ELSEVIER

Pharmacology, Biochemistry and Behavior 70 (2001) 31–41

PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR

www.elsevier.com/locate/pharmbiochembeh

Discrimination of gamma-hydroxybutyrate and ethanol administered separately and as a mixture in rats

Brian R. Metcalf[a,*], Jeanne M. Stahl[b], Joseph D. Allen[c], Dedra R. Woolfolk[b], Paul L. Soto[b]

[a]*Psychology Program, Hawaii Pacific University, 1188 Fort Street Mall, Honolulu, HI 96813, USA*
[b]*Department of Psychology, Morris Brown College, Atlanta, GA 30314, USA*
[c]*Department of Psychology, University of Georgia, Athens, GA 30302, USA*

Received 23 August 2000; received in revised form 10 April 2001; accepted 27 April 2001

Abstract

The physiological effects of gamma-hydroxybutyrate (GHB) are complex and not yet clearly defined. GHB has been labeled as a recreational drug and is reported to be frequently coabused with ethanol (ETH). Other studies have yielded discrepant results as to the interaction between GHB and ETH. Thus, the present study investigated extensively the discriminative stimulus of GHB and ETH and a mixture of the two compounds. Thirty male Long–Evans rats were divided into three groups and trained to discriminate doses of either 300 mg/kg GHB, 1000 mg/kg ETH, or a mixture (MIX: 150 mg/kg GHB + 500 mg/kg ETH) from vehicle on a two-lever fixed-ratio (FR) 10 schedule of food reinforcement. Dose–response curves were attained in each group with its respective training drugs. GHB and ETH did not cross-generalize in the ETH- and GHB-trained rats, respectively. However, when the effects of the MIX were tested in the GHB- and ETH-trained rats, a greater than additive response was observed. Testing also revealed that the MIX-trained rats did not perceive a novel stimulus but a near-equal contribution from GHB and ETH. This study provides evidence of a complex relationship between GHB and ETH and opposes previous work reporting cross-generalization between GHB and ETH. © 2001 Elsevier Science Inc. All rights reserved.

Keywords: Gamma-hydroxybutyrate (GHB); Ethanol; Drug discrimination; Cross-generalization; Mixture; GABA; Fixed ratio; Long–evans hooded rats

1. Introduction

Gamma-hydroxybutyrate (GHB) is a naturally occurring metabolite of gamma-aminobutyrate (GABA) that satisfies several criteria as a neurotransmitter. GHB has been demonstrated to be synthesized in neurons, released by depolarization, has chemical reuptake mechanisms in presynaptic endings, and has specific high-affinity receptors in several brain areas studied (Colombo et al., 1998; Maitre, 1997; Vayer et al., 1987). While the physiological function of GHB has yet to be clearly defined, it is known to have binding sites in the brain where it exerts GABA-like activity, increases brain opioids, induces changes in dopamine synthesis and release, and increases serotonin levels (Cash, 1994; Colombo et al., 1998). GHB is well absorbed orally, readily crosses the blood–brain barrier, and is subsequently metabolized to carbon dioxide and water without active metabolites (Mamelak, 1989; Vayer et al., 1987).

During the 1980s, GHB was widely available over-the-counter (OTC) in health food stores and was purchased largely by body-builders for its supposed ability to stimulate growth hormone release during slow wave, Stage 4 sleep to enhance muscle growth. By the early 1990s, GHB had gained popularity as a recreational drug that produced pleasant, alcohol-like (yet hangover free) euphoric feelings, as well as potent prosexual and hypnotic effects (Friedman et al., 1996; Maitre, 1997). Shortly thereafter, the United States Food and Drug Administration (FDA) issued a report warning of the potential abuse liability of GHB. In the late 1990s, the FDA banned the OTC sale of GHB due to potential GHB-related illnesses and symptoms among recreational users including nausea, vomiting, dizziness, tremors, seizures, diarrhea, incontinence, respiratory depression, unconsciousness, and coma. The only legal use of GHB in the United States has been with FDA exemp-

* Corresponding author. Tel.: +1-808-544-1473; fax: +1-808-544-9306.
E-mail address: bmetcalf@hpu.edu (B.R. Metcalf).

PII: S0091-3057(01)00579-2

tions for approved research protocols. Elsewhere, GHB has been studied as a means for inducing absence seizures, for use as an anesthetic, and for the treatment of narcolepsy (Mamelak et al., 1986). Researchers have investigated the potential use of GHB in ethanol (ETH) and opiate withdrawal (Gallimberti et al., 1989, 1992, 1993, 1994; Rosen et al., 1997).

The abuse liability representation of GHB, however, seems far from clear. While some researchers have noted the ability of users of GHB to develop tolerance and addiction (Galloway et al., 1997), others have not (Maitre, 1997). The few investigations of the rewarding properties of GHB through preference tests or self-administration in animals are likewise inconclusive with some reporting preference (Colombo et al., 1995a; Martellotta et al., 1997), and others failing to find reinforcing properties or self-administration beyond control levels (Beardsley et al., 1996; Maitre, 1997). Despite these inconsistent results in animal studies, and in light of the fact that recreational use of GHB is high, it appears that GHB has reinforcing properties and abuse potential for humans (Galloway et al., 1997).

Because of its chemical and pharmacological similarity to ETH, and because it is often taken by humans in conjunction with ETH, a better understanding of the relationship between these two drugs is particularly important and has prompted several investigations. Administration of GHB suppressed voluntary ETH drinking in rats (Fadda et al., 1988). Gallimberti et al. (1992) found that GHB reduced alcohol consumption and alcohol craving in humans in a clinical trial. GHB has also effectively alleviated symptoms of ETH withdrawal in both ETH-dependent rats (Fadda et al., 1989) and humans (Gallimberti et al., 1989). Cross-tolerance to motor-impairing effects between GHB and ETH in rats has been reported (Colombo et al., 1995d). The determinants of GHB's illicit use, its pharmacological similarity to ETH, and its apparent ability to relieve craving and signs of withdrawal from alcohol use are not clear.

Drug discrimination (DD) assays are one set of procedures that have been found to be particularly useful in characterizing these determinants for other drugs (Colpaert, 1986; Goudie and Leathley, 1993; Holtzman, 1990; Overton, 1982). DD assays involve training laboratory animals to emit one response when the training drug is administered and a different response after the vehicle is given. When a different test drug elicits behavior similar to that seen while under the training drug, an assumption is made that the animal's perception of the two drugs is similar. On the other hand, stimulus effects of drugs assumed to be perceived as different from the training drug produce behaviors consistent with those produced during the vehicle administration during training sessions.

There have only been a few published studies examining GHB using DD assays, and only two of these included tests for generalization to ETH. Winter (1981) found that ETH in doses ranging from 630 to 1260 mg/kg did not generalize to GHB in rats trained to discriminate 200 mg/kg GHB from saline on an fixed-ratio (FR) 10 schedule of water reinforcement. More recently, Colombo et al. (1995c), using a T-maze, food-reinforcing DD procedure with rats, found a narrow window of symmetrical cross-generalization between 1.0 g/kg of ETH and 300 mg/kg GHB with neither higher nor lower doses producing such effects. Furthermore, if the training dose of ETH was increased to 2.0 g/kg, generalization to GHB was not seen. Colombo et al. (1995b) also found antagonism of the discriminative stimulus effects of both 300 and 700 mg/kg doses of GHB after administration of the GHB-antagonist, NCS-382. Beardsley et al. (1996) evaluated the reinforcing effects and discriminative stimulus effects of GHB in tests compared to vehicle and a variety of drugs (ETH was not investigated). They found no evidence of self-administration by their monkeys beyond control levels, and in their study with rats, GHB did not generalize to PCP or heroin, nor did GHB attenuate the discriminative stimulus effects of cocaine in a two-lever choice DD task. Studies such as these reveal that GHB continues to be an interesting compound whose pharmacology is not easily classified within common classes of CNS-active drugs, even though some findings suggest parallel effects between GHB and certain other drugs (Fadda et al., 1988, 1989; Gallimberti et al., 1989, 1992, 1993, 1994; Rosen et al., 1997).

Together, these studies suggest that GHB may have important and unique discriminative stimulus properties that might entail a compound stimulus of ETH-, morphine-, GABA-, and serotonin-like effects. It has been shown that the discriminative stimuli produced by many drugs, when administered singly, exert their effects as multiple cues with different neurological components (Barry and Krimmer, 1979; Overton, 1987). This concept has been confirmed by several researchers who have shown that animals can be trained to distinguish drug mixtures from their vehicles (Gauvin and Halloway, 1993; Stolerman et al., 1991). Typically, with rats trained to discriminate a mixture from the nondrugged state, there is partial or full generalization to the individual drugs of the mixture suggesting that the components of compound drug-produced stimuli can be perceived separately (White and Stolerman, 1994). That is, the mixtures do not appear to produce stimuli unique from their constituents. Furthermore, each component of a mixture generally shows complete generalization to the mixture only at doses higher than the training dose of the mixture (Garcha and Stolerman, 1989; Mariathasan et al., 1991). Such discrimination testing between mixtures and vehicle is referred to as a "PLUS" discrimination (Gauvin and Halloway, 1993) or an "AND" discrimination, while tests between mixtures and their individual components is called an "AND/OR" discrimination (Stolerman and Mariathasan, 1990). This approach has been used to evaluate interactions between commonly abused mixtures, such as nicotine and ETH (Gauvin and Halloway, 1993), amphetamine and pentobarbital (Stolerman and Mariathasan, 1990), cocaine, caffeine, ephedrine, phenylpropanolamine (Gauvin et al.,

1989), and phentermine and fenfluramine (Shoaib et al., 1997). Researchers such as Shoaib et al. (1997) have shown that two similar drugs given as a mixture do not produce a novel cue as might be expected if the synergistic effect of the drugs work in a potentiative manner.

Due to the unclear relationship between ETH and GHB in terms of site of shared activity, potential stimulus cross-generalization, and the increasing use and abuse of these two drugs in humans, the present study represents a partial replication of the work by Winter (1981) and Colombo et al. (1995c) and an additional investigation of the level of generalization occurring during concurrent administration of ETH and GHB. Additionally, whether rats can discriminate between a "mixture" (MIX) dose made up of half of each of the individual training doses vs. a single, "whole" dose of either one alone was studied. This study was also conducted to determine whether the discriminative stimulus effects of ETH and GHB are additive or if they comprise a more complicated synergistic interaction based on potentiation.

2. Method

2.1. Animals

Thirty adult male Long–Evans hooded rats (Charles River Laboratories, Portage, MI), approximately 90 days old at the onset of training, were used. Rats were singly housed in standard laboratory cages with a 12-h light–dark cycle (lights on at 7:00 AM) with ambient temperature and relative humidity maintained at approximately 22°C and 60%, respectively. After several days of acclimation to the lab and daily handling by the research team, the free-feeding body weight and the estimated normal growth rate for each rat was determined. Rats were gradually (over two weeks time) reduced to and maintained at 90% of these weights for the duration of the study by means of daily supplemental postsession feedings. Water was provided continuously in the home cages.

Animals used in this study were maintained in facilities consistent with the guidelines of the American Association for the Accreditation of Laboratory Animal Care (AAALAC), and all experimentation was conducted in accordance with the regulations of the Atlanta University Center Institutional Animal Care and Use Committee.

2.2. Apparatus

Standard operant chambers (Med Associates, Fairfield, VT) were used. Each chamber was housed in a sound-attenuating cubicle with an air circulation fan. Each chamber contained a house light and two levers, one on each side of the intelligence panel and equidistant from a food cup which received 45-mg food pellets (P.J. Noyes, Lancaster, NH) from a food dispenser. A white noise generator was situated in the room to mask ambient sounds. The experiments were controlled by a microcomputer connected to the chambers through an interface using MED-PC (Med Associates) software programs.

2.3. Drugs

The drug vehicle solution (WAT) was tap water flavored with a commercially available, artificially sweetened, sugar-free beverage mix (Crystal Light Tropical Passions Strawberry–Kiwi mix, Kraft Foods, Rye Brook, NY) at the ratio indicated in the directions on the label. The vehicle was flavored to attenuate the presence of confounding taste cues since all solutions were administered orally by gastric intubation (gi) with 3-in. 15-gauge curved feeding needles (Braintree Scientific, Braintree, MA). ETH solutions (10%, w/v for the training solution) were prepared by diluting 99% ETH (Aldrich Chemicals, Milwaukee, WI) in vehicle. GHB (sodium salt, Sigma, St. Louis, MO) was dissolved in vehicle. Mixtures were a combined bolus of ETH and GHB solutions, administered as a single intubation. All solutions were administered in a volume of 10 ml/kg. These solutions were freshly made approximately once per week and were refrigerated when not in use. The method of gastric intubation was used rather than the more common intraperitoneal method by recommendation of a local veterinarian concerned about potentially irritating or necrotic effects of chronic administration of 10 ml/kg solutions containing ETH.

2.4. Discrimination training

Rats were trained to the location and operation of the pellet dispenser during an initial hour-long session during which the pellet dispenser periodically delivered pellets (approximately one per minute). In follow-up sessions, by the method of successive approximations, rats were trained during hour-long sessions to operate both levers for food reinforcement using a FR schedule that increased progressively from a FR 1 to a FR 10. This training was performed on alternating levers on a session-by-session basis to help prevent a lever preference bias from forming.

Upon mastery of the FR 10 schedule, rats were randomly assigned to one of three groups ($N = 10$ per group): GHB, ETH, or MIX. Rats were trained to discriminate GHB (300 mg/kg gi), ETH (1000 mg/kg gi), or MIX (150 mg/kg of GHB plus 500 mg/kg of ETH gi given in a single administration) from an equal volume of vehicle (WAT) using a FR 10 schedule of food reinforcement. Prior to each 15-min training session, rats were administered either their training drug dose or an equal volume of vehicle (WAT) and placed back in their home cages for 30 min before placement in the experimental chambers.

For half of the rats (determined randomly) in each group, the left lever was assigned the drug-appropriate lever, while the right was assigned for the other half. For each rat, the

opposite lever was assigned the WAT-appropriate lever. After drug or WAT administration, only responses on the stimulus-appropriate lever were reinforced, while responses on the nonappropriate lever were recorded but not reinforced. The chamber house lights were illuminated 1 min after the session programs were started, signaling the onset of the contingency, and staying on for the duration of the 15-min session. At the end of each session, the rats were removed and returned to their home cages where a food supplement was provided to maintain a 90% free-feeding body weight. Each animal received one training (or testing) session per day.

For each training session, the *daily* criteria for correct lever selection were defined as (a) emitting fewer than 20 responses prior to the first reinforcer (FRF < 20) and (b) emitting 90% or more of the total session responses on the stimulus-appropriate lever. After rats met the stimulus control criteria of eight consecutive sessions (four each for drugged and nondrugged states) satisfying the daily criteria, they began discrimination testing. Selection of training criteria by researchers is rather arbitrary, and this set of criteria was chosen as being a fairly common and stringent set. It proved to be a challenging one to train, but provided a strong base from which to compare effects. The adopted set of criteria are stringent enough that it is unlikely that chance factors alone would result in them being met.

There was some variation in the speed at which the training criteria were met. Initially, the ratio requirement on the stimulus-appropriate lever would reset to zero if the rat responded on the stimulus-inappropriate lever; i.e., 10 successive presses on the drug-appropriate lever were required for reinforcement. It was concluded that this was perhaps too stringent; therefore, all subsequent training and testing sessions were conducted such that the ratio requirement was not reset if an incorrect response was made.

2.5. Discrimination/generalization testing

After stimulus control was established for most animals (by Session 72), discrimination test sessions were begun for those rats having met the criteria. Dose–response curves were generated during 15-min sessions 30 min following administration of the drug. Testing sessions were identical to training sessions except (a) during some sessions, a novel drug or dose was administered, and (b) reinforcement was produced by whichever lever first accumulated 10 total responses, rather than by the predetermined drug-appropriate lever as in training trials. All rats were tested with vehicle; 75, 150, 225, 300, 450, 600, and 900 mg/kg of GHB, 250, 500, 750, 1000, 1500, 2000, and 3000 mg/kg ETH, and seven MIX doses consisting of a combination of one half of each of the respective doses of GHB and ETH, administered as a single unit. These doses were selected to closely approximate the doses used by Colombo et al. (1995c) and to maximize the number of mixture doses having a corresponding matching single dose of GHB and ETH for direct comparisons.

Each dose of each drug was tested once by every animal in all three groups and was administered in a randomized order to each rat. If a rat failed to select one lever or another (i.e., did not achieve at least one reinforcement) during a 15-min novel drug/dose test, that dose was repeated on another randomly determined session day. Tests were conducted for each rat approximately three times weekly with training sessions taking place during the intervening days. A pseudorandom alternating sequence of training and testing was used such that, on average, every other day was a testing day, and approximately every other testing day incorporated a novel drug/dose, but this was varied so as not to provide a reliable extraneous cue. For example, a typical 2-week session sequence for a rat originally trained with ETH was: ETH training, WAT test, WAT training, novel test, ETH training, WAT test, ETH training, novel test, WAT training, WAT test, ETH training, and novel test. To avoid the development of a lever bias, there were equal numbers of drug and vehicle sessions. Additionally, animals were not run in the same sequences, as it is possible for rats to discriminate the lever on which the previous animal had been responding, and potentially respond themselves on the basis of olfactory cues. If a rat did not meet the performance criteria for stimulus control during a training trial during this phase of discrimination testing, further novel testing was postponed until at least one successful training drug or WAT session was achieved. This test procedure and reinforcement schedule is in accordance with recommendations by others (Goudie and Leathley, 1993).

3. Results

During training and testing, some rats from each group died (two GHB-, four ETH-, and two MIX-trained rats). None of these deaths appeared to be directly caused by the drug doses, but by errors in the technique of administration. Furthermore, two rats (one each from GHB and ETH groups) achieved individual training criteria, but failed to reliably demonstrate adequate stimulus control throughout training and testing. No data from any of these animals are included.

The overall mean number of sessions to reach criteria (as described above) was approximately 50 days (S.D. = 20.9). The mean numbers of sessions (± S.E.M.) to reach criteria for GHB-, ETH-, and MIX-trained rats were 37.4 (± 5.0), 57.8 (± 11.5), and 56.6 (± 7.1), respectively. There was not a significant difference between these means as tested by a single-factor ANOVA [$F(2,17) = 2.29$, $P = .13$], likely due to the relatively small sample sizes. Although not statistically different, the ETH- and MIX-trained rats may have taken longer to acquire the discrimination than the GHB-trained group due to an initial error in formulating the ETH dose (and therefore also the MIX training dose since MIX doses

were prepared from the ETH dose solutions). It was discovered after a few weeks of training that our ETH solution was only 750 mg/kg rather than the 1000 mg/kg desired. Once corrected, all subsequent sessions were run using 1000 mg/kg ETH (and 500 mg/kg ETH component in the MIX training dose).

Training and testing data are presented and analyzed in a number of ways. During discrimination training and generalization test sessions, selection of a lever, as defined above, is a dichotomous or quantal measure (i.e., the rat either selects the drug- or the vehicle-appropriate lever). As is commonly done when using the procedure employed here, the proportion or percentage of rats from each training group selecting the drug-appropriate lever during sessions was calculated and plotted to form dose–response curves (see Fig. 1a–c). Assuming close to full discrimination accuracy during training, probability estimates for selection of the drug or vehicle lever for a group can be determined from the binomial theorem with an underlying probability of 50%. For example, using the test sample size of the GHB-trained group of $n=7$, if fewer than two rats (approximately 29%) chose the GHB-appropriate lever at a particular test dose level, then the treatment was considered to be essentially identical with the vehicle condition. If five or more rats selected the drug lever, then the treatment was considered to be subjectively identical to the training dose of GHB. Lever selections in the intermediate range were assumed to be different from both training conditions. Admittedly, problems arise with such an imprecise method of interpretation, especially with the relatively small sample

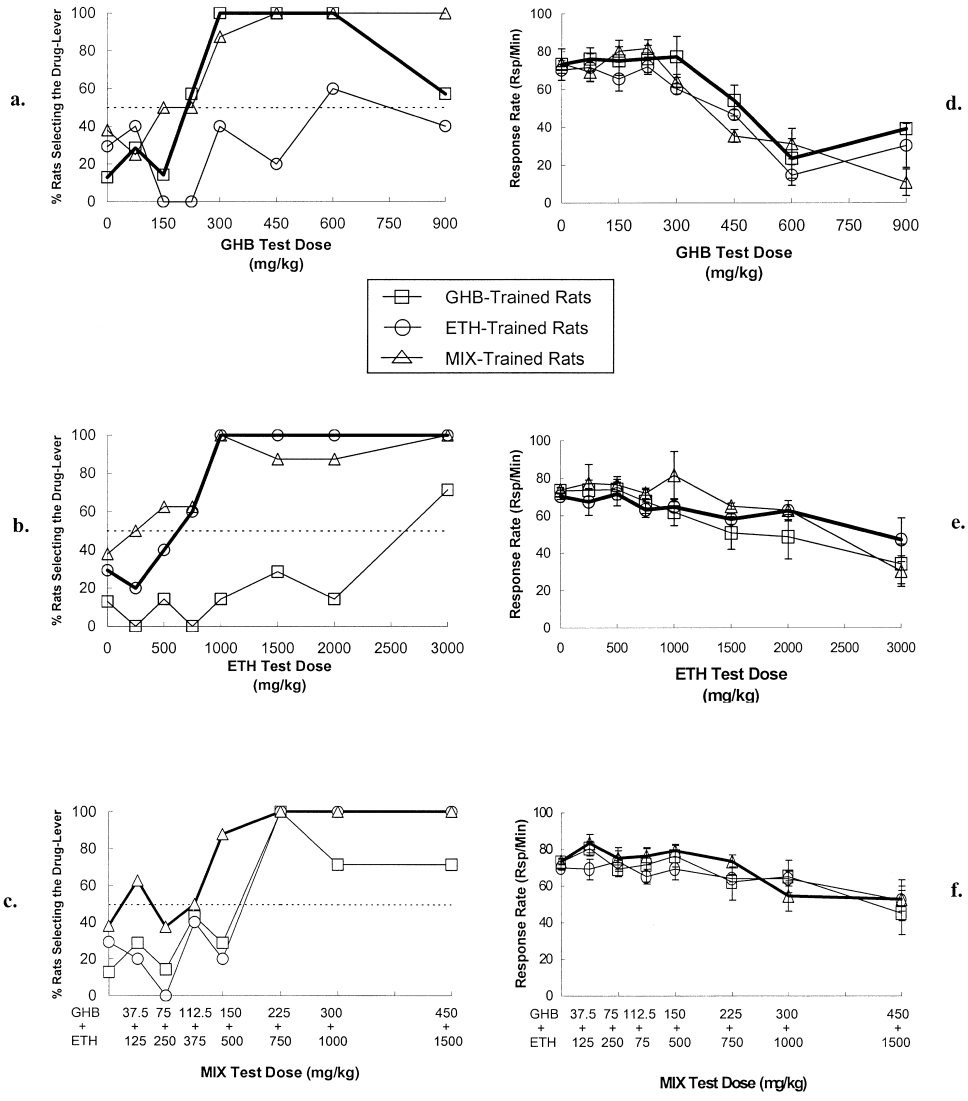

Fig. 1. Dose–response (bold lines), generalization curves, and response rates for rats tested with several doses of GHB (panels a and d), ETH (panels b and e), and MIX (panels c and f). Results of tests for groups following discrimination training with either 300 mg/kg GHB (–□–), 1000 mg/kg ETH (–○–), or a MIX (–△–) containing 150 mg/kg GHB + 500 mg/kg ETH vs. vehicle ($n=7$, 5, and 8, respectively) are shown. Panels a–c show the percentage of rats from each training group selecting their trained drug lever across the seven doses of each of the three drugs tested. Responding after vehicle (WAT) is also shown. Panels d–f show response rates (responses/min) during the 15-min test sessions across these same test doses for each training group.

sizes of this study (e.g., a single ETH-trained rat is worth 20% on this scale).

The total number of responses accumulated by an animal on both levers prior to the presentation of the first reinforcement is typically designated the first-reinforcement (FRF) value and can be used to determine the degree of accuracy of lever selection. For example, a FRF 10 value indicates that the animal in question made 10 responses on the lever delivering reinforcement and none on the other lever, therefore selecting the reinforcing lever with perfect accuracy (or with ''full strength''). In contrast, a FRF 19 value also indicates that the animal made 10 responses on the lever delivering reward, but nine responses in the meantime on the nonoperative lever prior to the first reinforcement. Such an animal has therefore also selected the operative lever, but with a very low degree of accuracy or ''strength.'' While FRF values >19 are not possible during testing sessions, a FRF value >19 during a training session indicates that the animal made 10 or more responses on the lever delivering no reward before making 10 responses on the reinforcing lever. By definition, such an animal has selected the inoperative lever in that session. Thus, FRF values of 20 or more represent incorrect lever selections during training trials (when only the training drug-appropriate lever will deliver reinforcements) and values of 19 or less represent correct lever selections during training and the degree of strength or accuracy of selections during testing. Binomial probabilities and FRF values for the results of all generalization and discrimination tests are included in Tables 1–3.

Fig. 1 depicts the dose–response curves and generalization results for all three drugs tested. The left panels (a–c) consist of dose–response (bold lines) and generalization curves for each of the three groups of drug doses tested with the percentage of rats in each training group selecting their trained drug-appropriate lever following the administration of each of the test doses employed. The respective response rates (responses/min) for each group are shown in the right panels (d–f). Group results of generalization tests at each dose level for each drug can be seen in Tables 1–3. Median FRF values (and ranges), the number and percentage of rats selecting the drug lever, and the one-tailed binomial probability of this number are presented for each training group for each drug dose tested. The descriptions and results of generalization tests for the three sets of drug solutions tested and the effects of administrations on response rates are presented below.

3.1. Gamma-hydroxybutyrate

In rats trained to discriminate 300 mg/kg GHB alone, different doses of GHB administered alone produced drug-appropriate responding (Fig. 1a). Full generalization was attained at the training dose and the next two higher doses, reflecting robust stimulus control by GHB in these rats. Levels of generalization following administration of the lower and the highest doses of GHB were lower, producing a characteristic dose–response curve. No dose of GHB, other than 600 mg/kg, resulted in drug-appropriate responding higher than 40% in ETH-trained rats, suggesting little or no generalization of the ETH stimulus effects to GHB. A high degree of generalization to the GHB stimuli was seen in MIX-trained rats. All doses of GHB 300 mg/kg and higher resulted in complete generalization in MIX-trained rats.

Table 1
Results of dose–response and generalization tests with GHB doses for all three training groups

	GHB dose (mg/kg)						
Group	75	150	225	300	450	600	900
GHB trained (n = 7)							
FRF[a] (median + range)	10 (10–16)	10 (10–18)	11 (10–18)	10 (10–12)	10 (10–12)	10 (10–14)	12 (10–19)
No. selecting DL (*n*)[b]	2 (7)	1 (7)	3 (7)	7 (7)	7 (7)	7 (7)	4 (7)
Binomial probability[c]	0.16	0.05	0.27	0.008	0.008	0.008	0.27
Percent selecting DL[d]	28.57	14.29	57.14	100.00	100.00	100.00	57.14
ETH trained (n = 5)							
FRF (median + range)	15 (10–16)	13 (10–17)	11 (10–14)	11 (11–16)	16 (10–19)	11 (10–13)	12 (10–15)
No. selecting DL (*n*)	2 (5)	0 (5)	0 (5)	2 (5)	1 (5)	3 (5)	2 (5)
Binomial probability	0.31	0.032	0.032	0.31	0.16	0.31	0.31
Percent selecting DL	40.00	0.00	0.00	40.00	20.00	60.00	40.00
MIX trained (n = 8)							
FRF (median + range)	12 (10–16)	10 (10–17)	10 (10–12)	10 (10–12)	12.5 (10–17)	10 (10–14)	10.5 (10–17)
No. selecting DL (*n*)	2 (8)	4 (8)	4 (8)	7 (8)	6 (6)	7 (7)	6 (6)
Binomial probability	0.11	0.27	0.27	0.035	0.016	0.008	0.016
Percent selecting DL	25.00	50.00	50.00	87.50	100.00	100.00	100.00

[a] Median number of responses prior to the first reinforcement.
[b] Number of rats selecting the drug lever (*n* = number earning at least one reinforcement).
[c] One-tailed binomial probability associated with the number of rats selecting the drug lever.
[d] Percentage of rats selecting the drug lever.

Table 2
Results of dose–response and generalization tests with ETH doses for all three training groups

	ETH dose (mg/kg)						
Group	250	500	750	1000	1500	2000	3000
GHB trained (n = 7)							
FRF[a] (median + range)	11 (10–18)	11 (10–17)	11 (10–15)	11 (10–18)	12 (10–15)	11 (10–17)	11 (10–15)
No. selecting DL (*n*)[b]	0 (7)	1 (7)	0 (7)	1 (7)	2 (7)	1 (7)	5 (7)
Binomial probability[c]	0.008	0.05	0.008	0.05	0.16	0.05	0.16
Percent selecting DL[d]	0.00	14.29	0.00	14.29	28.57	14.29	71.43
ETH trained (n = 5)							
FRF (median + range)	10 (10–13)	12 (10–16)	10 (10–12)	11 (10–18)	11 (10–12)	10 (10–11)	12 (10–19)
No. selecting DL (*n*)	1 (5)	3 (5)	2 (5)	5 (5)	5 (5)	5 (5)	5 (5)
Binomial probability	0.16	0.31	0.31	0.032	0.032	0.032	0.032
Percent selecting DL	20.00	40.00	60.00	100.00	100.00	100.00	100.00
MIX trained (n = 8)							
FRF (median + range)	15 (10–19)	11 (10–17)	10.5 (10–14)	12 (10–16)	13.5 (10–16)	13 (10–18)	13 (10–18)
No. selecting DL (*n*)	4 (8)	5 (8)	4 (8)	8 (8)	7 (8)	7 (8)	6 (6)
Binomial probabilty	0.27	0.22	0.27	0.004	0.035	0.035	0.016
Percent selecting DL	50.00	62.50	62.50	100.00	87.50	87.50	100.00

[a] Median number of responses prior to the first reinforcement.
[b] Number of rats selecting the drug lever (*n* = number earning at least one reinforcement).
[c] One-tailed binomial probability associated with the number of rats selecting the drug lever.
[d] Percentage of rats selecting the drug lever.

3.2. Ethanol

In rats trained to discriminate 1000 mg/kg ETH alone from vehicle, different doses of ETH administered alone produced drug-appropriate responding (Fig. 1b). Full generalization was attained at the training dose and all higher doses, reflecting robust stimulus control by ETH in these rats. Levels of generalization following the lower doses of ETH gradually rose until reaching asymptote at 1000 mg/kg ETH. No dose of ETH, other than the highest dose, resulted in drug-appropriate responding higher than 30% in GHB-trained rats, suggesting little or no generalization of the GHB stimulus effects to ETH at these lower doses. The highest dose of ETH (3000 mg/kg) tested produced 71% generalization. Similarly to tests with GHB, a high degree of generalization to the ETH stimuli was seen in MIX-trained

Table 3
Results of dose–response and generalization tests with MIX doses for all three training groups

	MIX [GHB + ETH] dose (mg/kg)						
Group	GHB → ETH →	37.5 + 125	75 + 250	112.5 + 375	150 + 500	225 + 750	300 + 1000	450 + 1500
GHB Trained (n = 7)								
FRF[a] (median + range)		12 (10–19)	10 (10–12)	11 (10–14)	10 (10–11)	13 (10–18)	12 (10–18)	12 (10–18)
No. selecting DL (*n*)[b]		2 (7)	1 (7)	3 (7)	2 (7)	7 (7)	5 (7)	5 (7)
Binomial Probability[c]		0.16	0.05	0.27	0.16	0.008	0.16	0.16
Percent selecting DL[d]		28.57	14.29	42.86	28.57	100.00	71.43	71.43
ETH trained (n = 5)								
FRF (median + range)		10 (10–16)	12 (10–13)	11 (10–18)	11 (10–18)	11 (10–12)	12 (10–18)	10 (10–12)
No. selecting DL (*n*)		1 (5)	0 (5)	2 (5)	1 (5)	5 (5)	5 (5)	5 (5)
Binomial probability		0.16	0.032	0.31	0.16	0.032	0.032	0.032
Percent selecting DL		20.00	0.00	40.00	20.00	100.00	100.00	100.00
MIX trained (n = 8)								
FRF (median + range)		11.5 (10–13)	11 (10–17)	11.5 (10–15)	11 (10–18)	10.5 (10–15)	10 (10–14)	11.5 (10–12)
No. selecting DL (*n*)		5 (8)	3 (8)	4 (8)	7 (8)	8 (8)	8 (8)	8 (8)
Binomial probability		0.22	0.22	0.27	0.035	0.004	0.004	0.004
Percent selecting DL		62.50	37.50	50.00	87.50	100.00	100.00	100.00

[a] Median number of responses prior to the first reinforcement.
[b] Number of rats selecting the drug lever (*n* = number earning at least one reinforcement).
[c] One-tailed binomial probability associated with the number of rats selecting the drug lever.
[d] Percentage of rats selecting the drug lever.

rats. All doses of ETH 1000 mg/kg and higher resulted in complete generalization in MIX-trained rats.

3.3. Mixture

In rats trained to discriminate MIX (150 mg/kg GHB + 500 mg/kg ETH) from vehicle, different doses of MIX also produced drug-appropriate responding (Fig. 1c). Increasing doses of the mixture engendered dose-related responding on the mixture-appropriate lever such that all doses higher than the training dose resulted in full generalization. Levels of generalization following the lower doses of MIX more or less gradually rose until reaching asymptote at the training MIX dose. Interestingly, tests of single administrations of the individual components making up the MIX training dose (i.e., 150 mg/kg GHB and 500 mg/kg ETH) resulted in partial generalization (approximately 50% and 63% mixture-appropriate lever selections, respectively, in MIX-trained rats). However, when 150 mg/kg GHB and 500 mg/kg ETH were administered jointly (as the MIX training dose), this compound stimulus produced the trained discriminative stimulus for the MIX group. This MIX training stimulus, however, did not generalize to the GHB or ETH training doses in rats trained to these stimuli, respectively (see Fig. 1c). In other words, half doses of the discriminative stimuli for the GHB- and ETH-trained rats (150 mg/kg GHB and 500 mg/kg ETH) can be combined to form a discriminative stimulus to which GHB and ETH will generalize. However, rats trained to the discriminative stimuli produced by 300 mg/kg GHB and 1000 mg/kg ETH will not generalize to the stimulus complex made of half of each (the MIX training dose). Individual doses larger than that contained in the MIX training dose produced near or complete substitution of the mixture stimulus.

Some larger doses of MIX did produce high levels of generalization in GHB-trained rats, however. The 225 mg/kg GHB + 750 mg/kg ETH mixture dose produced full generalization, while the two higher MIX doses resulted in moderately high levels of drug-appropriate responding (approximately 71%). Interestingly, the third highest MIX dose, which contained 75% of the amount of GHB in the GHB training dose, produced full generalization in GHB-trained rats while the two highest doses of MIX containing as much or more GHB as the GHB group training dose did not produce more than 71% generalization. Furthermore, in GHB-trained rats, the MIX dose containing 225 mg/kg GHB produced full generalization, yet 225 mg/kg GHB alone produced just 57% generalization. No other MIX doses produced more than about 40% generalization.

The three highest doses of MIX did produce complete generalization in ETH-trained rats. Interestingly, no other doses of MIX produced more than 40% generalization. Also interesting is the fact that the MIX dose containing 750 mg/kg ETH produced complete generalization in ETH-trained rats, while 750 mg/kg ETH alone produced just 60% generalization in these same rats. This is the same pattern seen for GHB-trained rats tested with GHB and MIX doses.

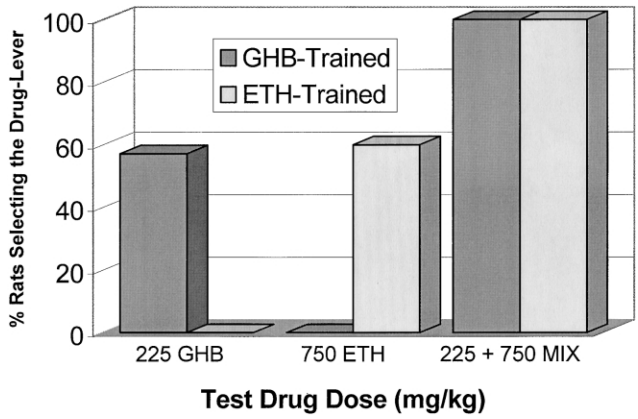

Fig. 2. The percentage of GHB- and ETH-trained rats selecting the trained drug lever following administration of 225 mg/kg GHB, 750 mg/kg ETH, and a MIX dose consisting of 225 mg/kg GHB + 750 mg/kg ETH.

Despite the lack of generalization between GHB and ETH, some evidence for potentiation between GHB and ETH can be seen in Fig. 2. Doses of 225 mg/kg GHB and 750 mg/kg ETH, each containing 75% of the doses used in training, produced approximately 60% generalization in GHB- and ETH-trained rats, respectively. In cross-generalization tests, the same doses yielded 0% drug-appropriate responding. However, when GHB- and ETH-trained rats were tested with the MIX dose of 225 mg/kg GHB and 750 mg/kg ETH, complete generalization resulted in both groups.

3.4. Response rates

As seen in the right panels (d–f) of Fig. 1, response rates for all groups following the lower five doses of all drugs tested were quite similar and approximated the response rates seen during test sessions following WAT administration. Response rates tended to drop off somewhat at the highest doses. In all groups, the two highest doses of GHB tested caused a marked decrease in responding and indeed, had to be retested by several rats who failed to respond enough for a single reinforcement the first time tested with the dose. The same was true of the highest dose of ETH (3000 mg/kg); this dose had to be retested in several rats.

The mean response rates (and S.D.) for GHB-trained rats following GHB, ETH, and MIX administrations (collapsed across Dose) were 61.46 (5.31), 59.60 (6.54), 68.79 (6.14) responses/min, respectively. Pairwise comparisons between these means using the Bonferroni adjustment for multiple comparisons revealed no significant difference between rates following GHB and ETH ($P = 1.00$), but that both of these solutions produced significantly lower responses rates than MIX ($P < .05$). To assess the effect of dose size, planned tests of within-subjects contrasts (using .05 P values) comparing rates at each dose level for each drug

with the rate following WAT administration revealed that GHB doses of 450 and 600 mg/kg marginally reduced response rates. Meanwhile, 900 mg/kg GHB, ETH doses of 1000 mg/kg and above, and the highest MIX dose all significantly reduced response rates for GHB-trained rats.

The mean response rates (and S.D.) for ETH-trained rats following GHB, ETH, and MIX administrations (collapsed across Dose) were 52.47 (4.64), 63.29 (3.32), 66.07 (4.68) responses/min, respectively. Based on pairwise comparisons of these means, response rate following GHB was significantly lower than rates following both ETH and MIX ($P < .05$), which did not differ significantly from each other. Planned tests of within-subjects contrasts revealed that only the two highest doses of GHB in ETH-trained rats resulted in reduced response rates compared to the rates following WAT administration ($P < .05$).

The mean response rates (and S.D.) for MIX-trained rats following GHB, ETH, and MIX administrations (collapsed across Dose) were 57.18 (3.54), 67.53 (3.08), 71.38 (3.58) responses/min, respectively. Pairwise comparisons between these means revealed a significant reduction ($P < .001$) in response rates caused by GHB in comparison to ETH and MIX, which did not differ significantly from each other. Planned tests of within-subjects contrasts revealed that the three highest doses of GHB and ETH, and the two highest doses of MIX all significantly reduced response rates for MIX-trained rats in comparison to rates following WAT ($P < .05$).

4. Discussion

One aim of the present study was to determine whether GHB and ETH, two commonly abused "social" drugs, share similar discriminative stimulus effects in rats. Three separate groups of rats were trained to discriminate either GHB, ETH, or a MIX from water. GHB produced dose-dependent increases in GHB-lever responding in the GHB-trained rats, but it did not substitute for the ETH stimulus in ETH-trained rats. Likewise, ETH yielded dose-dependent drug lever responding in ETH-trained rats but did not produce significant GHB-lever responding in GHB-trained rats. These findings suggest a lack of generalization between GHB and ETH and are in agreement with those of Winter (1981) who showed that ETH did not generalize to GHB in female CFN rats trained to discriminate 200 mg/kg GHB from saline. Our results do not support data reported by Colombo et al. (1995c), the study in which some aspects of the present study were modeled (i.e., same training doses, route of administration, strain and age of rats). The results of that study revealed that generalization between GHB and ETH occurred within a very narrow dose range (300 mg/kg GHB substituted for 1000 mg/kg ETH), whereas our study incorporated a wide range of doses, including doses close to the effective doses reported by Colombo et al. (1995c). The discrepancy in these results may be attributed to the different paradigms used. Colombo et al. (1995c) used the T-maze, while the present study utilized the two-lever operant responding task.

Despite the lack of cross-generalization between GHB and ETH, there was some evidence of a synergistic interaction between the two drugs when administered as a mixture in the GHB- and ETH-trained rats. In GHB-trained rats, a single dose of 225 mg/kg GHB resulted in about 60% of rats choosing the drug lever, while a single dose of 750 mg/kg ETH resulted in 0% of rats selecting the drug lever. However, when these doses were given at the same time as a mixture, 100% of the rats responded on the GHB-appropriate lever. The same was true in the ETH-trained rats. A total of 750 mg/kg ETH yielded about 60% of rats choosing the drug lever, while 225 mg/kg GHB produced no drug lever responding; however, the combination resulted in 100% of rats selecting the ETH-appropriate lever. If the effects were simply additive, the combination of GHB and ETH should have produced the same response that 225 mg/kg GHB and 750 mg/kg ETH did in the GHB- and ETH-trained rats, respectively.

It is unclear why cross-generalization did not occur between GHB and ETH despite a synergistic interaction between the two drugs when administered as a mixture. The neurobiology and neurochemistry involved in mediating the discriminative stimulus effects of both GHB and ETH are reported to be complex (Grant, 1999; Kostowski and Bieńkowski, 1999); however, the GABAergic system appears to be one common link between the two compounds. It is likely that GHB acts via the GABAergic system after its conversion to GABA, although it is possible that GHB may act directly on GABA receptors (Maitre, 1997). There is evidence that the stimulus effects of GHB are mediated differentially by $GABA_A$ and $GABA_B$ receptors at different doses (Lobina et al., 1999). In this particular study, the $GABA_B$ receptor agonist, baclofen, generalized with both a 300- and 700-mg/kg training dose of GHB. In addition, the $GABA_B$ receptor antagonist, CGP 35348, completely blocked the discriminative stimulus effects of the high dose, but only partially blocked the low dose of GHB. This study also reported that the positive $GABA_A$ modulator, diazepam, partially substituted for the low GHB training dose but failed to generalize to the high GHB training dose. Thus, the $GABA_B$ component of the GHB cue appears to be more salient at higher doses, causing possible overshadowing of the effects mediated by the $GABA_A$ component, which are more readily revealed at lower doses.

One of the primary receptor systems involved in mediating the discriminative stimulus effects of ETH is the $GABA_A$ system. Substitution studies have shown that some GABA-mimetic drugs (e.g., diazepam) acting through different sites within the $GABA_A$–benzodiazepine receptor complex may yield complete substitution for ETH at certain doses (Bieńkowski et al., 1997). However, both glutaminergic and serotonergic systems have been reported to play essential roles in the discriminative stimulus effects of ETH as well (Grant, 1999; Kostowski and Bieńkowski, 1999). In fact,

there is evidence that ETH produces a redundant stimulus complex, such that activation of independent receptor-mediated systems may serve as the basis for the discrimination. Such discriminations have been termed "redundant" because multiple features of the cue could serve as the basis for the discrimination. In addition, it has been suggested that the anxiolytic, sedative, atactic, and myorelaxant effects of ETH can play roles in the determination of its interoceptive stimulus (Kostowski and Bieńkowski, 1999). Notably, the contributions that each of these components make to the interoceptive ETH cue probably depend on various factors such as the time after drug administration, the testing paradigm, and the ETH training dose (Barry, 1991). For example, low doses of ETH tend to produce stimulant-like stimuli, while higher doses produce sedative/hypnotic-like effects. It is conceivable then that the complexity of the stimulus properties of both GHB and ETH could account for the results of the present study. Failure of ETH and GHB to generalize to each other might be due to a "mismatch" in the receptor systems mediating the stimuli during the time and circumstances of testing, while the synergistic effect of the combination of ETH and GHB may involve effects of ETH and GHB within a common GABAergic pathway.

Another aim of this study was to investigate the stimulus effects of a mixture of ETH and GHB since these drugs are frequently coabused (Galloway et al., 1997; Greenblatt, 1997). Mixture discrimination studies have been undertaken with ETH and various other substances in the past (for example, see Stolerman et al., 1999), but the present study appears to be the first done with GHB.

The apparent synergistic interaction between GHB and ETH may underlie the tendency for users to coabuse GHB and ETH. The complex nature of the stimulus properties of both GHB and ETH merits further investigation, and future studies should be extended to other paradigms that may reveal information about the reinforcing effects of GHB in combination with ETH.

Acknowledgments

This study was conducted in partial fulfillment of the requirements for the PhD degree by the first author, who wishes to thank the second author for her generosity and support in achieving this goal. We all also wish to thank Karen E. Brakke, Abena Abayomi, and Donna Floyd for their assistance. This project was conducted with support from grant no. 5R24DA07256 from the National Institute on Drug Abuse and Minority Institutions Research Development (NIDA/MIRD) Program awarded to the second author.

References

Barry H. Distinctive disciminative effects of ethanol. In: Glennon RA, Järbe TUC, Frankenheim J, editors. Drug Discrimination: applications to drug abuse research. National Institute of Drug Abuse Research Monograph Series 116 OHHS, Publ. No. ADM 92-1878. Washington: U.S. Government Printing Office, 1991. pp. 131–61

Barry HI, Krimmer EC. Differential stimulus attributes of chlordiazepoxide and pentobarbital. Neuropharmacology 1979;18:991–8.

Beardsley PM, Balster RL, Harris LS. Evaluation of the discriminative stimulus and reinforcing effects of gammahydroxybutyrate (GHB). Psychopharmacology (Berlin) 1996;127:315–22.

Bieńkowski P, Iwinska K, Stefanski R, Kostowski W. Discriminative stimulus properties of ethanol in the rat: differential effects of selective and non-selective benzodiazepine receptor agonists. Pharmacol, Biochem Behav 1997;58:968–73.

Cash CD. Gammahydroxybutyrate: an overview of the pros and cons for it being a neurotransmitter and–or a useful therapeutic agent. Neurosci Biobehav Rev 1994;18:291–304.

Colombo C, Agabio R, Balaklievskaia N, Diaz G, Lobina C, Reali R, Gessa GL. Oral self-administration of γ-hydroxybutyric acid in the rat. Eur J Pharmacol 1995c;285:103–7.

Colombo C, Agabio R, Bourguignon J, Fadda F, Lobina C, Maitre M, Reali R, Schmitt M, Gessa GL. Blockade of the discriminative stimulus effects of γ-hydroxybutyric acid (GHB) by the GHB receptor antagonist NCS-382. Physiol Behav 1995c;58:587–90.

Colombo C, Agabio R, Lobina C, Reali R, Fadda F, Gessa GL. Symmetrical generalization between the discriminative stimulus effects of gamma-hydroxybutyric acid and ethanol: occurrence within narrow dose ranges. Physiol Behav 1995c;57:105–11.

Colombo C, Agabio R, Lobina C, Reali R, Fadda F, Gessa GL. Cross-tolerance to ethanol and γ-hydroxybutyric acid. Eur J Pharmacol 1995d; 273:235–8.

Colombo C, Agabio R, Lobina C, Reali R, Gessa GL. Involvement of $GABA_A$ and $GABA_B$ receptors in the mediation of discriminative stimulus effects of γ-hydroxybutyric acid. Physiol Behav 1998;64:293–302.

Colpaert FC. Drug discrimination: behavioral, pharmacological, and molecular mechanisms of discriminative stimulus effects. In: Goldberg SR, Stolerman IP, editors. Behavioral analysis of drug dependence. London: Academic, 1986. pp. 161–93.

Fadda F, Mosca E, Colombo G, Gessa GL. Gamma-hydroxybutyric acid suppresses ethanol consumption in alcohol-preferring rats. Psychopharmacology (Berlin) 1988;96:107–11.

Fadda F, Mosca E, Colombo G, Gessa GL. Suppression by gamma-hydroxybutyric acid of ethanol withdrawal syndrome in rats. Alcohol Alcohol 1989;24:447–51.

Friedman J, Westlake R, Furman M. "Grievous bodily harm": gamma hydroxybutyrate abuse leading to a Wernicke–Korsakoff syndrome. Neurology 1996;46:469–71.

Gallimberti L, Canton O, Gentile N, Ferri M, Cibin M, Fadda F, Ferrara SD, Gessa GL. Gamma-hydroxybutyric acid for the treatment of alcohol withdrawal syndrome. Lancet 1989;2:787–9.

Gallimberti L, Ferri M, Ferrara SD, Fadda F, Gessa GL. Gamma-hydroxybutryic acid in the treatment of alcohol dependence: a double-blind study. Alcohol: Clin Exp Res 1992;16:673–6.

Gallimberti L, Cibin M, Pagnin P, Sabbion R, Pani PP, Pirastu R, Ferrara SD, Gessa GL. Gamma-hydroxybutyric acid for treatment of opiate withdrawal syndrome. Neuropsychopharmacology 1993;9:77–81.

Gallimberti L, Schifano F, Forza G, Miconi L, Ferrara S. Clinical efficacy of gamma-hydroxybutryic acid in treatment of opiate withdrawal. Eur Arch Psychiatry Clin Neurosci 1994;244:113–4.

Galloway GP, Frederick SL, Staggers FE, Gonzales M, Stalcup SA, Smith DE. Gamma-hydroxybutyrate: an emerging drug of abuse that causes physical dependence. Addiction 1997;92:89–96.

Garcha HS, Stolerman IP. Discrimination of a drug mixture in rats: role of training dose, and specificity. Behav Pharmacol 1989;1:25–31.

Gauvin DV, Halloway FA. The discriminative stimulus properties of an ethanol–nicotine mixture in rats. J Psychopharmacol 1993;7:52–62.

Gauvin DV, Harland RD, Michaelis RC, Halloway FA. Caffeine–phenylethylamine combinations mimic the cocaine discriminative cue. Life Sci 1989;44:67–73.

Goudie AJ, Leathley MJ. Drug-discrimination assays. In: Sahgal A, editor. Behavioural neuroscience: vol. 2. A practical approach. Oxford: Oxford University, 1993. pp. 145–67.

Grant KA. Strategies for understanding the pharmacological effects of ethanol with drug discrimination procedures. Pharmacol, Biochem Behav 1999;64:261–7.

Greenblatt JC, Gamma hydroxybutyrate (GHB) abuse in the United States. OAS working paper. The National Clearinghouse for Alcohol and Drug Information. Rockville, MD: Substance Abuse and Mental Health Services Administration, 1997, September.

Holtzman SG. Discriminative stimulus effects of drugs: relationship to potential for abuse. In: Adler MW, Cowan A, editors. Modern methods in pharmacology: vol. 6. Testing and evaluation of drugs of abuse. New York: Wiley-Liss, 1990. pp. 193–210.

Kostowski W, Bieńkowski P. Discriminative stimulus effects of ethanol: neuropharmacological characterization. Alcohol 1999;17:63–80.

Lobina C, Agabio R, Reali R, Gessa GL, Colombo G. Contribution of $GABA_A$ and $GABA_B$ receptors to the discriminative stimulus produced by gamma-hydroxybutyric acid. Pharmacol, Biochem Behav 1999;64:363–5.

Maitre M. The γ-hydroxybutyrate signaling system in brain: organization and functional implications. Prog Neurobiol 1997;51:337–61.

Mamelak M. Gammahydroxybutyrate: an endogenous regulator of energy metabolism. Neurosci Biobehav Rev 1989;18:187–98.

Mamelak M, Scharf MB, Woods M. Treatment of narcolepsy with gamma-hydroxybutyrate: a review of clinical and sleep laboratory findings. Sleep 1986;9:285–9.

Mariathasan EA, Garcha HS, Stolerman IP. Discriminative stimulus effects of amphetamine and pentobarbitone separately and as mixtures in rats. Behav Pharmacol 1991;2:405–15.

Martellotta MC, Fattore L, Cossu G, Fratta W. Rewarding properties of gamma-hydroxybutryic acid: an evaluation through place preference paradigm. Psychopharmacology (Berlin) 1997;132:1–5.

Overton DA. Application and limitations of the drug discrimination method for the study of drug abuse. In: Colpaert FC, Slanger JL, editors. Drug discrimination: applications in CNS pharmacology. Amsterdam: Elsevier, 1982. pp. 291–340.

Overton DA. Applications and limitations of the drug discrimination method for the study of abuse liability. In: Bozarth MA, editor. Methods of assessing the reinforcing properties of abused drugs. New York: Springer, 1987. pp. 291–340.

Rosen MI, Pearsall HR, Woods SW, Kosten TR. Effects of gamma-hydroxybutyric acid (GHB) in opioid-dependent patients. J Subst Abuse Treat 1997;14:149–54.

Shoaib M, Baumann MH, Rothman RB, Goldberg SR, Schindler CW. Behavioural and neurochemical characteristics of phentermine and fenfluramine administered separately and as a mixture in rats. Psychopharmacology (Berlin) 1997;131:296–306.

Stolerman IP, Mariathasan EA. Discrimination of an amphetamine–pentobarbitone mixture by rats in an AND–OR paradigm. Psychopharmacology (Berlin) 1990;102:557–60.

Stolerman IP, Mariathasan EA, Garcha HS. Discriminative stimulus effects of drug mixtures in rats. In: Glennon RA, Jarbe TUC, Frankenheim J, editors. Drug discrimination: applications to drug abuse research. National Institute of Drug Abuse Research Monograph Series 116, 1991. pp. 277–306.

Stolerman IP, Mariathasan EA, White JA, Olufsen KS. Drug mixtures and ethanol as compound internal stimuli. Pharmacol, Biochem Behav 1999;64:221–8.

Vayer P, Mandel P, Maitre M. Gamma-hydroxybutyrate, a possible neurotransmitter. Life Sci 1987;41:1547–57.

White J-AW, Stolerman IP. Antagonism of a nicotine plus midazolam discriminative cue in rats. Behav Pharmacol 1994;5:351–5.

Winter JC. The stimulus properties of γ-hydroxybutyrate. Psychopharmacology (Berlin) 1981;73:372–5.