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DOPAMINE MEDIATES COCAINE-INDUCED CONDITIONED TASTE AVERSIONS AS DEMONSTRATED WITH CROSS-DRUG PREEXPOSURE TO GBR 12909

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Abstract

Although cocaine readily induces taste aversions, little is known about the mechanisms underlying this effect. It has been suggested that its inhibitory effects at one of the monoamine transporters may be mediating this suppression. Using the cross-drug preexposure preparation, the present series of studies examined a possible role of dopamine (DA) in this effect. Male Sprague-Dawley rats were exposed to cocaine (18 mg/kg; Experiment 1) or the selective DA transporter (DAT) inhibitor GBR 12909 (50 mg/kg; Experiment 2) prior to the pairing of a novel saccharin solution with injections of GBR 12909 (32 mg/kg), cocaine (18 mg/kg) or vehicle in a conditioned taste aversion (CTA) procedure. Preexposure to cocaine attenuated aversions induced by itself but not aversions induced by GBR 12909 (Experiment 1). Conversely, preexposure to GBR 12909 attenuated aversions induced by itself and cocaine (Experiment 2). This asymmetry suggests that cocaine and GBR 12909 induce CTAs via similar, but non-identical, mechanisms. These data are discussed in the context of previous work demonstrating roles for dopamine, norepinephrine and serotonin in cocaine-induced CTAs.

Keywords

cocaine; GBR 12909; conditioned taste aversions; US preexposure; dopamine

1. INTRODUCTION

Cocaine, like other drugs of abuse, has been shown to have both rewarding (Kosten et al., 1997; Nomikos and Spyraki, 1988; Wise et al., 1992) and aversive (Ettenberg, 2004; Ferrari et al., 1991; Goudie et al., 1978) effects with the balance of these effects determining its abuse potential (see Hunt and Amit, 1987; Kohut and Riley, 2010; Riley et al., 2009). Understanding the basis for these affective properties may be important in understanding the vulnerability to cocaine abuse. Cocaine's action as a dopamine transport inhibitor (DAT) appears to be largely involved in its rewarding effects (Ritz et al., 1987; Rocha et al., 1998; Sora et al., 2001). The mechanism underlying its aversive effects is less understood, although a few reports have implicated cocaine's dopaminergic actions in these effects as well.

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For example, Freeman and colleagues recently reported parallels between aversions induced by cocaine and another dopamine reuptake inhibitor, i.e., vanoxerine (GBR 12909; see Freeman et al., 2005). Specifically, they reported that aversions were induced by both compounds and at the highest dose tested (50 mg/kg) those induced by GBR 12909 were comparable to cocaine in the rate of acquisition and degree of the aversion. In a more direct assessment, Hunt et al. (1985) reported that the dopamine (DA) receptor antagonist pimozide given immediately prior to taste aversion conditioning with cocaine blocked cocaine-induced CTAs (Hunt et al., 1985). Similarly, Serafine et al. (2011) have shown that the nonselective DA antagonist haloperidol blocked aversions induced by cocaine. The fact that cocaine-induced CTAs can be blocked by direct pharmacological antagonism of DA receptors suggests that dopaminergic action (produced by inhibition of DAT by cocaine) is a necessary component in its ability to induce taste aversions.

If DA is involved in cocaine-induced aversions, it might be expected that a history with cocaine would impact subsequent aversion learning induced by other compounds that increase DA levels (and vice versa). This prediction is based on work utilizing the cross-drug preexposure preparation (De Beun et al., 1996; Gommans et al., 1998; Serafine and Riley, 2009) in which exposure to one compound is given prior to aversion conditioning with another. Under such conditions, aversions to the second compound are often weakened, an effect commonly interpreted as being due to cross tolerance (or adaptation) to the shared aversion-inducing effects of the two drugs (Berman and Cannon, 1974; Jones et al., 2009; LeBlanc and Cappell, 1974; Simpson and Riley, 2005; Serafine and Riley, 2009; 2010; for reviews and alternative interpretations, see Cappell and LeBlanc, 1977; Randich and LoLordo, 1979; Riley and Simpson, 2001). In one of the first demonstrations of the use of this procedure for investigations of common stimulus properties, De Beun and colleagues (1993) reported that CTAs induced by the selective serotonin (5-HT) agonist 8-OHDPAT were blocked by preexposure to compounds that also had 5-HT agonist activity (for the same receptor subtype, e.g., 5-HT_{1A}; see De Beun et al., 1993). Given that these compounds (ipsapirone, buspirone, RU-24969, sertraline, d-amphetamine, LSD, metergoline and idazoxane) were effective in blocking 8-OHDPAT-induced CTAs, De Beun and colleagues concluded that cross-drug preexposure could be used to assess the commonalities in aversion-inducing mechanism between different compounds (De Beun et al., 1993). Since this demonstration, several other investigations have also utilized this procedure to examine common mechanisms in the aversive effects of various compounds (see De Beun et al., 1996; Gommans et al., 1998; Jones et al., 2009; Kayir et al., 2008; Olivier et al., 1999; Van Hest et al., 1992; Serafine and Riley, 2009; 2010).

To assess the possible role of DA in aversions induced by cocaine, in the present series of experiments animals were exposed to cocaine (18 mg/kg) prior to aversion conditioning with the selective DAT inhibitor GBR 12909 (Experiment 1). Conversely, in Experiment 2, a different set of animals were exposed to GBR 12909 (50 mg/kg) prior to aversion condition with cocaine. Given the relative selectivity of GBR 12909's action as a DAT inhibitor, any attenuation of aversions would implicate DA in the aversive effects of cocaine.

2. GENERAL METHOD

2.1. Subjects

The subjects were experimentally naïve, male Sprague-Dawley rats (Harlan Sprague-Dawley, Indianapolis, Indiana), approximately 75 days old and between 250 and 350 g at the start of the experiments. Procedures recommended by the National Institutes of Health Guide for the Care and Use of Laboratory Animals (1985), the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research (2003) and the Institutional

Animal Care and Use Committee at American University were followed at all times. Animals were handled daily approximately 2 weeks prior to the initiation of the study to limit the effects of handling stress during conditioning and testing.

2.2. Drugs and Solutions

Cocaine hydrochloride (generously provided by the National Institute on Drug Abuse) and GBR 12909 bismethanesulfonate monohydrate (synthesized at the Chemical Biology Research Branch of the National Institute on Drug Abuse) were each dissolved in distilled water at a concentration of 10 mg/ml. All injections were administered subcutaneously (SC). All drug doses are expressed as the salt. Saccharin (sodium saccharin, Sigma) was prepared as a 1 g/l (0.1%) solution in tap water.

2.3. Apparatus

All subjects were individually housed in hanging wire-mesh cages on the front of which graduated Nalgene tubes could be placed for fluid presentation. Subjects were maintained on a 12:12 light-dark cycle (lights on at 0800h) and at an ambient temperature of 23 °C. Except where noted, food and water were available *ad libitum*.

2.4. Procedure

2.4.1. Habituation—Following 24-h water deprivation, subjects were given 20-min access to tap water daily. This procedure was repeated until consumption stabilized, i.e., subjects approached and drank from the tube within 2 s of its presentation and water consumption was within 2 ml of the previous day for a minimum of 4 consecutive days. Throughout each study, fluid was presented in graduated 50-ml Nalgene tubes and measured to the nearest 0.5 ml by subtracting the difference between the pre- and post-consumption volumes.

2.4.2. Preexposure—Water consumption for all subjects was recorded and averaged over the last 3 days of habituation. Animals were then ranked on average water consumption and assigned to a preexposure condition (drug or vehicle). Five hours following their regular 20-min water access, animals were injected with drug or vehicle every 4^{th} day for a total of 5 days (five total drug or vehicle injections). No injections were given during intervening days. Water consumption was monitored throughout this phase.

2.4.3. Conditioning—Conditioning began 4 days following the final preexposure injections. On Day 1 of conditioning, all subjects were given 20-min access to the novel saccharin solution. Immediately following this presentation, animals from each preexposure condition were rank ordered based on saccharin consumption and assigned to a treatment group (either vehicle or drug) such that overall consumption was comparable between groups. Subjects received an injection of either distilled water or drug approximately 20 min after access to the saccharin solution. The 3 days following this initial saccharin presentation were water-recovery days during which animals were given 20-min access to tap water (no injections followed this access). This alternating procedure of conditioning and water recovery was repeated for a total of four complete cycles. Following the last water-recovery session after the fourth conditioning trial, animals were given 20-min access to saccharin in a final aversion test after which no injections followed.

2.5. Statistical Analysis

During drug preexposure, the differences in mean water consumption were analyzed for each experiment using a 2 × 20 repeated measures ANOVA with a between-subjects variable of Preexposure Drug and a within-subjects variable of Preexposure Day. Where appropriate, subsequent one-way ANOVAs and Tukey post-hoc analyses were run to

examine group differences on individual days. During conditioning, the differences in mean saccharin consumption were analyzed for each experiment using a 2 × 3 × 5 mixed-model ANOVA with the between-subjects variables of Preexposure Drug and Conditioning Drug and a within-subjects variable of Trial (1–4 and the final aversion test). CTAs can be defined in a number of ways (see Anderson et al., 1982; Dacanay and Riley, 1982; MacPhail, 1982 for examples); however, given that the procedure requires the use of intermediate doses (see below) and that CTAs are more likely to be detected when they are defined as differences from controls (for evidence of this, see Dacanay et al., 1984) the present assessment used this between-group comparison (treated subjects vs. controls) to determine aversions. Where appropriate, subsequent one-way ANOVAs were run for individual trials and Tukey post-hoc analyses were used to examine mean saccharin consumption differences between groups on each individual trial. All significance levels were set at $p \leq 0.05$.

3. EXPERIMENT 1

Experiment 1 assessed the effects of cocaine preexposure on aversions induced by GBR 12909. In other work assessing the effects of cross-drug preexposure on aversion learning, both attenuating and potentiating effects have been reported. That is, preexposure to Compound A can weaken or strengthen the aversion induced by Compound B (De Beun et al., 1996; Gommans et al., 1998; Serafine and Riley, 2009; see also Riley and Simpson, 2001). Since preexposure can result in either effect, it is necessary to use doses during conditioning that induce intermediate aversions (to detect potentiation or attenuation). In this context, cocaine has been reported to induce intermediate aversions at a dose of 18 mg/kg (Freeman et al., 2005; Serafine and Riley, 2009; 2010). Therefore, this dose was used during conditioning in the present experiment. Preexposure to cocaine (18 mg/kg) has been shown to attenuate aversions induced by itself (18 mg/kg; Serafine and Riley, 2009; 2010). Therefore, this dose was chosen for preexposure. GBR 12909 induces intermediate aversions at a dose of 32 mg/kg (Freeman et al., 2005). Since higher doses have been reported to cause complete suppression of consumption (i.e., 50 mg/kg; see Freeman et al., 2005), this intermediate dose was used for conditioning. Importantly, the use of such doses often results in a relatively weak aversion (see Freeman et al., 2005 for a dose response assessment).

Following water habituation, subjects ($n = 51$) were rank ordered on consumption (across 3 days) and assigned to a preexposure condition. Subjects were given injections of either cocaine (18 mg/kg) or vehicle (matched in volume) every 4th day for a total of five preexposures. Four days following the last preexposure injection, subjects were given 20-min access to the novel saccharin solution. Following saccharin consumption, rats were injected with either 18 mg/kg cocaine, 32 mg/kg GBR 12909 or vehicle (matched in volume to GBR 12909), yielding six experimental groups, specifically, cocaine-cocaine (COC-COC; $n = 9$), cocaine-GBR 12909 (COC-GBR; $n = 8$), cocaine-vehicle (COC-VEH; $n = 8$), vehicle-vehicle (VEH-VEH; $n = 8$), vehicle-GBR 12909 (VEH-GBR; $n = 9$) and vehicle-cocaine (VEH-COC; $n = 9$). The first series of letters in each group designation refer to the drug given during preexposure; the second series of letters refer to the drug given during conditioning.

4. RESULTS

4.1. Preexposure

The 2 × 20 repeated measures ANOVA revealed a significant effect of Preexposure Day [$F(19, 931) = 10.125$, $p < 0.001$] and Preexposure Drug [$F(1, 49) = 4.603$, $p = 0.037$], but no significant Preexposure Drug x Preexposure Day interaction [$F(19, 931) = 1.307$, $p = 0.170$]. Regarding the effect of Preexposure Day, all subjects (regardless of preexposure

drug) increased consumption over the preexposure phase. Regarding the main effect of Preexposure Drug, all subjects preexposed to cocaine drank significantly more than subjects preexposed to vehicle. The average consumption for subjects preexposed to cocaine was 16.2913 ml (+/− 0.8288). The average consumption for subjects preexposed to vehicle was 15.4896 (+/− 0.5930). Although the basis for these differences is not known, it is possible that indirect DA agonist activity (as a result of DAT inhibition) may have impacted overall drinking either directly or indirectly via compensation (see Amato et al., 2008; De Carolis et al., 2010; Milella et al., 2010 for examples using polydipsia). Interestingly, such increases in consumption during preexposure with other compounds have also been reported (see Serafine & Riley, 2009).

4.2. Conditioning

The 2 × 3 × 5 mixed-model ANOVA revealed significant effects of Trial [F (3,180) = 18.130, $p < 0.001$], Preexposure Drug [F (1,44) = 13.921, $p = 0.001$] and Conditioning Drug [F (2,44) = 18.307, $p < 0.001$] and significant Trial x Conditioning Drug interaction [F (8, 180) =5.360, $p < 0.001$]. In relation to the significant Trial effect, consumption significantly increased from Trial 1 to Trial 2 and then significantly decreased on Trials 3, 4 and 5. In relation to the significant effect of Preexposure Drug, subjects preexposed to cocaine drank significantly more than those preexposed to vehicle. In relation to the significant Conditioning Drug effect, subjects conditioned with drug (cocaine and GBR 12909) drank significantly less than those conditioned with vehicle. Although there was no significant three-way interaction involving Preexposure Drug, an examination on the final exposure to saccharin (a final aversion test after four conditioning trials) revealed significant group differences [F (5, 50) = 10.267, $p < 0.001$] (there were no differences on Trial 1). Tukey post-hoc analyses revealed that subjects preexposed and conditioned with cocaine (Group COC-COC) drank significantly more than subjects in Group VEH-COC ($p = 0.042$), indicating a US preexposure effect. Subjects in Group COC-GBR, however, did not differ significantly in consumption compared to subjects in Group VEH-GBR ($p = 0.997$), indicating no significant effect of cross-drug preexposure (see Figure 1).

5. EXPERIMENT 2

As described, there was no effect of preexposure to cocaine on the aversion induced by GBR 12909. Although the basis for this effect is not known, there are several possibilities. First, the failure of cocaine exposure to attenuate GBR 12909-induced aversions could reflect the fact that there is no overlap in the aversive effects of the two drugs. If there is no overlap, such a history would not be expected to impact subsequent aversions. The failure to see any attenuating effects in Experiment 1, however, may be a function of the relative degree of overlap of the stimulus properties of cocaine and GBR 12909. According to this explanation, the two drugs may have similar, but non-identical, stimulus properties and it is the degree of the overlap that impacts any attenuating effects. That is, a compound like cocaine has multiple actions (general monoamine transport inhibition) and aversions may be induced by any one (or some combination) of all these actions. On the other hand, GBR 12909 has one selective action (i.e., DAT inhibition) and it is this action which likely mediates its aversive effects. Preexposure to cocaine with its multifaceted action may induce tolerance to monoamine transport inhibition sufficiently enough to attenuate cocaine-induced CTAs but may not induce tolerance to DAT inhibition alone significantly enough to attenuate aversions to GBR 12909 whose aversive effects are completely DAT mediated. Although cocaine preexposure may not affect aversions induced by GBR 12909, it is possible that the reverse serial presentation (GBR 12909 exposure before cocaine conditioning) may result in an attenuation (if DAT inhibition is playing some role in their aversive effects). In this case, when animals are exposed to GBR 12909, tolerance to the effects of DAT inhibition will

occur given that there is no other effect of GBR 12909. If cocaine-induced CTAs are mediated to any degree by this same mechanism, preexposure (and the accompanying tolerance) should result in an attenuation of cocaine-induced CTAs. It is interesting in this context that such asymmetrical cross-drug preexposure effects have been reported for other combinations of drugs (De Beun et al., 1996; Gommans et al., 1998; Goudie and Thornton, 1975; see also Riley and Simpson, 2001; see Grakalic and Riley, 2002; Serafine and Riley, 2009; 2010 for examples with cocaine) and are generally interpreted as evidence of similar, but non-identical, mechanisms responsible for the induction of CTAs by the two compounds.

Given the abovementioned reports of asymmetry with cocaine in the cross-drug preexposure design (with compounds acting on norepinephrine [NE]; Serafine & Riley, 2009), Experiment 2 examined the effects of preexposure to the highly selective DAT inhibitor GBR 12909 on aversions induced by itself and the relatively nonselective monoamine transport inhibitor cocaine. The same dose of cocaine used in Experiment 1 was used in Experiment 2, given that it induces intermediate aversions during conditioning (Freeman et al., 2005). GBR 12909 has not been reported using the cross-drug preexposure design, and the intermediate dose used in Experiment 1 for conditioning induced CTAs that were weaker than those induced by the intermediate dose of cocaine. Given that a more robust attenuating effect is generally seen when higher doses are used during preexposure (see Riley and Simpson, 2001 for an overview), a larger dose of GBR 12909 (50 mg/kg) was used in Experiment 2 during preexposure while the same intermediate dose (32 mg/kg) used in Experiment 1 was used during conditioning.

Following water habituation in the present experiment, subjects (n = 48) were rank ordered on consumption (across 3 days) and assigned to a preexposure condition. Subjected were given injections of either GBR 12909 (50 mg/kg) or vehicle (matched in volume) every 4th day for a total of five preexposure injections. Four days following the last preexposure injection, subjects were given 20-min access to the novel saccharin solution. Following saccharin consumption, they were injected with either 18 mg/kg cocaine, 32 mg/kg GBR 12909 or vehicle (matched in volume to GBR 12909), yielding six experimental groups, specifically, GBR 12909-vehicle (GBR-VEH; n = 8), GBR 12909-GBR 12909 (GBR-GBR; n = 8), GBR 12909-cocaine (GBR-COC; n= 8), vehicle-vehicle (VEH-VEH; n = 8), vehicle-GBR 12909 (VEH-GBR; n = 8) and vehicle-cocaine (VEH-COC; n = 8). The first series of letters in each group designation refer to the drug given during preexposure; the second series of letters refer to the drug given during conditioning.

6. RESULTS

6.1. Preexposure

The 2 × 20 repeated measures ANOVA revealed a significant effect of Preexposure Day [F (19,874) = 11.450, $p < 0.001$] and Preexposure Drug [F (1, 46) = 11.296, $p = 0.002$] as well as a significant Preexposure Drug x Preexposure Day interaction [F (19, 874) = 6.499, $p < 0.001$]. Subsequent one-way ANOVAs and Tukey post-hoc analyses comparing Preexposure Drug on each Preexposure Day revealed that subjects preexposed to GBR 12909 drank significantly more than subjects preexposed to vehicle on Days 6, 8, 10, 12–14, and 16–20 (all p's < 0.012). These days do not all correspond to preexposure injections, which took place on Days 1, 5, 9, 13 and 17. Overall, all subjects increased consumption over the preexposure phase. The average consumption for subjects preexposed to GBR 12909 was 16.1796 ml (+/−1.1593). The average consumption for subjects preexposed to vehicle was 14.9229 (+/−0.4533). As with cocaine preexposure, it is possible that this represents some direct or compensatory response to repeated exposure (see above).

6.2. Conditioning

The 2 × 3 × 5 mixed-model ANOVA revealed significant effects of Trial [F (4,168) = 14.395, $p < 0.001$], Preexposure Drug [F (1,42) = 34.707, $p = 0.001$] and Conditioning Drug [F (2,42) = 15.354, $p < 0.001$] and significant Trial x Conditioning Drug [F (8, 168) = 8.649, $p < 0.001$], Trial x Preexposure Drug [F (4,168) = 8.627, $p < 0.001$] and Trial x Preexposure Drug x Conditioning Drug [F (8,168) = 3.378, $p < 0.001$] interactions. Since there was a significant Trial x Preexposure Drug x Conditioning Drug interaction, one-way ANOVAS for each trial and Tukey post-hoc analyses were run on individual groups for individual trials. There were no significant differences on Trial 1 (even after differences were seen in consumption during preexposure; see above). On Trial 2, subjects in Group VEH-COC drank significantly less than subjects in Group VEH-VEH ($p = 0.014$), indicating a significant cocaine-induced CTA. Subjects in Group VEH-GBR did not differ from subjects in Group VEH-VEH, indicating that GBR 12909 was not effective in inducing aversions after only a single conditioning trial. Interestingly, subjects in Group GBR-COC also drank significantly more than subjects in Group VEH-COC, indicating a significant cross-drug preexposure effect on this trial ($p = 0.004$). These differences were maintained on Trial 3. On this trial, subjects preexposed and conditioned with GBR 12909 (Group GBR-GBR) drank significantly more than subjects in Group VEH-GBR ($p = 0.004$), although Group VEH-GBR did not drink less than the vehicle control (VEH-VEH). These differences were all maintained on Trial 4. In addition, on this trial, Group VEH-GBR drank significantly less than Group VEH-VEH (indicating a significant GBR 12909-induced CTA; $p = 0.018$). These differences were all maintained on the final aversion test (see Figure 2).

7. General Discussion

Although preexposure to cocaine did not attenuate GBR 12909-induced aversions, GBR 12909 did significantly attenuate aversions induced by cocaine. The lack of attenuation of GBR 12909-induced aversions following cocaine preexposure could be interpreted as evidence that the two compounds do not share common aversion-inducing mechanisms. On the other hand, it is important to consider that cocaine has several actions and GBR 12909 only has one. Therefore, it is possible that, even though they may share a common mechanism, cocaine's actions on DAT may not induce tolerance to DAT inhibition sufficiently enough to attenuate aversions induced by a specific DAT inhibitor (see above). This latter interpretation is supported by the fact that GBR 12909 attenuated cocaine-induced CTAs, implicating a shared mechanism (i.e., DAT inhibition) in aversions induced by both compounds. A role for DA in cocaine-induced aversions has also been supported in other preparations directly assessing the effects of DA antagonists (i.e., pimozide and haloperidol) on aversions induced by cocaine (see Hunt et al., 1985; Serafine et al., 2011; though see also Gale, 1984). For example, when haloperidol at a non-aversive dose is administered immediately after saccharin administration (but prior to cocaine), cocaine-induced CTAs (18 mg/kg) are significantly attenuated (Serafine et al., 2011). Although DA appears involved in such aversions, the relative role of each DA receptor subtype in this phenomenon remains to be determined given the relative lack of selectivity for D_2 over other receptor subtypes with these particular compounds (Vangveravong et al., 2010; see also Serafine et al., 2011 for a discussion).

It is important to consider that although cross-drug preexposure has been used to determine whether compounds induce CTAs via similar mechanisms, this preparation does not identify which specific shared effect mediates the aversions. That is, although the shared neurochemical action between cocaine and GBR 12909 is DAT inhibition (and the resulting increase in DA), how such increases in DA induce an aversive effect is not known for cocaine or for most drugs in general (see Riley et al., 2009; Riley, 2011 for a discussion). The aversive effects may be considerably downstream from DA, e.g., stress, sickness,

novelty or disruption of homeostasis. As such, this procedure does not identify the specific aversion-inducing effects shared by the two compounds, only that there is a shared effect.

The abovementioned possibility raises another concern for the singular use of the cross-drug preexposure preparation in assessing underlying mechanisms for aversion learning. For example, although DA activity must be the initial trigger in GBR 12909's aversive effects, this may not be the case for cocaine that has general inhibitory effects on all of the monoamines. With cocaine, any of its neurochemical actions (alone or in combination) could initiate its downstream aversive effects. Thus, it is possible that the aversive effects of cocaine and GBR 12909 are similar but are initiated via different biochemical activity. Such a position is unlikely the case with cocaine given that other collateral evidence supports a role for DA in its aversive effects. However, it does point to the fact that the cross-drug preexposure design simply indicates, but does not identify, a shared stimulus property. Interestingly, DA agonists have been shown to induce nausea and vomiting (see Perez-Lloret et al., 2010). It is possible that the indirect agonist activity of DA on its receptors (from DAT inhibition by cocaine or GBR 12909) could also cause nausea under the present conditions. It is possible that sickness could be the underlying mechanism shared between these compounds that is responsible for the induction of CTAs (for a general overview of the role of sickness in aversion learning, see Parker, 1995).

To demonstrate an effect of drug preexposure on drug-induced aversions, it is important that such aversions are evident in naive subjects. In both Experiments 1 and 2, GBR 12909 induced weaker aversions relative to cocaine (see above; see also Freeman et al., 2005). Importantly, GBR 12909-induced aversions were only evident relative to vehicle-injected controls. Although this difference from controls was defined here as an aversion, there is an alternative interpretation to these effects with GBR 12909 that does not assume the acquisition of a taste aversion. Specifically, animals injected with GBR 12909 may have differed from controls due to the fact that GBR 12909 interfered with the habituation of neophobia, thus limiting intake relative to controls that increased as control subjects habituated to the novel saccharin. This possibility would account for the fact that these subjects differed from controls but not from their own baseline (see Mitchell et al., 1977). Accordingly, conclusions regarding the effects of cocaine or GBR 12909 on *aversions* induced by GBR 12909 may be premature. However, given that GBR 12909 induces dose-dependent CTAs (see Freeman et al., 2005), it is likely that the results seen here are a product of its aversive effects and not simply a function of the compound's interference with the attenuation of neophobia. Assessments of the effects of drug preexposure with higher conditioning doses of GBR 12909 would provide more convincing evidence of the ability of drug preexposure to impact aversion learning with this compound.

Although DA may be involved in cocaine's aversive effects, recent work from our laboratory has also implicated roles for NE and 5-HT. In relation to NE, the NE antagonists prazosin and propranolol significantly *potentiate* cocaine-induced CTAs (Freeman et al., 2008), suggesting that NE may limit the aversive effects of cocaine. Antagonizing NE activity removes this inhibition allowing cocaine to induce stronger aversions. In support of this, Serafine and Riley (2009) have recently shown that preexposure to cocaine potentiated aversions induced by the NE transporter (NET) inhibitor desipramine (Serafine and Riley, 2009), suggesting that tolerance to cocaine during preexposure (likely through its actions on NET) resulted in the weakening of any inhibitory effects NE may have on desipramine's aversive effects (for similar investigations and findings with mice; see Jones et al., 2009). Although collectively this work suggests that NE activity may limit the aversive effects of cocaine (and desipramine), preexposure to desipramine attenuated cocaine-induced CTAs (Serafine and Riley, 2009). That is, tolerance to NET inhibition (via preexposure) weakened cocaine-induced aversions, an effect at odds with the aforementioned potentiating effects

with the reverse serial presentation, i.e., cocaine preexposure before desipramine. The basis for these different effects is not clear.

The role of 5-HT in cocaine-induced aversions is basically unknown. To date, no direct pharmacological studies have been conducted to assess the effects of 5-HT receptor activation (or antagonism) on the aversive effects of cocaine. Our laboratory has recently used the cross-drug preexposure preparation to assess the effects of exposure to the selective serotonin reuptake inhibitor (SSRI) fluoxetine on aversions induced by cocaine (and vice versa; Serafine and Riley, 2010; see also Jones et al., 2009 for a similar assessment with mice). Under these conditions, fluoxetine preexposure did not attenuate cocaine-induced CTAs, suggesting that the two do not share a common aversive effect, although higher doses of fluoxetine may attenuate cocaine-induced aversions. Interestingly, cocaine preexposure did attenuate fluoxetine-induced aversions. This asymmetry suggests that 5-HT may play some role in cocaine-induced aversions, but perhaps not a primary one. Preexposure to cocaine results in the adaption to all of its stimulus effects and to a degree that can affect aversions induced by other compounds with this action. However, preexposure to this single action is not sufficient to affect aversions induced by cocaine whose aversive effects may be multifaceted. It will be important to assess the effects of direct antagonism of 5-HT on cocaine-induced aversions to determine the role of 5-HT in cocaine's aversive effects.

The present experiments sought to confirm a role of DA (via DAT inhibition) in cocaine's aversive effects through the use of cross-drug preexposure. Although the effect was asymmetrical, the results of these experiments with cocaine and GBR 12909 do implicate a common mechanism in aversions induced by the two drugs (i.e., DAT inhibition). That DAT inhibition is integral to the induction of aversions by both compounds suggests that increases in extracellular DA mediate CTAs induced by each. Such a conclusion is supported by work with DA antagonists (see above). In the absence of collateral data from work using direct antagonism, such a conclusion would be limited, since the cross-drug preexposure preparation does not identify the specific shared mechanism, but only determines that there is a shared stimulus property. As such, the present findings, like other results of cross-drug preexposure, should be examined in the context of work from other procedures investigating the mechanism underlying cocaine-induced CTAs.

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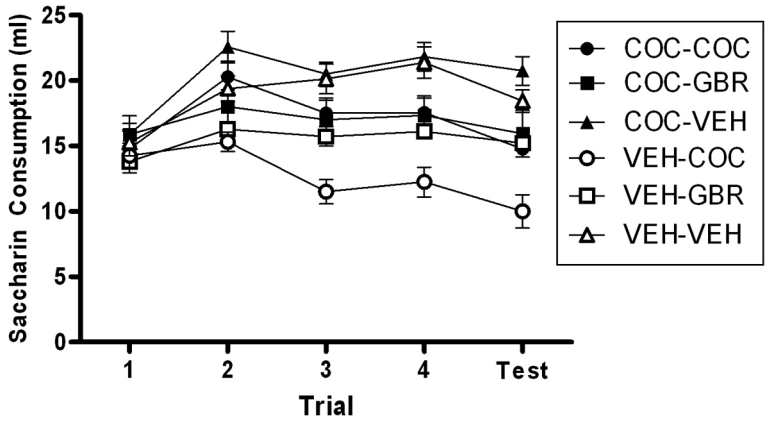

Fig. 1.

Mean (± SEM) saccharin consumption (ml) for all subjects in groups preexposed to cocaine or vehicle and conditioned with cocaine (18 mg/kg), GBR 12909 (32 mg/kg) or vehicle. All cocaine-preexposed subjects (regardless of conditioning drug) drank significantly more than vehicle-preexposed subjects (regardless of conditioning drug) on Trials 2 and 3. All drug-conditioned subjects (collapsed across preexposure condition) drank significantly less than all vehicle-conditioned subjects (collapsed across preexposure condition) on Trials 2, 3 and 4. Since no significant three-way interaction was observed, no post-hoc analyses were run on individual groups.

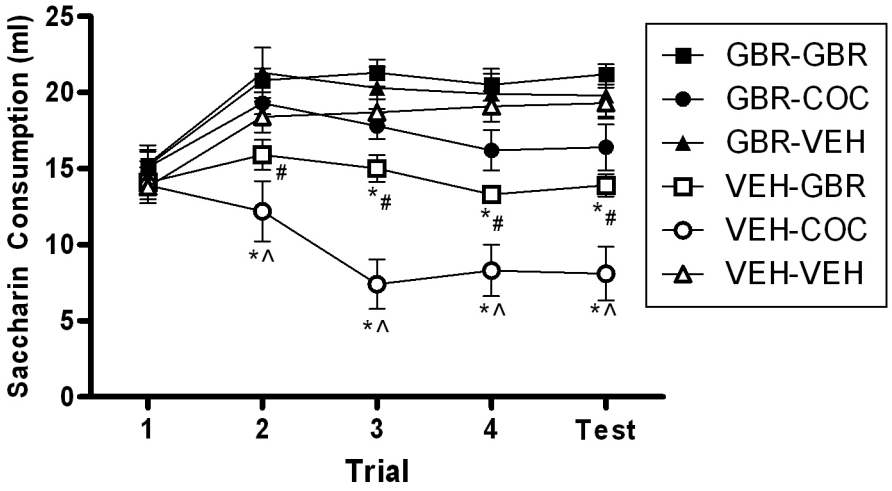

Fig. 2.

Mean (±SEM) saccharin consumption (ml) for all subjects in groups preexposed to GBR 12909 (50 mg/kg) or vehicle and conditioned with cocaine (18 mg/kg), GBR 12909 (32 mg/kg) or vehicle. *Significantly different from Group VEH–VEH; #Significantly different from Group GBR–GBR; ^Significantly different from Group GBR–COC.