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Diazepam and Discriminative Motor Control: Acute, Chronic and Withdrawal Effects

JOHN W. CULBERSON, MAISY TANG,[1] CHYAN E. LAU AND JOHN L. FALK[2]

Department of Psychology—Busch, Rutgers University, New Brunswick, NJ 08903

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CULBERSON, J. W., M. TANG, C. E. LAU AND J. L. FALK. *Diazepam and discriminative motor control: Acute, chronic and withdrawal effects*. PHARMACOL BIOCHEM BEHAV **35**(2) 419–427, 1990.—Rats were trained to hold a force transducer operated with a paw so that it remained between upper and lower limits of a force band for a continuous 1.5-sec period to deliver each food pellet. Acute doses of diazepam impaired measures of this performance. Separate groups received chronic diazepam injections (6 mg/kg, IP) either presession (Before Group) or postsession (After Group), or presession vehicle (Vehicle Group). The After and Vehicle Groups demonstrated that neither chronic postsession diazepam, nor time alone, altered motor performance. The performance of the Before Group was affected by the daily diazepam, and although tolerance to the impairment developed, it was incomplete. Late in the chronic-administration phase (after 75 days) a toxic effect of the suspending agent became evident in all groups as a decrease in work rate, although the other performance indices were not affected. The withdrawal of diazepam from the Before Group led to improved performance which returned to the original baseline level.

Motor performance Diazepam Chronic benzodiazepine Diazepam withdrawal

THE investigation of drug effects on motor behavior has emphasized the analysis of unlearned, relatively global behavior (e.g., general activity, rotorod) with little attention paid to quantifiable measures on specifically trained discriminative motor topographies. Early studies of drug effects on continuous lever (8) and head (5) positioning steadiness tasks were promising, but little pursued. We have used a similar task with rats, a discriminative motor control technique, in which holding a force transducer for a short, continuous period within a force band is reinforced by the delivery of a food pellet. The acute, chronic and withdrawal effects of various drugs on this fine motor control performance have been studied in rats (11, 22, 23, 37, 40, 41), monkeys (1, 10, 20, 35) and humans (3,46).

Although the benzodiazepines have various behavioral effects, the muscle-relaxant and sedative-hypnotic components raise the question of the extent to which this class of agents might affect motor behavior. A previous study explored the acute, chronic and withdrawal effects of the ultrashort-acting benzodiazepine agent midazolam on discriminative motor control (41). In the present study, the aim was to explore the generality of the effects observed with midazolam by using diazepam, another benzodiazepine which has been employed extensively as a therapeutic agent.

METHOD

Animals

Nine male, albino, adult rats of the Holtzman strain with a mean initial body weight of 382.2 g (range: 377–388 g) were used. They were housed individually in stainless-steel cages in a temperature-regulated room with a daily cycle of illumination from 700–1900 hr. Animals were naive and were reduced to 80% of their ad lib body weights by limiting daily food rations. Food necessary for maintaining these weights was made available in the living cages immediately after daily experimental sessions. Water was available continuously in the living cages.

Drug

Diazepam was suspended in a vehicle consisting of distilled water and 2 mg/ml of Agent K (BioServ, Inc., Frenchtown, NJ), ultrasonicated in an ice bath for 5 min, and injected (IP) in a volume of 1 ml/kg body weight. [Three animals, Y16, Y17 and Y22 received diazepam and vehicle injections using an inadequate concentration of Agent K (1 mg/ml) during the dose-effect determinations and for the first 2 weeks of the chronic-administration phase.]

Serum Diazepam Analysis

Blood samples (100 μl) were taken from the tail tip. Samples were centrifuged for 5 min at 13,700 × g. The clear serum layer was analyzed for diazepam and its metabolites using a single extraction procedure (21). For calibration standards, 25 μl of the internal standard (1 μg/ml demoxepam) and 50 μl of the drug standard were pipetted into a 15-ml conical centrifuge tube and

[1]Present address: Department of Neuropathology (Neuroscience), Harvard Medical School, Boston, MA 02115.
[2]Requests for reprints should be addressed to John L. Falk.

evaporated to dryness under a stream of nitrogen. A 50-μl blank serum sample, 100 μl of 1 M borate buffer (pH 9.0) and 2.5 ml of diethyl ether were then added to the sample, vortex-mixed for 30 sec, followed by centrifuging at room temperature for 5 min at 1100 × g. The ether layer was carefully transferred to a 5-ml conical centrifuge tube and evaporated under nitrogen. The residue was resuspended in 50 μl of the mobile phase and then 100 μl of n-hexane was added in order to remove the colloidal lipids. The mixture was vortex-mixed for 2 sec and the hexane layer was removed immediately with a disposable pipette. Samples for drug serum analysis were prepared identically except drug standards were not added. Instead, serum samples (50 μl) were added after the internal standard was initially evaporated to dryness. Separation was performed on an Ultrasphere C18 column (5 μm particle size, 150 × 2.0 mm i.d., Altex, San Ramon, CA), and the mobile phase consisted of methanol, acetonitrile and 0.056 M sodium acetate buffer which had been adjusted to pH 4.0 (47.5:9:43.5). The flow rate was set at 0.3 ml/min and normally operated at a pressure of 2000 p.s.i.

Apparatus

The experimental space for evaluating discriminative motor control performance was a Plexiglas chamber (25 × 30 × 30 cm) with stainless-steel front and rear panels and a floor consisting of parallel-mounted, spaced, stainless-steel rods. The operandum was a stainless-steel lever mounted 2.5 cm from the floor. It was surrounded by a Plexiglas shield with a 1 cm wide × 4 cm high slot so that access to it was limited to a single paw. The front edge of the operandum was recessed 1.2 cm from the front surface of the shield to prevent nose-poking or responses other than paw actuation from operating the lever. The operandum was suspended by a phosphor-bronze leaf spring (0.20 mm thick), and its shaft rested on a drive rod connected to a force transducer (model UC3 strain gauge, Statham Instruments, Oxnard, CA) through a load cell (Statham model UL4). The voltage output from the force transducer was conveyed to a customized signal control box (Tri-Tech Services, Hamilton Square, NJ) and sorted into one of three signal regions: above, below or within a window defined by preset lower and upper voltage limits. These limits corresponded to applied forces of 0.147 N (15 g force) and 0.265 N (27 g force), respectively, incident at the paw-placement region of the operandum. A buffer was set so that a minimum force of 0.015 N (1.5 g force) was required for signal recognition. A Commodore Pet 4016 microcomputer was programmed in assembly language to sample signal input once every 10 msec. When the force applied by the animal was within the 0.147 to 0.265 N band, an audio feedback signal (Sonalert SC648H, P. R. Mallory, Indianapolis, IN) was turned on.

Discriminative Motor Control Measures

A continuously applied in-band force (within the 0.147–0.265 N window) lasting 1.5 sec was required for the delivery of a 45-mg food pellet (BioServ, Inc.). If the applied force left the band before 1.5 sec had elapsed, then this timer was reset. Thus, the behavior reinforced by food pellet delivery was holding the force transducer steadily operated within the force band for a continuous, set period of time. Ordinarily, a session was terminated when the 50th pellet had been delivered, but a session was also terminated if 30 min had elapsed without operation of the transducer. This happened rarely and was drug-dose related. These occurrences are noted in the relevant figure legends.

In each session, the raw measures of motor behavior were: Session Time (the time taken to earn 50 food pellets), Total Response Time (the amount of the session time that the transducer was held operated above the minimum recognition threshold of 0.015 N), In-Band Time (the amount of the session time that the transducer was held operated within the force-band window) and Entrances (the number of times during a session that applied force entered the band from either the lower or upper set limits). Except in the case of Entrances, these raw measures are not useful characterizations of motor performance. For example, the In-Band Time measure is more informative when compared with the minimum total In-Band Time that would produce the delivery of 50 food pellets (1.5 sec/pellet × 50 pellets = a Minimum Possible In-Band Time of 75 sec). Therefore, a measure of In-Band Efficiency is calculated by taking the ratio of these two values:

$$\text{In-Band Efficiency} = \frac{\text{Minimum Possible In-Band Time}}{\text{In-Band Time}}$$

Also, raw In-Band Time can be viewed in relation to the Total Response Time in a session. Thus, Tonic Accuracy measures the proportion of the total response time of a session that is spent in band:

$$\text{Tonic Accuracy} = \frac{\text{In-Band Time}}{\text{Total Response Time}}$$

Work Rate is simply the proportion of the Session Time that the animal spent operating the transducer:

$$\text{Work Rate} = \frac{\text{Total Response Time}}{\text{Session Time}}$$

As indicated above, the Entrances measure is simply a count of the number of times during a session that the applied force enters the band from either its upper or lower limit.

$$\text{Entrances} = \text{total number of entrances into the force band}$$

A perfectly efficient performance would yield an In-Band Efficiency of 1.00. The Tonic Accuracy approaches 1.00 as the total time spent responding approaches the time spent in band. It measures an aspect of discriminative motor control that is somewhat different than that measured by In-Band Efficiency: Although a high proportion of session operandum holding might be within the appropriate force band, if the holding times are frequently of too short a duration to produce pellet delivery, then Tonic Accuracy could be high although In-Band Efficiency is low. Because Work Rate can approach a value of 1.00 or zero, the previous measures can approximate 1.00 or zero in complete independence of Work Rate. Although they often covary, Entrances and In-Band Efficiency are independent measures. For example, relative inefficiency could indicate that the in-band holding times often fall just short of the appropriate hold time; such a performance would not yield a high Entrances measure.

Procedure

Animals were trained to hold the force transducer operated within the force band by the method of successive approximation to the required response topography. During the initial training period, animals were permitted to earn up to 100 food pellets per session. As session-to-session performance stabilized, the number of pellets was reduced to 50 per session. Daily sessions were given for the duration of the experiment. After approximately 5 months, session performance reached a stable baseline with respect to the measures calculated. Then, acute dose-effect functions for diazepam (1.5–6.0 mg/kg) were determined. Animals were injected IP 30 min before a session. Doses were administered in the following sequence: 0, 1.5, 3.0, 6.0, 0 mg/kg. Doses were separated by 7–10 days.

After the determination of the acute dose-effect function, animals were assigned to one of 3 groups (N = 3 each) for the duration of the experiment. The Before Group received a daily injection of 6.0 mg/kg diazepam 30 min before each session and an injection of Agent K vehicle immediately after each session. The After Group received a daily injection of Agent K vehicle 30 min before each session and an injection of 6.0 mg/kg diazepam immediately after each session. The Vehicle Group received a daily injection of Agent K vehicle before and immediately after each session. As animals completed the initial acute dose-effect determination, they were assigned sequentially to treatment groups in order to insure that each group received an equal number of animals. Immediately following assignment to a treatment group, each animal began the chronic-injection phase of the experiment.

A blood sample was taken from each animal 1 hr after the 6.0 mg/kg diazepam injection for the determination of serum diazepam and its metabolites. This was the last drug dose given in the determination of the acute dose-effect relation. A second blood sample was taken from each animal 1 hr after receiving an injection of 6.0 mg/kg diazepam after they had been on the chronic-injection regimen for about 70 days. Animals in the Before and After Groups also had occasional blood samples taken just before a session to determine whether there was any residual diazepam or metabolite remaining from the previous day's injection.

After about 4 months of chronic diazepam injection, animals in the Before and After Groups had injections of Agent K substituted for the drug to assess the effects of withdrawal from diazepam.

RESULTS

Acute Effects of Diazepam

Figure 1 shows the diazepam dose-effect relations for 6 animals on the two measures of motor behavior most sensitive to drug-produced performance decrements. With the exception of rats D18 and D4, In-Band Efficiency showed clear decrements at either 3 or 6 mg/kg, or both doses of diazepam. Except for D18, Entrances were increased by diazepam at the 3 and 6 mg/kg doses, usually in a dose-related manner. Data from only 6 animals are shown since analysis of serum diazepam levels indicated that the initial drug solution suspensions for Y16, Y17 and Y22 were inadequate. As noted in the above section (cf., see the Drug section), 1 mg/ml of Agent K provided insufficient suspension of the drug. This concentration was used for these animals during the initial dose-effect determination phase, as well as during the first 2 weeks of the chronic-administration phase. Consequently, these data are not presented. The inadequacy of the suspension was determined by analyzing the serum samples taken 1 hour after the 6 mg/kg dose given in the initial phase. These 3 animals showed diazepam levels of 34 ng/ml or less. As other animals had not yet been phased into the experiment, we were able to correct the suspension. The corresponding serum samples for the subsequent 6 animals, for which the corrected suspension concentration was used, revealed a much greater diazepam level (94–255 ng/ml; mean = 190 ng/ml) as shown in the left side of Table 1. These latter values agree with the levels reported for IP injection of 5 mg/kg diazepam in the rat (13).

One animal (D18) failed to complete the session following the 6 mg/kg dose (Fig. 1, point a). This dose was selected as the maximum to be administered during the acute phase of the experiment, and the daily dose to be used in the chronic-administration phase.

Effects of Chronic Administration and Withdrawal

Figures 2 and 3 show the values for individual animals of the four performance measures. At points A, the means of the last 10

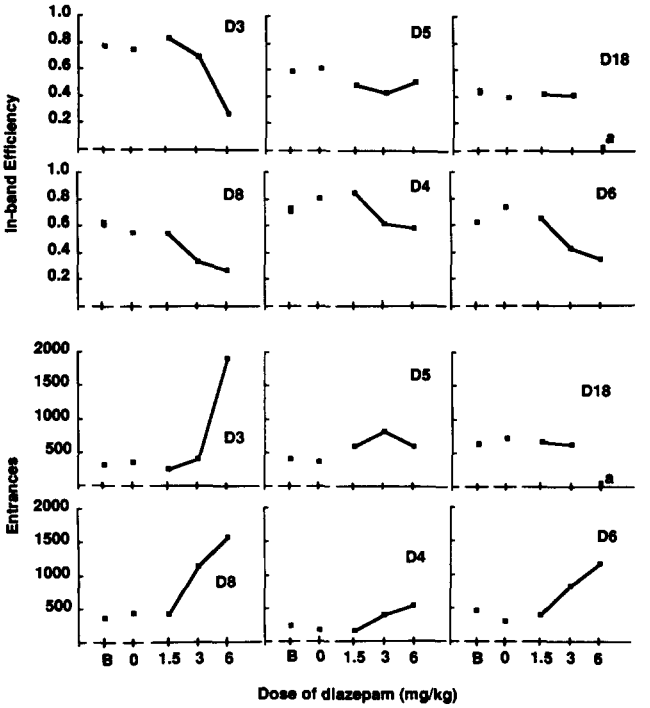

FIG. 1. Dose-effect relation for diazepam (IP, 30 min postinjection) for two measures of motor performance. Each drug point based on data from single determinations, except 0 mg/kg (vehicle), which is the mean of 2 injections given at the beginning and end of the dose-effect determination. B = mean (SEs are small and lie within the plotted point dimensions) of 10 baseline sessions, 2 preceding each dose. a = animal did not finish session at this dose.

baseline sessions before the start of the chronic phase are shown. During the chronic phase, animals received two injections daily, one 30 min before the session and the other immediately after: Before Group (Diazepam-Vehicle), After Group (Vehicle-Diazepam) and Vehicle Group (Vehicle-Vehicle). For the chronic-administration phase, session values are plotted for the first 14 days; mean values for each animal for the last 10 days of this chronic phase are plotted at points B. During the withdrawal phase, all animals received the Vehicle-Vehicle injection regimen. The drug was withdrawn from the Before and After Groups after 100–136 days of chronic administration (mean = 120 ± 6 days). Individual session values are plotted for 10–14 days after withdrawal of the drug. Animals in the Vehicle Group received injections for the number of days indicated on each axis. The length of the chronic regimen for this group was limited by the toxicity of the vehicle as explained below.

Vehicle Group. The Vehicle Group was stable on all measures (D6 revealed a few deviant sessions) with the exception of Work Rate over the course of the experiment. Although Work Rate remained unaffected during the initial 75 days of the chronic phase, at point B Y17 had decreased to 77% of baseline point A; D4 and D6 were affected at about chronic days 77 and 79, respectively.

Before Group. The Before Group animal D5 decreased to 76% of its baseline on In-Band Efficiency with the first chronic injection and remained at that level throughout most of the chronic phase (cf., point B). D3 was similar, although values remained near baseline for the first 3 days of chronic drug administration and were at 88% of baseline at point B. Following correction of the inadequate suspending agent on chronic day 17, serum drug values

TABLE 1

SERUM LEVELS (ng/ml) OF DIAZEPAM (DZ) AND N-DESMETHYLDIAZEPAM (DesDZ) 1 HR AFTER IP INJECTION OF 6.0 mg/kg DIAZEPAM IN THE ACUTE AND CHRONIC (AFTER ABOUT 70 DAILY IP INJECTIONS OF EITHER 6.0 mg/kg DIAZEPAM OR VEHICLE) PHASES

		Acute Phase Determ.		Chronic Phase Determ.	
Rat	Group	DZ	DesDZ	DZ	DesDZ
Y17	Vehicle	i	i	146	43
D4	Vehicle	248	156	180	58
D6	Vehicle	170	71	159	—
Y22	Before	i	i	274	52
D5	Before	128	60	151	37
D3	Before	94	61	104	42
Y16	After	i	i	210	80
D18	After	255	120	267	51
D8	After	244	143	262	24

i = inadequate vehicle (sample not counted); — = value undetectable.

for Y22 were within the expected range. Subsequently, chronic diazepam administration was continued for 120 days. A decrement in In-Band Efficiency persisted during the 4 months of chronic administration at 76% of baseline (cf., point B). Upon withdrawal, In-Band Efficiency returned to the original baseline level of point A either immediately (Y22, D3) or slowly (D5).

The Entrances measure presented a similar picture. There was an increase in session Entrances during the initial chronic days which correlated with the decreased In-Band Efficiency (D3, D5). This increase persisted throughout the chronic administration period, so that at points B the values were 188% and 118% of baseline points A for D5 and D3, respectively. For Y22, the corresponding value was 179% of baseline. All 3 animals in the Before Group returned to the baseline level of Entrances immediately after withdrawal of diazepam. No signs of an abstinence effect from withdrawal were evident in motor performance.

The Tonic Accuracy measure differed somewhat from the above measures in both the time course and extent of the drug effects on motor performance. With the first chronic-phase injection, D5 decreased in Tonic Accuracy to 84% of baseline; subsequent session values were variable, but stabilized to 86% of baseline at point B. D3 exhibited decrements in Tonic Accuracy for sessions 4–6 of the chronic phase and then returned to baseline performance. Y22 stabilized at 80% of baseline (point B). Upon drug withdrawal, D5 and Y22 returned to baseline. D3 had recovered baseline performance prior to withdrawal and remained unaffected by withdrawal.

Work Rate remained approximately at baseline levels during the initial 2 weeks of the chronic phase for the 2 animals (D3, D5) for which these data were available. However, Work Rate for Y22 and D3 during the 4 months of chronic administration displayed a decrement similar to that seen in the Vehicle Group. All 3 animals showed an additional decrease in Work Rate during the withdrawal phase.

After Group. Occasional serum samples taken approximately 24 hr after diazepam injection from these animals and those in the Before Group revealed no detectable levels of diazepam or metabolite. Hence, any chronic performance decrements cannot be explained by an accumulation of drug or active metabolite in the serum as a result of daily dosing.

The In-Band Efficiency measure revealed some decrements in the performance of D8 which persisted throughout the chronic phase (89% of baseline at point B). Y16 and D18 showed no decrease in this measure as a result of chronic dosing. Upon withdrawal of the drug, In-Band Efficiency for D8 returned to the baseline level for about a week and then declined. By the 11th day of withdrawal, the animal failed to complete the session. Withdrawal produced no notable change in this measure for Y16 or D18.

The Entrances measure displayed a similar picture. At point B, D8 was at 130% of the baseline level, while Y16 and D18 showed no decrement in performance. Withdrawal produced effects similar to those observed for In-Band Efficiency: a temporary improvement for D8 and no notable change for Y16 or D18.

Tonic Accuracy remained quite stable for this group, showing little or no decrement at point B. Likewise, the values after withdrawal remained relatively stable.

For the 2 animals measured during the initial 2 weeks of the chronic phase (D18, D8), Work Rate remained stable. However, as was the case for some animals in the other groups, Work Rate began to decrease after about 85 days of chronic administration, dropping to 75% and 67% of baseline, respectively (point B). Y16 showed a 51% decrement. Upon withdrawal, Work Rate either remained stable, but low (Y16), or remained low and unstable (D18, D8).

Vehicle Toxicity

As noted in the above results, most animals late in the chronic-administration phase, or in the withdrawal phase, showed a marked decrease in Work Rate. Inasmuch as these late decreases occurred in all groups it suggested the possible toxicity of the suspending agent. Weight gains at the same time indicated an edematous condition since food rations were controlled within narrow limits. The reductions in Work Rate did not appear in any animal earlier than the 75th day of the chronic phase. Other measures of performance were not adversely affected. One animal died and 6 others were sacrificed in a moribund condition at the end of the experiment. Discounting the 20–30 days for the 3 animals that received the lower concentration of Agent K (1 mg/ml) initially, the health of all but 2 animals was seriously compromised after 87–137 days of chronic Agent K (2 mg/ml) administration. Postmortem examination showed excessive fluid in the peritoneal cavities. Elevated blood urea nitrogen values indicated renal failure. Histological examination of liver, spleen and kidney sections revealed an occlusion of glomerular capillaries and an accumulation of foreign material within the spleen. Liver damage was minimal. In order to confirm that Agent K by the IP route of administration was the toxic factor, two additional animals were reduced to 80% body weight and given 2 injections daily (separated by 0.5 hr) of Agent K (2 mg/ml). One died after 101 days and the other after 115 days; similar pathological changes were noted.

Serum Levels of Diazepam and Desmethyldiazepam

Table 1 shows the serum drug levels in the acute and chronic phases of the experiment. Diazepam levels were comparable in the two phases. Although metabolite levels varied greatly, these data suggest that the diazepam/desmethyldiazepam ratio may increase after chronic treatment.

DISCUSSION

Acute Effects of Diazepam

In the present discriminative motor control procedure, performance was stable and highly overtrained before the acute dose-effect relation was determined. Most animals showed impaired performance in the 3–6 mg/kg diazepam dose range. Acute

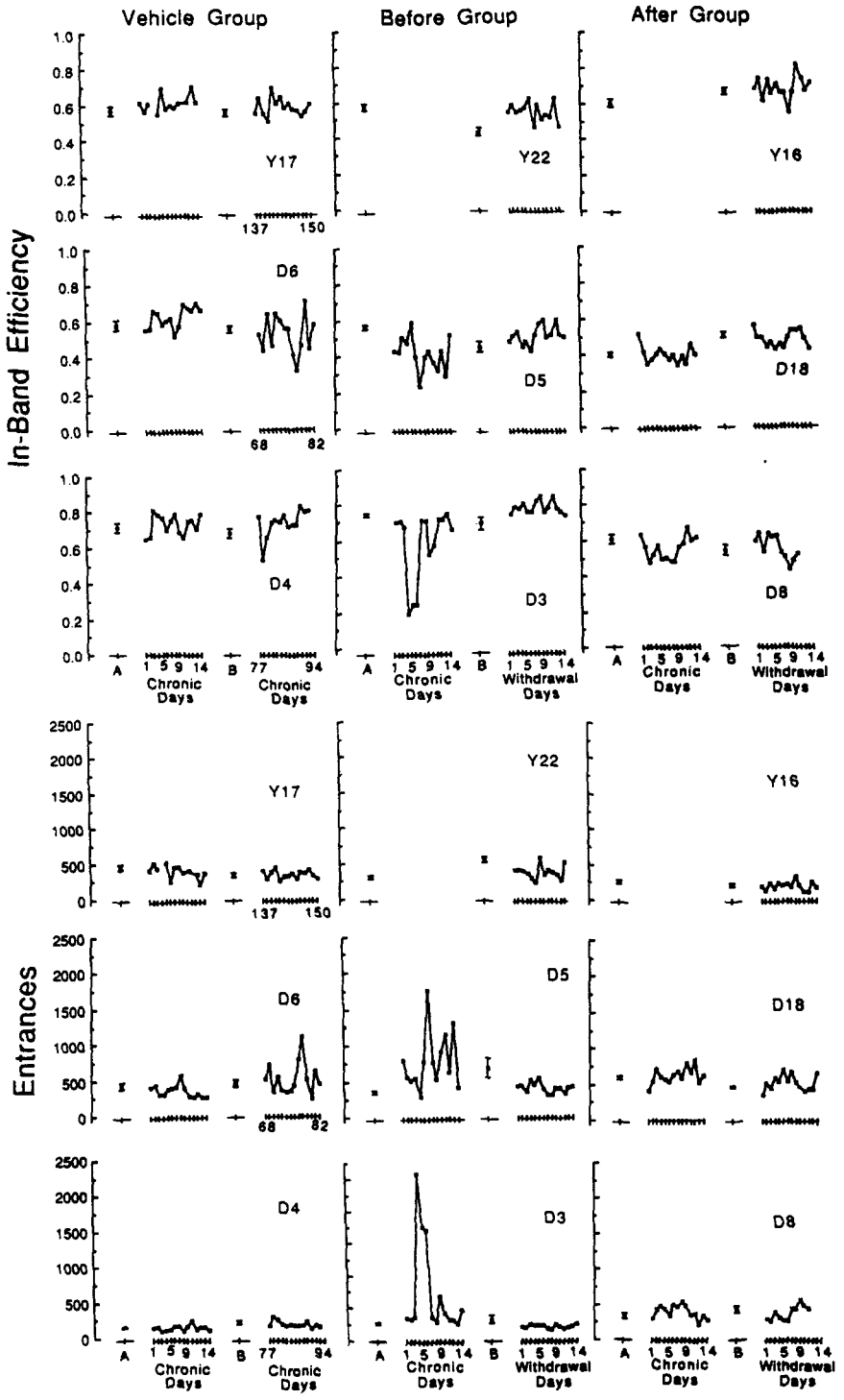

FIG. 2. Mean (±SE) and successive-session values for motor performance measures (In-Band Efficiency, Entrances) of individual rats in 3 groups. BEFORE GROUP: Diazepam (6 mg/kg, IP 30 min presession)—Vehicle IP postsession. AFTER GROUP: Vehicle IP 30 min presession—Diazepam (6 mg/kg, IP postsession). VEHICLE GROUP: Vehicle IP 30 min presession—Vehicle IP postsession. A = mean (±SE) of 10 sessions before start of chronic, daily injections. B = Mean (±SE) of last 10 sessions of chronic phase. (SEs not visible lie within plotted point borders.)

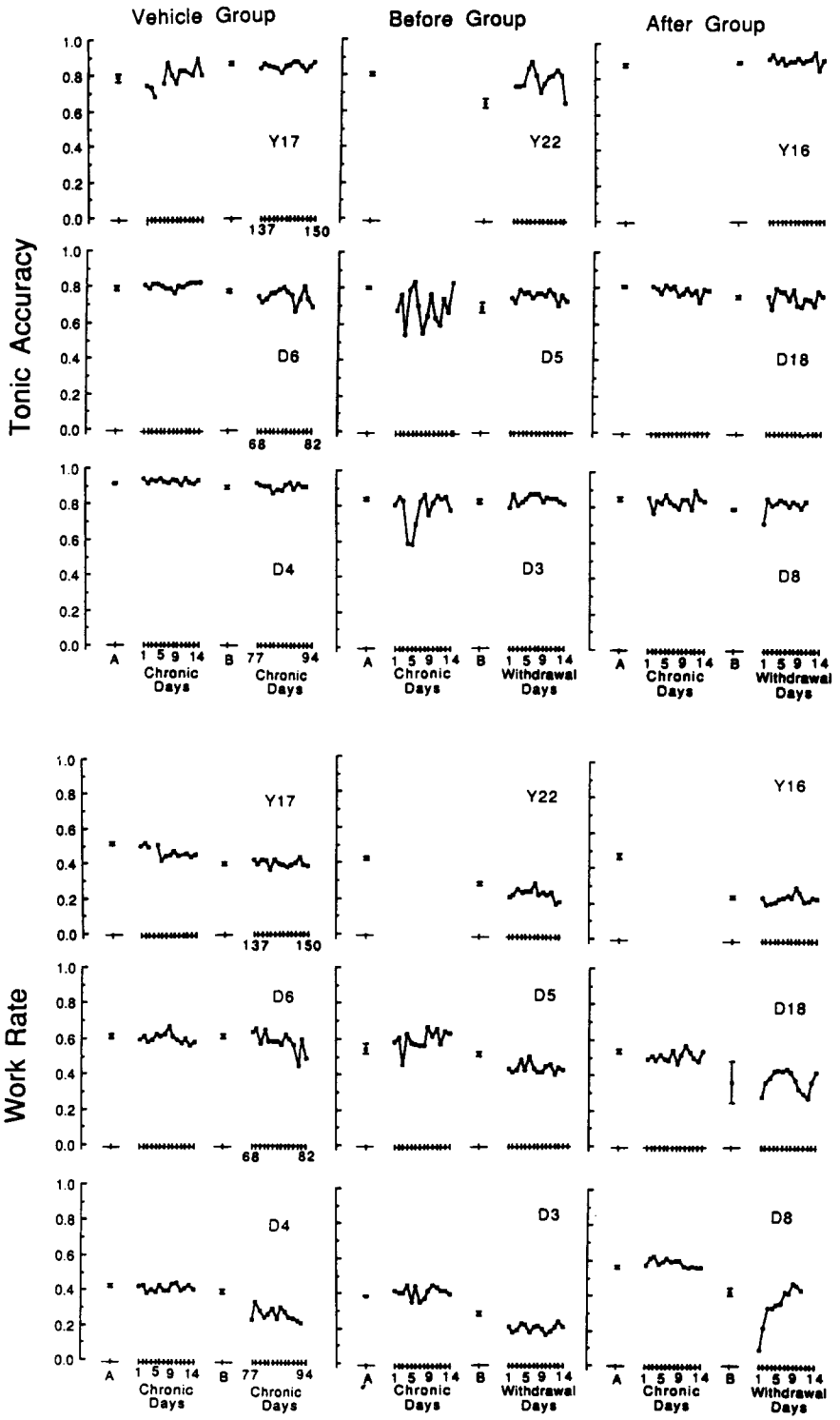

FIG. 3. Mean (± SE) and successive-session values for motor performance measures (Tonic Accuracy, Work Rate) of individual rats in 3 groups. BEFORE GROUP: Diazepam (6 mg/kg, IP 30 min presession)—Vehicle IP postsession. AFTER GROUP: Vehicle IP 30 min presession—Diazepam (6 mg/kg, IP postsession). VEHICLE GROUP: Vehicle IP 30 min presession—Vehicle IP postsession. A = Mean (± SE) of 10 sessions before start of chronic, daily injections. B = Mean (± SE) of last 10 sessions of chronic phase. (SEs not visible lie within plotted point borders.)

administration of diazepam to rats tested on the rotorod (39,44) or elevated runway (4) tasks produced motor performance impairments within the same dose range. Compared to the effects of midazolam on the same discriminative motor control procedure (41), diazepam yielded qualitatively similar results, with most animals showing dose-related impairments more clearly than was the case with midazolam.

In humans, psychomotor performances such as reaction time, tracking, digit-symbol substitution and cancellation were affected by diazepam, as well as other benzodiazepines (47). In general, performance on tasks requiring repetitive-act speed and rapid reaction times were more readily compromised by acute administration of benzodiazepines than were those demanding accuracy (18, 36, 43, 45). The acquisition of new behavior is also impaired by acute diazepam administration [e.g., (14)]. The greater sensitivity of acquisition, as opposed to steady-state performance, to disruption by diazepam in humans was shown by requiring a subject to either perform a well-practiced response sequence part of the time or acquire a new sequence (42). On the acquisition component, diazepam (IV in a 70 kg subject) increased the error rate slightly with a 5 mg dose, more with 10 mg, and markedly with 15 mg, but only a slight effect on the performance component finally occurred at the 15 mg dose. Further, psychomotor performances were more frequently and definitively impaired when functions were assessed soon after drug administration (24,47).

With the present technique, fine-motor control, rather than speed, was assessed, but acute-dose performance decrements were detected. Animals were tested soon after drug administration, the period during which other studies also found impaired performance. Our animals were trained to a long-term stability criterion, nevertheless, they remained sensitive to acute diazepam dosage. The acquisition of new behavior was not a necessary condition for the demonstration of impairment by diazepam. But perhaps the doses employed were greater than those common in human therapeutics.

Chronic Effects of Diazepam

Throughout the period of chronic administration (up to and including point B of Figs. 2 and 3), the Vehicle Group maintained a discriminative motor control performance close to the original baseline value (cf., points A) on all 4 measures. (Animal Y17 was an exception on one measure with a decreased Work Rate at point B.) For the After Group, behavior closely approximated the baseline measures throughout the chronic-administration phase, except for the final Work Rates (point B), which will be discussed in the following section. (Animal D8, however, displayed a small decrement in In-Band Efficiency and Entrances throughout most of the chronic-administration phase.) Summarizing the performance of these two groups: with the exception of late disruptions in Work Rate, neither time alone, chronic vehicle administration, nor the postsession administration of diazepam had appreciable effects on performance through point B.

The Before Group displayed a rather different picture. During the first 2 weeks of chronic administration, there was a disruption in In-Band Efficiency, Entrances and Tonic Accuracy, but not in Work Rate. These disruptions were maximal only after a few days of chronic dosing. Some tolerance occurred to these decrements, but it was incomplete (cf., points B). Two of the 3 animals showed decrements in Work Rate at point B.

A similarly designed study using chronic administration of midazolam, rather than diazepam, yielded similar results with respect to In-Band Efficiency, Entrances and Tonic Accuracy performances (41). Performance was disrupted initially by chronic, presession administration of 3 mg/kg SC midazolam. Tolerance to these performance decrements developed, but it was incomplete. As measured in the rat, both midazolam and diazepam are highly lipophilic and have comparable brain uptakes (2). Rat brain half-lives increased as a function of dose and were roughly comparable for midazolam and diazepam (38). Although midazolam is ultrashort-acting in both rats and humans, diazepam is long-acting in humans, with a half-life of 20–70 hr (17), but quite short-acting in the rat. At the 3 mg/kg dose, the serum half-life of midazolam was 0.92 hr for the rat (41), while at 5 mg/kg the plasma half-life of diazepam was 0.88 hr (13). The respective metabolites are also rapidly eliminated in the rat (12, 13, 31). For the rat, then, these two benzodiazepines are pharmacokinetically quite similar agents. Further, with long-term dosing, the benzodiazepines reveal no changes in their kinetics (16), nor did we find a systematic change for diazepam (cf., Table 1). In concert with the similarity of acute kinetics for midazolam and diazepam, and the lack of altered kinetics by long-term dosing, the finding of comparable effects on discriminative motor control with chronic administration of these agents might be expected.

In rats, tolerance to the disruption of rotorod performance by diazepam developed after 6 days of 3 mg/kg/day diazepam administration (39). In humans, although acute doses of the benzodiazepines can impair performances, tolerance to impairment can develop with a chronic dosing regimen (26). However, psychomotor performance deficits may still remain during long-term diazepam administration at therapeutic levels (9,25), or despite the development of tolerance to the subchronic administration of higher doses (36). For example, in testing wherein the performance level of a digit-symbol substitution task had not yet reached full efficiency, daily therapeutic-level diazepam doses for 6 weeks prevented the development of the improved performance observed in a placebo group as a function of practice (27). These reports of incomplete development of tolerance to repeated doses of diazepam agree with the present results and with the similar outcome of chronic administration of midazolam (41).

In humans, given the long plasma half-lives of diazepam and desmethyldiazepam (30), the incomplete tolerance to the performance deficits produced by daily diazepam dosing could perhaps be attributed to the accumulation of active drug and metabolites (15) during the chronic administration phase. However, for the rat, neither presession nor postsession sampling in the present experiment revealed evidence of any progressive metabolite accumulation in serum which could be appealed to in order to explain the incomplete development of tolerance.

In one respect, the diazepam results differ from those obtained with midazolam on the discriminative motor control procedure. Midazolam, in comparison to diazepam, is notable not only for its generally greater potency, but also for its more pronounced sedative effects (19). With midazolam, the first chronic-phase session in the Before Group produced a considerable decrease in Work Rate; tolerance developed with succeeding sessions, so that by the fourth chronic session Work Rate had returned to the baseline level (41). The corresponding chronic phase in the present experiment did not show this initial decrease in Work Rate with a progressive development of tolerance. As suggested previously (41), Work Rate decrement may be an index of sedation for sedative-hypnotic agents, whereas the other indices measure aspects of performance that are more specifically motoric. Work Rate in the chronic phase, then, could be interpreted as indicating the more pronounced sedative component of midazolam compared to diazepam.

Chronic Effects: Work Rate and Agent K

As described above, Agent K affected animals in all groups

adversely, but not before the 75th day of the chronic-administration phase of the experiment. The adverse effect became evident as a decrease in Work Rate at that time. Other performance measures were spared, and the effect of Agent K could be distinguished from diazepam effects since the two control groups (Vehicle and After Groups) were included in the design. Further, the specificity and latency of Agent K as the toxic component in this experiment was confirmed by the inclusion of two additional animals subjected only to Agent K treatments. Inasmuch as Work Rate was the only measure affected during the latter part of the chronic-administration phase and the withdrawal phase, the design and the results permit valid interpretations of the effects of chronic diazepam dosing and its withdrawal on the other measures of motor function.

Withdrawal of Diazepam

It is well-known from placebo-controlled, double-blind studies that withdrawal from prolonged use of benzodiazepines (including diazepam) at therapeutic dose levels can result in an abstinence syndrome, particularly upon abrupt drug discontinuance (7, 33, 34). With prolonged misuse of diazepam (60–120 mg daily) in 10 patients, withdrawal produced anorexia, insomnia and agitation, as well as a number of motor effects: palpable tremor, myoclonus and Quinquaud's sign (32). No such adverse motor effects were observed in the present experiment when diazepam was withdrawn; rather, the impaired motor performance of the Before Group animals returned to baseline levels. Similarly, in normal human subjects, McLeod and his associates (27) found that discontinuance after 6 weeks of 15 mg diazepam/day led to improved performances on a number of psychomotor tests. Patients in the clinical studies cited above who showed the abstinence syndrome generally had taken greater daily amounts of diazepam for a period of years. Although the animals in the present study were exposed to diazepam for a comparatively long period of time, perhaps the dose level, or the difference between human and rat half-lives of diazepam and its metabolites, were important factors precluding the demonstration of disturbed motor performance upon withdrawal. Considering these possible explanations, it is most likely that dose, rather than pharmacokinetics, was the critical determinant. First, we have shown a withdrawal effect on discriminative motor control upon the discontinuance of chronic dosing with midazolam (41), an agent whose pharmacokinetics (as discussed above) are quite similar to those of diazepam in the rat. Second, much greater daily diazepam doses [e.g., 133 mg/kg/day (28)] than the one used in the present experiment are apparently required for the frank demonstration of physical dependence in the rat (6,29).

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