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Differentiating the discriminative stimulus effects of gamma-hydroxybutyrate and ethanol in a three-choice drug discrimination procedure in rats

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Abstract

Anecdotal reports indicate that GHB produces subjective effects similar to those of ethanol. However, recent investigations comparing the discriminative stimulus effects of GHB to those of ethanol suggest that the subjective effects of these substances may differ considerably. To explore further potential differences between GHB and ethanol, 16 male Sprague–Dawley rats were trained in a three-lever drug discrimination procedure to discriminate ethanol (1.0 g/kg, experiment 1; 1.5 g/kg, experiment 2) and GHB (300 mg/kg) from vehicle. Dose–response functions determined with both training compounds revealed a clear dissociation between the discriminative stimulus effects of these drugs. As expected, the GHB precursors gamma-butyrolactone and 1,4-butanediol produced full substitution for GHB. In addition, the $GABA_B$ receptor agonist baclofen substituted for GHB, whereas the benzodiazepine flunitrazepam and the NMDA receptor antagonist ketamine engendered greater responding on the ethanol-lever. GHB's discriminative stimulus effects were blocked by the $GABA_B$ receptor antagonist CGP-35348 but only partially blocked by the putative GHB receptor antagonist NCS 382. These findings are consistent with previous reports of GHB's discriminative stimulus effects in two-choice drug discrimination procedures and provide additional evidence that these effects are distinct from those of ethanol.

Keywords: Gamma-hydroxybutyrate; Gamma-butyrolactone; 1,4-butanediol; Flunitrazepam; Ketamine; Baclofen; CGP-35348; NCS-382; Drug discrimination; Rats

1. Introduction

Gamma-hydroxybutyrate (GHB) occurs naturally in the mammalian nervous system where it is a putative neurotransmitter with proposed affinity for either a $GABA_B$ metabotropic receptor (Carter et al., 2003) or a specific GHB metabotropic receptor (Snead, 1977). In some European countries GHB has been used in alcohol and opiate detoxification (Gallimberti et al., 1989; Gallimberti et al., 1993) and in 2002 the United States Food and Drug Administration approved GHB (under the trade name Xyrem®) for the treatment of cataplexy in narcoleptic patients (Fuller and Hornfeldt, 2003; Fuller et al., 2005). The abuse of GHB is also a significant health concern and this drug has been characterized as a "date-rape" drug in the popular media (Schwartz et al., 2000).

Human users report that GHB produces feelings of euphoria and sedation that presumably resemble the effects of ethanol and other central nervous system depressants (Couper and Logan, 2001; Miotto et al., 2001; O'Connell et al., 2000). Based on these reports, and assuming that subjective effects of the drugs are similar in humans and non-humans, one would expect to find strong generalization between GHB and ethanol in non-human animals tested in drug discrimination procedures. Several studies have used such procedures to characterize the discriminative stimulus effects of GHB (e.g., Winter, 1981; Colombo et al., 1995a; Colombo et al., 1995b; Colombo et al., 1998; Lobina et al., 1999; Metcalf et al., 2001; Carter et al., 2003; Wu et al., 2003; Koek et al., 2004; Baker et al., 2004; Baker et al., 2005). It is now well accepted that GHB can be readily established as a discriminative stimulus and that the metabolic precursors of GHB, gamma-butyrolactone (GBL) and 1, 4-butanediol (1,4-BDL), produce stimulus generalization in animals trained to discriminate GHB from vehicle (Baker et al.,

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2005). Furthermore, the discriminative cue produced by GHB appears to be mediated by actions at $GABA_B$ receptors (Carter et al., 2003; Carter et al., 2004; Baker et al., 2005).

Results regarding stimulus generalization between GHB and ethanol are somewhat inconsistent. Colombo et al. (1995b) demonstrated cross-generalization between GHB and ethanol, but only within a narrow dose range. More recent investigations using different procedures have demonstrated only partial substitution between GHB and ethanol (Metcalf et al., 2001; Baker et al., 2004; Baker et al., 2005). To date, no one has examined whether animals can learn to discriminate among GHB, ethanol, and vehicle in a three-choice discrimination procedure. Such a procedure may detect differences in drug effects that are not evident in two-choice drug discrimination procedures. For example, research from our laboratory and elsewhere has demonstrated that drugs that show substantial cross-generalization in two-choice (drug versus vehicle) discrimination procedures may be readily discriminated in three-choice (drug 1 versus drug 2 versus vehicle) procedures (Bowen et al., 1997; Bowen and Grant, 1998; Makhay et al., 1998; Baker and Taylor, 1997; Goodwin and Baker, 2000; Goodwin et al., 2003).

The discriminative stimulus effects of ethanol have been examined extensively in two-choice (ethanol-vehicle) procedures. These investigations have consistently found that ethanol's discriminative stimulus effects are mediated by multiple receptor systems. Stimulus generalization to ethanol has been reported with $GABA_A$ positive modulators, including benzodiazepines, barbiturates, and neuroactive steroids (Barry and Krimmer, 1977; York, 1978; Ator et al., 1993; Grant et al., 1996), competitive and non-competitive NMDA receptor antagonists (Grant et al., 1991; Grant and Colombo, 1992; Sanger, 1993; Shelton and Balster, 1994), and 5-HT_1 receptor agonists (Signs and Schechter, 1988; Grant and Colombo, 1993a,b,c; Grant et al., 1997).

In an effort to provide detailed information about the neurochemical mechanisms underlying ethanol's discriminative stimulus properties and the similarity of those properties to those of other drugs, a few studies have implemented three-choice discrimination procedures (Gatto et al., 1995; Bowen et al., 1997; Bowen and Grant, 1998). Two of these investigations reported that ethanol can be discriminated from the non-competitive NMDA receptor antagonist dizocilpine (Gatto et al., 1995; Bowen and Grant, 1998) and one study demonstrated that ethanol can be discriminated from the $GABA_A$ positive modulator pentobarbital (Bowen et al., 1997). When rats were trained to discriminate dizocilpine from ethanol, this essentially eliminated the NMDA receptor component of the ethanol cue, without altering the $GABA_A$ or 5-HT_1 mediated effects. In contrast, when rats were trained to discriminate pentobarbital from ethanol, thereby eliminating the $GABA_A$ component, the NMDA antagonism component of the ethanol cue was also diminished. Moreover, the results of these three-choice discrimination investigations clearly indicate that the pharmacological effects of ethanol involved in establishing discriminative stimulus control may be modified by the discrimination training conditions to the extent that a particular receptor system appears to be no longer involved in the discriminative stimulus effects of the drug (Bowen et al., 1997).

The principle aim of the present investigation was to use a three-choice drug discrimination procedure to determine whether rats could discriminate among GHB, ethanol, and vehicle. Because most prior studies involving two-choice training procedures have reported only partial generalization between GHB and ethanol (Metcalf et al., 2001; Baker et al., 2004; Baker et al., 2005), we assumed that it would be possible to establish this discrimination. It was, and once this discrimination was established we examined whether it was based on qualitative differences between GHB and ethanol by examining stimulus generalization to substances previously shown to substitute for GHB (GBL, 1,4 -BDL, baclofen) or ethanol (flunitrazepam, ketamine) in two-choice drug discrimination investigations.

2. Methods

2.1. Subjects

Sixteen male Sasco Sprague Dawley rats (Charles River, Portage, MI) were individually housed in polycarbonate cages with corn cob bedding in a colony maintained with a 12-h light/dark cycle (lights on 0700 to 1900) and constant temperature (20 °C±2°) and humidity (50%±5%). Animals were experimentally naïve, approximately 60 days old, and weighed approximately 250 g at the beginning of the study. Water was freely available in the home cages, and commercial rodent diet was restricted to maintain body weights at 80–85% of free-feeding levels, accounting for age-related growth. Animals were maintained according to the general principles of animal husbandry outlined by the National Research Council (1996) and the experimental protocol was approved by the Institutional Animal Care and Use Committee of Western Michigan University.

2.2. Apparatus

Experimental sessions were conducted in eight operant testing chambers (MED Associates, Georgia, VT) measuring 30×31×24 cm and housed within sound- and light-attenuating cubicles. The test chambers were equipped with three retractable levers on the front panel, a food pellet delivery mechanism located above the center lever, and a 28-V house light located at the top of the rear panel. Dustless precision food pellets (45 mg, product # F0021, Bioserv®, Frenchtown, NJ) were used as reinforcers. MED-PC (version 4.0 for Windows) instrumentation and software were used to control experimental events and to record data.

2.3. Drugs

Gamma-hydroxybutyrate (National Institute on Drug Abuse, Bethesda, MD) and ethanol (AAPER Alcohol and Chemical Company, Shelbyville, KY) were administered by intragastric (IG) delivery 30 min before training or test sessions. Gamma-butyrolactone, 1,4-butanediol, (±)-baclofen, flunitrazepam, and ketamine-hydrochloride (Sigma Chemical Company, St. Louis, MO) were administered by intraperitoneal (IP) injection 15 min prior to test sessions. NCS-382 (6,7,8,9 Tetrohydro-5-[*H*]

benzocycloheptene-5-ol-4-vlideneacetic Acid, sodium salt; National Institute on Drug Abuse, Bethesda, MD) and CGP-35348 (Novartis Pharma, Basel, Switzerland) were administered by IP injection 40 min prior to test sessions.

To mask the taste of the training stimuli, GHB and ethanol solutions were prepared in strawberry–kiwi flavored Crystal Light® (Kraft Food, Ryebrook, NY) prepared with deionized water according to package instructions. These substances were administered at a volume of 10 ml/kg. Flunitrazepam was suspended in methyl-cellulose and administered at an injection volume of 2 ml/kg. All other drugs were dissolved in sterile 0.9% saline and administered at an injection volume of 1 ml/kg. Drug doses were calculated based on the weight of the salt (GHB, flunitrazepam-salt, (±)-baclofen, ketamine-hydrochloride, NCS-382) base (CGP-35348) or liquid (GBL, 1,4-BDL, ethanol). During training, Crystal Light® dissolved in deionized water was administered by IG delivery at a volume of 10 ml/kg 30 min prior to sessions when neither GHB nor ethanol was administered (i.e., Crystal Light® in deionized water constituted the "vehicle").

2.4. Training procedures

All training and test sessions occurred during the light phase of the light/dark cycle at approximately the same time of day five or six days per week. Training procedures were similar to those described by Goodwin and Baker (2000). In experiment 1, eight rats were trained to discriminate among GHB (300 mg/kg, IG 30 min), ethanol, (1.0 g/kg, IG 30 min) and vehicle. In experiment 2, eight other rats were trained to discriminate among GHB (300 mg/kg, IG 30 min), ethanol 1.5 g/kg, IG 30 min), and vehicle. In both experiments, training sessions were 20 min in duration and a resetting fixed-ratio 10 (FR 10) schedule was arranged for condition-appropriate responses. Under this schedule, 10 consecutive responses on the condition-appropriate lever were required for food delivery; each response on one of the other levers reset the response requirement. Responses on the center lever were reinforced (with food delivery) following vehicle administration in all animals. Responses on the right lever following GHB administration and responses on the left lever following ethanol administration were reinforced for half of the animals in each training group. Conditions were reversed for the remaining animals. To reduce the influence of olfactory stimuli on lever pressing, each lever was wiped with isopropyl alcohol before each session (Extance and Goudie, 1981). The training procedures varied slightly between experiments 1 and 2, and they are described separately below.

2.4.1. Experiment 1

Prior to discrimination training, eight preliminary training sessions were conducted in which only the condition-appropriate lever was present in the chamber; this is a variant of the "errorless" drug discrimination training procedure. These sessions were conducted in the following order: VVVGGEEG for four animals and VVVEEGGG for the other four animals (V=vehicle, G=GHB, E=Ethanol). When all animals were responding reliably under all three-stimulus conditions, drug discrimination training began with all three levers present in the chamber. The three putative discriminative stimuli (i.e., GHB, ethanol, and vehicle) were administered in a pseudo-random order, with the limitation that no animal received more than two consecutive sessions under the same stimulus condition. The FR schedule was gradually increased from FR 1 to FR 10. Once animals were reliably responding on the FR 10 schedule under all three-stimulus conditions, the criterion for discrimination was set at a minimum of 80% condition-appropriate responses before delivery of the first reinforcer. Each rat had to meet this criterion on a minimum of 8 of 10 consecutive training sessions before testing began.

2.4.2. Experiment 2

In this experiment, an effort was made to reduce the number of training sessions required to establish the GHB-vehicle-ethanol discrimination. To do so, rats were first trained to discriminate one drug (GHB 300 mg/kg or ethanol 1.5 g/kg) from vehicle, and subsequently trained to discriminate the other drug from vehicle. Then the three-condition discrimination was introduced. Although to our knowledge no one has directly compared the number of sessions required to meet the training criterion in a drug–drug-vehicle discrimination when subjects are first trained to discriminate one drug from vehicle as opposed to when they are trained from the beginning to discriminate among the two drugs and vehicle, Porter et al. (2005) recently reported that the former procedure led to very rapid acquisition of a clozapine–chlorpromazine-vehicle discrimination in rats. Therefore, we hypothesized that acquisition would be faster in experiment 2 than in experiment 1. In addition to changing how acquisition was arranged, we used a larger training dose of ethanol (1.5 g/kg) in experiment 2 than in experiment 1. This was done because larger drug doses are more easily discriminated and may yield more specific cues than smaller ones (Stolerman, 1993), assumedly increasing the likelihood of rapid acquisition of the GHB-ethanol-vehicle discrimination in experiment 2 and also increasing the likelihood of clear separation of the GHB and ethanol cues in generalization tests.

In Experiment 2, six preliminary training sessions were conducted (VVDDVD) with only the condition-appropriate lever present to establish responding on each lever. Following preliminary training, discrimination training commenced with only two levers present in the chamber and the response requirement was gradually increased from 1 to 10. For half of the animals beginning training under GHB and vehicle conditions, the left lever was designated the GHB-lever and the right lever was designated the vehicle lever. Likewise, for half the animals beginning training under the ethanol and vehicle conditions, the left lever was designated the ethanol-lever and the right lever was designated the vehicle lever. Conditions were reversed for the remaining animals in each subgroup.

When each rat's discrimination performance satisfied the above-mentioned discrimination criterion (80% condition-appropriate responses prior to first reinforcer for a minimum of 8 out of 10 consecutive training sessions) in the first two-lever drug discrimination, training commenced with the second drug condition and vehicle, with only the vehicle lever and second drug-lever present during these training sessions. After

the criterion was met for the second two-lever drug discrimination, discrimination training began with all three-stimulus conditions and all three levers present in the chamber. The order of presentation of the three-stimulus conditions was random, with the restriction that no animal received more than two consecutive training sessions under the same stimulus condition.

2.5. Testing procedures

In both experiments 1 and 2, stimulus generalization tests commenced for each animal after the discrimination criterion was reached. For each compound examined, test sessions were conducted once or twice per week, and doses were tested in a counterbalanced order across subjects. Between stimulus generalization tests, animals received at least one training session under each stimulus condition, and they were required to meet the minimum criterion of 80% condition-appropriate responses under all three training conditions before each test. Test sessions ended when 10 consecutive responses were made on any lever or after 20 min, whichever occurred first. No reinforcers were delivered during test sessions and each animal was removed from the chamber immediately upon completion of 10 consecutive responses on any lever.

2.5.1. Experiment 1

Stimulus generalization tests were conducted with vehicle, GHB (75–300 mg/kg) and ethanol (0.5–4.0 g/kg) to determine dose–response functions with each training drug. Subsequently, stimulus generalization tests were conducted with the GHB precursors, GBL (37.5–150 mg/kg) and 1,4-BDL (50–400 mg/kg), and with the benzodiazepine flunitrazepam (1.0–4.0 mg/kg). Finally, tests of stimulus antagonism were conducted with the purported GHB antagonist NCS-382 (50–200 mg/kg), and the $GABA_B$ antagonist CGP-35348 (100–400 mg/kg), each administered in combination with 300 mg/kg GHB. The training condition conducted prior to each dose tested was counterbalanced among subjects.

2.5.2. Experiment 2

Stimulus generalization tests were conducted with vehicle, GHB (75–300 mg/kg), and ethanol (0.25–1.5 g/kg) to determine dose–response functions with each training drug. Subsequently, stimulus generalization tests were conducted with flunitrazepam (.0–4.0 mg/kg), the $GABA_B$ agonist baclofen (1.0–10.0 mg/kg), and the NMDA receptor antagonist ketamine (1.0–20.0 mg/kg). The training condition conducted prior to each dose tested was counterbalanced among rats.

2.6. Data analysis

Dose–response functions are presented as the group mean percent of total responses made on the condition-appropriate lever during test sessions. Response rate is expressed as the mean total number of responses per second during test sessions. Data from tests in which animals did not emit at least 10 total

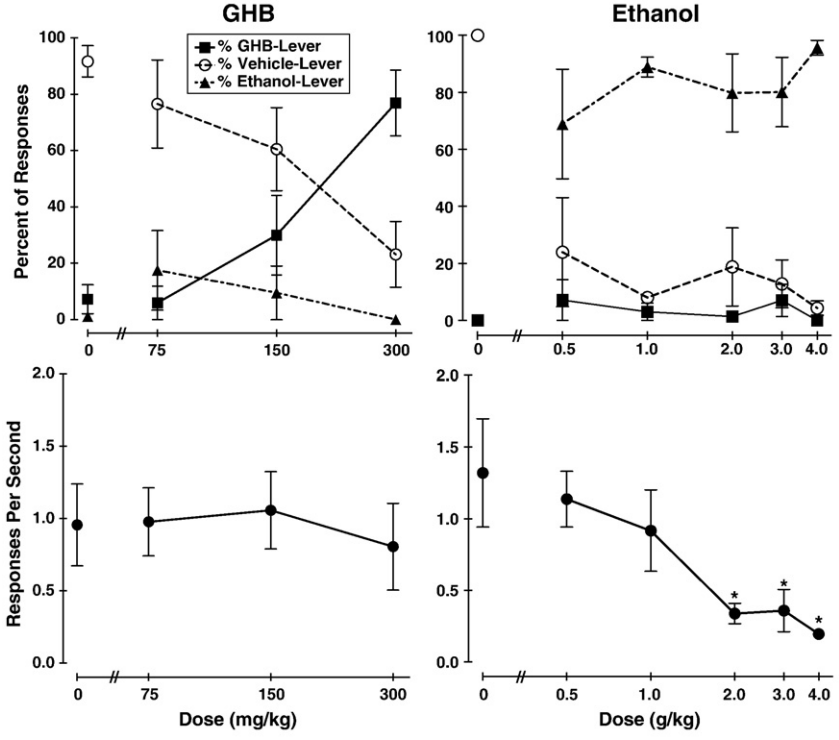

Fig. 1. Results of stimulus generalization tests with GHB (left) and ethanol (right) in rats ($n=7$) trained to discriminate among GHB (300 mg/kg), ethanol (1.0 g/kg) and vehicle. The top graphs depict the average percentage of responses on each of the three levers and the bottom graphs depict the average overall response rate (responses per second) during stimulus generalization tests. Points represent means, and error bars represent the standard error of the mean. Asterisks indicate response rates that were significantly different from vehicle control.

responses were not graphed or statistically analyzed. Drug-appropriate responding above 80% was considered as evidence for complete substitution, or stimulus generalization. Complete antagonism was defined as less than 20% drug-appropriate responses. Response rate data were statistically analyzed using repeated measures analyses of variance, and Dunnett's tests were conducted to compare the different dose levels to the vehicle control. All statistical analyses were conducted and graphs were created using Prism GraphPad (version 4.0) software (San Diego, CA).

3. Results

3.1. Experiment 1

All eight animals met the criterion for discrimination among GHB (300 mg/kg), ethanol (1.0 g/kg), and vehicle conditions. The mean (±S.E.M.) number of sessions required to meet criterion was 98.3 (±8.3) (median=107; range: 70–103). The dose–response functions for GHB and ethanol are depicted in Fig. 1. Both GHB and ethanol produced dose-dependent increases in the percentage of responses on the GHB- and ethanol-appropriate levers, respectively. Higher doses of ethanol (2.0–4.0 g/kg) substituted for the training dose of ethanol, but also suppressed responding in a dose-dependent manner. An ANOVA of the response rates from ethanol tests yielded statistical significance ($F_{5,36}=3.66$, $p<0.05$) with significant decreases following the three higher doses (2.0, 3.0, and 4.0 g/kg) compared to vehicle control ($p<0.05$).

Fig. 2 illustrates the results of stimulus generalization tests with GBL, 1,4-BDL, and flunitrazepam in experiment 1. Both GHB precursors generally produced dose-dependent increases in GHB-lever responses with full substitution at the highest dose tested. The benzodiazepine flunitrazepam produced substantial ethanol-lever responding, but failed to produce full stimulus generalization for ethanol, even at the highest dose tested (70% ethanol-lever responses at 4.0 mg/kg). Only two of the five animals tested showed complete stimulus generalization to ethanol with 4.0 mg/kg flunitrazepam; the remaining three made between 40 and 60% of their responses on the ethanol-lever. Response rates were not significantly decreased by either GHB precursor or by flunitrazepam. Higher doses of flunitrazepam were not tested due to difficulties maintaining this compound in solution at higher concentrations.

The results of stimulus antagonism tests with CGP-35348 and NCS-382 administered in combination with GHB (300 mg/kg) are depicted in Fig. 3. The discriminative stimulus effects of GHB were completely blocked by all three doses (100, 200, 400 mg/kg) of the $GABA_B$ antagonist CGP-35348. The effects of this drug combination on response rate were not statistically significant. The GHB antagonist NCS-382 attenuated GHB-appropriate responding but did not completely block the GHB stimulus at the doses tested (25, 50, 100 mg/kg).

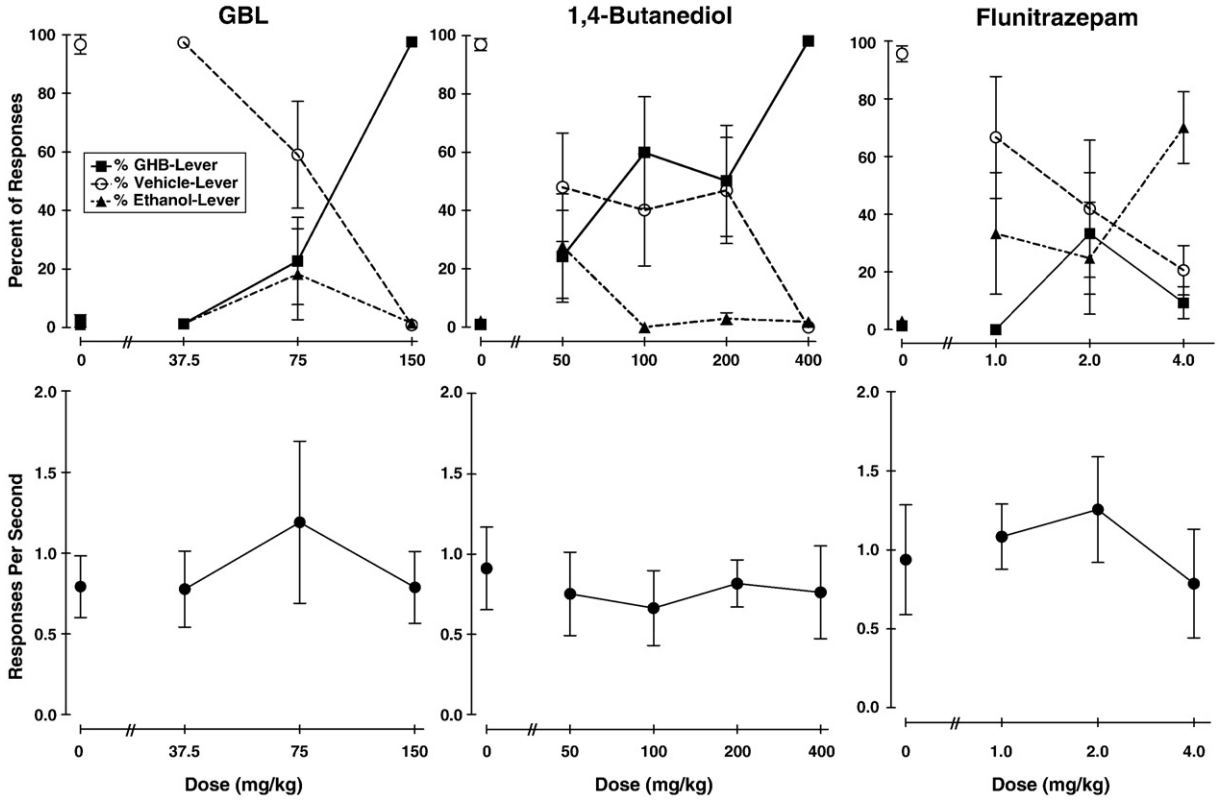

Fig. 2. Results of stimulus generalization tests with GBL ($n=6$–7), 1,4-BDL ($n=6$–7), and flunitrazepam ($n=5$) in rats trained to discriminate among GHB (300 mg/kg), ethanol (1.0 g/kg) and vehicle. See Fig. 1 for details.

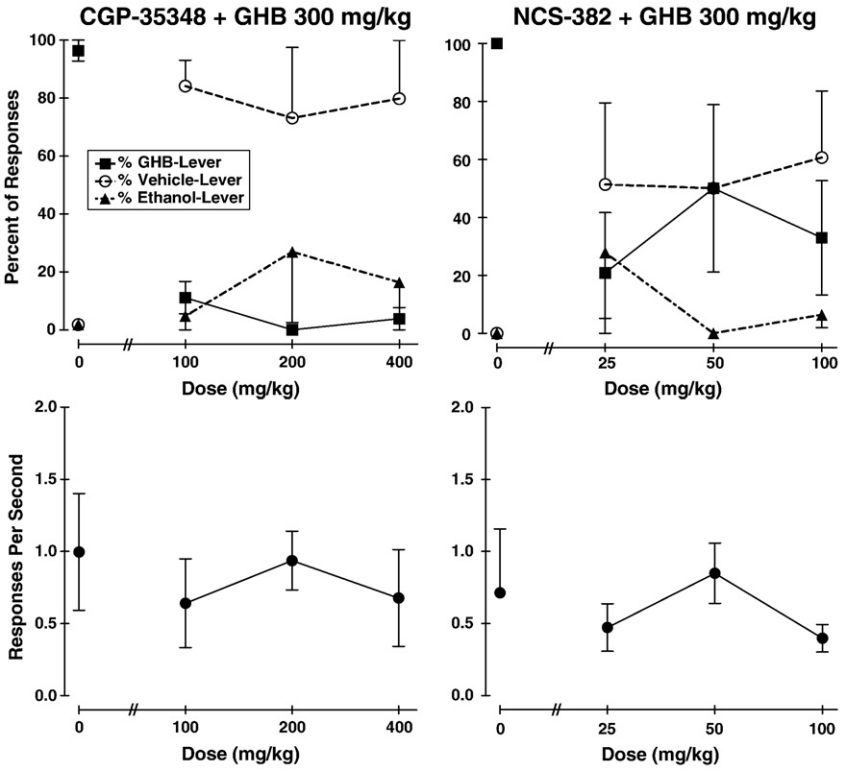

Fig. 3. Results of antagonism tests with CGP 35348 ($n=4$) and NCS-382 ($n=4$) administered in combination with 300 mg/kg GHB in rats trained to discriminate among GHB (300 mg/kg), ethanol (1.0 g/kg), and vehicle. See Fig. 1 for details.

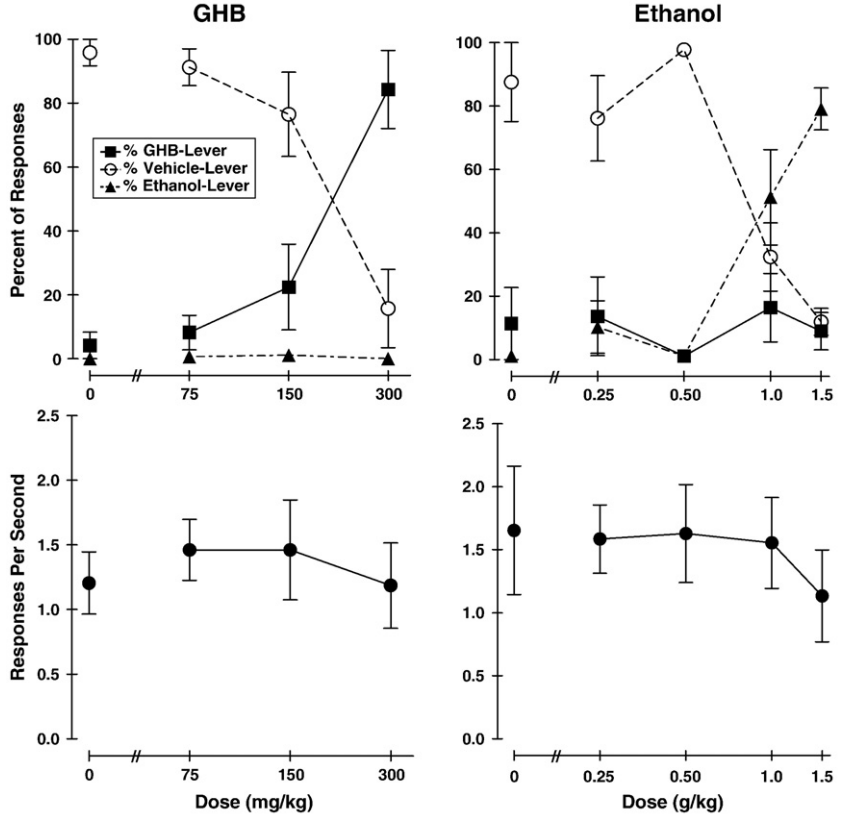

Fig. 4. Results of stimulus generalization tests with GHB and ethanol in rats ($n=8$) trained to discriminate among GHB (300 mg/kg), ethanol (1.5 g/kg), and vehicle. See Fig. 1 for details.

3.2. Experiment 2

The first training drug was GHB for four animals and ethanol for the other four animals. The order in which discriminations were established did not appear to influence the number of sessions required to meet the final (i.e., GHB-ethanol-vehicle) discrimination criterion. As a group, the eight rats met the first discrimination criterion in an average of 35.13 (±6.71; range 19–78) sessions and the second discrimination criterion in an average of 50.63 (±8.14; range 16–85) sessions. Following the presentation of all three training conditions, the animals required an average of 65.5 (±8.9; range 30–115) additional sessions to meet the discrimination criterion. Thus, the average total number of sessions to meet the final criterion for the GHB-ethanol-vehicle discrimination was 151.3 (±10.8; range 121–195). This number is higher than the average total number of sessions required to meet the criterion in experiment 1. Under conditions as arranged in the present study, and contrary to our hypothesis, training rats under two-stimulus conditions (drug-vehicle) prior to training them under all three-stimulus conditions slowed, not accelerated, acquisition of the GHB-ethanol-vehicle discrimination.

The dose–response functions for GHB and ethanol in experiment 2 are shown in Fig. 4. As in experiment 1, both training drugs produced dose-dependent increases in the percentage of drug-appropriate responses. A lower dose of ethanol (0.25 g/kg) was examined in this experiment, and it (like the 0.5 g/kg dose) produced minimal drug-lever responding. None of the GHB or ethanol doses tested significantly suppressed responding in these animals. Higher doses of ethanol were not tested in experiment 2 because they were found to significantly disrupt responding in experiment 1.

Fig. 5 illustrates the results of stimulus generalization tests with baclofen, flunitrazepam, and ketamine. The $GABA_B$ receptor agonist, baclofen, produced an average of 80% GHB-lever responses at the 3.0 mg/kg dose, although higher doses (6.0, 10.0 mg/kg) produced slightly less GHB-appropriate responding. It is important to note that there was considerable variability across subjects in their response to baclofen. Four of the eight animals made more than 90% of their responses on the GHB-lever following 3.0 mg/kg baclofen, while the other four made between 53 and 79% of their responses on the GHB-lever. Following the 6.0 mg/kg dose, five animals exhibited full stimulus generalization to GHB, two animals distributed their responding about equally between the GHB and ethanol-levers, and one animal responded entirely on the vehicle lever. Following 10 mg/kg baclofen, four animals exhibited full stimulus generalization to GHB. Of the remaining four animals, one emitted 56% of its responses on the GHB-lever, one responded exclusively on the ethanol-lever, one responded exclusively on the vehicle lever, and one failed to make any responses. An ANOVA of the response rates during baclofen tests yielded statistical significance

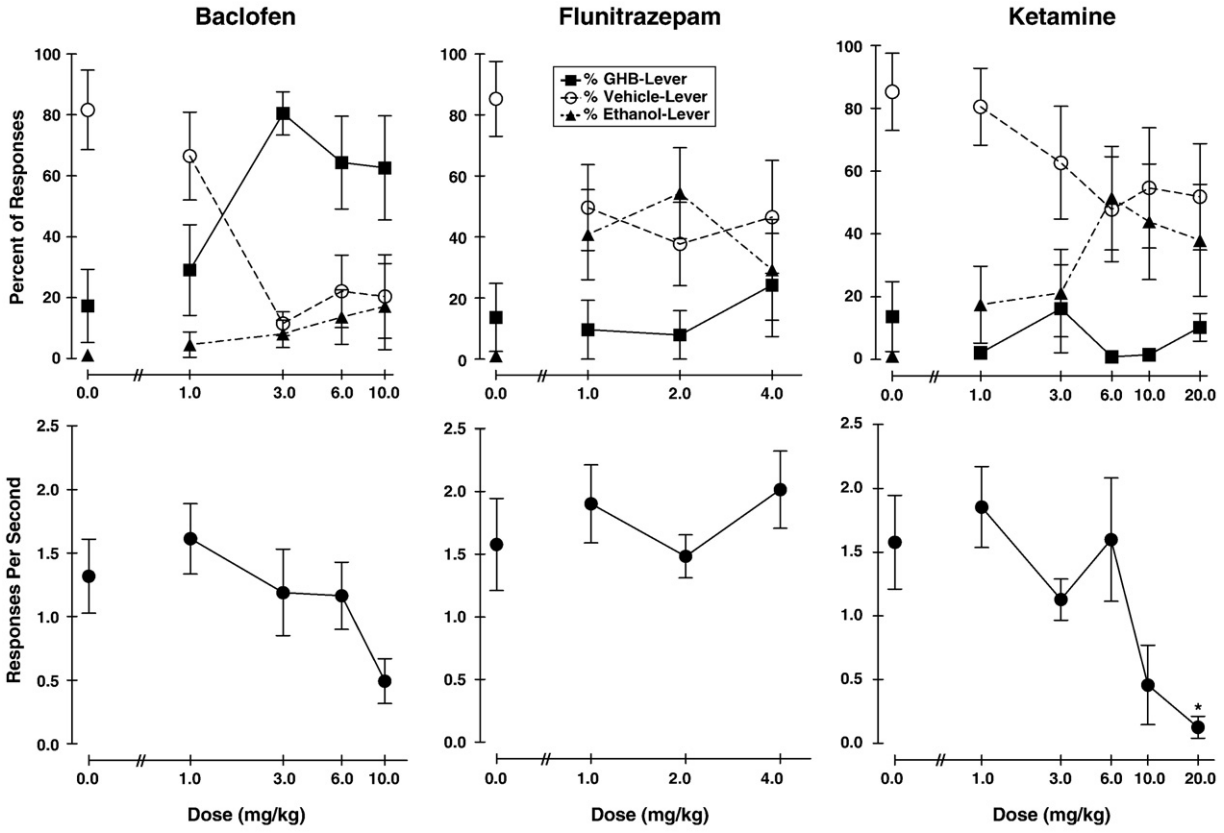

Fig. 5. Results of stimulus generalization tests with baclofen (n=7–8), flunitrazepam (n=6–8), and ketamine (n=6–8) in rats trained to discriminate among GHB (300 mg/kg), ethanol (1.5 g/kg), and vehicle. See Fig. 1 for details.

($F_{4,39}=3.01$, $p<0.05$), with significant differences between the 1.0 mg/kg and 10.0 mg/kg dose ($p<0.05$).

Testing with flunitrazepam resulted in substantial variability across subjects and the drug failed to substitute fully for ethanol in experiment 2, producing at most only 54% ethanol-appropriate responding (at a dose of 2.0 mg/kg). In contrast to the results of experiment 1, only one of six animals generalized completely to ethanol following 4.0 mg/kg flunitrazepam; one responded exclusively on the GHB-lever, two responded exclusively on the vehicle lever, one distributed its responses between the ethanol and vehicle lever, and one distributed its responses among all three levers. Flunitrazepam did not significantly decrease response rates relative to vehicle control in these animals. Higher doses were not tested due to difficulties maintaining flunitrazepam in solution at higher concentrations.

The NMDA receptor antagonist ketamine produced only partial substitution to ethanol. Only three of the six animals tested exhibited full generalization to ethanol following 6.0 mg/kg ketamine and two exhibited full generalization to ethanol following 10 and 20 mg/kg ketamine. Most animals distributed their responses between the ethanol-lever and the vehicle lever, with little to no responding on the GHB-lever. Response rates were significantly reduced by ketamine ($F_{5,39}=4.52$, $p<0.005$), with significant differences between the 20.0 mg/kg and 0, 1.0, and 6.0 mg/kg doses ($p<0.05$).

4. Discussion

The results of both experiments 1 and 2 indicate that GHB and ethanol produce distinguishable discriminative stimulus effects, even though human users report that their subjective effects are similar (Couper and Logan, 2001; Miotto et al., 2001; O'Connell et al., 2000). In both experiments, all subjects learned to discriminate among these substances and vehicle. Moreover, in generalization tests, no dose of GHB occasioned substantial ethanol-appropriate responding and no dose of ethanol occasioned substantial GHB-appropriate responding. These results suggest that the rats were distinguishing between the training doses of GHB and ethanol based on qualitative differences in the cues produced by these drugs, as opposed to quantitative differences between them.

Porter et al. (2005) previously reported rapid acquisition of a clozapine–chlorpromazine-vehicle discrimination when rats were trained under procedures similar to those of experiment 2. Moreover, a higher training dose of ethanol (1.5 g/kg) was used in experiment 2 than an experiment 1 (1 g/kg), and higher training doses typically establish stronger stimulus control (Stolerman, 1993). Given these considerations, we hypothesized that the GHB-ethanol-vehicle discrimination would be acquired in fewer sessions in experiment 2 than in experiment 1. This hypothesis was not confirmed; the initial training of animals under two stimulus conditions (drug-vehicle) prior to training under all three-stimulus conditions failed to accelerate the acquisition of the GHB-vehicle-ethanol discrimination.

In fact, this strategy significantly increased the number of sessions required to meet the final discrimination criterion. Perhaps this was due to considerable overlap in the discriminative stimulus effects of GHB and ethanol. If the stimulus effects of these two substances were distinctly different, then stimulus control would likely have been established within the first 10 sessions that all three levers were present in experiment 2, because the animals had already met the criterion for stimulus control under both of the drug-vehicle training conditions. The dissociation of GHB's and ethanol's discriminative effects may require successive contact with all three-stimulus conditions and substantial learning under each of those conditions. The extent to which preliminary discrimination training under two-stimulus conditions facilitates or hinders the subsequent establishment of discriminative stimulus control under three-stimulus conditions may depend on the particular drugs and/or doses employed as the stimulus conditions. This possibility warrants further investigation.

As expected, GBL and 1,4-BDL produced complete stimulus generalization to GHB. This is consistent with our previous studies which assessed the same doses of these substances in rats trained to discriminate GHB from vehicle using two-lever drug discrimination methods (Baker et al., 2004; Baker et al., 2005). This result is also consistent with the generally held assumption that GBL and 1,4-BDL are rapidly converted in the body to GHB, and it is the latter substance that is primarily responsible for the behavioral effects of the precursors (Nicholson and Balster, 2001). The fact that flunitrazepam did not substitute for GHB, but produced significant ethanol-lever responding (experiment 1), is consistent with previous findings that other benzodiazepines do not substitute for the discriminative stimulus effects of GHB (Colombo et al., 1998; Baker et al., 2005). Differences in the extent of flunitrazepam substitution for ethanol between experiment 1 and experiment 2 may be due to the different training doses of ethanol employed in the two experiments, although this was not examined systematically. In the present study, baclofen substituted for GHB but ketamine did not; both results are also consistent with previous reports involving two-choice discrimination procedures (Winter, 1981; Carter et al., 2004; Baker et al., 2005).

Results from stimulus generalization tests in both experiments suggest that the discriminative stimulus effects of GHB and ethanol may be mediated through different pharmacological mechanisms. The substitution of baclofen for GHB and the partial substitution of flunitrazepam and ketamine for ethanol in the present study suggest that $GABA_B$ receptor actions may generate a salient component of the discriminative stimulus effects of GHB, whereas ethanol's discriminative stimulus effects appear to be mediated, at least in part by $GABA_A$ and NMDA glutamate receptors. This latter conclusion is consistent with previous reports (e.g., Butelman et al., 1993).

Testing with the $GABA_B$ antagonist CGP-35348 and the putative GHB antagonist NCS-382 also yielded findings similar to those of our previous studies using two-choice discrimination procedures (Baker et al., 2005). An early study suggested that NCS-382 antagonized the discriminative stimulus effects of GHB (Colombo et al., 1995a), but more recent investigations suggest that the GHB antagonist effects of NCS 382 are limited (Carter et al., 2003; Koek et al., 2004; Koek et al., 2005). NCS-382 has been reported to substitute for GHB in pigeons (Koek

et al., 2004) but not in rats (Baker et al., 2005). NCS-382 did not strongly antagonize GHB as a discriminative stimulus in the present study; we did not examine whether this compound substituted for either training drug. The $GABA_B$ antagonist, CGP-35348, did significantly reduce GHB-appropriate responding in the present study. These findings are consistent with previous results from two-choice GHB-vehicle discrimination studies (Colombo et al., 1998; Baker et al., 2005).

Because ethanol produces partial substitution for GHB (Metcalf et al., 2000; Metcalf et al., 2001; Baker et al., 2004), the cues mediating discrimination of these substances appear to be similar, but not identical. The present findings clearly demonstrate that the stimulus effects of 300 mg/kg GHB and 1.0 or 1.5 mg/kg ethanol are dissociable. Like ethanol, GHB may produce a complex cue, mediated by multiple components. The three-choice drug discrimination procedure has proven to be a useful method for assessing the various neurochemical components of the ethanol discriminative cue (Gatto et al., 1995; Bowen et al., 1997; Bowen and Grant, 1998). When animals are trained to discriminate ethanol from vehicle, the ethanol discriminative cue appears to have GABAergic, glutaminergic, and serotonergic components. However, the extent to which each of these components contributes to ethanol discrimination clearly depends upon the training conditions employed. For example, the NMDA receptor antagonists PCP and ketamine substitute for ethanol (Butelman et al., 1993), although ethanol exhibits only partial substitution for PCP (Balster et al., 1992). Evidence for asymmetrical generalization between PCP and ethanol suggests that NMDA antagonism may be a sufficient but not a necessary component of the ethanol discriminative cue.

In contrast, when rats were trained to discriminate the NMDA antagonist dizocilpine from both ethanol and vehicle, PCP did not substitute for ethanol (Bowen and Grant, 1998). Thus, the relative importance of NMDA receptor antagonism in mediating ethanol's discriminative cue depends on the drug stimuli used during discrimination training. The present findings are generally consistent with this conclusion. In a previous study from our laboratory, ketamine and flunitrazepam both produced partial substitution in rats trained to discriminate GHB from vehicle (Baker et al., 2005). In the present study, these substances produced very little GHB-appropriate responding, but also failed to substitute completely for ethanol.

Although the procedure is rarely used, an innovative method for assessing the influence of training conditions on the discriminative stimulus effects of drugs involves training a drug versus "other" condition, in which the "other" condition may consist of vehicle or one or more different drugs. For example, Koek et al. (2005) recently demonstrated that rats could be trained in a two-lever procedure to discriminate between GHB (stimulus 1) and vehicle (stimulus 2) (group 1); among GHB (stimulus 1), baclofen or vehicle (stimulus 2) (group 2), and; among GHB (200 mg/kg), diazepam (1 mg/kg) or baclofen (3.2 mg/kg) or vehicle (stimulus 2) (group 3). In generalization tests, GHB produced over 80% GHB-appropriate responding in all groups. Diazepam produced 68% GHB-appropriate responding in group 1, 30% in group 2, and 5% in group 3. Baclofen produced 84% GHB-appropriate responding in group 1, but less than 30% in groups 2 and 3. Clearly, the particular stimulus conditions under which rats were initially trained strongly influenced the extent to which diazepam and baclofen produced effects similar to those of GHB. Such findings have important implications for attempts to delineate the neuropharmacological mechanisms of drug action based on the results of drug discrimination procedures. Specifically, the manner in which drug discrimination studies are conducted may strongly influence the extent of cross-generalization between drugs, and hence speculations regarding similarities and differences in neurochemical activity.

In summary, the results of this study clearly demonstrate that the discriminative stimulus effects of GHB (300 mg/kg) and ethanol (1.0 or 1.5 mg/kg) are dissociable, at least in rats. Our findings also suggest that the neuropharmacological mechanisms mediating the subjective effects of these drugs may differ. Finally, this study lends further support for the utility of three-choice drug discrimination procedures in characterizing the discriminative stimulus effects of drugs with complex cue properties.

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