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Spontaneous and precipitated withdrawal after chronic intragastric administration of gamma-hydroxybutyrate (GHB) in baboons

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Abstract *Rationale:* γ -Hydroxybutyrate (GHB) is a current drug of abuse that may produce physical dependence. *Objectives:* The present study characterized the behavioral effects of chronic GHB in baboons ($n=4$), and evaluated whether signs of withdrawal occurred (1) after administration of the GABA-B antagonist CGP36742 during chronic GHB administration (precipitated withdrawal) and (2) following discontinuation of chronic GHB administration (spontaneous withdrawal). *Methods:* Water (vehicle) and then GHB was continuously infused via intragastric (IG) catheters. GHB administration was initiated at 350

mg/kg per day, and the dose was increased by 100 mg/kg over 4 days to 750 mg/kg per day. Food pellets were available 20 h/day under a fixed ratio (FR5 or 10) schedule of reinforcement. Observation sessions and a 2-min fine motor task were conducted during vehicle and GHB administration. CGP36742 (32 and 56 mg/kg, IM) was administered during vehicle and chronic GHB administration. After a total of 32–36 days GHB administration was abruptly discontinued. Blood samples were collected during all interventions and analyzed for GHB content. *Results:* Chronic GHB decreased food-maintained behavior, disrupted performance of the fine motor task, and produced ataxia, muscle relaxation, tremors and jerks. At the end of GHB administration, plasma levels of GHB ranged from 486 to 2080 $\mu\text{mol/L}$. Administration of CGP36742 during chronic GHB administration produced increases in aggression, self-directed behaviors, vomit/retch, tremors and/or jerks, which is consistent with a precipitated withdrawal syndrome. Similar signs were observed when GHB administration was discontinued. Seizures were not observed. *Conclusions:* These data indicate that chronic GHB administration produced physical dependence and that activation of the GABA-B receptor may be important for GHB physical dependence.

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Introduction

Abuse of gamma-hydroxybutyrate (GHB) is a growing public health concern. GHB (Xyrem®) is approved for the treatment of narcolepsy. The therapeutic dose is 3–9 g/night administered in two equally divided doses (21.5–64.3 mg/kg) given 2–4 h apart (Cook 2003). Under these conditions, tolerance does not appear to develop to the hypnotic effects of GHB and symptoms of withdrawal were not reported when GHB were discontinued (Mamelak et al. 1986; Cook 2003). A recent review concluded that

GHB is generally effective and well tolerated by patients when therapeutic doses were maintained and there was little evidence of abuse of GHB during treatment (see Beghe and Carpanini 2000). In addition to its therapeutic use for narcolepsy, GHB has been evaluated as a treatment for opiate and for alcohol withdrawal (Gallimberti et al. 1989, 1992; Addolorato et al. 1998a,b). In these studies, signs of physical dependence or withdrawal were not reported when GHB was discontinued if the patients were maintained on the clinically prescribed dose regimen (25–150 mg/kg). However, some patients took up to 6–7 times the prescribed dose, reported “craving” for GHB, and had symptoms that are consistent with a withdrawal syndrome upon discontinuation of GHB (Addolorato et al. 1999, 1996).

GHB has emerged as a “club drug” and its abuse is well documented (Nordenberg 2000; O’Connell et al. 2000). Users are typically young adults who take GHB orally for its euphoric and intoxicating effects. Adverse reactions of GHB include dizziness, nausea, vomiting, weakness, seizures, confusion, hallucinations, agitation, bradycardia, respiratory suppression, unconsciousness, and coma. GHB-related deaths have also been reported (DEA/ODE 2000). There are now numerous case reports of a withdrawal syndrome in those that abuse high doses of GHB (Dyer et al. 2001; Mason and Kerns 2002; Rosenberg et al. 2003), which reportedly resembles withdrawal from sedative-hypnotics and alcohol (for review, see McDonough et al. 2004). Because of its known abuse, GHB has a dual classification as a Schedule I substance under the controlled substances act for non-medical use, and as a Schedule III substance for medical use.

The behavioral effects of GHB are often compared to that of classic sedative-hypnotic drugs (e.g. benzodiazepines and barbiturates) (for review, see Nicholson and Balster 2001). The mechanisms by which GHB produces its effects are not fully understood. While some effects of GHB appear to be via the GHB-specific binding site in the brain (Maitre 1997), it appears that GABAergic mechanisms may be important for some of the behavioral effects of GHB (see Nicholson and Balster 2001). GHB does not bind to GABA-A receptors, which are the primary site of action for classic sedative-hypnotic drugs, but acts as a weak agonist at GABA-B receptors. GABA-B antagonists blocked the discriminative stimulus effects (Colombo et al. 1998; Lobina et al. 1999), sedative-hypnotic effects (Carai et al. 2001), and locomotive effects (Nissbrandt and Engberg 1996) of GHB, and prevent GHB-induced absence seizures (Snead 1996). In baboons, the GABA-B receptor antagonist CGP36742 blocked GHB-induced decreases in food-maintained behavior and GHB-induced disruption of performance of a fine motor task in baboons (Goodwin et al. *in press*). A high dose of GHB (320 mg/kg) also produced signs of sedation/muscle relaxation and abdominal discomfort, which were decreased by CGP36742 pretreatment. Based on these findings, it seems likely the GABA-B receptor may play a role in GHB physical dependence (Wong et al. 2004).

The current study evaluated the behavioral effects and physical dependence potential of GHB in baboons. First, behavior during chronic intragastric (IG) administration of water (vehicle) and then GHB was characterized. The characterization of behavior during chronic GHB may be particularly important for evaluating its physical dependence potential, as some behaviors that are typically viewed as signs of withdrawal (e.g. tremors, loss of appetite, impairment of motor function) are reported effects of GHB itself. Second, a GABA-B antagonist was administered during chronic GHB administration to determine if it would precipitate a withdrawal syndrome. Third, GHB administration was terminated after an additional 10 days of dosing, and evidence for GHB spontaneous withdrawal was evaluated.

Materials and methods

Subjects

Subjects were four adult male baboons (*Papio anubis*) that weighed 27–35 kg at the beginning of the study. The baboons had been implanted with IG catheters using methods described previously (Lukas et al. 1982), and had participated in a study evaluating the effects of acute doses of GHB and potential antagonists (Goodwin et al. *in press*) before beginning the current study. Prior to that study, baboons HA and PF were experimentally naive. Baboon KH had previously received acute doses of cocaine, quinpirole, morphine, buprenorphine, and alcohol, but had no history of chronic drug administration. Baboon DM had a history of cocaine self-administration (Weerts and Griffiths 2003). As described below, all baboons had 20 h per day access to food pellets. Baboons also received supplemental feedings of one to two pieces of fresh produce and a children’s chewable multi-vitamin at noon each day. Tap water was continuously available from an automatic watering system attached to the cage, and the volume of water consumed was recorded at the same time each morning before the experimental session.

Baboons were anesthetized with ketamine hydrochloride (HCl) (150–300 mg) every 2–3 weeks to permit weighing, physical examinations, and cage washing. During the physical examination, routine catheter maintenance procedures were performed (see Weerts et al. 1998). Blood samples were also collected (see below). Ketamine was administered as needed to repair catheters or equipment; days on which this occurred were excluded from the analysis. If a catheter broke subcutaneously, catheters were repaired as described previously (Lukas 1983) under halothane anesthesia. The protocol was approved by the JHMI Animal Care and Use Committee and followed the *Guide for the Care and Use of Laboratory Animals* (National Academy of Sciences 1996). Facilities were maintained in accordance with USDA and AALAC standards.

Apparatus

Baboons were housed singly in standard primate cages, which also served as the experimental chambers. Cages were equipped with a bench, which ran along the side of one wall, a waterspout, which was attached to the front of the cage, and an aluminum intelligence panel, which was mounted on the rear wall as described previously (Weerts et al. 1998). The panel contained a Lindsley pull operandum (Med-Associates, Georgia, Vt., USA), a colored jewel light, and a hopper for delivery of food pellets. Catheters were protected by a vest and tether system (Lukas et al. 1982) that permitted the baboon free movement inside the cage. The tether was connected to the infusion system via an 18-g liquid swivel and strain-relief system (Instec-Soloman, Plymouth Meeting, Pa., USA) mounted on top of the cage. Drug and vehicle solutions were infused into the catheter using a peristaltic pump (Model 1201 or 1203; Harvard Apparatus, South Natick, Mass., USA). Pellet feeders, water bottles, and peristaltic pumps were located on a grating above the cages. Room ceiling lights were brightly illuminated for 13 h/day (6:00 A.M.–7:00 P.M.) and dimly illuminated for the remaining 11 h/day.

Experimental sessions were controlled and data were collected using personal computers with Med Associates (East Fairfield, Vt., USA) software and instrumentation. The behavioral observation data were collected using laptop personal computers and observation software (Noldus Information Technology, Wageningen, Netherlands). The fine motor task used custom-made Plexiglas trays onto which six 3-cm cups with 1-cm high rims were mounted. The task was timed using a stopwatch and results were recorded using a pen and paper checklist.

General procedures

Food reinforcement procedure

Pellets were available during daily 20-h sessions that began at approximately 10:00 A.M. An unlimited number of food pellets were available during the session under a fixed ratio (FR) schedule of pellet delivery on the Lindsley operandum. Pellet availability was correlated with continuous illumination of the jewel light above the operandum. Each response required both a pull and then a release of the operandum. The FR requirement for each baboon was based on the following criteria: (1) the number of pellets delivered per day was relatively stable (i.e. no increasing or decreasing trends), (2) the pellets delivered were consumed and (3) the number of pellets delivered per day was sufficient to maintain normal body weights. Using these criteria, three baboons (DM, HA and PF) were maintained on an FR10 and the fourth baboon (KH) an FR5 schedule of pellet reinforcement. Throughout the study, the cage pans were inspected to determine if pellets obtained were also consumed. If pellets were found in the pans, the number was recorded in the daily record. If food intake de-

creased to below 125 g for 3 consecutive days (see Results), supplementation with biscuits (200 g) was initiated and maintained until pellet intake returned to normal levels. During the study, baboon HA developed staph dermatitis; he was treated with an antibiotic on days 18–27 of the withdrawal phase.

Observations

Effects of GHB were evaluated during systematic observation periods. The occurrence of six postures and 21 behaviors were recorded on a laptop computer. Behaviors included agonistic behaviors (e.g. yawn, threat, bruxism, lip smack), self-directed behaviors (e.g. scratch, groom, nose wipe, nose rub, masturbation) and motor activity (e.g. locomotion). In addition, a range of behaviors to assess sedation (e.g. sitting with eyes closed, lying down), muscle relaxation (e.g. lip droop), and motor coordination (e.g. ataxia), as well as those shown previously to be indicative of a benzodiazepine withdrawal syndrome (e.g. tremors, jerks, vomit/retch, head-below-torso-posture, rigidly braced posture) were recorded. A table with complete definitions of the behaviors and postures that were used in the current study has been published previously (Weerts et al. 1998).

Baboons were habituated to the observation procedures before the study began. Observations were conducted at the same time of day (e.g. 10 A.M.) and were 20 min long except on days in which the GABA-B antagonist or its vehicle was administered when observations were 60 min (see below). An observer sat in front of the cage with a laptop computer and recorded the frequency and duration of observed behaviors and postures in “real time”. The list of all possible behavioral signs and postures that could be scored were displayed on the computer screen. Observers could type comments at any time. Any instance of vomit/retch or diarrhea that was observed outside of the observation periods was also recorded in the daily records by animal care technicians. Reliability observations were conducted between all pairs of observers both before and during the study. The overall reliability for agreement of occurrence and nonoccurrence of all behaviors and postures between all observers was greater than 90%.

Fine motor task

A fine motor task was conducted at the same time each day as described previously (Weerts et al. 1998). Briefly, one raisin (baboons HA, KH, PF) or M&M (baboon DM) was placed in each of the six equally spaced cups on a tray that was presented to the baboon at the front of the cage. Using a stopwatch, the observer presented the tray for 120 s or until all six items were retrieved, whichever occurred first. A checklist was completed which indicated the occurrence or non-occurrence of tremor, lipdroop, and incoordination, the time (s) to retrieve all six items, the total

number retrieved/dropped, and any comments about overt behaviors observed during the task.

Drugs

Gamma-hydroxybutyric acid (GHB) sodium salt (Sigma-Aldrich and NIDA Drug Supply Program) was dissolved in distilled water. CGP36742 (3-aminopropyl-*n*-butyl-phosphinic acid) was dissolved in sterile water and injected intramuscularly at a volume of 2–2.5 ml. All drug doses were based on the salt.

Experimental design

Phase 1: continuous IG infusion of water or GHB

GHB or water was continuously infused into the IG catheter at a rate of 0.38 ml/min. The volume infused over the 24-h period was approximately 550 ml. First, distilled water (vehicle) was continuously infused via the IG catheter for a period of 2 weeks, and baseline levels of food-maintained behavior and performance on the fine motor task were determined. Observations were conducted during the vehicle condition ($n=5$ per baboon) to determine baseline levels of observed behaviors. GHB administration was initiated at a dose of 350 mg/kg per day. The dose was then progressively increased by 100 mg/kg per day over 4 days to the final dose of 750 mg/kg per day GHB, which was then maintained for 4 weeks. The actual volume (ml) infused was recorded at the same time each day (8:30 A.M.), and then the drug bottle was refilled. If the entire volume had not infused by 8:30 A.M. (e.g. pump failed), a bolus dose of GHB equivalent to the missing dose or up to 2 g GHB (which ever was less) was delivered immediately, and then the chronic administration was continued; this occurred on days 8 and 31 of administration for baboon HA. Observations were conducted for the first 14 days of GHB administration, and then five additional observations were conducted in the last week of GHB administration (i.e. between days 28 and 36).

Phase 2: effects of acute administration of the GABA-B antagonist CGP36742 during chronic administration with GHB (precipitated withdrawal test)

The GABA-B antagonist CGP36742 was administered during vehicle and during GHB chronic administration. The doses of CGP36742 (32 and 56 mg/kg IM) used previously blocked acute GHB (320 mg/kg)-induced reductions in food-maintained behavior and disruptions of performance of the motor task (Goodwin et al. [in press](#)). In the current study, the antagonist or its vehicle were injected IM 5 min before the pellet session started (10 A.M. \pm 15 min). Observers recorded behaviors for 60 min (as described above) and then presented the fine motor task immediately after the observation. During chronic GHB,

each antagonist dose was administered once. The first dose was given after 2 weeks of chronic GHB administration and the second dose was given 8–10 days after the first dose. Each antagonist test was preceded by a vehicle test 1–3 days before. Three baboons (DM, KH and PF) received 56 mg/kg first and one baboon (HA) received 32 mg/kg first. Chronic GHB administration was continued for an additional 10 days after the last antagonist dose before proceeding to phase 3.

Phase 3: effects of abrupt discontinuation of chronic administration with GHB (spontaneous withdrawal)

Chronic GHB administration was discontinued and distilled water (vehicle) replaced the GHB solution at 9 A.M. Distilled water was then continuously infused via the IG catheter at the same rate/volume as GHB. The total length of GHB administration was: 32 days for PF, 35 days for KH and 36 days for DM and HA. The difference in number of days between baboons was to allow at least 5 days of GHB administration after a medical check. After GHB administration was terminated, observations were conducted at 6 h, 24 h and then daily (between 9:30 and 10:30 A.M.) for at least the first 14 days and until behaviors were similar to those recorded during the vehicle baseline.

Evaluation of GHB in blood

Blood samples were collected during regularly scheduled medical examinations (see above). For each sample, approximately 5 ml of blood was collected from a saphenous vein of the baboon using a vacutainer with a lithium heparinized tube. Immediately after the sample was obtained it was centrifuged at 3200 rpm for 12 min. The plasma was drawn off and transferred to two separate polypropylene tubes and frozen. Samples were shipped on dry ice for subsequent analysis. GHB levels in plasma were determined by stable-isotope dilution gas chromatography-mass spectrometry as previously described (Gibson et al. [1990](#)) using d_6 -GHB as internal standard. Blood samples were collected on 2 separate days of chronic GHB administration for all baboons; The first sample was collected on 1 day between days 14 and 16 for all baboons except DM, who had a sample on day 8 instead days 14–16. The second sample was collected on 1 day between days 26 and 29 of GHB administration. Blood samples were also collected during the baseline vehicle condition 2–4 weeks before GHB administration began.

Data analysis

A single-subject design (Sidman [1960](#)) was used in which each baboon served as his own control. For each baboon, z -scores were calculated for each variable for (1) the observations during the vehicle condition that preceded drug administration ($n=5$) and (2) the observations con-

ducted at the end of chronic GHB administration ($n=5$). Behavioral changes were judged as significantly increased or decreased by GHB when compared to the vehicle control z -scores. For behaviors in which an increase was predicted (e.g. lying down, and head-below-torso posture, lip droop) significance was determined if the observed frequency of that behavior exceeded the vehicle control z -score above which only 5% of the scores would be expected to fall (i.e. a one-tailed test). For all other behaviors that were predicted to increase or decrease, the z -scores delineated the top 2.5% and the bottom 2.5% (i.e. a two-tailed test).

The incidence of spontaneous withdrawal behaviors scored after drug was discontinued was judged as significant if the observed frequency of the behavior exceeded the z -scores for both the vehicle baseline and chronic GHB administration. The incidence of precipitated withdrawal behaviors scored following administration of the CGP36742 was judged as significant if the frequency of behavior exceeded the z -score for the vehicle baseline ($n=5$) and also exceeded the range of values for a) the paired vehicle observations conducted during chronic GHB administration and b) the observation for each CGP36742 dose during chronic vehicle administration. Behaviors that were significantly changed according to the above criteria were used to assign withdrawal scores (see legends for Figs. 3 and 4 for description). Thus, both time-related and drug-produced changes in behavior were accounted for in the analysis.

Results

During the vehicle baseline, the mean number of pellets delivered ranged from 345.0 to 412.8 over the 20-h session, and pellets delivered were also consumed. GHB administration began at 350 mg/kg and was increased by 100 mg/kg

per day over the next 4 days to a final dose of 750 mg/kg on day 5. As shown in Fig. 1, the number of pellets delivered decreased within 4–7 days of GHB administration and remained low over 2 weeks in all four baboons. Two baboons also did not eat all pellets delivered on days 11–25. When the response ratio was lowered to 1, they did work at a higher rate, but again did not consume all pellets delivered. All baboons received supplemental biscuits when pellet intake was low (see [Materials and methods](#)), but did not always consume all biscuits given in the first 25 days. After 25–35 days of GHB administration, food-maintained responding increased in two baboons, but remained low in the other two baboons. As shown in Table 1, body weights decreased in three of the four baboons during GHB administration and weight loss was between 5% and 7% of their total body weight. Body weights typically fluctuate (+ or –) by 1–2% under non-drug control conditions.

During vehicle, all baboons completed the fine motor task daily. For three baboons (KH, HA and PF), the time (s) to retrieve all six of the food items ranged from a mean (± 1 SD) of 7.5 ± 1.4 to 8.3 ± 2.2 s. In the fourth baboon (DM), time to complete the task was slower and performance was more variable (mean 11.84 ± 3.86). As shown in Fig. 2, performance of the task was relatively unaffected during the first 5 days of GHB administration. Although performance of the task was slower on day 1 for PF, the time to complete the task was not significantly altered on most days for this baboon. For the other three baboons, performance of the task was disrupted in two baboons after 1 week and in a third baboon after 2 weeks of GHB administration. Disruptions of the task included (1) days in which baboons attempted to do the task without being able to retrieve all six food items in the maximum time of 120 s, (2) days in which baboons were non-responsive and would not engage in the task and (3) days in which baboons completed the task, but more slowly. Performance improved in all baboons after about

Fig. 1 Effects of GHB on food-maintained behavior across consecutive days of chronic administration. Data shown are the number of pellets delivered (*filled circles*) and number of pellets not eaten (*open diamonds*). The data point over “V” represents the mean ± 1 SD of pellets delivered and consumed during the baseline condition that preceded chronic GHB administration. The shaded area represents 95% confidence interval based on the calculated z -scores; data points outside the shaded area were considered significantly increased or decreased. Days on which baboons received ketamine or doses of the antagonist are not shown

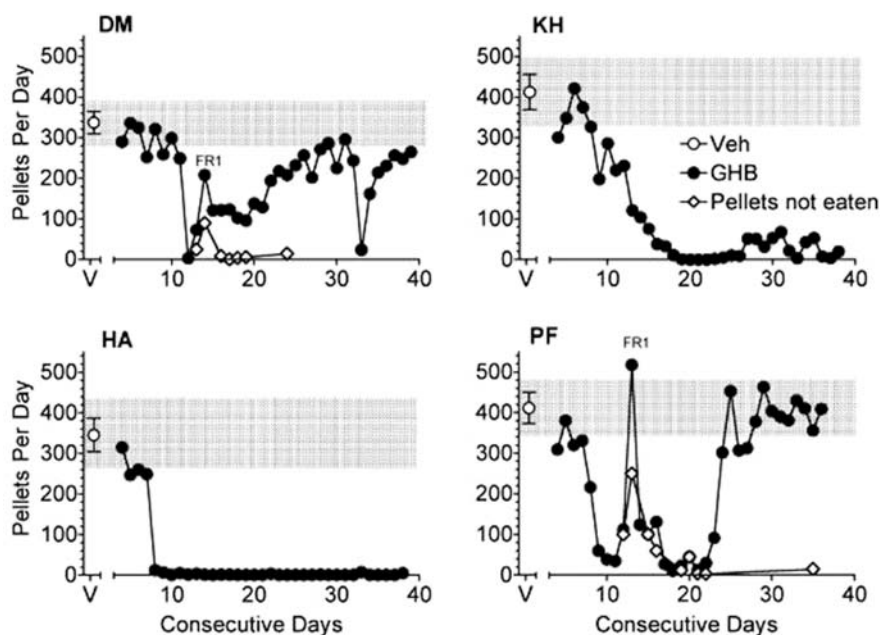


Table 1 Amount of GHB ($\mu\text{mol/L}$) in plasma during chronic IG administration of vehicle (control) and 750 mg/kg GHB in individual baboons (DM, HA, KH and PF). Control blood samples were obtained for each baboon during chronic vehicle condition that preceded GHB administration. GHB samples were collected after 2

weeks (first sample) and again after 4 weeks (second sample) of chronic GHB administration, except as noted. Data are the average of the duplicate samples analyzed by two different laboratories. Body weights (*BW*) shown were obtained at the same time as blood samples. *ND* represent non-detectable level

Baboon	Control		Chronic GHB (750 mg/kg per day)			
	BW (kg)	GHB ($\mu\text{mol/L}$)	BW (kg)	GHB ($\mu\text{mol/L}$) 1st sample	BW (kg)	GHB ($\mu\text{mol/L}$) 2nd sample
DM	27.5	ND	27.9	224 ^a	26.9	486
HA	38.7	ND	36.8	2,350	35.9	2,080
KH	30.2	ND	29.7	1,383	28.7	1,595
PF	27.7	ND	25.7	1,770	26.4	1,109

^a1st sample for DM was after only 1 week of administration

25 days of chronic administration, but time (sec) to complete the task was still longer on most days for two baboons.

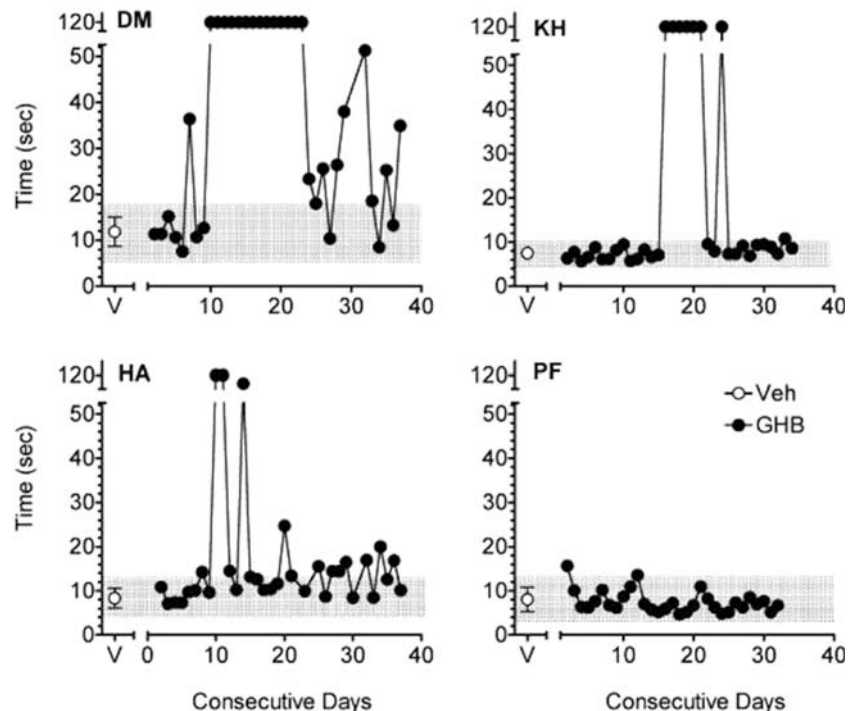
Data from observation sessions and the fine motor task revealed some behavioral changes at different time points during chronic GHB administration. Specifically, in the first 5 days during the GHB dose escalation, increases in resting postures (lying down, sitting with eyes closed) were observed in three of four baboons. One baboon (KH) showed retching on day 2 (450 mg/kg GHB), and day 3 (550 mg/kg) of the dose escalation phase, and a second baboon (PF) retched on day 13. Vomit/retch was not observed during vehicle in these baboons. Vomit or retch was not observed during the last 5 days of GHB administration in any of the baboons. Ataxia and lip droop (a sign of muscle relaxant effects), which was not observed under vehicle, was observed in three baboons (HA, KH and PF) after 7–10 days of GHB administration and continued to be observed throughout administration in two baboons (HA and

KH). Limb tremors were not observed under baseline conditions, but were observed on most days of the fine motor task after 20 and 23 days of GHB administration in baboons DM and HA, respectively. Jerks were observed also on some days in these baboons.

Analysis of baboon plasma by isotope dilution assay revealed that chronic administration of GHB produced high levels of GHB in blood (Table 1). During chronic administration with 750 mg/kg GHB, the group mean (\pm SEM) level of GHB in blood was 1496.3 (445.3) $\mu\text{mol/L}$ for the first sample and was 1364.8 (333.3) $\mu\text{mol/L}$ GHB for the second sample. One baboon (DM) had lower GHB blood levels when compared to the other baboons.

When the GABA-B antagonist CGP36742 was administered during chronic GHB, withdrawal scores were dose-dependently increased. As shown in Fig. 3, withdrawal scores were 1–4 for 32 mg/kg and were 3–7 for 56 mg/kg CGP36742. Vomiting and retching were observed in two baboons at 32 mg/kg and in all four baboons after 56 mg/kg

Fig. 2 Effects of GHB on performance of the fine motor task across consecutive days of chronic administration. Data shown are the time (s) to retrieve six food items (raisins or M&Ms) from a tray presented at the front of the cage for individual baboons (designated DM, KH, HA and PF). The maximum time allowed for retrieving all six raisins was 120 s. The data point over “V” represents the mean \pm 1 SD for tasks conducted during the baseline condition that preceded chronic GHB administration. Other details as in Fig. 1



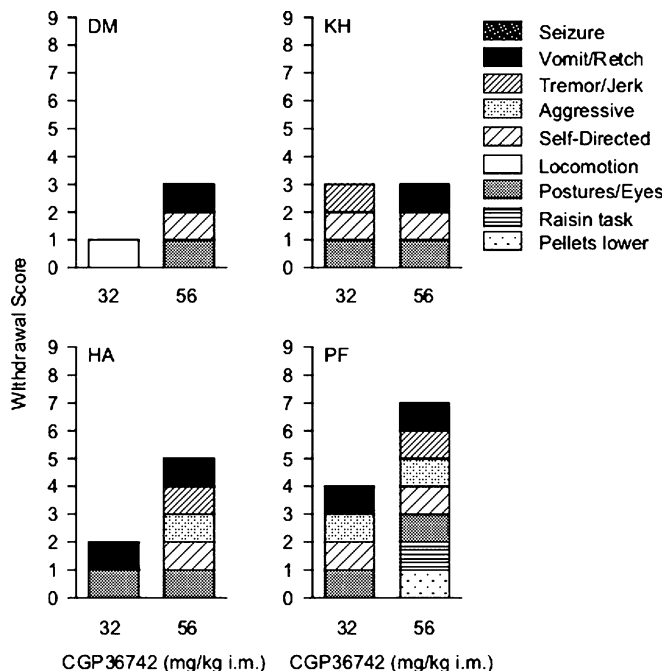


Fig. 3 CGP36742-precipitated withdrawal scores during chronic GHB administration. Twenty-minute observations and the fine motor task were conducted after IM administration of vehicle, 32 and 56 mg/kg CGP36742. The particular measure was scored as positive, if the value exceeded all three of the following: **a** the frequency of behavior recorded for each dose CGP36742 alone during the distilled water baseline, **b** the z-scores for behavior for IM vehicle during the distilled water baseline ($n=5$), and **c** the two IM vehicle control tests during chronic GHB administration. The maximum possible score that could be obtained was 9. Points were assigned as follows: 1 point for each of seven categories of behavior recorded during observations; 1 point for increased duration or refusal of the fine motor task conducted at the end of the observational session; and 1 point if the number of pellets delivered in the first 2–6 h of the 20-h session was decreased

CGP36742. Similarly, tremors and/or jerks were increased in one baboon at 32 mg/kg and in two baboons at 56 mg/kg CGP36742. Seizures were not observed in any of the baboons. Mild signs of withdrawal such as increases in aggression, self-directed behaviors, disruption of the fine motor task and increased time spent in abnormal postures and sitting with eyes closed were also observed. When administered alone, 32 and 56 mg/kg CGP36742 did not produce any overt behavioral effects and food-maintained behavior was similar to vehicle control.

Behavioral signs consistent with a withdrawal syndrome were observed in all four baboons when GHB administration was discontinued. As shown in Fig. 4, peak withdrawal scores of 5 or 6 were obtained in three of the four baboons within 48 h after drug was discontinued, and effects decreased over time. In these three baboons, increases in limb tremors, body tremors, body jerks, aggression, self-directed behaviors and disruption of the fine motor task were apparent 6 h after drug was discontinued. Limb tremors and jerks were no longer observed after 4–8 days. Vomiting was also observed in two baboons in the first 8 days. The fourth baboon (DM) had lower withdrawal scores, which ranged from 1 to 3. This baboon did

not have tremors/jerks or vomiting, but milder signs of increased self-directed behaviors, disruption of the fine motor task and suppression of food intake were observed. Seizures were not observed in any of the baboons.

Before GHB was discontinued, two baboons were working for over 200 pellets and consuming them. In the other two baboons, GHB suppressed operant responding and pellets remained low (range 1–68) at the end of chronic GHB administration (Fig. 5, closed circles). When GHB administration was discontinued, the number of pellets delivered and consumed (open circles) returned to control range within 10–28 days. For the two baboons that were working for pellets at the end of GHB administration, the number of food pellets delivered was decreased in one baboon (DM) and the other baboon (PF) did not eat all pellets delivered for the first 2 weeks after GHB administration was terminated (open diamonds).

Discussion

Numerous case reports indicate that discontinuation of GHB in individuals that abuse high doses of GHB produces a wide range of symptoms that may be indicative of a withdrawal syndrome. The most common symptoms are insomnia, agitation, anxiety, loss of appetite and tremor (Friedman et al. 1996; Galloway et al. 1997; Price 2000), although severe agitation, hallucinations, dysphoria, increased blood pressure and tachycardia have been described (Craig et al. 2000; Hutto et al. 2000). Some of these same behaviors (e.g. agitation, tremor, loss of appetite) have also been reported as adverse effects of high doses of GHB.

Relatively few studies have evaluated whether GHB produces tolerance and/or physical dependence. Tolerance to the motor-incoordinating effects of 1 g/kg GHB in a rotorod task appeared to develop when dosing was repeated over 9 days in rats (Colombo et al. 1995). A second study in mice found that GHB dose-dependently decreased locomotion and tolerance developed to these effects with repeated administration (200 mg/kg for 14 days) (Itzhak and Ali 2002). A third study reported that when rats ($n=2$ per group) were administered increasing doses of GHB (0.25–2 g/kg) every 3 h for 3, 4, 5 or 6 days, viewer-rated intoxication scores decreased with repeated administration and withdrawal scores increased when GHB administration was discontinued (Bania et al. 2003). A fourth study did not find evidence of physical dependence in rats administered up to 4.8 g/kg per day GHB for 5 days (Cook et al. 2002).

In humans and laboratory animals, decreased appetite and/or food intake is a hallmark of withdrawal from sedatives. In the current study, chronic GHB administration suppressed operant responding for food, and decreased consumption of food (i.e. baboons did not eat all pellets delivered or biscuits given), and produced weight loss. Thus, it is not entirely clear if the low levels of food-maintained behavior observed after GHB was discontinued was related to withdrawal or was a slow recovery from the hypophagic effects of GHB itself. However, some

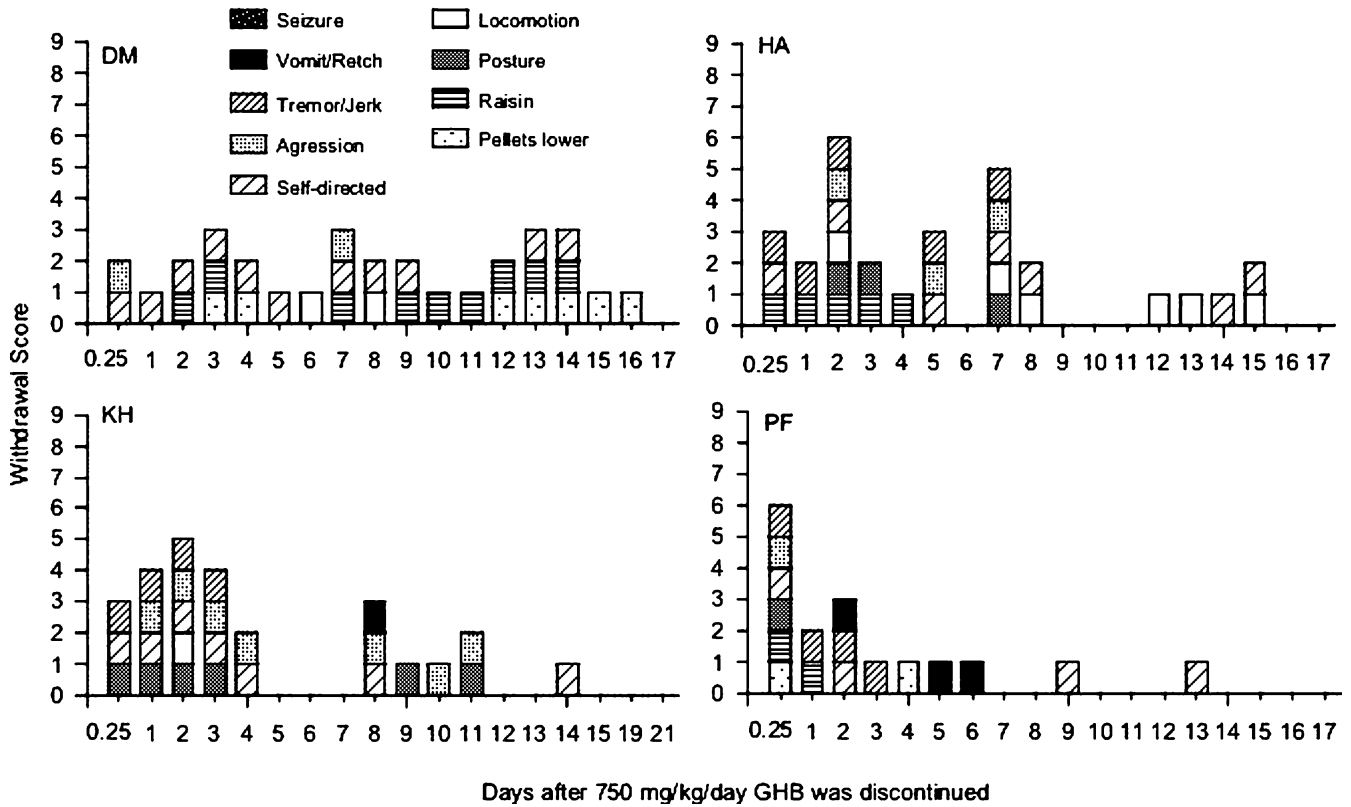


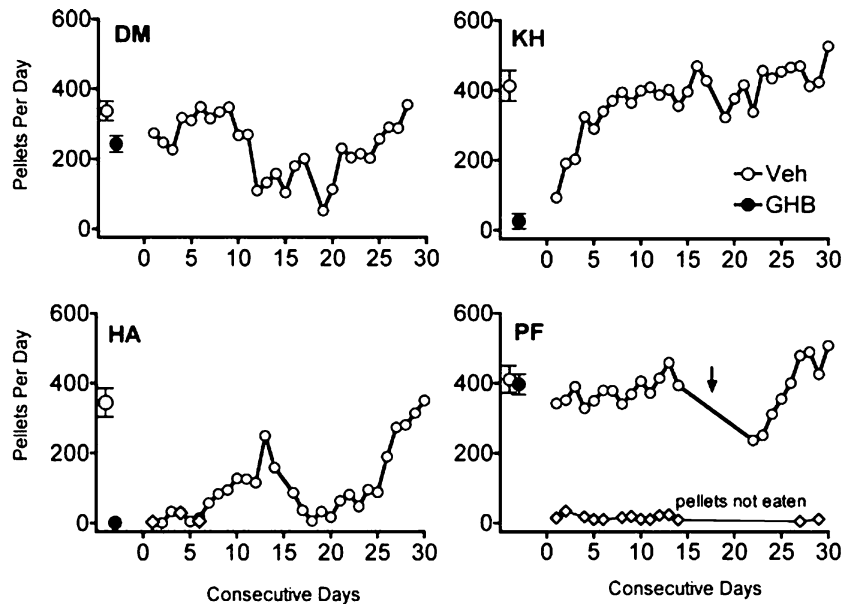
Fig. 4 Spontaneous withdrawal scores over consecutive days following abrupt termination of GHB administration in individual baboons. Scores were determined from behaviors recorded during the 20-min observation sessions and the 2-min fine motor tasks. The particular measure was scored as positive if it was decreased

(pellets) or increased (all other behaviors) when compared to the z-scores for both of the following: **a** the control observations conducted during the vehicle baseline ($n=5$), and **b** the control observations conducted at the end of the chronic GHB administration ($n=5$). Other details as in Fig. 3

animals did show tolerance to the effects of GHB on food-maintained behavior. In two baboons, pellets per day initially decreased and then increased after about 20 days. However, pellets per day remained suppressed throughout GHB administration for two of the four baboons. Performance of the fine motor task was disrupted after 10–15

days of GHB in three baboons, and then also improved after 20–25 days, which suggests a slow development of GHB tolerance. However, tolerance was not evident for other behaviors. For example, the decreases in food-maintained behavior coincided with the observation of behaviors indicative of muscle relaxation (lipdroop) and

Fig. 5 Effects of discontinuation of GHB administration on food-maintained behavior. The data points before “0” represent the mean ± 1 SD of pellets delivered and consumed during the vehicle baseline condition that preceded GHB administration (open circle) and the last 5 days of administration with 750 mg/kg per day GHB (filled circle). Data shown after “0” are the number of pellets delivered (open circles) and number of pellets not eaten (open triangles) over consecutive days after vehicle was substituted for GHB. An arrow indicates when the catheter broke and a subcutaneous resplicing of the catheter was performed; data for days on which this occurred as well as the following 5 days of recovery were omitted



motor incoordination (ataxia). Lip droop and ataxia were observed in two of the four baboons throughout GHB administration. Tremors and jerks were observed consistently in three baboons at end of GHB administration. High doses of GHB produce EEG changes that are similar to those seen in petit mal seizures in rhesus monkeys (100–200 mg/kg IV) (Snead 1978), and can result in tremors and jerks in baboons (320–420 mg/kg IG) (Goodwin et al. *in press*).

When chronic GHB was discontinued in the current study, behaviors consistent with a withdrawal syndrome were observed and effects peaked in the first 6–48 h. Aggressive behavior, self-directed behavior and withdrawal-related postures were increased 6 h after GHB was discontinued. In three of the four baboons, the number and severity of tremors and jerks was increased beginning 6 h after GHB was discontinued and returned to baseline with in 4–8 days. Seizures were not observed. Vomiting was also observed in two baboons in the first 8 days. Thus, the onset and time course of withdrawal appear to be very similar to those described in case reports of human GHB abusers (Dyer et al. 2001; McDonough et al. 2004).

When compared to rodent studies on physical dependence, the total GHB dose in the current study was high (20–29 g/day), the period of administration was longer (over 30 days), and GHB was administered continuously 24 hr/day into the stomach. This dosing procedure maintained high blood levels of GHB (285–2350 $\mu\text{mol/L}$ or 23–244 $\mu\text{g/ml}$). In rhesus monkeys, acute administration of GHB (100–1000 mg/kg, IV) produced dose-related EEG changes and hypothermia with GHB blood levels of 145–2298 $\mu\text{g/ml}$. The threshold dose for EEG changes was 100–200 mg/kg and doses of 300 mg/kg or higher produced a trancelike stupor. The values for GHB in blood in our study overlapped those detected by Snead, and were much higher than peak blood levels (range 30–103 $\mu\text{g/ml}$) reported for chronic therapeutic GHB doses (3–4.5 g) (Scharf et al. 1998; Borgen et al. 2004). In the current study, higher withdrawal scores and more severe signs during spontaneous withdrawal were observed in baboons with the higher blood levels of GHB (115–244 $\mu\text{g/ml}$). The severity of physical dependence for other sedative-hypnotics (e.g., benzodiazepines) is a function of the dose and duration of treatment (Woods et al. 1992). It is likely this is also the case for GHB. Thus, high doses and/or longer periods of GHB dosing may be critical for GHB physical dependence.

When administered chronically, classic sedative-hypnotics produce tolerance and physical dependence (for reviews see Woods et al. 1992; Griffiths and Weerts 1997). Administration of a competitive benzodiazepine receptor antagonist (e.g. flumazenil) following chronic benzodiazepine treatment precipitates withdrawal symptoms such as vomiting, anorexia, impairment of motor function, autonomic signs, tremors, jerks and seizures. A qualitatively similar withdrawal syndrome occurs following abrupt termination of benzodiazepine administration. Thus, it is generally accepted that benzodiazepine physical dependence is mediated primarily via the GABA-A benzodiazepine receptor (for review see Woods et al. 1992). In the

current study, administration of the GABA-B antagonist CGP36742 during chronic GHB produced dose-related increases in behaviors consistent with a precipitated withdrawal syndrome. The number of withdrawal-related behaviors observed and severity of withdrawal was related to the dose of the antagonist administered.

In conclusion, the observation of both a precipitated and a spontaneous GHB withdrawal syndrome provides evidence that baboons were physically dependent on GHB. The observed GHB withdrawal syndrome was not markedly different than that observed for other sedatives. Thus, when using scales previously developed for benzodiazepine and barbiturate physical dependence (Yanagita and Takahashi 1970; Weerts et al. 1998; Kaminski et al. 2003), the GHB withdrawal syndrome in the current study can be characterized as mild to moderate. This is the first study to clearly demonstrate spontaneous and antagonist precipitated GHB withdrawal. The precipitation of withdrawal by the GABA-B receptor antagonist CGP 36742 suggests that the GABA-B receptor may play a prominent role in GHB physical dependence.

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