptimal group. Bottom panel: mean $\pm$ SEM percent optimal choices 30 min after pre-treatment with vehicle or disulfiram for the optimal group ($n = 27$; light gray bars) and suboptimal group ($n = 12$; dark gray bars). $*p < 0.05$ compared to vehicle for the suboptimal group

Fig. 2.

Mean $\pm$ SEM percent choice of P1, P2, P3, or P4 options. Top panel: percent choice 120 min after pre-treatment with disulfiram for the optimal group (left panel; $n = 29$) and suboptimal group ($n = 10$; right panel). Bottom panel: percent choice 30 min after pre-treatment with disulfiram for the optimal group (left panel; $n = 27$) and suboptimal group (right panel; $n = 12$)

Fig. 3.

Disulfiram dose- and time-dependently increased DA tissue levels in the striatum but not frontal cortex. Mean $\pm$ SEM DA levels (pg/mg tissue) in the frontal cortex (A) and striatum (B) 120 min after pre-treatment with vehicle or disulfiram (10 or 50 mg/kg, i.p.). Data for the 30-min pre-treatment interval are shown in the bottom panels for the frontal cortex (C) and striatum (D). * $p < 0.05$ compared to vehicle. Sample size was $n = 7-8$

Fig. 4.

Disulfiram dose- and time-dependently decreased NE tissue levels in the striatum. Mean $\pm$ SEM NE levels (pg/mg tissue) in the frontal cortex (A) and striatum (B) 120 min after pre-treatment with vehicle or disulfiram (10 or 50 mg/kg). Data for the 30-min pre-treatment interval are given in the bottom panels for the frontal cortex (C) and striatum (D). * $p < 0.05$ compared to vehicle. Sample size was $n = 8$ for all groups

Table 1

Reward and punishment received for the various response options in the rGT

Group A	Hole 1	Hole 4	Hole 5	Hole 2
Group B	Hole 2	Hole 5	Hole 4	Hole 1
Choice	P1	P2	P3	P4
Reward (# pellets)	1	2	3	4
Punishment duration	5 s	10 s	30 s	40 s
Punishment probability	0.1	0.2	0.5	0.6
Rewards possible	295	411	135	99

Table 2

Summary of treatment conditions. Rats first received treatments in “Disulfiram 1,” then “Disulfiram 2,” then in the “Neurochemical study.” *DR* dose-response, *min* minutes, *N* sample size

	N	Disulfiram 1 (min)	Disulfiram 2 (min)	Neurochemical study (mg/kg)
Replication 1	18	DR 30	DR 120	
Replication 2 *	23	DR 120	DR 30	10 or 50
Neurochemical study *	25			10 or 0

* Rats were used in previous studies (see in text)

Table 3

Effect of disulfiram on other measures of the rGT at either the 30- or 120-min pre-treatment time for the optimal ($n = 29$, 120 min; $n = 27$, 30 min) and suboptimal ($n = 10$, 120 min; $n = 12$, 30 min) groups. Data presented are mean $\pm$ SEM

Variable	Group	Doses (mg/kg)					
		Vehicle	10	25	50	75	100
30-min pre-treatment							
Trials	Optimal**	106 ± 6.9	113 ± 6.7	115 ± 6.4	112 ± 5.7	115 ± 5.6	111 ± 5.7
	Suboptimal	75 ± 3.4	71 ± 3.6	73 ± 2.3	76 ± 3.4	77 ± 5.5	67 ± 4.5
% premature	Optimal**	11 ± 1.5	11 ± 1.6	11 ± 1.8	11 ± 1.6	10 ± 1.4	10 ± 1.6
	Suboptimal	16 ± 2.4	19 ± 2.1	17 ± 1.9	16 ± 2	17 ± 2.3	17 ± 3.6
Reward perseverative	Optimal	0.01 ± 0.004	0.001 ± 0.001	0.02 ± 0.01	0.005 ± 0.002	0.007 ± 0.002	0.013 ± 0.01
	Suboptimal	0.015 ± 0.01	0.04 ± 0.02	0.002 ± 0.001	0.02 ± .01	0.03 ± 0.02	0.03 ± 0.03
Punishment perseverative	Optimal	0.052 ± 0.01	0.047 ± 0.01	0.055 ± 0.01	0.048 ± 0.004	0.049 ± 0.01	0.05 ± 0.01
	Suboptimal	0.048 ± 0.01	0.042 ± 0.01	0.050 ± 0.01	0.049 ± 0.01	0.051 ± 0.01	0.048 ± 0.01
Choice latency	Optimal	1.13 ± 0.14	1.21 ± .19	1.26 ± 0.20	1.26 ± 0.20	1.27 ± 0.18	1.29 ± 0.19
	Suboptimal	1.0 ± 0.15	0.86 ± 0.12	1.09 ± 0.16	1.1 ± 0.14	1.06 ± 0.16	1.27 ± 0.20
Collect latency	Optimal	3.08 ± 1.22	1.44 ± 0.06	3.05 ± 1.60	1.46 ± 0.06	1.49 ± 0.08	1.95 ± 0.36
	Suboptimal	1.28 ± 0.07	1.26 ± 0.09	1.24 ± 0.08	1.24 ± 0.07	1.20 ± 0.06	2.89 ± 1.60
% omissions	Optimal	0.82 ± 0.36	1.15 ± 0.61	1.36 ± 0.55	1.58 ± 0.81	1.34 ± 0.54	1.12 ± 0.34
	Suboptimal	1.40 ± 0.98	3.6 ± 2.48	1.12 ± 0.63	0.73 ± 0.28	1.05 ± 0.35	2.81 ± 2.46
120-min pre-treatment							
Trials	Optimal**	113 ± 7.88	115 ± 7.64	108 ± 5.25	112 ± 5.62	104 ± 4.31	107 ± 6.79
	Suboptimal	67 ± 6.37	71 ± 5.13	81 ± 9.23	77 ± 6.9	73 ± 5.7	75 ± 5.51
% premature	Optimal**	13 ± 1.6	12 ± 1.4	11 ± 1.2	11 ± 1.2	14 ± 1.4	14 ± 1.7
	Suboptimal	20 ± 3.8	17 ± 2.8	20 ± 2.4	18 ± 2.7	20 ± 2.9	16 ± 2.8
Reward perseverative	Optimal	0.45 ± 0.13	0.38 ± 0.11	0.45 ± 0.13	0.46 ± 0.15	0.64 ± 0.18	0.41 ± 0.11
	Suboptimal	0.02 ± 0.01	0.05 ± 0.02	0.03 ± 0.02	0.03 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
Punishment perseverative	Optimal	0.056 ± 0.01	0.049 ± 0.01	0.049 ± 0.01	0.052 ± 0.004	0.052 ± 0.01	0.049 ± 0.005
	Suboptimal	0.051 ± 0.01	0.063 ± 0.01	0.053 ± 0.01	0.058 ± 0.01	0.050 ± 0.01	0.058 ± 0.01
Choice latency	Optimal	0.95 ± 0.12	0.92 ± 0.11	0.96 ± 0.12	0.99 ± 0.15	0.95 ± 0.12	1.01 ± 0.15
	Suboptimal	1.36 ± 0.16	1.33 ± 0.21	1.08 ± 0.12	1.04 ± 0.06	1.02 ± 0.12	1.26 ± 0.13

A significant effect of group ($p < 0.05$)