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Effects of γ-hydroxybutyrate (GHB) and its metabolic precursors on delayed-matching-to-position performance in rats

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Abstract

The purpose of the present study was to provide further information about the effects of γ-hydroxybutyrate (GHB) on memory. Initially, the acute effects of gamma-butyrolactone (GBL, 75-200 mg/kg IP), 1,4-butanediol (1,4-BD, 100–300 mg/kg IP), and ethanol (1.0–3.0 g/kg, oral), as well as GHB (100–300 mg/kg IP), were examined in rats responding under a delayed-matching-to-position (DMTP) procedure with delays from 0 to 32 s. Acute administration of all four drugs reduced the number of trials completed and also reduced accuracy during delay trials, but not during trials without a delay. Some tolerance developed to the disruptive effects of GHB following exposure to 300 mg/kg/day for 29 consecutive days. These data indicate that GHB can disrupt working memory and speed of responding, and that tolerance can develop to these effects. Moreover, the acute effects of GHB under the DMTP procedure resemble those of its metabolic precursors, GBL and 1,4-BD, and of the prototypical CNS depressant drug, ethanol.

Keywords: GHB; GBL; 1,4-BD; Ethanol; Delayed-matching-to-position; Memory; Tolerance; Rats

1. Introduction

γ-Hydroxybutyrate (GHB) is both a precursor of GABA and a neurotransmitter in the mammalian central nervous system. As a neurotransmitter, GHB is thought to bind to either a $GABA_B$ receptor (Carter et al., 2003) or a specific GHB metabotropic receptor (Snead, 2000). Artificially synthesized by Laborit in 1960 (Tunnicliff, 1997), GHB is now used in some countries for the treatment of cataplexy and is being evaluated as a treatment for alcohol and opiate addiction (Fuller and Hornfeldt, 2003; Fuller et al., 2004; Gallimberti et al., 1989; Gallimberti et al., 1993). Nevertheless, GHB poses potential health risks (Galloway et al., 1997). GHB has been recognized as a "date rape" drug by the news media and historically has been popular among drug users at "rave parties" (Schwartz et al., 2000), although recent findings suggest that the drug is most often used at private residences these days (Barker et al., 2007). The two precursors of GHB, GBL and 1,4-BD, are found in some commercial solvents and might pose risks similar to those of GHB (Nicholson and Balster, 2001; Tarabar and Nelson, 2004). For example, their is a case report of 1,4-BD-induced intoxication in a person who intentionally ingested "liquid ecstasy" (Lora-Tamayo et al., 2003).

Memory impairment is one of the potential adverse effects of GHB. In an early study with humans, Grove-White and Kelman (1971) found significant short-term memory deficits in a digit recognition task after intravenous administration of GHB to healthy individuals when the retention interval was 20 s, but not when it was 4 s. Carter et al. (2006), who examined the effects of a broad range of orally-administered GHB doses (2–18 g/70 kg) in a complex test battery, also reported GHB-induced memory impairment, although it was weaker than that produced by triazolam (0.5 and 1 mg/70 kg) or pentobarbital (200 and 400 mg/70 kg).

Nonhuman research affords an opportunity to explore the effects of GHB on memory under tightly controlled conditions, and some relevant investigations have appeared. For example,

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Nakamura et al. (1987) found that acute exposure to 125 mg/kg GHB reduced the number of responses made by monkeys performing a go/no go discrimination, but had no effects on errors. Response rates decreased whereas number of errors increased when 250 mg/kg was administered, which suggested working memory interference at this dose. A study in which the effects of chronic exposure to GHB (5 or 30 mg/kg) were examined in mice tested in a hole-board apparatus also indicated that the drug impaired working memory (García et al., 2004). Similarly, in another study that examined the behavior of adolescent rats in a Morris water maze, Sircar and Basak (2004) found that repeated exposure to GHB impaired learning and memory at doses of 50 and 100 mg/kg, but not at 10 mg/kg. Interestingly, memory impairments were not observed during the first three daily exposures to GHB, but were observed during the fourth session. García et al. (2004) also administered GHB chronically but did not report whether the drug's effects differed across the 15 days of daily administration, precluding a comparison of the acute and chronic effects of GHB.

A recent study from our laboratory (Laraway et al., 2008) found that acute administration of GHB (100–500 mg/kg) reduced the response rates, but not the accuracy, of rats responding under fixed-consecutive-number (FCN) 8 and 16 schedules. The highest doses produced general behavioral incapacitation. Results were similar regardless of whether FCN 8 or FCN 16 schedules were arranged or whether an exteroceptive stimulus change was correlated with completion of the response requirement on the work lever (e.g., completion of the eighth response on a designated lever under the FCN 8 schedule). These results suggest that GHB produced nonselective behavioral disruption, but did not impair working memory. Substantial tolerance developed to the effects of GHB when the rats were exposed to daily doses of 200 or 300 mg/kg. Laraway et al. speculated that a possible reason for the absence of memory impairment in their study was that all of the schedules studied established strong, stable baseline levels of responding that were relatively resistant to the disruptive effects of GHB until the rats received doses that essentially eliminated responding. They proposed that examining behavior under a procedure in which behavior is under some conditions weakly controlled by the prevailing schedule and stimulus conditions and other conditions more strongly controlled may allow for more sensitive detection of GHB's effects on memory.

To provide further information about GHB's effects on memory, the present study examined the pre- and post-chronic effects of GHB in rats responding under a delayed-matching-to-position (DMTP) procedure. DMTP is a well-established animal model of working memory and provides a potential method for separating specific memory impairment from nonspecific drug-induced disruption of behavior (Baron et al., 1998; Dunnett, 1993; Pontecorvo and Clissold, 1993). In DMTP as typically arranged with rats, one of two or more response levers, defined by their position, is initially presented as the sample. It is then withdrawn, a delay ensues, the lever that initially was presented and one or more alternative levers are presented as choice levers, and responding on the choice lever presented in the same location as the sample lever is rewarded.

By arranging a substantial range of delay lengths each session (e.g., 0–30 s in rats), DMTP allows drug-induced reductions in accuracy to be determined as a function of delay. When the delay is nonexistent or very short (e.g., 0–1 s), accuracy in the absence of drug is high and it is assumed that memory processes are not involved in the control of behavior. As delays increase in length, memory processes come into play and baseline accuracy decreases. Comparable drug-induced accuracy reductions regardless of delay length are taken as evidence of nonspecific drug effects, not selective memory impairment (Han et al., 2000; Robinson et al., 2000; Sloan et al., 2006). In contrast, when disruption is evident only at longer delays, selective memory impairment is evident. Even stronger evidence of selective memory impairment is present when no disruption is evident when delays are nonexistent or very short and the degree of disruption increases directly with delay length at larger values (Dunnett, 1993).

The main objective of the present investigation was to assess the acute effects of GHB and its precursors on the performance of rats under a DMTP procedure. For purposes of comparison, ethanol was also examined. Ethanol was selected due to its prototypical CNS depressant properties, which reportedly are similar to those of GHB (Freese et al., 2002; National Institute on Drug Abuse, 2000). A secondary objective was to examine the development of tolerance to the effects of GHB following chronic exposure to the drug.

2. Materials and methods

2.1. Subjects

Subjects were eight male Sprague–Dawley rats (Charles River Laboratories, Portage, MI) approximately six months of age at the beginning of the study. The age of the rats was based on availability; they were initially purchased at three months of age for use in a student research laboratory but were not needed for that purpose. They were individually housed in polycarbonate cages with corncob bedding in a colony maintained under a 12-h light/12-h dark cycle with consistent temperature (20 ±2 °C) and humidity (50±5%). The rats were maintained at 80–85% of their free-feeding weights and had continuous access to water in their home cages. Animal care was in accordance with the *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996) and our university's Institutional Animal Care and Use Committee approved the study.

2.2. Apparatus

Training and testing were conducted using eight operant conditioning chambers (MED Associates, Georgia, VT), each measuring 28 cm long by 21 cm wide by 21 cm high. Each chamber contained two retractable levers, two white stimulus lights located above the levers, a food receptacle equipped with a head entry detection device, a 28-V white house light to illuminate the chamber, and a fan to mask noise and provide ventilation. A minimum force of 0.14 N was required to operate the levers. Food

pellets (45 mg BioServ #F0021, Frenchtown, NJ) served as reinforcers. Programming of experimental events and data recording were arranged using MED-PC version 4.0 software (Med Associates) installed on an IBM-compatible computer.

2.3. Preliminary training procedures

Training began with a single 1-h session during which no levers were present and food was presented every 60 s regardless of the subject's behavior. Subsequently, subjects were exposed to five 40-min lever-press training sessions, one session per day across five consecutive days. These initial training sessions began with the illumination of the house light and random insertion of one of two levers. A response on the inserted lever immediately retracted the lever, turned on both stimulus lights, and produced a food pellet. The detection of a head entry into the food receptacle turned off the stimulus lights. Five seconds after this occurred, the other lever was inserted into the chamber and a response on that lever immediately retracted the lever, turned on both stimulus lights, and produced a food pellet. Trials continued in this manner for a period of 40 min, with left and right lever insertions alternating, until all subjects were reliably responding on both levers.

2.4. Delayed-matching-to-position training

Following initial lever-press training, subsequent training was conducted according to a discrete-trial DMTP procedure described by Dunnett (1993). A no-delay procedure was used initially. Here, each trial began with illumination of the house light and random insertion of one of the two levers, which constituted the sample lever. A press on the inserted (i.e., sample) lever resulted in the retraction of the sample lever. Following retraction of the lever, a head entry into the food pellet receptacle initiated the immediate insertion of both levers. Both levers at that point constituted choice levers. A response on the lever that corresponded to the sample lever constituted a matching (correct) response, whereas a response on the lever that did not correspond to the sample lever constituted a nonmatching (incorrect) response. Each matching response produced immediate retraction of both levers, turned on the stimulus lights above the levers, and delivered a food pellet. A head entry into the food receptacle turned off the stimulus lights and initiated the next trial. A nonmatching response immediately retracted both levers and initiated a 5-s timeout, during which the house light and the stimulus lights were not illuminated. In an attempt to prevent response bias, a correction procedure was arranged following nonmatching responses. In this procedure, the same (e.g., left) sample lever was reintroduced across successive trials until a correct (matching) response occurred. Once a matching response occurred, the sample lever for the next trial was selected at random.

All subjects were required to achieve an overall accuracy of $\geq$90% correct responses for three consecutive training sessions, then 2-, 4-, and 8-s delay trials, as well as no-delay trials, were arranged each session. Here and throughout the balance of the study, delay values for individual trials were selected at random, with the provision that all delays (including no delay) occurred approximately the same number of times each session. When all subjects achieved $\geq$90% accuracy for three consecutive sessions, 2-, 4-, 8-, 16-, 24,- and 32-s delay trials, along with no-delay trials, were arranged each session. Training continued with these terminal values until all subjects met the three-consecutive-session accuracy criterion, then acute drug testing commenced. Once the terminal delay values were in place, daily 40 min sessions were conducted 5 days a week at approximately the same time each day.

2.5. Acute drug tests

Once all subjects met the training criterion, they were tested with a range of acute doses of GHB (100, 200, 300, 400 mg/kg), GBL (75, 150, 200 mg/kg), 1,4-BD (100, 200, 300 mg/kg) and ethanol (1.0, 2.0, 3.0 g/kg). The drugs were tested in that order. For each drug, the doses of interest were administered in a random order and each dose was tested once. At least two training sessions separated test sessions. In all cases, the session just before drug administration was preceded by vehicle injection.

2.6. Chronic GHB treatment

When acute tests were completed, all subjects were chronically exposed to a GHB dose of 300 mg/kg/day for 29 consecutive days. After the 29-day GHB treatment, subjects were administered substitution tests with each of five GHB doses (100, 200, 300, 400, and 500 mg/kg) in both an ascending (100–500 mg/kg) and descending (400–100 mg/kg) series. Therefore, substitution tests were conducted twice with each dose, with the exception of 500 mg/kg. When doses less than 300 mg/kg were tested, supplemental doses were administered following test sessions to equal a total daily dose of 300 mg/kg.

2.7. Drugs

Gamma-hydroxybutyrate (National Institute on Drug Abuse, Bethesda, MD), gamma-butyrolactone, and 1,4-butanediol (Sigma Chemical Company, St. Louis) were dissolved in sterile water and were administered by IP injection at a volume of 1 ml/kg body weight 15 min before test sessions. Initial injections of GHB appeared to be irritating; the rats sometimes contracted and squealed, but after a few injections they adapted and did not show obvious signs of pain. Because of its irritating properties when injected, ethanol (AAPER Alcohol and Chemical Company, Shelbyville, KY) was diluted with sterile water and administered by oral gavage at a volume of 10 ml/kg 30 min before test sessions. Sterile water (1 ml/kg) as control was administered by IP injection 15 min before vehicle-control sessions. Drug concentrations were calculated based on the weight of the salt (GHB) or liquid (GBL, 1,4-BD, ethanol).

2.8. Data analyses

Mean percent correct responses (accuracy) and mean number of trials completed at each delay (0–32 s) were plotted as a function of acute drugs and doses. Accuracy data from subjects

that failed to emit at least one response at each delay were excluded from data analyses. For the data amenable to parametric analyses, all accuracy data were subjected to arcsine transformations prior to statistical analyses, (McNaughton, 1993). A two-factor repeated-measures ANOVA for accuracy data was then conducted with delay and drug dose as the two factors. For number of trials completed, Friedman's repeated-measures ANOVA on ranks was conducted with drug dose as the main factor. The alpha level for all statistical tests was set at $p<.05$. All ANOVA tests were followed by post hoc Student–Newman–Keuls (SNK) tests. Only significant post hoc tests are reported. Graphs were plotted with GraphPad Prism 5 (GraphPad Software, San Diego, CA) and statistical analyses were done with Sigma Stat 3.1 (Systat Software, Point Richmond, CA).

3. Results

3.1. Acute drug effects on DMTP performance

Fig. 1a depicts accuracy during vehicle-control sessions (0 mg/kg), as well as the acute effects of GHB on accuracy (percent correct responses) at each delay value. In this graph, data are presented as the group average percent correct at each delay value following the administration of each GHB dose. In the absence of drug, the mean percent correct exceeded 90% at all delay values and accuracy was not systematically affected by delay value. GHB at doses of 100–300 mg/kg did not affect accuracy during trials with no delay between the retraction of the sample lever and insertion of the choice levers. The 100 and 200 mg/kg doses of GHB did not obviously reduce accuracy during delay trials, but the 300 mg/kg dose did so. Following acute administration of 200 mg/kg GHB, accuracy was slightly lower during trials with longer delays (i.e., 24, 32 s) compared to trials with shorter delays (i.e., 2, 4 s). Accuracy following acute administration of 300 mg/kg GHB was roughly equivalent at all delays. Because the 400 mg/kg dose of GHB disrupted responding in more than half of the subjects, data for this dose were not included in the statistical analyses (below) or in Fig. 1. A two-way ANOVA of the data shown in Fig. 1a revealed a significant main effect of GHB dose [$F(3,18)=7.855$, $p=0.001$; SNK tests, 0, 100, 200 versus 300 mg/kg, all $ps<.05$] and a significant delay effect [$F(7,42)=7.816$, $p<0.001$; SNK tests, 0 versus 8–32 s, all $ps<.05$], although no significant drug × delay interaction was found. As shown in Fig. 1b, Friedman's repeated-measures ANOVA on ranks revealed a significant effect of GHB on the mean total number of trials completed [$\chi^2_{(3)}=17.92$, $p=0.001$; SNK test, 0 versus 300 mg/kg of GHB was significant, all $ps<0.05$], with the number of trials completed inversely related to dose.

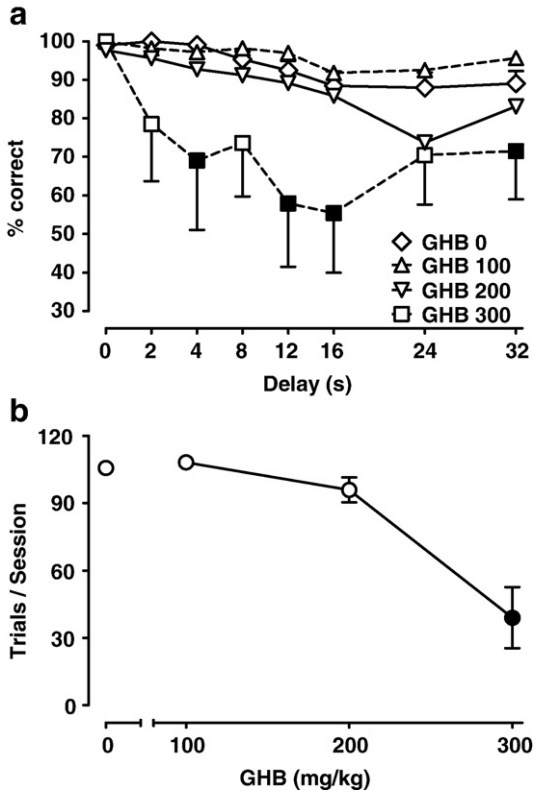

Fig. 1. Acute effects of GHB (100, 200, 300 mg/kg) on DMTP performance before chronic administration of GHB (300 mg/kg). (a) Data points represent the average percent correct matching responses at a specific delay and different symbols represent effects of different doses. (b) Data points indicate the mean number of trials completed at each dose. In all graphs, the vertical lines depict standard errors. Filled symbols indicate mean values (±SEM) that are significantly different from control ($p<0.05$).

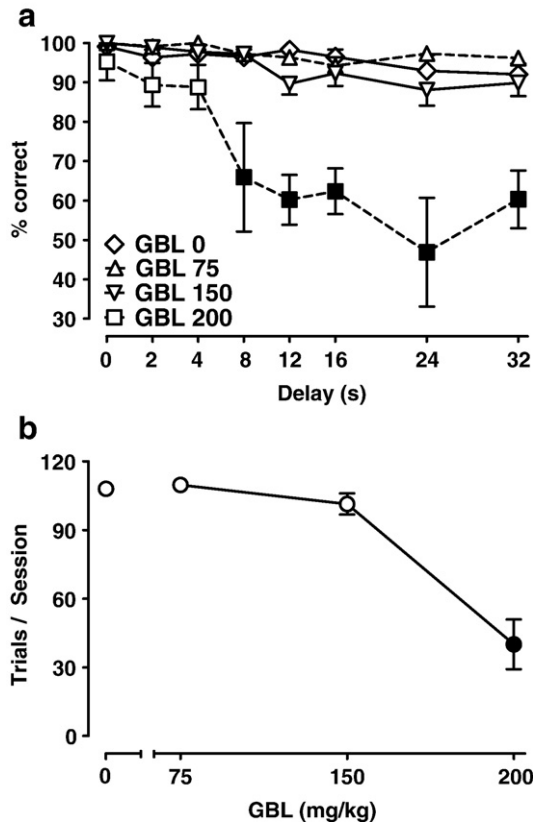

Fig. 2. Acute effects of GBL (75, 150, 200 mg/kg) on DMTP performance. See Fig. 1 for details. Filled symbols indicate mean values (±SEM) that are significantly different from control ($p<0.05$).

Fig. 2a illustrates the acute effects of GBL on matching accuracy at each delay. There was a significant drug effect [$F(3,18)=21.999$, $p<0.001$], delay effect [$F(7,42)=12.991$, $p<0.001$] and drug × delay interaction following the administration of GBL [$F(21,126)=3.416$, $p<0.001$]. Only 200 mg/kg GBL reduced accuracy and these effects were more pronounced at longer delays than at shorter delays. As shown in Fig. 2b, there was also a significant effect of GBL dose on the number of trials completed [$\chi^2_{(3)}=18.346$, $p=0.001$; SNK test, 0 versus 200 mg/kg of GBL, $p<0.05$], with the number of trials completed inversely related to dose.

Fig. 3a depicts the acute effects of 1,4-BD on accuracy at each delay. With respect to mean percent correct responses there was a significant main drug effect [$F(4,20)=3.294$, $p=0.032$], a significant delay effect [$F(7,35)=15.395$, $p<0.001$], and a significant drug × delay interaction [$F(28,140)=1.623$, $p<0.036$]. Like GHB and GBL, 1,4-BD impaired accuracy to a greater extent at longer delays compared to shorter delays (SNK test, all $ps<0.05$). The effects of 1,4-BD on the number of trials completed (Fig. 3b) were also significant [$\chi^2_{(4)}=18.576$, $p=0.001$; SNK test, 0 versus 300 and 400 mg/kg of 1,4-BD, all $ps<0.05$], with the number of trials completed inversely related to dose.

Fig. 4a illustrates the acute effects of ethanol on accuracy at each delay. As with the other drugs tested, with ethanol there was a significant main effect of ethanol dose [$F(3,18)=12.395$, $p<0.001$], a significant delay effect [$F(7,42)=20.747$, $p<0.001$], and a significant drug × delay interaction [$F(21,126)=2.756$, $p<0.001$]. The higher doses of ethanol (2.0 and 3.0 g/kg) impaired accuracy more at longer delays than at shorter delays (SNK test, all $ps<0.05$). Fig. 4b depicts the number of trials completed following each ethanol dose. As with GHB and its precursors, ethanol significantly reduced the number of trials completed [$\chi^2_{(3)}=14.909$, $p=0.002$; SNK test, 0 versus 3 g/kg of ethanol, all $ps<0.05$].

Tables 1–5 provide information about the overall number of trials with a correct response and the total number of trials completed by individual rats (and the group mean) when subects were exposed to pre-chronic GHB, GBL, 1,4-BD, ethanol, and post-

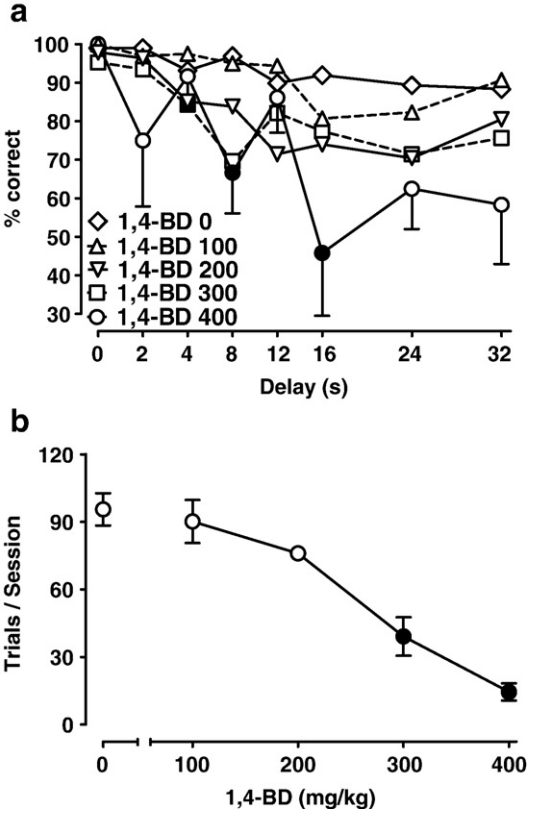

Fig. 3. Acute effects of 1,4-BD (100, 200, 300, 400 mg/kg) on DMTP performance. See Fig. 1 for details. Filled symbols indicate mean values (±SEM) that are significantly different from control ($p<0.05$).

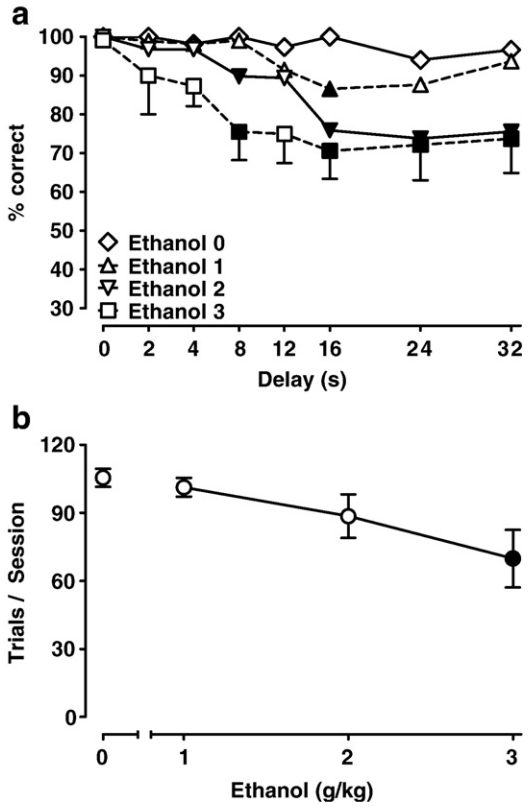

Fig. 4. Acute effects of ethanol (1.0, 2.0, 3.0 g/kg) on DMTP performance. See Fig. 1 for details. Filled symbols indicate mean values (±SEM) that are significantly different from control ($p<0.05$).

Table 1
Effects of pre-chronic GHB on total trials with a correct response, and, after the /, total trials completed in 40-min sessions

Subject	GHB (mg/kg)			
	0	100	200	300
1	107/112	113/114	103/108	2/8
2	103/111	108/113	106/113	100/111
3	108/111	105/110	91/105	42/51
4	88/99	95/102	79/87	0/0
5	93/104	99/105	56/79	5/12
6	99/108	107/111	83/102	8/11
7	99/103	107/108	104/106	41/45
8	93/101	97/102	61/69	58/70
Mean	98.8/106.1	103.9/108.1	85.4/96.1	32.0/38.5

Table 2
Effects of GBL on total trials with a correct response, and, after the /, total trials completed in 40-min sessions

Subject	GBL (mg/kg)			
	0	75	150	200
1	111/114	112/113	104/109	35/50
2	107/111	107/112	103/107	42/52
3	111/113	105/106	103/107	13/24
4	91/97	99/105	78/92	11/16
5	108/110	109/110	93/102	12/24
6	92/109	99/106	103/110	44/57
7	110/111	111/112	111/111	87/98
8	95/97	105/108	65/73	0/2
Mean	103.1/107.8	105.9/109.0	95.0/101.4	30.5/40.4

Table 4
Effects of ethanol on total trials with a correct response, and, after the /, total trials completed in 40-min sessions

Subject	Ethanol (g/kg)			
	0	1	2	3
1	114/116	112/113	103/110	46/65
2	109/110	106/110	103/109	98/109
3	110/113	103/111	62/87	88/100
4	77/80	85/92	78/85	43/55
5	103/107	91/104	21/33	22/46
6	106/109	91/107	90/105	75/105
7	108/109	105/107	100/104	86/91
8	98/100	89/93	92/97	0/0
Mean	103.1/105.5	97.8/104.6	81.1/91.3	57.3/71.4

chronic GHB, respectively. Appropriate vehicle-control data are also presented in these tables. At sufficiently high doses, each drug substantially reduced the total number of trials completed, as well as the total number of trials with a correct response. At the highest dose tested, the mean total number of trials completed was 36, 37, 15, 68, and 39% of the vehicle-control level for pre-chronic GHB, GBL, 1,4-BD, ethanol, and post-chronic GHB, respectively.

Because the number of trials completed at a given delay, as well as the total number of trials completed, might influence accuracy, two-way repeated-measures analysis of variance, with drug dose and delay length as factors, was applied to the trials completed for each drug. Results indicated that drug dose, but not length of delay, significantly influenced the number of trials completed for all drugs.

Drug dose and delay length did not interact significantly with respect to GHB and 1,4 BD, but with GBL and ethanol there were significant interactions between these factors. Statistical results were as follows: GHB (dose $F[3]=22.143$, $p<.001$; delay length $F[7]=1.109$, $p>.05$; interaction $F[21]=0.908$, $p>.05$); GBL (dose $F[3]=45.06$, $p<.001$; delay length $F[7]=0.287$, $p>.05$; interaction $F[21]=2.268$, $p<.01$); 1,4 BD (dose $F[4]=20.582$, $p<.001$; delay length $F[7]=1.805$, $p>.05$; interaction $F[28]=0.942$, $p>.05$), ethanol (dose $F[3]=45.06$, $p<.001$; delay length $F[7]=0.287$, $p>.05$; interaction $F[21]=2.268$, $p>.05$).

3.2. Chronic effects of GHB

The results of GHB dose–response determinations following chronic GHB (300 mg/kg) administration are depicted in Fig. 5.

Table 3
Effects of 1,4-BD on total trials with a correct response, and, after the /, total trials completed in 40-min sessions

Subject	1,4-BD (mg/kg)				
	0	100	200	300	400
1	101/101	104/106	33/48	35/39	27/31
2	39/49	80/90	68/69	74/81	13/17
3	107/112	109/112	87/102	23/29	19/21
4	90/97	53/60	68/75	29/34	6/10
5	87/95	28/39	58/86	0/0	0/0
6	105/110	103/111	87/99	54/63	15/20
7	106/109	106/110	102/105	10/23	8/17
8	85/91	89/93	9/17	34/41	0/0
Mean	90.0/95.5	84.0/90.1	64.0/75.1	32.4/38.8	11.0/14.5

One subject died for reasons unrelated to the present study before the start of chronic treatment; chronic data are for seven rats. The behavior of more than half of the rats was severely disrupted at 500 mg/kg, therefore, data for this dose were not analyzed and are not reported.

Fig. 5a depicts the chronic effects of GHB on accuracy at different delays. A two-way ANOVA of these data revealed a significant main effect of GHB dose [$F(3,15)=3.758$, $p=0.034$] and of delay [$F(7,35)=10.622$, $p<0.001$]. As was the case with acute administration (Fig. 1a), no significant dose × delay interaction was obtained following chronic GHB treatment (Fig. 1b). SNK tests revealed significant differences when 100–300 mg/kg were compared to 400 mg/kg, but only at the 32-s delay interval (all $ps<0.05$). Such tests also revealed significant differences in 0-s accuracy versus accuracy with 16-, 24- and 32-s delays, all $ps<0.05$ within 300 and 400 mg/kg.

A significant main drug effect [$\chi^2_{(3)}=13.500$, $p=0.004$; SNK test, 100 versus 300 and 400 mg/kg of GHB, all $ps<0.05$] was found when the number of trials completed as a function of GHB dose (100–400 mg/kg) was assessed following chronic GHB treatment (Fig. 5b). SNK tests revealed significance when 100 versus 400 mg/kg of GHB were compared ($p=0.001$). Two-way repeated-measures analysis of variance, with drug dose and delay length as factors, indicated that post-chronic GHB dose ($F[3]=15.702$, $p<.001$), but not delay length ($F[7]=0.501$, $p>.05$), significantly influenced the number of trials completed, and these factors did not interact significantly ($F[21]=1.2$, $p>.05$).

To determine if chronic exposure to 300 mg/kg GHB induced tolerance to different doses, a two-way ANOVA comparison of

Table 5
Effects of post-chronic GHB on total trials with a correct response, and, after the /, total trials completed in 40-min sessions

Subject	GHB (mg/kg)			
	100	200	300	400
2	66/76	101/106	104/106	79/84
3	97/105	99/102	86/100	24/31
4	72/84	67/70	57/63	17/24
5	86/94	88/96	11/18	33/46
6	92/103	74/85	52/63	34/52
7	91/97	58/62	71/74	10/12
8	66/75	47/52	25/31	0/0
Mean	81.4/90.6	76.3/81.9	58.0/65.0	28.1/35.6

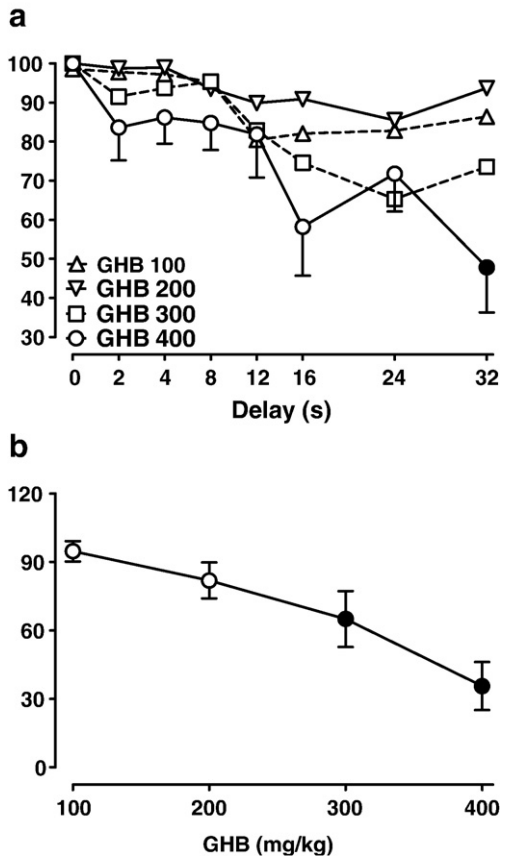

Fig. 5. Post-chronic effects of GHB (100, 200, 300 mg/kg) on DMTP performance. Filled symbols indicate mean values (±SEM) that are significantly different from 100 mg/kg GHB.

acute and chronic effects of GHB was conducted, with dose and acute versus chronic GHB administration as the two factors. Comparisons were restricted to three doses of GHB (100, 200, 300) and seven subjects. A significant main dose effect [$F(2,11)=5.135$, $p=0.024$] was obtained, but no significant acute versus chronic effects were found. Nevertheless, there was a significant dose × acute versus chronic interaction effect [$F(2,11)=16.244$, $p<0.001$]; SNK tests, pre- versus post- within 100, 200, and 300 mg/kg, all $ps<0.05$. Thus, chronic exposure to GHB appeared to induce moderate tolerance. This is evident when comparing accuracy and the number of trials completed during acute tests with GHB (Fig. 1) versus chronic tests with GHB (Fig. 5). Following acute administration of 200 and 300 mg/kg GHB, the average overall accuracy during delay trials was lower prior to chronic exposure compared to these effects following chronic exposure. In addition, animals completed an average of 39 trials following acute administration of 300 mg/kg GHB prior to chronic exposure. Following chronic exposure, animals completed an average of 65 trials in tests with this dose of GHB. Furthermore, 400 mg/kg GHB completely suppressed responding during acute tests (data not shown), while the rats completed an average of 42 trials following this dose during tests following chronic exposure.

4. Discussion

The present study evaluated the effects of GHB and its metabolic precursors with the DMTP procedure to determine if these drugs impair working memory. When this procedure is used, selective memory impairment is often inferred when disruption of accuracy is confined to trials in which a delay interval is present and when the degree of disruption is directly related to delay length (Han et al., 2000; Robinson et al., 2000). In the present study, acute administration of GHB and its precursors produced greater disruption at longer delays than at shorter ones, which suggests that these drugs selectively impaired working memory. For example, GHB significantly impaired accuracy at ≥100 mg/kg, but only during trials in which a delay (>0 s) was arranged. Moreover, the degree of disruption was greater at long delays (24, 32 s) than at shorter ones (2, 4 s). Consistent with some previous studies (e.g., García et al., 2004; Sircar and Basak, 2004), these results suggest that GHB can disrupt working memory.

It is important to point out, however, that reductions in accuracy associated with GHB (and the other drugs) were accompanied by reductions in the number of trials completed relative to vehicle-control levels. The number of trials completed could have been reduced by increases in the time elapsed from 1) the insertion of the sample lever to the occurrence of a response on that lever (leading to sample lever retraction), 2) the retraction of the sample lever to the occurrence of a nose insertion after the programmed delay expired (leading to insertion of both levers), 3) the insertion of both levers and a response on one of those levers, and 4) the presentation of food to the occurrence of a nose insertion (initiating the next trial). Both 2 and 3 would functionally increase the delay interval on a given trial, which would be expected to reduce accuracy. Whether reduced accuracy produced through such a mechanism is evidence of memory impairment *per se* is questionable. Unfortunately, we did not determine how closely obtained delays approximated nominal delays and can only recommend that this be done in future studies.

Delay-dependent decreases in accuracy similar to those produced by GHB were also found with 1,4-BD and GBL. That the three drugs produced similar effects is to be expected, given that both GBL and 1,4-BD are metabolic precursors of GHB (Nicholson and Balster, 2001), but the present results with GBL and 1,4-BD are nonetheless noteworthy, given that there appear to be no prior studies of the effects of either drug on memory. GBL was found to be slightly more potent than GHB in the present study, and this finding is consistent with the results from drug discrimination studies (Baker et al., 2005; Carter et al., 2006).

Consistent with the suggestion that the behavioral effects of GHB resemble those of ethanol (Freese et al., 2002; National Institute on Drug Abuse, 2000), ethanol (1–3 g/kg) produced delay-dependent reductions in accuracy in the present study. This finding agrees with previous reports indicating that ethanol can impair working memory in DMTP procedures (Escher and Mittleman, 2004; Melia et al., 1990).

The differential effects of GHB and its precursors at different delay lengths were not a function of the number of trials completed,

because delay length did not significantly affect the number of trials completed. Although the number of trials completed was significantly reduced by 300 mg/kg GHB, indicating the drug produced nonselective behavioral disruption, no significant differences were found between the number of trials completed at different delay intervals. This indicates that nonselective drug effects that differed as a function of delay could not account for the present findings. Nonetheless, drug-induced decreases in the number of trials completed might have indirectly decreased accuracy by functionally increasing the time elapsed from the retraction of the sample lever to the insertion of both levers and a response on one of those levers. As noted previously, this could have occurred if nose-pokes, which were required for insertion of the comparison levers, occurred well after the programmed delay expired, or if responses to the comparison levers occurred substantially after their insertion.

Finally, and significantly, the present study indicates that administration of 300 mg/kg GHB for 29 consecutive days resulted in some tolerance to the accuracy- and rate-reducing effects of the drug. This is consistent with preclinical and clinical observations suggesting that tolerance frequently develops with repeated exposure to GHB (e.g., Colombo et al., 1995; Galloway et al., 1997; Laraway et al., 2008; Miotto et al., 2001; Van Sassenbroeck et al., 2003). For example, in the present study 400 mg/kg severely disrupted responding with initial acute administration, but not when administered following chronic exposure to 300 mg/kg GHB. Lower doses (200 and 300 mg/kg) also reduced accuracy and the number of trials completed to a greater extent prior to chronic exposure. Although tolerance was observed in the present study, the half-life of GHB in rats is 60 min (Snead, 1977), therefore, more frequent administrations might well have produced greater tolerance.

Because the present study was designed, in part, to examine the development of tolerance to GHB, subjects received a substantial number of drug injections (>60). Repeated exposure to GHB and other drugs almost certainly affected our findings and it would be useful to expose drug-naïve rats to procedures comparable to those that we used. For example, 3 g/kg ethanol, a dose in the hypnotic range, produced only a 20% reduction in trials completed in the present study. It is highly probable that it would produce far more disruption in drug-naïve rats. Be that as it may, the present findings, like those of most but not all prior studies, suggest that GHB can impair working memory, as well as generally disrupt learned behavior. Therefore, memory impairment should be recognized as one of several risks that GHB poses for recreational users, who expose themselves to high doses of the drug.

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