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Effects of sarin on the operant behavior of guinea pigs☆

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Abstract

The present study evaluated the dose–response effects of subacute exposure to sublethal doses of the organophosphorus (OP) chemical warfare nerve agent (CWNA) sarin (GB) on the operant behavior of guinea pigs. Dietary restricted guinea pigs, trained to respond for food under a progressive ratio (PR) schedule of reinforcement, were injected five times per week (Monday–Friday) for 2 weeks with fractions (0.1, 0.2, and 0.4) of the established LD_{50} of GB (42 μg/kg). Changes in body weight, whole blood (WB) acetylcholinesterase (AChE) levels, and operant performances were monitored over the 2 weeks of GB exposure and for an additional 2 weeks following the termination of exposures. There were dose-related changes in body weight and WB AChE levels throughout the exposure and post-exposure periods. Several parameters of PR performance were disrupted during exposure to 0.4 LD_{50} GB, however, concurrent weight loss indicated the presence of overt toxicity. PR performance recovered following the termination of exposures. Lower doses (0.1 and 0.2 LD_{50}) of GB failed to produce reliable effects on operant performance during the exposure period. Overall responding decreased during exposure to 0.4 LD_{50} GB, resulting in reduced response rates and break points. The decrease in overall response rates was attributed to an increase in pausing since there was no decrease in running rate. Motor effects of 0.4 LD_{50} GB were evident as an increase in the proportion of lever press durations ≥ 1.0 s. In the present study, doses of GB lower than 0.4 LD_{50} produced no marked alteration of operant performance in guinea pigs, although WB AChE levels were maximally inhibited to 20% of control.

Keywords: Sarin; GB; Nerve agents; Operant behavior; Progressive ratio; Guinea pigs

1. Introduction

Recent world events such as the terrorist attacks perpetrated against Japanese civilians, the possible exposure of US soldiers to sarin (GB) during the 1991 Persian Gulf War, and efforts to destroy aging chemical weapon stockpiles have increased interest in the effects of repeated low level exposure to organophosphorus (OP) chemical warfare nerve agents (CWNA). CWNA (e.g., sarin, soman, and VX) are highly toxic OP compounds that are chemically related to the OP insecticides commonly used for pest control and have similar toxicological profiles [11]. The acute effects of OP compounds are a result of the disruption of normal communication within the nervous system through the irreversible binding of acetylcholinesterase (AChE), the enzyme responsible for the degradation of the neurotransmitter acetylcholine (ACh). The inability to degrade ACh leads to excessive accumulation of this neurotransmitter at central and peripheral synapses resulting in over stimulation of postsynaptic membranes [75]. The cholinergic signs and symptoms resulting from exposure can include miosis, headache, nausea, dizziness, anxiety and restlessness, muscle fasciculations and weakness, tremor, incoor-

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dination, emesis, abdominal cramps, diarrhea, sweating, salivation, tearing, rhinorrhea, and phlegm [11]. The life-threatening effects of high doses (dose that produces acute cholinergic signs and symptoms) of these agents include unconsciousness and coma, seizures, respiratory depression, and death due to cardiorespiratory collapse if the poisoning is not promptly and aggressively treated. The acute effects of high doses of these agents and their neuropharmacological bases are well characterized [50]. Characterization of the effects of repeated low dose (dose that produces minimal cholinergic signs and symptoms) exposure to these agents, however, is sparse [69].

Behavioral incapacitation often results from acute exposure to relatively high doses of OP compounds [9,29,32,86]; moreover, subtle behavioral alterations may be evident following repeated low dose exposure [33,49]. For example, a recent report indicated a failure of GB-exposed guinea pigs to habituate to certain aspects of functional observation battery testing [38]. Other reports have indicated that long-term changes in EEG patterns [12,85], alterations of the temporal patterning of operant responding [39,40], supersensitivity to the acute effects of anticholinergic drugs [53], performance decrements on a compensatory tracking task [6], impaired acquisition of a spatial navigation task [66,67], increased startle response (M.L. Sipos, personal communication), and development of attention deficits [27] result from repeated sublethal exposure to OP compounds in rodents and nonhuman primates.

Current medical research to evaluate the toxic effects of CWNA is focused on the use of a guinea pig model [1,38,76]. The guinea pig is considered to be a more valid rodent model of human exposure than either the rat or mouse [2,24,41,79,80]. This is partly due to the relatively low concentration of plasma carboxylesterases (CaE) present in guinea pigs compared with rats and mice [47], making the guinea pig's response to the toxic effects of CWNA more similar to that of nonhuman primates and, presumably, humans. CaE are another esterase that OP compounds irreversibly bind with and have an important role in the detoxification process because they stoichiometrically reduce the amount of an OP compound available to inhibit AChE [25,45–48]. The lower concentrations of CaE in the guinea pig contribute to an increased sensitivity to the lethal effects of CWNA as well as to increased efficacy of pretreatment drugs [22,30] compared with other rodent species. Furthermore, toxicokinetic studies reported that the concentration time profile of an OP compound in guinea pig resembles that of the marmoset monkey more closely than that of the rat [2].

The present study evaluated the dose–response effects of subacute exposure to sublethal doses of GB on progressive ratio responding of guinea pigs. The range of doses chosen was based on the results of previous studies [1,38] that showed 0.4 LD_{50} GB was the maximum tolerated dose (MTD) that could be administered for 2 weeks without producing signs of acute cholinergic toxicity. The progressive ratio schedule requires the animal to emit an increasing number of responses to obtain each successive reinforcer and was selected because it represents acquired behavior, provides a measure of motivation [36,56,57], and has been demonstrated to be sensitive to the effects of AChE inhibitors [26,84]. An additional consideration was that the progressive ratio schedule has been suggested to potentially provide more information than a fixed-ratio schedule [68].

2. Methods

2.1. Animals

Forty-four male Hartley guinea pigs (Crl:(HA)BR) weighing 250±20 g, were obtained from Charles River Laboratories (Kingston, NY). Upon arrival they were quarantined for 5 days and observed for evidence of disease. Animals were housed individually in polycarbonate cages in a temperature (21±2 °C) and humidity (50±10%) controlled colony room maintained on a 12-h light-dark cycle with lights on at 0600 h. Food and water were available ad libitum in home cages. Animals were implanted subcutaneously (sc) with sterile transponders (IPTT-200; BioMedic Data Systems Inc., Seaford, DE) for animal identification and body temperature monitoring. Animals were allowed to acclimate to the colony room (>1 week) and to reach 375 g body weight before their feed was restricted to 80% of daily recommended diet (60 g/kg). Daily feedings occurred in the afternoon not less than 1 h after behavioral sessions were conducted.

2.2. Apparatus

Sixteen conventional rodent operant conditioning chambers (30.5 × 24.1 × 29.2 cm [D × W × H]; Med-Associates, Georgia, VT) were used. Each chamber was enclosed in a ventilated, light- and sound-attenuating cubicle and equipped with two response levers (requiring approximately 0.22 N to operate), an opening centered between the levers through which 45-mg food pellets (Bio-Serv, Frenchtown, NJ) could be delivered, and a cue light above each lever. The food trough contained an infrared emitter-detector pair for monitoring entries. Illumination of the chamber was accomplished via a house light mounted on the wall opposite the response levers. White noise was generated from a speaker located beneath the house light. Reinforcement contingencies and data collection were accomplished with 0.01 s resolution using a Pentium microcomputer running MED-PC® software (Med-Associates, Georgia, VT).

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Subjects were acclimated to the operant test chambers for 1 week by placing them in the chambers daily for 10 min. During acclimation sessions, the house and cue lights were

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2.4. Sarin administration

Isopropyl methylphosphonofluoridate (sarin; GB) was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD) and was diluted in sterile saline to achieve the desired concentrations (0.1, 0.2, 0.4 LD_{50}, LD_{50}=42 μg/kg [38]; equivalent doses of 4.2, 8.4, and 16.8 μg/kg, respectively). The dilute nerve agent was aliquoted into serum vials (one for each day), sealed with Teflon septa and stored at −80 °C until the day of injection. Injections were administered sc between the shoulder blades in a volume of 1.0 ml/kg body weight. Beginning one week prior to GB exposures, all animals received daily (M-F) saline injections to acclimate them to the injection procedures. For the next 2 weeks, GB or saline was administered daily (M-F). Behavioral sessions began 30 min after each injection.

2.5. AChE activity

Blood samples (0.5–1.0 ml) were collected weekly (Friday) via toenail clips [82] from all animals at six time points. Blood samples were collected approximately 30 min after behavioral sessions had ended. The first collection time was after the final saline injection to establish baseline AChE levels and the last collection period was 17 days after the last exposure. Whole blood (WB) AChE activity was determined by an automated method using a COBAS/FARA clinical chemistry analyzer (Roche Diagnostics, Nutley, NJ). The analytical procedure was based on the manual method of Ellman et al. [23] and modified for the COBAS/FARA system [35] using acetylthiocholine as substrate.

2.6. Data analysis

Break point denotes the highest ratio requirement completed to earn the last reinforcer of a session and is identical to the number of reinforcers obtained in a session due to the progressive-ratio algorithm implemented. Overall rate of responding was expressed as responses per second and determined by dividing the total number of left lever responses by the session duration (in s). Correlations among break point, response rate, and total responses were high [mean correlations (std. dev.) of 0.96 (0.122), 0.99 (0.004), and 0.97 (0.113) for break point–response rate, break point–total responses, and response rate–total responses, respectively]; therefore, effects reported for one measure will be representative of effects on the others, unless otherwise noted. Individual lever press durations (LPDs) and true interresponse times (IRTs) [54] were used for the following analyses. Pause duration was defined as the proportion of session time occupied by interresponse times (IRTs) $\geq$5.0 s [20]. Hold duration was defined as the proportion of total lever press duration occupied by lever presses $\geq$1.0 s. Running rate was computed from IRT distributions, eliminating IRTs classified as pauses (IRTs $\geq$5.0 s).

GB effects on AChE activity, break point, and response rate were expressed as a percentage of baseline performance. Two-way (Treatment X Block) ANOVAs were conducted for each dependent variable with repeated measures based on the mean of blocks of 5 sessions. Due to violations of assumptions of homogeneity of variance, break point and response rate data were log transformed prior to ANOVA; hold and pause data were arcsine transformed prior to ANOVA. Physiological indices of exposure (weight change, body temperature, and AChE activity) were analyzed untransformed. WB AChE data were analyzed by two-way repeated measures ANOVA with treatment as the between subject's factor and collection time as the repeated measure. For all analyses, Hyunh–Feldt's procedure was used to adjust for violations of assumptions of sphericity of repeated measures and adjusted P values are reported. Main effects of block were evaluated using Bonferroni's procedure. Main effects of treatment were evaluated with Tukey's procedure. Significant interactions were followed by tests of simple main effects. For all analyses, $\alpha=0.05$.

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3. Results

3.1. WB AChE Activity

Exposure to fractions of the LD_{50} of GB resulted in dose-dependent inhibition of WB AChE (Fig. 1). The two-way repeated measures ANOVA of percent control WB AChE activity revealed a significant main effect of Treatment [$F(3,37)=104.48$, $P<0.001$], a significant main effect of Collection Time [$F(5,185)=123.40$, $P<0.001$], and a significant Treatment × Collection Time interaction [$F(15,185)=30.38$, $P<0.001$]. By the end of the first week of GB exposure, WB AChE levels were inhibited to 62%, 19%, and 5% of control for the 0.1, 0.2 and 0.4 LD_{50} groups, respectively. These values were significantly different from each other as well as from those of the control group ($P<0.001$). Following the second week of exposure WB AChE levels where maximally inhibited to 40%, 16%, and 2% of baseline control values for the 0.1, 0.2, and 0.4 LD_{50} groups, respectively. Post hoc tests of the main effects of Treatment revealed that AChE levels of each group were significantly different from those of the other groups ($P<0.001$) with the exception that those from the 0.4 LD_{50} and 0.2 LD_{50} groups did not differ. WB AChE levels had returned to baseline control values by 17 days following the last exposure to GB.

3.2. Weight changes

There were dose-related changes in the body weight gains of the guinea pigs over the course of the GB exposure period and the post-exposure assessment period. Fig. 2 shows the average weight gain (±SEM) per session (calculated as the animal's weight on day n minus the animal's weight on the day prior to the first day of baseline). A two-way repeated measures ANOVA of the average weekly weight gain revealed significant main effects of Treatment [$F(3,40)=5.56$, $P<0.01$], Block [$F(5,200)=508.72$, $P<0.001$],

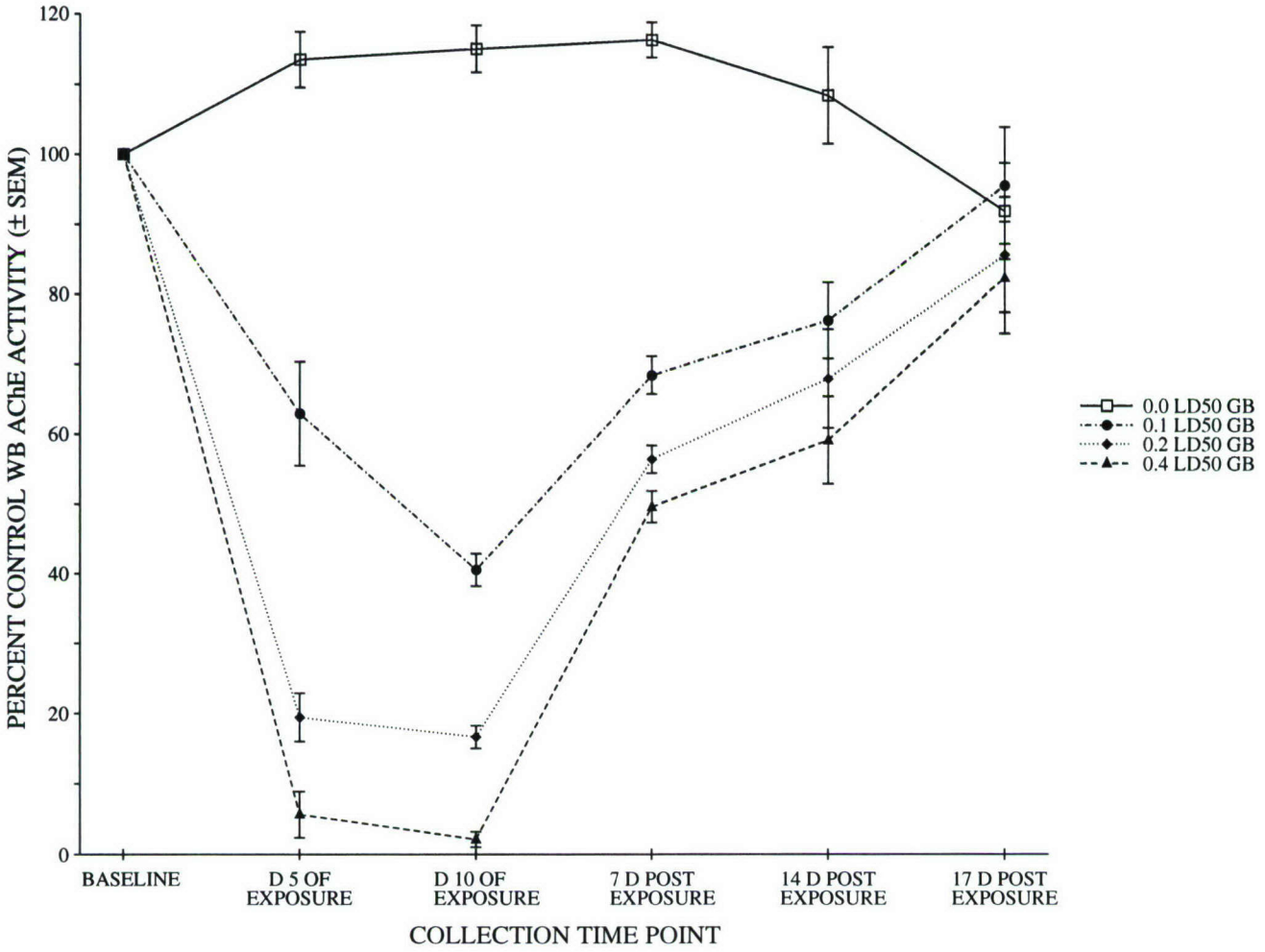

Fig. 1. WB AChE levels as a function of collection time. Blood was collected via toe-nail clip on Fridays during GB exposure and AChE levels were determined using standard measures. Values are expressed as mean±SEM with a minimum of $n=10$ samples (each run in triplicate) for each data point. Following the fifth and 10th days of GB injections, WB AChE levels exhibited a dose–response relationship with each exposure group differing significantly from all other groups ($P<0.001$). One week following the termination of GB injections, WB AChE levels began to recover and by 17 days following the last exposure they had recovered to control levels.

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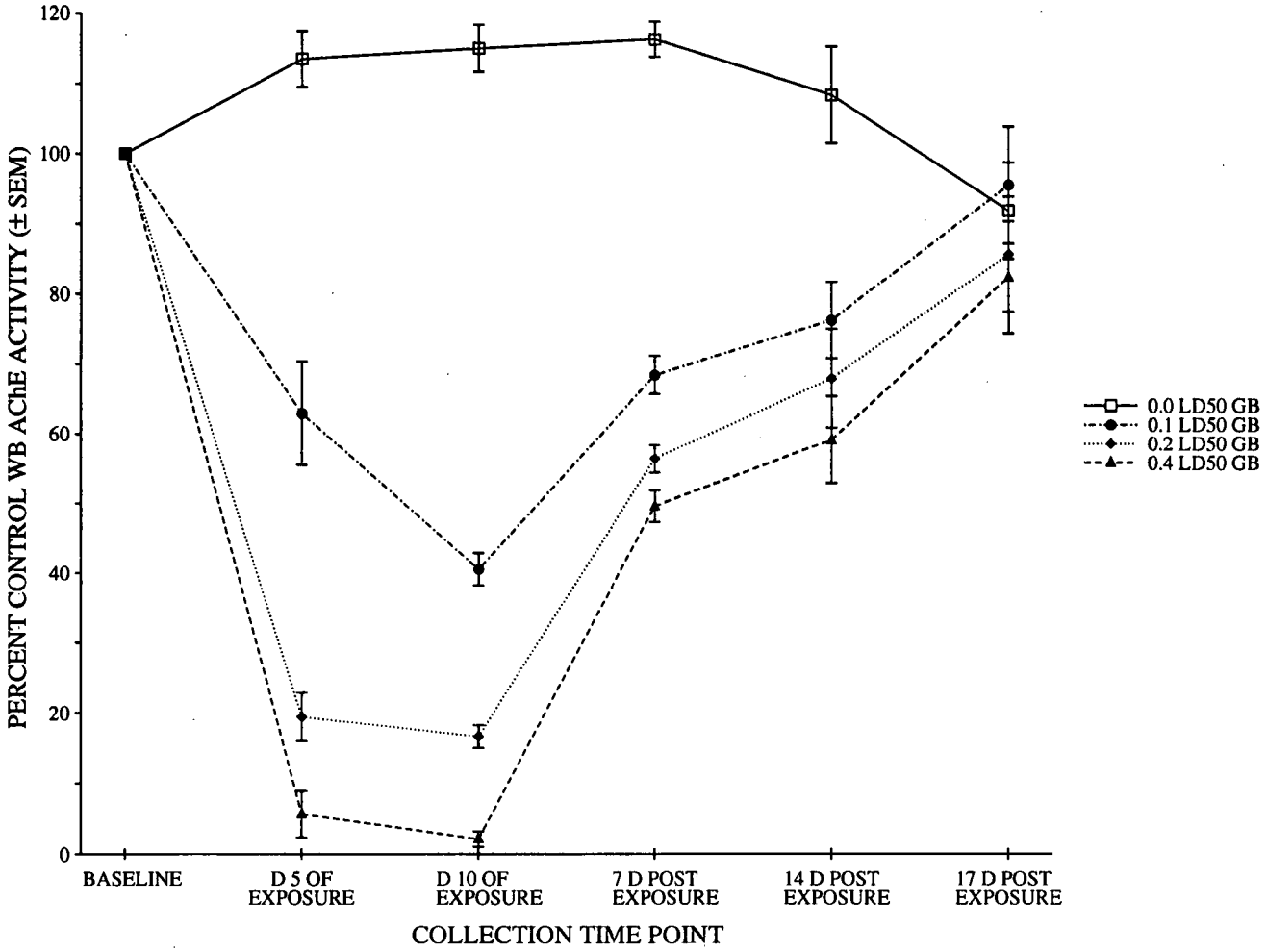

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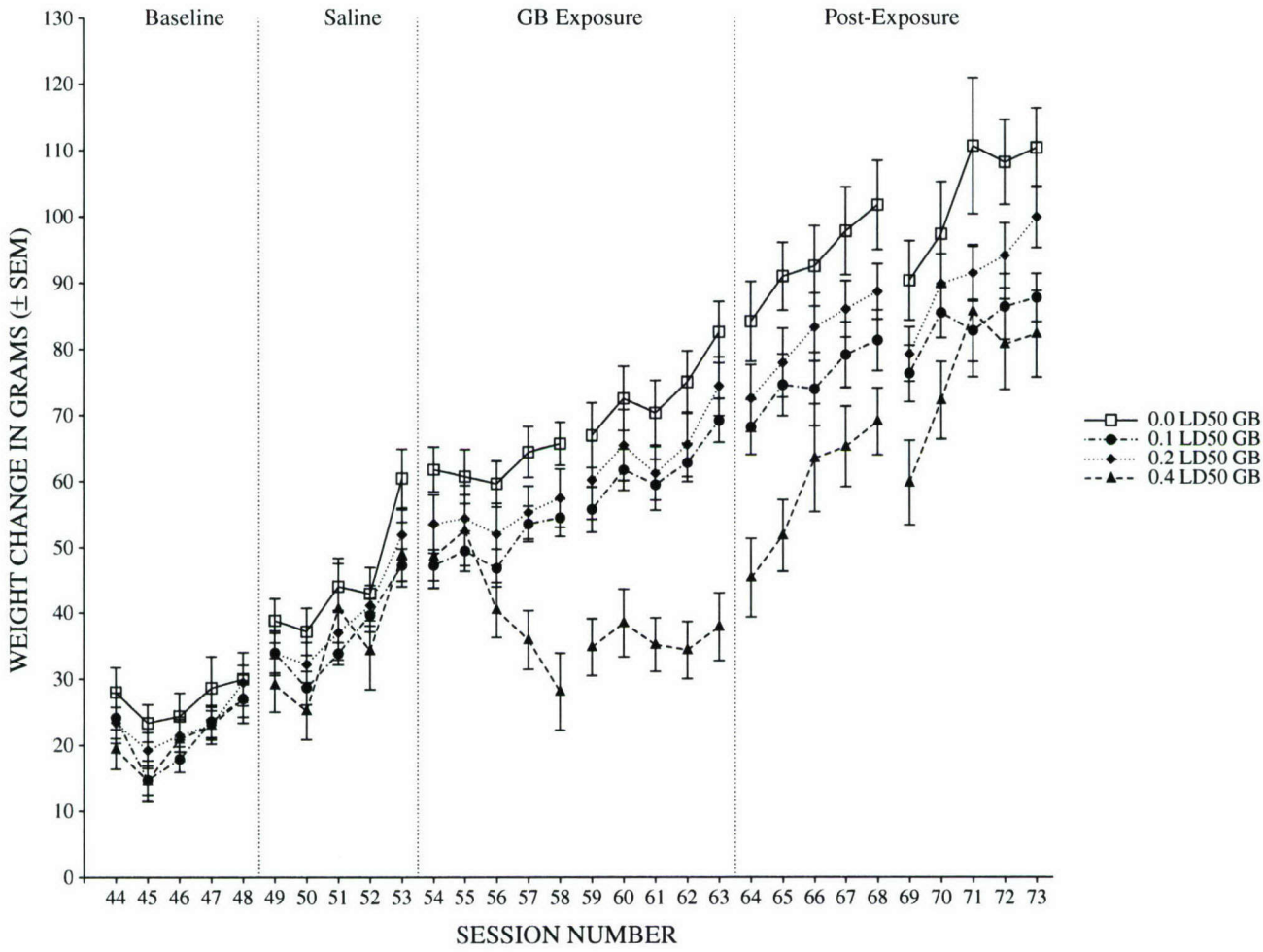

Fig. 2. Weight change as a function of session number. Weight change is the deviation between body weights prior to a given session and initial weight, determined prior to session 43. Values represent mean ± SEM with $n = 11$ for each data point. There is a dose-related change in weight gain; the 0.4 LD_{50} GB animals gained significantly less weight than animals receiving saline ($P < 0.005$).

and a significant Treatment × Block interaction [$F(15, 200) = 7.88$, $P < 0.001$]. The control animals gained more weight throughout the 6 weeks of assessment than did the 0.4 LD_{50} animals. The difference in weight gain between the control animals and the 0.4 LD_{50} animals became apparent during the initial week of GB exposure, and this difference remained until the experiment was terminated. As seen in Fig. 2, animals exposed to 0.4 LD_{50} GB lost weight during the first week of exposure. Differences in weight gain between the 0.4 LD_{50} animals and all other GB-exposed animals were evident during the second week of GB exposure. The 0.4 LD_{50} animals' weight gains were also below those of the 0.2 LD_{50} animals during the first post-exposure week.

3.3. Body temperature

Body temperatures were collected daily prior to behavioral testing (approximately 23 h post-exposure) and the weekly group means (± SEM) are presented in Table 1. A two-way repeated measures ANOVA revealed significant main effects of Treatment [$F(3,40) = 2.89$, $P < 0.05$] and Block [$F(5,200) = 11.61$, $P < 0.001$], however, the Treatment × Block interaction failed to reach conventional levels of significance [$F(15,200) = 1.68$, $P > 0.13$]. The main effect of Treatment revealed that the temperatures from the 0.4 LD_{50} animals were lower than those of the 0.2 LD_{50} animals ($P < 0.05$). The main effect of Block revealed that the temperatures during the 2 weeks of post-exposure assessment were lower than the previous 4 weeks, but not different from each other. The statistically significant effects on temperatures are suspected to be due primarily to the decrease in recorded temperatures for the 0.4 LD_{50} animals during the 2 weeks of post-exposure assessment and may have been due to transponder reliability.

3.4. Molar analysis of progressive ratio performance

As seen in the upper panel of Fig. 3, the 0.4 LD_{50} dose of GB resulted in decreased break points during the 2-week exposure period. There were no significant main effects of Treatment or Block; however, there was a significant

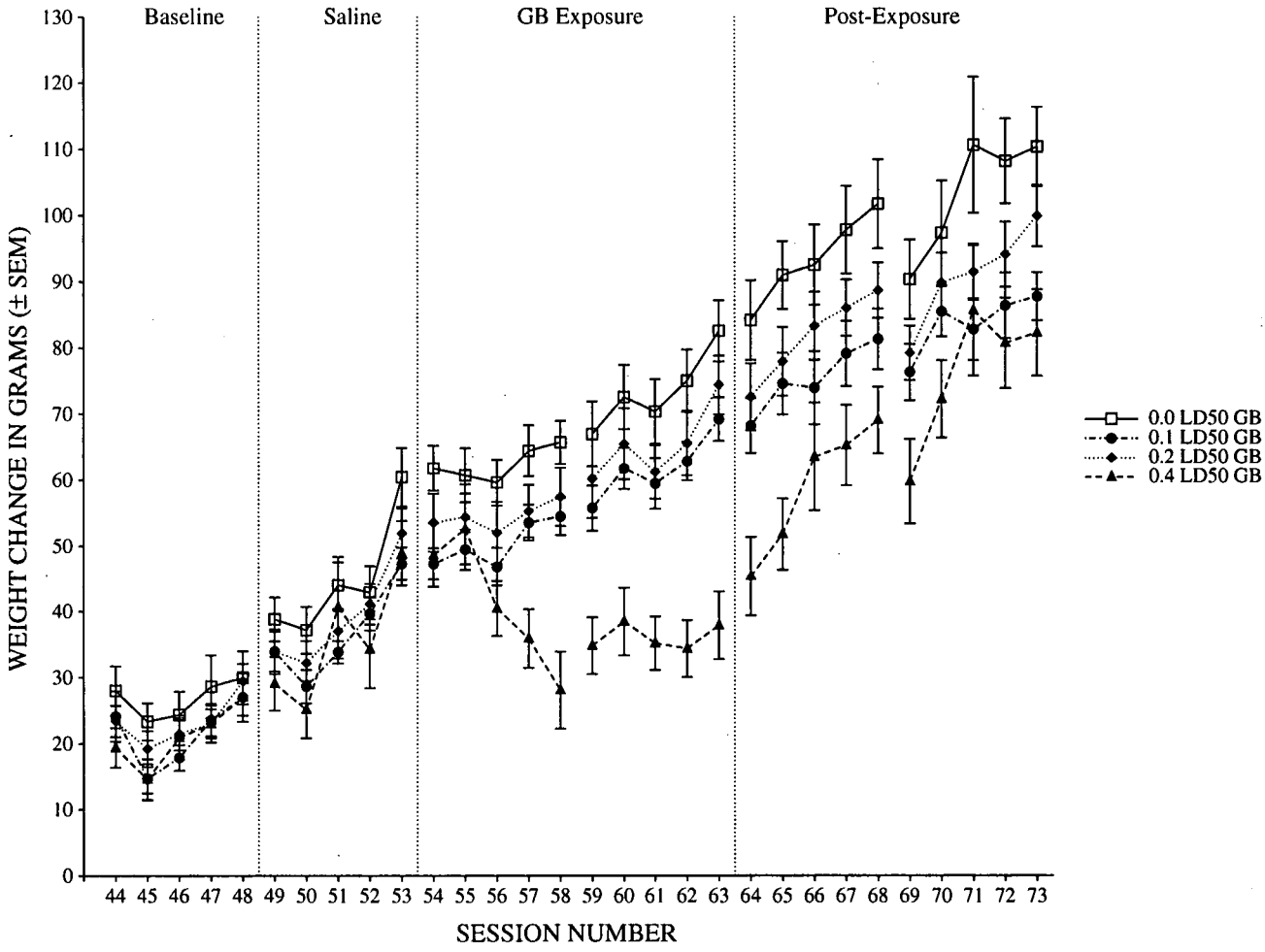

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Table 1
Effect of GB exposure on body temperature in guinea pigs

Observation	GB dose group			
Period	Control	$0.1 \times LD_{50}$	$0.2 \times LD_{50}$[a]	$0.4 \times LD_{50}$[a]
Baseline[b]	101.18 (0.13)	100.85 (0.12)	101.28 (0.15)	100.97 (0.07)
Saline[b,c]	101.17 (0.10)	100.92 (0.09)	101.35 (0.14)	101.03 (0.11)
GB week 1[b,c]	101.16 (0.15)	101.09 (0.09)	101.32 (0.12)	101.04 (0.13)
GB week 2[b,c]	101.16 (0.13)	100.88 (0.08)	101.25 (0.11)	100.89 (0.22)
Post week 1	100.97 (0.13)	100.81 (0.10)	101.18 (0.13)	100.30 (0.40)
Post week 2	100.83 (0.11)	100.76 (0.12)	101.15 (0.14)	100.48 (0.21)

Values are mean body temperature (°F) measured 1 h prior to behavioral sessions (~30 min prior to injection). Numbers in parentheses are SEM.
[a] Indicates significantly different from each other ($P<0.05$).
[b] Indicates significantly different from post week 2 ($P<0.05$).
[c] Indicates significantly different from post week 1 ($P<0.05$).

Treatment × Block interaction [$F(5,200)=3.01$, $P<0.01$]. Break points of the 0.4 LD_{50} GB animals were significantly less than those of all other groups during the first week of exposure ($P<0.05$) and significantly less than those of the control group during the second week of exposure ($P<0.04$). Break points for the 0.4 LD_{50} animals during the first week of exposure were also significantly less than their own pre-exposure and post-exposure break points

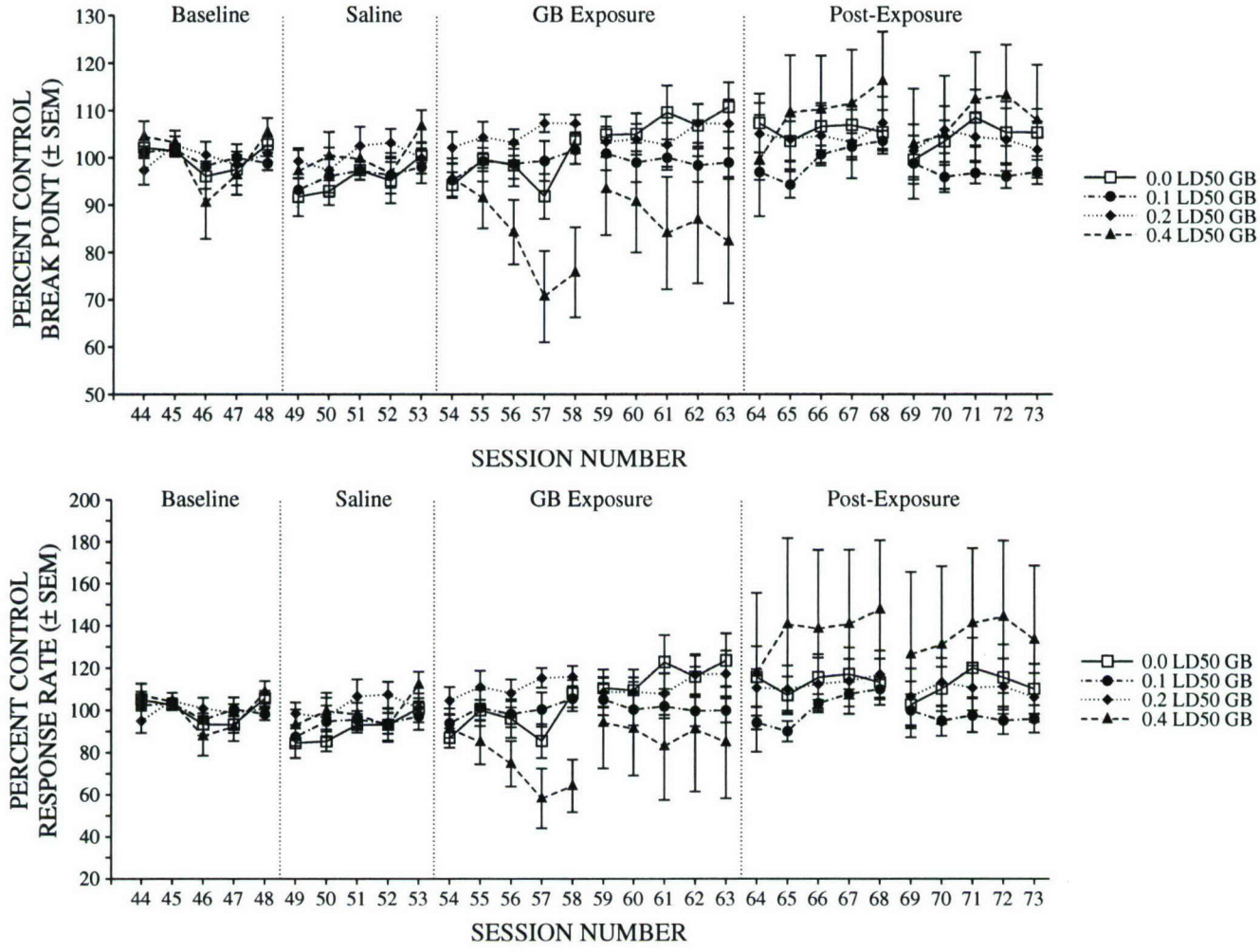

Fig. 3. Break point (upper) and overall response rate (lower) of guinea pigs responding under a PR schedule of food reinforcement expressed as a percentage of baseline values. Values are mean ± SEM with $n=11$ for each data point. Break points for animals receiving 0.4 LD_{50} GB were significantly lower than those for the animals receiving either 0.1 or 0.2 LD_{50} GB ($P<0.05$) during the first week of exposures. During the second week of GB injections, the break points for animals receiving 0.4 LD_{50} GB were significantly less than for those receiving saline ($P<0.05$). Due to the high correlation between break points and response rates, a similar pattern of results was obtained for overall response rates. See text for additional details.

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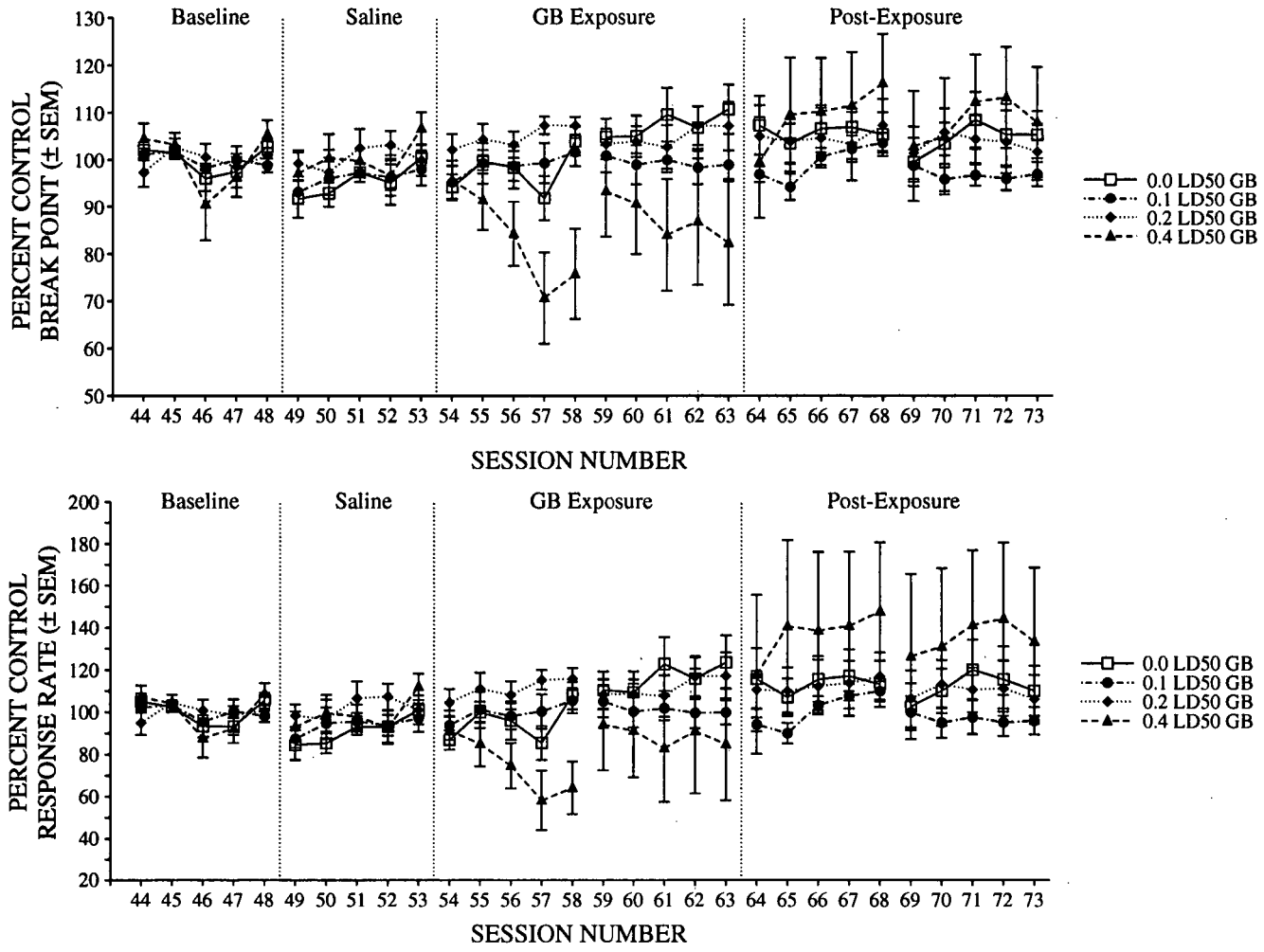

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($P<0.005$). Similarly, break points from these animals during the second week of exposure were significantly less than those from the post-exposure period ($P<0.002$).

Overall response rates tended to increase slightly over the time course of the study shown in the lower panel of Fig. 3, although this was not statistically significant ($P>0.05$). Due to the high correlation between break point and response rate, the pattern of effects noted for break points is consistent with the pattern of effects found for overall response rates with the exception that during the first week of exposure response rates for the 0.4 LD_{50} animals were not different from those of the control group ($P=0.086$).

3.5. Molecular analysis of progressive ratio performance

Running rate, expressed as a percentage of baseline performance is presented in Fig. 4. Running rate tended to increase across Blocks [$F(5,200)=4.61$, $P<0.01$]; however, due to adjustments made for multiple comparisons no differences between blocks could be stated. There were no main effects of Treatment nor was there a significant Treatment × Block interaction, indicating that within ratio responding was not disrupted.

Exposure to 0.4 LD_{50} GB produced an increase in the proportion of IRTs ≥ 5.0 s (Fig. 5). There were significant main effects of Treatment [$F(3,40)=3.50$, $P<0.03$] and Block [$F(5,200)=4.65$, $P<0.004$], and a significant Treatment × Block interaction [$F(15,200)=2.76$, $P<0.005$]. Overall, pause durations of the 0.4 LD_{50} group were significantly different from those of the 0.1 LD_{50} group. Pause durations during baseline were significantly lower than those during the first week of exposure ($P<0.01$) and during the second week post-exposure ($P<0.005$). During both weeks of GB exposure, pause durations for the 0.4 LD_{50} group were significantly greater than those for the control and 0.1 LD_{50} groups. Pause durations of the 0.4 LD_{50} group during the first week of GB exposure were significantly greater compared with their own values from both the baseline and saline blocks ($P<0.01$). Similarly, the pause durations of this group during the second week of GB

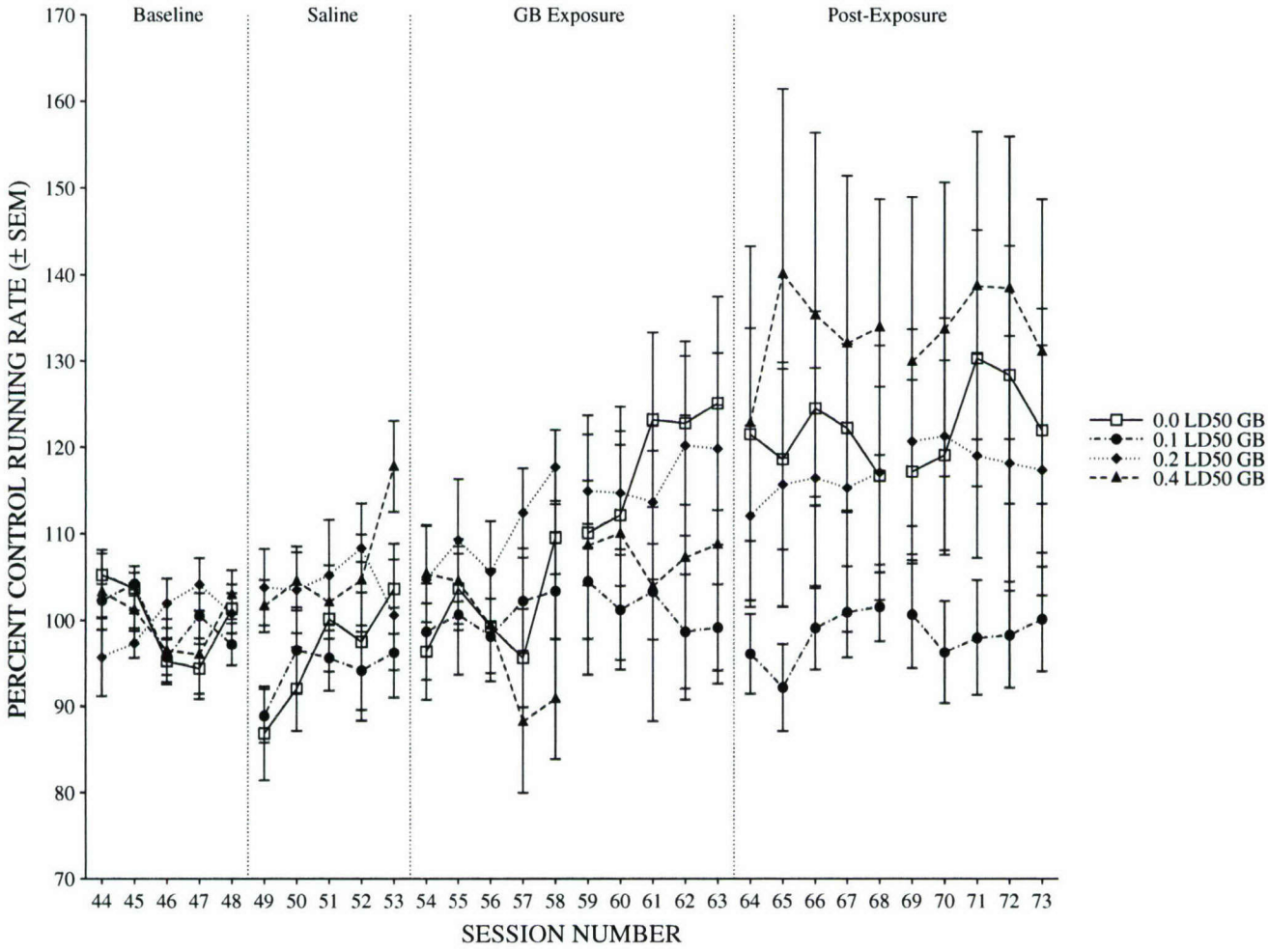

Fig. 4. Percent control running rate computed from IRT distributions eliminating pauses (IRTs ≥ 5.0 s). Values are mean ± SEM with $n=11$ for each data point. As seen in the figure, there was a trend toward increasing running rates during the GB exposure and post-exposure periods. There were no statistically reliable effects of Treatment, Block, or Treatment × Block interaction on running rates.

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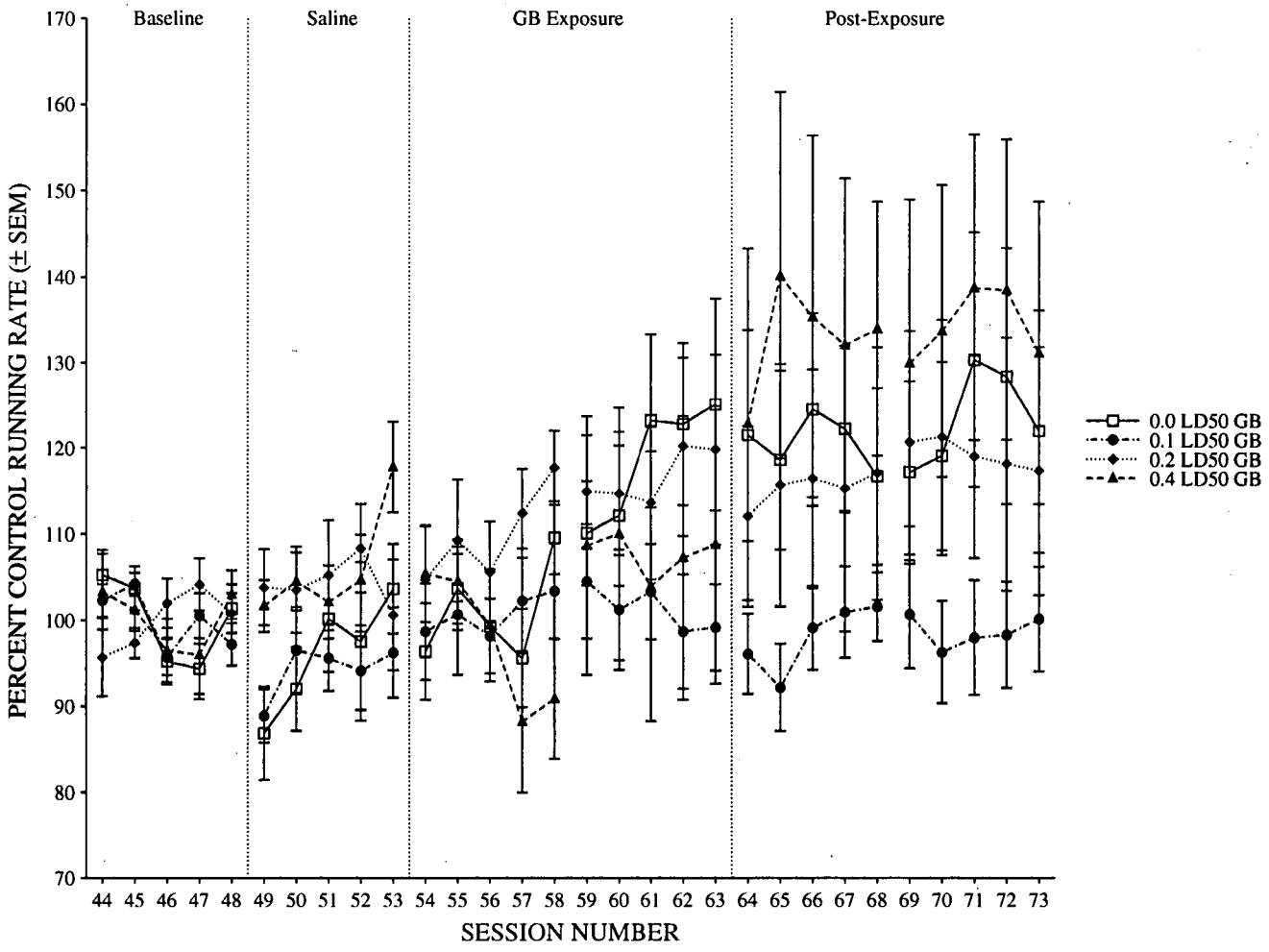

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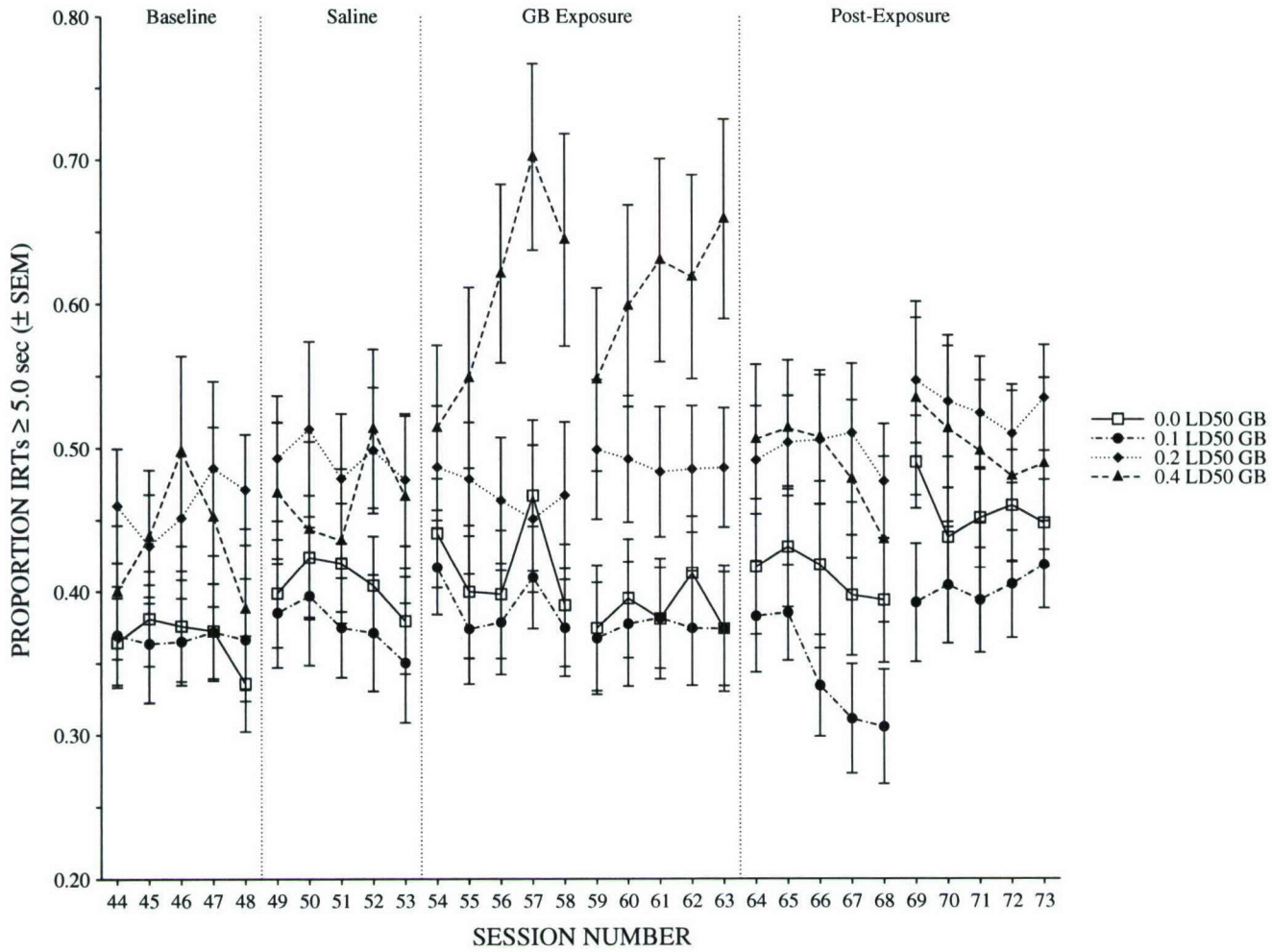


Fig. 5. Pause duration (proportion of session time occupied by pauses ≥ 5.0 s) for each exposure group as a function of session number. Pause duration increased for guinea pigs exposed to 0.4 LD_{50} GB during the 2 weeks of injection and were significantly higher than those from animals receiving saline or 0.1 LD_{50} GB ($P<0.03$).

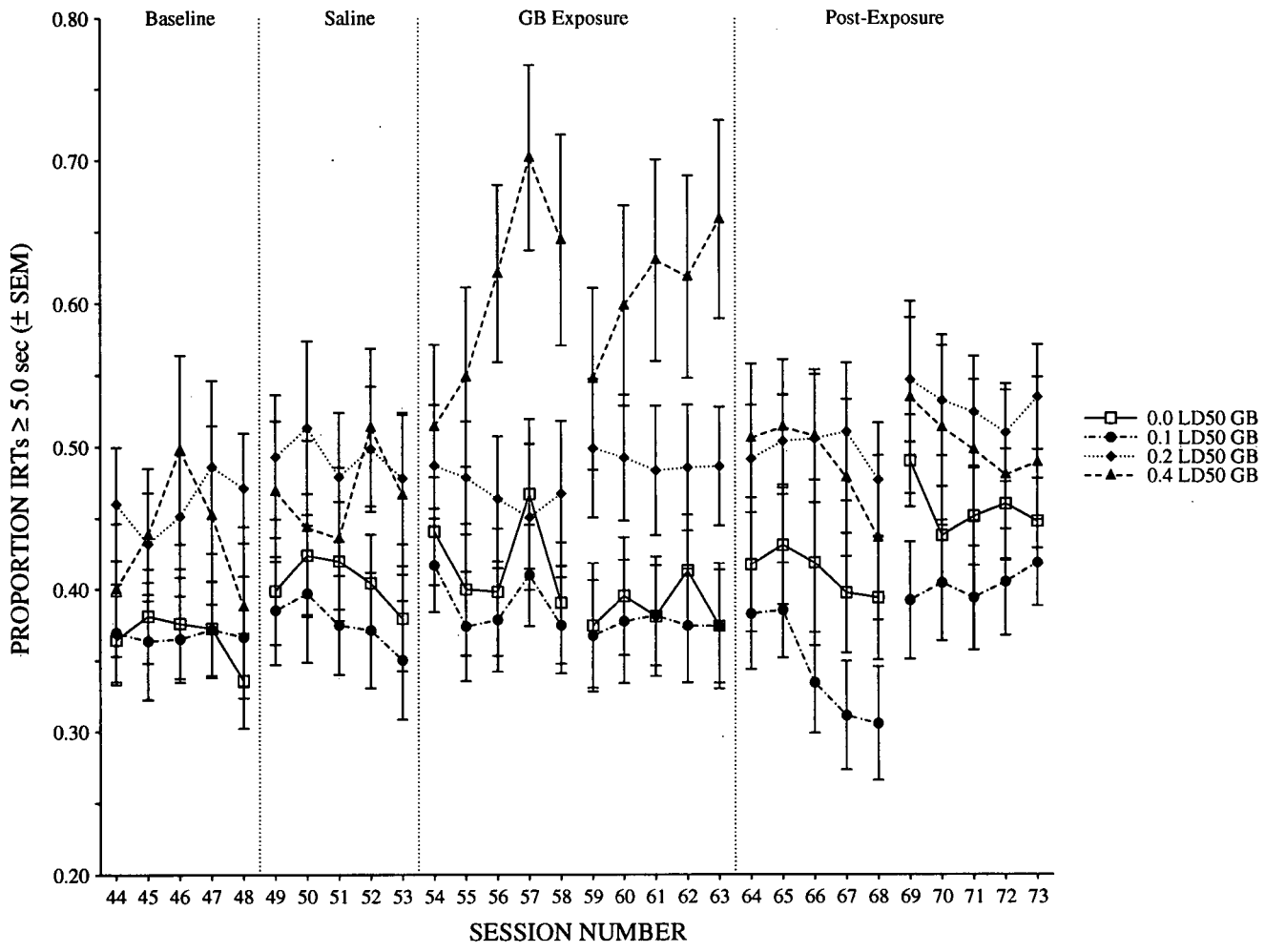
exposure were significantly different from those generated by this group during the baseline, saline, and both post-exposure blocks ($P<0.04$).

The effects of GB exposure on hold duration (proportion of LPDs ≥ 1.0 s) are displayed in Fig. 6. The main effect of Treatment was not significant [$F(3,40)=0.39$, $P>0.7$] nor was there a main effect of Block [$F(5,200)=1.20$, $P>0.31$]. However, there was a significant Treatment × Block interaction [$F(15,200)=3.44$, $P<0.001$]. Hold durations for the 0.4 LD_{50} group during the first week of GB exposure increased significantly when compared with those from their own baseline and saline blocks ($P<0.01$). Similarly, during the second block of GB exposure, hold durations for this group were increased when compared with their own baseline, saline and second post-exposure blocks ($P<0.03$).

4. Discussion

In the present investigation, repeated exposure to 0.4 LD_{50} GB produced a decrease in break points and response rates of guinea pigs responding for food under a PR schedule of reinforcement during the exposure period. The reduction in response rates was attributed to increased pausing, since running rate increased slightly over the same time period. Motor effects of 0.4 LD_{50} GB were evidenced as an increase in the proportion of lever presses that exceeded 1.0 s in duration (hold duration). Behavior recovered rapidly following the termination of exposure. There was little evidence that doses of GB lower than 0.4 LD_{50} resulted in behavioral alterations as measured in the present study. GB exposure produced dose-related inhibition of WB AChE activity throughout the exposure period but had recovered by 17 days post-exposure. Weight loss was also evident in those animals receiving 0.4 LD_{50} GB, despite supplemented daily rations (to accommodate for decreased reinforcement rate) and no evidence to indicate decreased food consumption in their home cages.

The weight loss of the animals exposed to 0.4 LD_{50} GB in the present study is inconsistent with previous investigations [1,38]. In these earlier studies, this dose did not produce weight loss; however, unlike animals in the present



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4. Discussion

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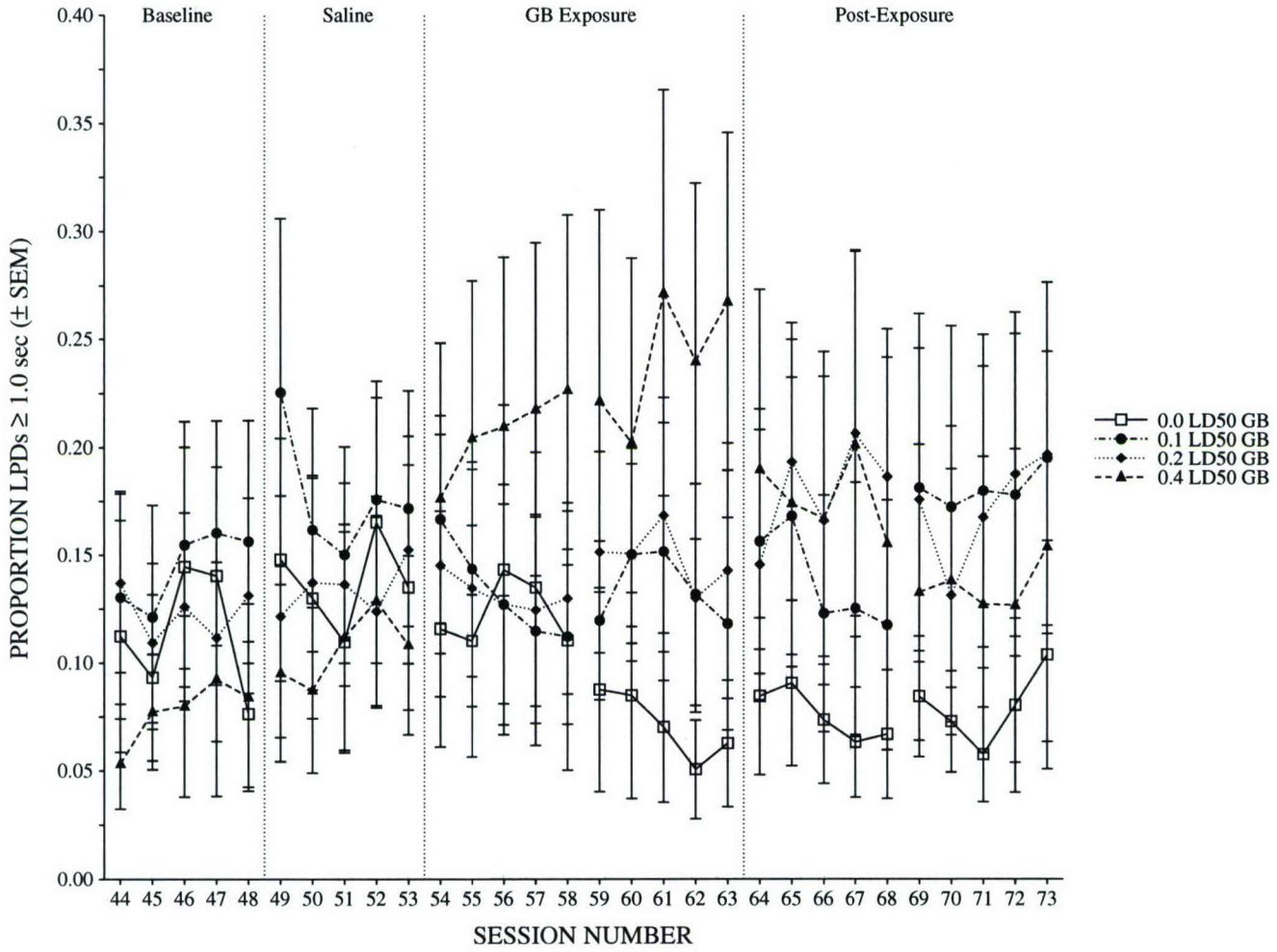

Fig. 6. Hold duration (proportion of total response time occupied by lever presses ≥ 1.0 s) as a function of session number for each GB exposure group. Hold durations were increased during the 2 weeks of GB injections for guinea pigs exposed to 0.4 LD_{50} as compared with their own baseline performance.

study, animals in earlier studies were not maintained under dietary restriction nor were they subjected to an extended pre-exposure training period. One possible explanation for the weight loss of the animals exposed to 0.4 LD_{50} GB in the present study is that older animals have been shown to be more sensitive to the acute effects of CWNA [28,58,77]. In those studies, the LD_{50} values for 120 day old rats and mice were approximately 0.6 of the LD_{50} values for 30 day old animals. Others [74] have observed a decrease in the LD_{50} values of soman in water-deprived animals as compared to non-deprived animals. The guinea pigs in the present study were approximately 130 days old when exposures began and the reference LD_{50} values [38] were based on animals approximately 4–6 weeks old (based on standard growth data). The influence of age on the acute toxicity of CWNA in guinea pigs is (to the authors' knowledge) unknown, however, studies are currently underway to examine the influence of age and dietary restriction on the acute lethality of CWNA in this species.

In contrast to the present data, there was no reported weight loss for rats maintained under restricted fluid intake when exposed to approximately 0.5 LD_{50} soman, another OP nerve agent [74]. Others, however, have reported the development of tolerance to decreases in both food and water consumption of deprived animals following chronic administration of the OP diisopropyl fluorophosphate (DFP) [15,71]. It has also been reported that tolerance develops to the suppression of feeding following chronic administration of the OP paraoxon when animals were given restricted access to a liquid diet [3]. Additionally, challenge by acute administration of chlorpyrifos following chronic DFP resulted in transient weight loss of food restricted animals [14]. Furthermore, repeated exposure to 0.5 LD_{50} but not 0.4 LD_{50} GB resulted in decreased weight gain in ad lib fed guinea pigs [38] and there was no evidence for the development of tolerance to this effect. The reasons for these discrepancies in effects of subacute administration of GB on weight gain from this laboratory are, at present, unclear; however, previous reports suggest that under restricted feeding conditions weight loss typically occurs following repeated exposure to OPs.

The reduction in break points in the present study is consonant with the effects of acute administration of both centrally and peripherally acting carbamates on PR respond-

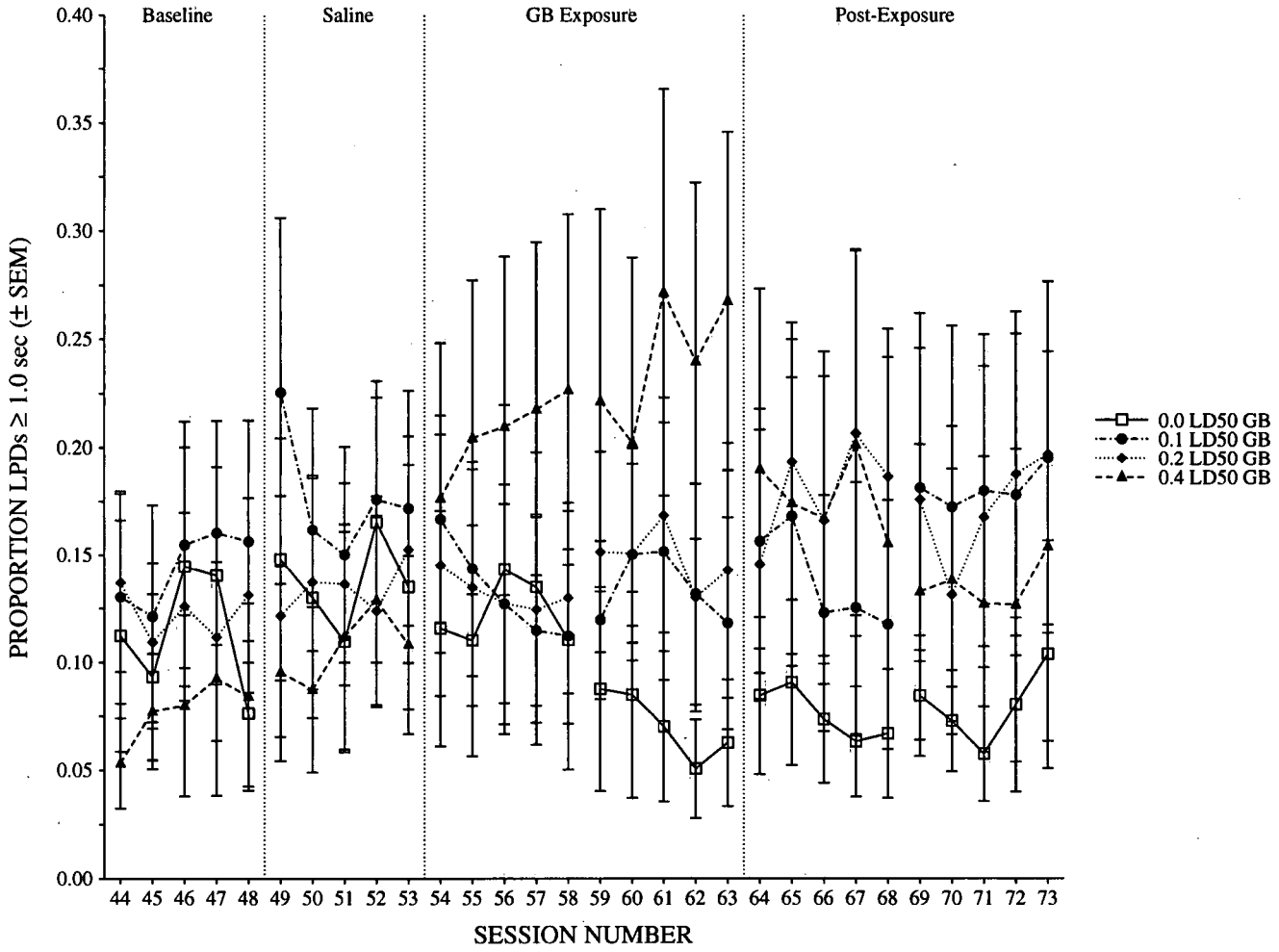

Fig. 6. Hold duration (proportion of total response time occupied by lever presses ≥ 1.0 s) as a function of session number for each GB exposure group. Hold durations were increased during the 2 weeks of GB injections for guinea pigs exposed to 0.4 LD_{50} as compared with their own baseline performance.

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ing [26,84]. Similarly, the transient decrease in responding following GB exposure in the present investigation is consistent with the acute effects of soman on behavior maintained under FR schedules of reinforcement [9,10,32]. In those studies, acute exposure to soman reduced responding during the session immediately following exposure and there was no evidence of response suppression thereafter. Furthermore, repeated soman administration has been reported to initially suppress schedule controlled behavior [39,40,74]; however, with repeated administration behavior recovers to baseline levels indicating the possible development of behavioral tolerance. The evidence of behavioral tolerance from the present data is scant; however, the effects of 0.4 LD_{50} GB seen in Figs. 3 and 5 indicate a reduction in the maximal effect during the second week of exposures as compared with the first week of exposures and this is consistent with the weight gain data presented in Fig. 2. This moderate increase in responding during the second week of exposures may be due to behavioral tolerance, since this effect has been reported previously for rats responding under simple [55,74,73,72] or multiple [39,40,53] schedules of reinforcement following exposure to soman and other OP compounds. However, the animals were not exposed during the weekends and there was opportunity for a 2-day recovery between testing weeks and this may have led to the diminished maximal effect observed during the second week of exposures. The importance of both the dosing and testing intervals in the development of behavioral tolerance to organophosphates are well known [3,70,87] and it has been shown that as there is a positive relationship between the interval between dosing and testing and the rate of development of behavioral tolerance [87]. Weekend recovery effects on behavior are most prominently seen in Figs. 3 and 5 between sessions 58 and 59. Hulet et al. [38] reported a 17% increase in RBC AChE levels in animals exposed to 0.4 LD_{50} GB following weekend recovery using an identical dosing regimen and species.

The increased pausing in the 0.4 LD_{50} GB animals of the present study is consistent with the effects of acute [29] and repeated [27] administration of soman on trials worked in a match to sample procedure. Similarly, acute administration of physostigmine, a carbamate cholinesterase inhibitor, resulted in increased session completion times of monkeys working under repeated acquisition [59] and match to sample procedures [60] when session length was controlled by number of trials. However, when session length was determined by elapsed time, acute administration of physostigmine produced a decrease in percent task completed [26].

The present data indicate that behavioral performances were not disrupted by repeated exposure to 0.2 LD_{50} GB despite WB AChE activity being inhibited by approximately 80% following the first week of exposure (Figs. 1 and 3). In guinea pigs, using an identical dosing regimen, red-blood cell (RBC) AChE activity was inhibited by 20% following a single sc dose of 0.2 LD_{50} GB, whereas RBC AChE activity was inhibited by 43% following a single sc dose of 0.4 LD_{50} GB (M.R. Roberson, personal communication). Hulet et al. [38] reported a 65% inhibition of RBC AChE activity following the administration of the second daily dose of 0.4 LD_{50} GB to guinea pigs. It has been suggested that the rate of inhibition of AChE activity is an important determinant for the appearance of behavioral effects following OP exposure [8,15,33,69]. In humans, the onset of symptoms following acute exposure to OP compounds is correlated with inhibition of RBC ChE activity by approximately 50–80% [31,69]; however, following repeated administration of lower doses over several days there was no correlation between the onset of symptoms and RBC ChE activity [31]. In rhesus monkeys, the acute administration of soman, in doses sufficient to inhibit serum AChE by 70%, produced performance deficits [6,7,33]; however, repeated administration of lower doses of soman did not result in performance deficits until serum AChE activity was inhibited by 80–90% [4,5,8,33]. In marmosets, the acute administration of GB at doses ranging 0.13–0.55 LD_{50} resulted in RBC AChE or WB ChE inhibitions ranging from 45% to >99%; behavioral effects appeared only at doses producing $\geq$75% WB ChE [88] or $\geq$88% RBC AChE inhibition [21]. In rats exposed daily to soman at 0.5 LD_{50}, behavioral effects (increased flinch threshold, decreased locomotor activity, decreased reinforcement rate FI 30s schedule) and physiological signs (hypothermia) were present following the third consecutive day of exposure when RBC AChE activity was 22% of control [74]. Additionally, in rats exposed repeatedly to chlorpyrifos, behavioral effects (increased choice latency and nosepoke IRT) were evident at WB ChE activities less than 25% of control [13].

The failure to detect behavioral effects following daily exposure to 0.2 LD_{50} GB despite significant reductions in WB AChE activity combined with the appearance of behavioral effects in animals exposed to 0.4 LD_{50} GB concurrently with overt signs of toxicity (weight loss) raises questions concerning the experimental model chosen. The guinea pig is the rodent species of choice for investigations interested in the acute lethality of CWNA as well as those investigating prophylactic and/or medical countermeasures for CWNA exposure; this is due partly to lower levels of CaE [25,45–48,58] and to the greater similarity of the guinea pig toxicokinetic profile for OP compounds to primate species [2]. The guinea pig has been used successfully as subjects in behavioral toxicology [1,22,52, 64,65,78] and behavioral pharmacology [37,63,62,81] experiments. Furthermore, the available evidence suggests that guinea pigs are able to perform behavioral tasks if appropriate consideration is given to species differences [42,61].

The schedule of reinforcement that behavior is maintained under has been shown to be a critical determinant of the effects of drugs and toxicants [17,19,51]. Behavior maintained under fixed-ratio schedules of reinforcement is typically less sensitive to the effects of drugs and toxicants

ing [26,84]. Similarly, the transient decrease in responding following GB exposure in the present investigation is consistent with the acute effects of soman on behavior maintained under FR schedules of reinforcement [9,10,32]. In those studies, acute exposure to soman reduced responding during the session immediately following exposure and there was no evidence of response suppression thereafter. Furthermore, repeated soman administration has been reported to initially suppress schedule controlled behavior [39,40,74]; however, with repeated administration behavior recovers to baseline levels indicating the possible development of behavioral tolerance. The evidence of behavioral tolerance from the present data is scant; however, the effects of 0.4 LD_{50} GB seen in Figs. 3 and 5 indicate a reduction in the maximal effect during the second week of exposures as compared with the first week of exposures and this is consistent with the weight gain data presented in Fig. 2. This moderate increase in responding during the second week of exposures may be due to behavioral tolerance, since this effect has been reported previously for rats responding under simple [55,74,73,72] or multiple [39,40,53] schedules of reinforcement following exposure to soman and other OP compounds. However, the animals were not exposed during the weekends and there was opportunity for a 2-day recovery between testing weeks and this may have led to the diminished maximal effect observed during the second week of exposures. The importance of both the dosing and testing intervals in the development of behavioral tolerance to organophosphates are well known [3,70,87] and it has been shown that as there is a positive relationship between the interval between dosing and testing and the rate of development of behavioral tolerance [87]. Weekend recovery effects on behavior are most prominently seen in Figs. 3 and 5 between sessions 58 and 59. Hulet et al. [38] reported a 17% increase in RBC AChE levels in animals exposed to 0.4 LD_{50} GB following weekend recovery using an identical dosing regimen and species.

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when compared to behavior maintained under fixed-interval schedules [17–19,51]. Progressive ratio schedules have been used relatively infrequently to investigate drug and toxicant effects on behavior, when compared to fixed-ratio schedules. However, in evaluating the effects of pyridostigmine bromide, a peripherally acting carbamate ChE inhibitor, on schedule-controlled behavior response rates were suppressed to a greater extent at equivalent doses when behavior was maintained by a FR 50 schedule [83] than when maintained under a PR 5 schedule [84] suggesting that behavior maintained under PR schedules may be less sensitive to drug effects than FR schedules.

The coincident appearance of response suppression and weight loss in animals exposed to 0.4 LD_{50} GB in the present study leads to problems in interpreting the behavioral effects [43]. Clearly, it could be argued that the animals were administered doses that induced overt toxicity and hence render the behavioral data superfluous. Indeed, given the data from other rodent species [58,77], it is highly probable that the age of the animals in the present study determined, in part, the appearance of signs of overt toxicity not seen in other studies with guinea pigs using an identical dose and dosing regimen [1,38]. However, laboratory records did not indicate a reduction in food consumption during the exposure period of the present study. Another contributing factor to the weight loss may have been water intake. In the guinea pig, food consumption is highly dependent upon water availability [34] and food intake is reduced when water deprivation is imposed [16,34]. Although not measured in the present study, the available literature suggests that fluid intake may be suppressed following exposure to OPs [15,71]. Therefore, it is extremely probable that the behavioral effects observed were a result of suppressed consumption of food and/or water due to ChE inhibition.

In summary, repeated exposure to 0.4 LD_{50} GB for 2 weeks (M-F) resulted in reduced AChE activity, weight loss, and decreased schedule controlled behavior of guinea pigs. Doses below 0.4 LD_{50} were without substantial behavioral effects despite maximal reductions in WB AChE activity to 20% of control. The effects on operant performance and body weight were rapidly reversed upon the termination of exposures despite prolonged depression of AChE activity. The behavioral effects of 0.4 LD_{50} GB noted in the present study are consistent with those reported previously [38] for guinea pigs. Clearly, the blood ChE levels observed in the present study would, in an occupational setting, prompt observation of the individual and removal from the workplace. However, the present data do not elucidate the issue of whether doses that produced no change in ongoing performance or weight gain have behavioral effects that would only be revealed by environmental [44] and/or pharmacological [89] challenges or whether exposures at these levels for an extended period would result in the manifestation of behavioral and/or systemic toxicity.

Acknowledgements

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