ELSEVIER

European Journal of Pharmacology 385 (1999) 89–97

ejp

www.elsevier.nl/locate/ejphar

Specificity of chronic effects of diazepam on responding of rats under fixed-ratio schedules

James B. Smith

School of Pharmacy, Mercer University — Atlanta, 3001 Mercer University Drive, Atlanta, GA 30341, USA

Received 17 August 1999; received in revised form 11 October 1999; accepted 15 October 1999

Abstract

Behavioral effects of diazepam were studied in rats responding in different daily sessions using different operant chambers and manipulanda. In one experiment, *key* pressing was maintained in a first session under a 40-response fixed-ratio schedule; *lever* pressing was maintained in a second session under a 40-response fixed-ratio schedule; and a third session consisted of a multiple schedule comprising both *key* and *lever* pressing maintained under a 40-response fixed-ratio schedule. In a second experiment, the first session consisted of a multiple schedule with both *key* and *lever* pressing maintained under a 40-response fixed-ratio schedule and the second session consisted of *lever* pressing maintained under a 40-response fixed-ratio schedule. After studying effects of widely spaced injections of diazepam (0.3–3.0 mg/kg) on responding for each separate schedule, animals received 1.7 mg/kg/day diazepam in order to study chronic effects of the diazepam on behavior among the different schedule-conditions. In both experiments, tolerance to rate-decreasing effects of diazepam in a particular schedule component did not extend to any other schedule component when the manipulandum or chamber was different, but did extend to other schedule components when the manipulandum or chamber was the same. These results suggest that behavioral effects of chronically administered diazepam were influenced not only by pharmacologic processes, but also by learned relationships among its interoceptive effects, reinforcement contingencies, and particular behavioral environments.

Keywords: Diazepam; Tolerance; Drug discrimination; Fixed-ratio; (Rat)

1. Introduction

In many instances, the initial effects of drugs diminish during chronic administration so that a particular dose becomes more ‘‘tolerated’’, and increasingly larger doses are required for reproducing earlier effects. Additionally, physiologic activities sometimes adjust and seem to ‘‘depend’’ on continued drug presence, and these newly adjusted physiologic activities are disrupted if the drug becomes unavailable. These two processes of drug tolerance and dependence can develop for important biological effects of many drugs, and an appreciation of these processes can help an understanding of drug overuse. For example, even though there are clear instances of metabolic tolerance (e.g., for sleeping time after barbiturates), and for ‘‘tachyphylaxis’’ based on altered cellular activity (e.g., cardiovascular effects of sympathomimetic amines), there are many more instances when altered behavioral effects of drugs are not based exclusively on identified cellular mechanisms, but are influenced as well by characteristics of the behavior being affected by the drug. In particular, a number of previous experiments have demonstrated behavioral influences on tolerance for both non-operant and operant behavior by comparing effects of drugs when they are differentially associated with discriminable features of specific environmental circumstances. In the case of non-operant behavior, there are associative influences on the effects of repeatedly administered drugs for analgesia (Siegel, 1976; Epstein et al., 1989; Taiwo et al., 1989; Tiffany et al., 1992), anorexia (Hunt et al., 1990; Caggiula et al., 1991; Wolgin and Benson, 1991), anticonvulsant effects (Löscher et al., 1991; Mana et al., 1992; Tietz, 1992), neuroendocrine response (Caggiula et al., 1991), and lethality (Melchior, 1990; Tsibulsky and Amit, 1993). For operant behavior, there are numerous reports that tolerance which has developed in one set of circumstances does not typically extend- or generalize- to similar performance by the same individual in a different set of circumstances (Siegel, 1989; Smith, 1991a,b; Advokat and McInnis, 1992; Ehrman et al., 1992).

Experiments in this laboratory have studied influences on drug tolerance using a ‘‘multi-environment’’ procedure

0014-2999/99/$ - see front matter
PII: S0014-2999(99)00717-7

in which operant responding of the same subjects is studied in varied experiments at different times each day. In these experiments, a drug that is administered *before* a later daily session is necessarily administered *after* earlier sessions of the same day. This procedure permits measurement of behavior both *before* and *after* drug administration in the same subject during the same day and provides a way to systematically study influences of specific environmental features on expression of tolerance. In one experiment (Smith, 1990), for example, tolerance that developed to the disruptive effects of phencyclidine and diazepam on lever pressing maintained under an FR schedule of food presentation in one experimental chamber did not extend to FR lever pressing maintained in a different experimental chamber. Moreover, after disruptive effects of diazepam eventually diminished for FR responding in the second session, the resulting tolerance did not extend to the disruptive effects of the diazepam on lever pressing maintained under a DRL schedule in a different component of a multiple schedule in the very same chamber. In this instance, therefore, tolerance did not extend across different environmental circumstances either for different or for similar response topographies and schedules of reinforcement, suggesting that tolerance was not a generalized process associated with presence of drug at its site of pharmacological activity alone, but was also influenced by behavioral processes as well.

Benzodiazepines remain among the most widely prescribed and clinically-used drugs in our culture (Woods and Winger, 1995; Woods et al., 1992; Perrine, 1996), and diazepam has representative cellular (Holt et al., 1997), behavioral (Costello et al., 1991; Cole, 1992; Löscher and Hönack, 1992; McMillan, 1992) and discriminative (Nierenberg and Ator, 1990; Tang and Franklin, 1991) effects of benzodiazepines. The present experiments were designed to characterize the influence of behavioral processes on tolerance to effects of this drug using a ''multi-environment'' procedure.

Lever and key pressing of rats was maintained under a 40-response FR schedule of food delivery in both the same and in different environments. The purpose of this procedure was to study expression of tolerance for diazepam when responding on the *same* manipulandum was maintained under the *same* schedule in the *same* experimental chamber in a different session and when responding on a *different* manipulandum was maintained under the *same* schedule in a *different* experimental chamber.

2. Methods

2.1. Subjects

Animals used in this study were maintained in accordance with guidelines of the Animal Care Committee of Mercer University and of the ''Guide for Care and Use of Laboratory Animals'' of the Institute of Laboratory Animal Resources, National Research Council, Department of Health, Education and Welfare, Publication Number (NIH)85-23, revised 1985.

Ten experimentally naive male Charles River CD albino rats (F344) were approximately 120 days old at the beginning of the experiment. Five animals were used in each of two experiments. Water was continuously available in home cages and experimental chambers, and animals were maintained at approximately 300 g body weight with a diet of Noyes Pellets Formula A and Purina Rat Chow. Reinforcers were single 45 mg pellets and supplemental feeding always occurred at least 45 min after completion of experimental sessions (Bacotti, 1976).

2.2. Apparatus

Experiments were conducted with Gerbrands Model C Rat Chambers (23 cm long $\times$ 20 cm wide $\times$ 20 cm high; Gerbrands). Features of chambers for each of two procedures are illustrated in Fig. 1. In general, chambers contained a response lever (Gerbrands G6312 or LVE/BRS 121-05) or key (G6315, Gerbrands); a recessed food cup (F7020, Gerbrands) mounted on the same wall; and a water bottle and speaker. The food cup was connected to a solenoid-operated pellet dispenser (G5100, Gerbrands). Each chamber was enclosed in a larger sound attenuating box, and control and recording of all scheduled events used computers and cumulative stepping recorders (Model C-3, Gerbrands).

2.3. Behavioral procedure — Experiment 1

Lever and key pressing were trained by selectively reinforcing desired features of behavior in each chamber, and responding was initially maintained under a 1-response FR schedule delivering food pellets in the presence of a 7-w white light and either a buzzer (lever) or tone (key). Subsequently, responding of all rats was maintained in three sessions each day.

Session 1 began at 0800 h and lasted 30 min; Session 2 began at 1200 h and lasted 30 min; and Session 3 began at 1600 h and lasted approximately 1 h. In Session 1, *key* pressing was maintained in the presence of a tone under an FR40 schedule (Fig. 1, top left); in Session 2, *lever* pressing was maintained under FR40 in the presence of a buzzer in a different chamber (Fig. 1, top middle); and in Session 3, key (tone) and lever pressing (buzzer) were maintained sequentially under a multiple schedule (Fig. 1, top right). The multiple schedule of the third daily session used the same chamber as the FR40-lever schedule in the second daily session. Each component of the multiple schedule lasted 30 min and was separated by a 2-min period during which visual and auditory stimuli were turned off, reinforcers were not delivered, and injections were occasionally administered. Experimental sessions

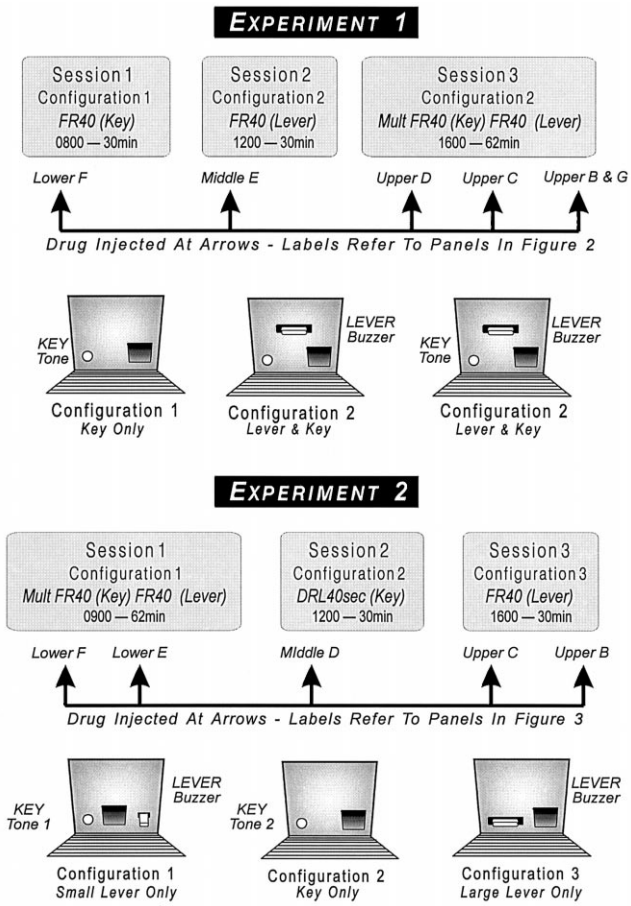

Fig. 1. Multi-environment procedure for Experiment 1 (top) and Experiment 2 (bottom). Responding was separately maintained for five animals under each procedure. The second and third sessions of Experiment 1 used the same chamber; all other sessions used different chambers. All schedule components lasted 30 min, and multiple schedule components occurred only once each session and were separated by a 2-min timeout period during which all auditory and visual stimuli were off and during which injections occasionally occurred (indicated by arrows). Injections also sometimes occurred immediately prior to individual schedules (indicated by arrows).

were conducted Monday–Friday, and animals responded under the described conditions without receiving any drug until variability of daily response rates was within 20% for two successive weeks.

2.4. Behavioral procedure — Experiment 2

Session 1 began at 0900 h and lasted approximately 1 h, and Session 2 began at 1600 h and lasted 30 min. Responding in Session 1 was maintained under a multiple schedule comprising key (higher pitch tone) and lever (buzzer) pressing under FR40 schedules (Fig. 1, bottom left); and lever pressing in Session 2 (buzzer) was maintained under an FR40 schedule (Fig. 1, bottom right). Each component of the multiple schedule lasted 30 min and was separated by a 2-min period during which visual and auditory stimuli were turned off, reinforcers were not delivered, and injections were occasionally administered. Specific details of daily sessions and determination of stable control responding were the same as in Experiment 1.

2.5. Pharmacological procedure

Diazepam (5 mg/ml; Steris Laboratories, Phoenix) was dissolved in a solution of 40% propylene glycol, 10% ethanol, 5% sodium benzoate, and 1.5% benzoic acid. This solution was further diluted with propylene glycol and sodium chloride and injected IM in a volume of 0.5 ml/kg body weight. Similar volumes of diluent served as control injections. After initial training, each animal received 3–5 injections of each of several doses of diazepam (0.3–3.0 mg/kg) 2–3 times monthly in mixed order immediately prior to each experimental session. When animals received drug prior to an earlier daily session during these initial widely spaced injections, they were not studied on that day in subsequent sessions. This prevented unplanned pairings of drug and behavior. Each animal also received control injections once weekly, and the average effects of these control injections were used for comparing pre-drug control responding with effects of both acutely and chronically administered diazepam. An intermediate dose of diazepam (1.7 mg/kg) was selected for chronic administration be-

cause it markedly decreased, but did not completely suppress, responding.

2.6. Chronic diazepam administration — Experiment 1

After determination of widely spaced dose-effects, animals received 1.7 mg/kg/day diazepam for 16 sessions immediately *after* all responding in the third daily session (Fig. 1 — top, right-most arrow). Then, animals received 1.7 mg/kg/day for 48 sessions *before* the lever-pressing component of the third daily session (Fig. 1 — top; second-from-right arrow); for 20 sessions *before* the key-pressing component of the third daily session (Fig. 1 — top; third-from-right arrow); for 20 sessions *before* the second daily session (same chamber; Fig. 1 — top; fourth-from-right arrow); for 24 sessions *before* the first daily session (Fig. 1 — top; left-most arrow); then once again for 72 sessions *after* all responding of the third daily session (Fig. 1 — top; right-most arrow). During chronic administration of drug prior to earlier sessions of the day, subjects continued to perform in later sessions that same day and occasionally received probe injections of diazepam before a session. Diazepam was administered at noon on Saturdays and Sundays during repeatedly daily injections, and differences in the number of weeks for each drug condition depended on asymptote for behavioral effects.

2.7. Chronic diazepam administration — Experiment 2

After determination of acute effects of diazepam from widely-spaced drug administration, a second group of animals received 1.7 mg/kg/day diazepam for 16 sessions immediately *after* all responding in the second daily session (Fig. 1 — bottom, right-most arrow). Then, animals received 1.7 mg/kg/day for 48 sessions *before* the second daily session (Fig. 1 — bottom; second-from-right arrow); for 60 sessions *before* the lever-pressing component of the first daily session (Fig. 1 — bottom; second-from-left arrow; including occasional probe sessions); and for 40 sessions *before* the key-pressing component of the first daily session (Fig. 1 — bottom; left-most arrow). The regimen for daily drug administration and determination of asymptotic effects was the same as in Experiment 1.

3. Results

3.1. Control performance — Experiment 1

Responding was readily controlled and maintained for both the lever and the key in separate experimental chambers, and rates and patterns of both responding and food delivery were comparable to those commonly reported for similar schedules and parameters presented individually (Ferster and Skinner, 1957). FR lever pressing occurred at 1.07–1.77 responses per second (total range both chambers), and there were generally 60–70 reinforcers per schedule component. FR key pressing occurred at 1.63–2.67 responses per second, and there were generally 75–90 reinforcers per schedule component.

3.2. Effects of diazepam — Experiment 1

FR responding for the first group of rats decreased as acutely-administered dose of diazepam increased for all three experimental sessions (Fig. 2, panel A), and none of

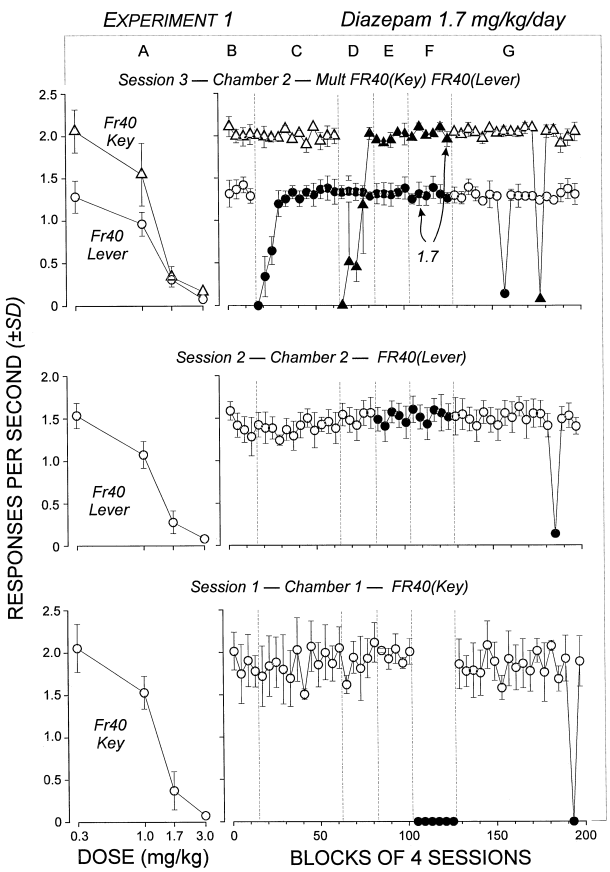

Fig. 2. Effects of acute (panel A) and repeated daily administration (panels B–G) of diazepam on responses per second (±1 SD) under FR40 schedules. Diazepam (1.7 mg/kg) was administered daily *after* responding of both sessions (panel B); *before* the FR lever pressing component of the third session (upper-panel C-filled circles); *before* the FR key pressing component of the third session (upper-panel D-filled triangles); *before* the FR lever pressing schedule of the second session (middle-panel E-filled triangles); *before* the FR key pressing schedule in the first session (lower-panel F); and then once again *after* the third session (Panel G). 1.7 mg/kg diazepam was also administered twice before the third session during the period of chronic administration prior to the first daily session (upper-panel F-arrows). Effects are shown for single sessions only, and not for blocks of sessions, after these probe injections. Points are always open when diazepam followed responding and are filled when diazepam preceded responding at any time during a day. Thus, for example, when diazepam was injected immediately prior to responding of the first daily session (bottom row), it was also injected 5 h prior to responding during the second session (middle row).

this responding was affected when diazepam (1.7 mg/kg) was subsequently injected at the end of the third session for 4 weeks (Fig. 1 — top, right-most arrow and Fig. 2, panel B). Four weeks was sufficient for diminution of muscle-relaxant effects of this dose during 30-min handling periods just after drug administration, indicating occurrences of dispositional or functional changes in pharmacologic sensitivity independent of operant responding during experiments. When 1.7 mg/kg diazepam was then injected prior to FR lever pressing in the third daily session for these rats (Fig. 1 — top, second-from-right arrow), responding was initially decreased as it had been after acute injections of that dose (Fig. 2, compare upper-panel C, filled points with the same dose in upper-panel A). These effects indicated that dispositional or functional changes in pharmacologic sensitivity that might have occurred during the preceding 20 daily injections outside behavioral circumstances did not extend to suppressive effects of diazepam on FR responding. During subsequent repeated daily administration of 1.7 mg/kg diazepam, however, suppression of FR lever pressing diminished, and responding occurred at pre-drug control rates after 20 days.

When 1.7 mg/kg diazepam was next administered prior to FR key pressing of the first component of the multiple schedule of the third session of the day (Fig. 1 — top, third-from-right arrow), responding was again suppressed as it had been after acute administrations of that dose (Fig. 2, upper-panel D, filled triangles). Just as for lever pressing in the same third session, however, suppressed key pressing also recovered during repeated daily administration of drug, and when diazepam was subsequently administered prior to lever pressing of the individual schedule in the same chamber during the second session of the day, responding was *not* suppressed, but occurred near control levels (Fig. 2, center-panel E, filled circles).

There had been no effects of diazepam on FR key pressing in the first daily session throughout the period of diazepam injections prior to responding in later sessions. Then, when diazepam was administered immediately prior to key-pressing during the first session of the day, responding was completely suppressed for up to 20 sessions (Fig. 2, lower-panel F, filled circles). It is noteworthy, however, that responding was not affected by additional injections of 1.7 mg/kg diazepam prior to the third session (Fig. 2, upper-panel F, arrows), indicating that animals remained tolerant in that environment and suggesting that suppressed responding in Session 1 did not result from age-related changes in benzodiazepine pharmacodynamics.

After it was evident that suppressed key pressing in the first session was stable, diazepam was once again administered at the end of the third daily session. Thus, administration of diazepam was not terminated, but occurred at the end rather than at the beginning of the day (Fig. 2, panel G). After 28 days of administering diazepam at the end of the day, injections were once again occasionally made prior to selected schedule components. When these probe injections were made, lever and key pressing were suppressed for both the multiple and individual schedules (Fig. 2, upper- and center-panel G, filled points). Consequently, even though behavioral effects of diazepam diminished when its repeated daily administration occurred prior to operant responding, those behavioral effects recurred when chronically administered diazepam was experienced at the end of the day in an environment that was not associated with operant reinforcement.

3.3. Control performance — Experiment 2

Control responding for the second group of rats was also readily controlled and maintained under the FR schedule in separate experimental chambers, and rates and patterns of both responding and food delivery were comparable to those commonly reported for similar schedules and parameters presented individually. FR *lever* pressing occurred at 1.00–1.93 responses per second (total range both chambers), and there were generally 66–83 reinforcers per schedule component. FR *key* pressing occurred at 0.77–1.38 responses per second, and there were generally 58–72 reinforcers per schedule component.

3.4. Effects of diazepam — Experiment 2

FR responding decreased as acutely-administered dose of diazepam increased during both fixed-ratio sessions (Fig. 3, upper- and lower-Panel A). These effects were similar to those observed for diazepam in different subjects responding in Experiment 1 (see also Fig. 2).

There were no observable effects on operant responding in any chamber when 1.7 mg/kg diazepam was injected after the second daily session for 4 weeks (Fig. 1 — bottom, right-most arrow and Fig. 3, panel B). Moreover, just as for Experiment 1, 4 weeks was sufficient for development of tolerance to these sedative effects. Then, when diazepam was injected prior to FR lever pressing in the second daily session, responding was decreased almost as much as it had been after widely-spaced single injections (Fig. 3, compare upper-panel C, filled circles, and upper-panel A). Suppressed lever pressing recovered within 10 sessions, however, and occurred at control levels for approximately 40 sessions.

When diazepam was then administered prior to FR lever pressing in the latter half of the first daily session (Fig. 1, lower panel, left), responding was suppressed in a manner similar to that after earlier widely-spaced injections of 1.7 mg/kg (Fig. 3, lower-panel D, filled circles). Suppressed FR responding worsened and occurred at zero levels even though FR responding later in the day during the second session occurred at control rates after a probe injection of 1.7 mg/kg diazepam (Fig. 3, arrow-indicated point in upper-panel D). This latter observation is noteworthy because it indicates that animals remained tolerant to effects of diazepam in the chamber used for Session 2 and

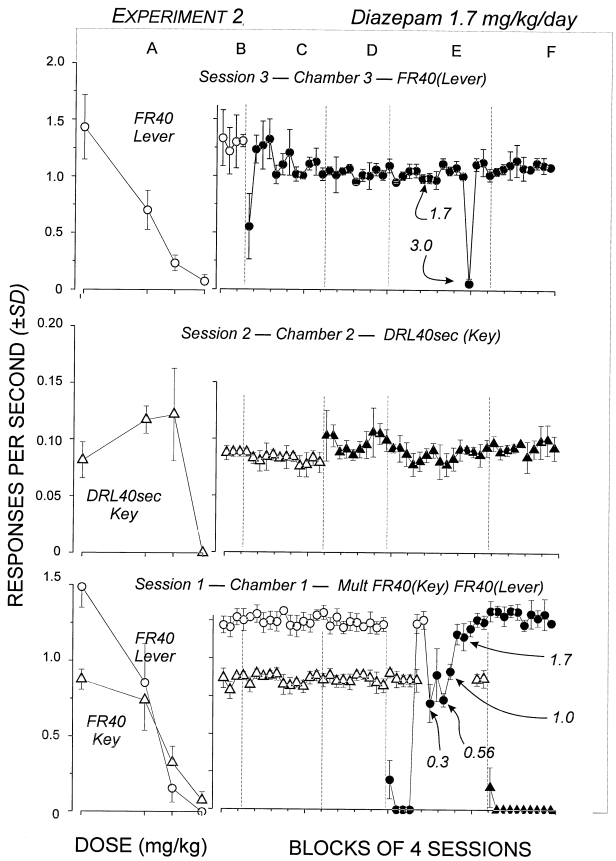

Fig. 3. Effects of acute (panel A) and repeated daily administration (panels B–F) of diazepam on responses per second (±1 SD) under FR40 schedules. Diazepam (1.7 mg/kg) was administered daily *after* responding of both sessions (panel B); *before* the FR lever pressing schedule of the second session (upper-panel C-filled circles); *before* the FR lever pressing component in the first session (lower-panel D); and then *before* the FR key pressing component in the first session (lower-panel E). Probe injections are labelled with dose, and several points are omitted from lower-panel D in order to make clearer effects of different doses of diazepam on lever pressing. Points are always open when diazepam followed responding and are filled when diazepam preceded responding at any time during a day.

suggests that suppression in Session 1 did not result from age-related changes in benzodiazepine pharmacodynamics. FR lever pressing in the first session occurred at control levels during probe substitutions of saline (Fig. 3, lower-panel D, open circles), indicating that responding had not altered under non-drug conditions, but was occurring at near-zero levels as a result of the presence of diazepam.

After it was evident that suppressed lever pressing in the first session was stable, the dose of diazepam prior to that session was decreased and then once again increased as responding stabilized at each larger dose. Lever pressing during the first session eventually occurred near control level after 1.7 mg/kg diazepam (Fig. 3, arrow-indicated filled circles in lower-panel D). Responding was suppressed by a probe administration of 3.0 mg/kg prior to Session 2, however, indicating that animals remained sensitive to diazepam (Fig. 3, upper-panel D, arrow). Then, when 1.7 mg/kg diazepam was finally injected prior to key pressing in the first component of the first daily session, that responding was suppressed as it had been after widely-spaced injections at the beginning of the experiment (Fig. 3, compare lower-panel E, filled triangles). Thus, recovery of lever pressing that accompanied the gradual increase of dose did not extend to key pressing in the same experimental chamber.

4. Discussion

Responding was readily maintained in different experimental chambers by FR schedules of food presentation, and rates and patterns of performance were comparable to those reported for similar schedules and parameters when presented individually (Sidman, 1956; Ferster and Skinner, 1957), as well as when studied for extended periods using ''multi-environment'' procedures (Smith, 1991a).

When diazepam was initially administered acutely, FR responding was decreased at all doses in a manner that was comparable to previously-reported effects of benzodiazepines on responding maintained under FR schedules of food presentation (Fowler et al., 1993).

There were no systematic effects on operant responding when 1.7 mg/kg diazepam was subsequently given *after* all daily sessions, and gross motor sedative effects observed disappeared within 2 weeks. Then, when diazepam was administered *before* responding in the last session each day, responding was suppressed as it had been after acute administration. This responding recovered to control levels over approximately 3–4 weeks of continued diazepam administration and remained stable for an additional month. These results are consistent with previous results in our laboratory that physiologic changes accompanying diminished sedative effects outside experimentally-defined operant circumstances are not sufficient to attenuate suppressive effects on reinforced responding *within* those operant circumstances (Smith, 1991a).

When subjects next received 1.7 mg/kg diazepam immediately prior to responding in an earlier session conducted in a different chamber or using a different manipulandum, behavior was again suppressed even though tolerance had developed to chronically-administered drug in a later daily session. However, when subjects received diazepam immediately prior to responding in either the same chamber or on the same type manipulandum, responding was not disrupted, but occurred within control range. Thus, diminished effects of diazepam in one component of a multiple schedule, for example, did not insure its diminished effects in even the same experimental session when response topography was markedly different, whereas diminished drug effects were observed in a different session when major features of reinforcement contingencies were

the same. These results are consistent with theory that control of operant behavior is influenced by both experimenter-controlled and experimenter-uncontrolled pairings of response requirements and their associated stimuli (Morse and Skinner, 1957; Mackintosh, 1983) and that internal, drug-produced stimuli can be as important as external stimuli in reinforcement contingencies that control operant behavior (Thompson and Pickens, 1971). According to this theory, it is not only experimenter-controlled relations among stimuli, responses, and reinforcers, but rather the collective presence of all stimuli, responses, and reinforcers, that comprise ''reinforcement contingencies''. When using operant procedures, for example, it is a common concern to try and eliminate unplanned, ''accidental'' pairings between reinforcers and inappropriate response topographies in order to avoid large variations in response rate that can complicate analysis and interpretation of data. Additionally, in the same manner that careless training of response topography can result in orderly, yet unintended, variation in response rate, unplanned pairings among responses, *stimuli*, and reinforcers can result in orderly, yet unintended, discriminative influences on behavior. In the present experiment, the complete set of reinforcement contingencies comprised both chamber-related and drug-related stimuli, and alteration of either of these sets of stimuli altered discriminative influences on measured behavior and reduced the likelihood of responding until discriminative control was reestablished following reinforced practice in the presence of the new drug-related condition. Thus, when chronically-administered drug preceded responding in a different chamber or on a different manipulandum, FR responding was markedly decreased until continued reinforcement in the presence of new drug- and chamber-related stimuli completed a transition to new stimulus–response–reinforcer relations controlling behavior. These kinds of transitional changes are typically called ''learning'', and because pharmacologic processes associated with diazepam presumably remained the same throughout extended periods in the present experiment, it seems more appropriate to describe present results in terms of ''learning'' transitions instead of in terms of ''tolerance'', or even ''learned'' or ''behavioral'' tolerance, to pharmacologic effects of the drug.

A consideration of present results in terms of ''learning'' is consistent with the view that reinforcement processes are why tolerance does not develop to the discriminative effects of drugs. For example, there is no loss of discriminative control by opioids (Young et al., 1991), ketamine (Rocha et al., 1996), or chlordiazepoxide (Bronson, 1993) when subjects continue to experience selective, ''training-dose'' reinforcement throughout chronic drug administration, but there is a loss of discriminative control by these drugs when ''training-dose'' reinforcement is suspended during repeated drug administration. Moreover, there is even an *increased* sensitivity to interoceptive effects of fentanyl during its continued administration provided that reinforcers are selectively associated with smaller doses (Colpaert et al., 1980). One interpretation of these findings is that functional relations between subjective drug effects and external reinforcers are as important for drug discrimination as functional relations between subjective drug effects and internal drug receptors. More specifically, this view emphasizes that functional relations among stimuli, responses, and reinforcers *are* the ''reinforcement contingencies'' that influence not only discriminative effects of drugs, but other behavioral effects of drugs as well. This ''reinforcement contingency'' analysis of drug discrimination is consistent with another widely-discussed view that temporal pairings between drug-related internal and other external stimuli result in ''memory states'' that not only *are* the basis of interoceptive effects of drugs but also constitute a psychological ''mechanism of action'' for analgesic effects of opiates (Colpaert, 1996) and important clinical effects of benzodiazepines (Colpaert and Koek, 1996).

In addition to describing behavioral processes that could mediate the initial effects of chronically-administered diazepam on responding in different environmental situations, ''reinforcement contingency'' theory also describes behavioral processes for three additional notable features of the present findings. The first of these features was the complete absence of ''tolerance'' to effects of diazepam for up to 40 daily administrations in both Experiment 1 (Fig. 2, lower panel, column F) and Experiment 2 (Fig. 3, lower panel, column E). Response suppression from diazepam had diminished after approximately 16 daily administrations in earlier phases of both experiments, and 40 sessions would clearly seem an adequate time for a similar ''learning transition''. In both experiments, however, suppression of responding was protracted only when reinforcers were available in other daily sessions or multiple schedule components. Even though lost reinforcers in single daily sessions can be sufficient for responding to recover from suppression during chronically-administered drug (Schuster et al., 1966), lost reinforcers are *not* sufficient for diminished suppression when reinforcers are also available during other periods of daily experimental sessions (Smith, 1986). In terms of ''reinforcement contingencies'', then, effects of chronically-administered drug in the present experiment were influenced not only by stimulus discrimination comprising learned associations between drug-related stimuli and reinforcers, but by deprivation-related associations between responses and lost reinforcers as well.

A second notable feature of the present results was effects in Experiment 2 of reducing, and then gradually increasing, the daily response-suppressing dose of diazepam. During the experimental phase depicted in Fig. 1 (lower panel, arrow at ''lower D'') and Fig. 3 (lower panel, column D), suppression of responding in Session 1 did not diminish for 16 sessions of daily diazepam even though responding in Session 2 was not affected by a

probe administration of the same dose. Thus, subjects were not somehow pharmacologically more sensitive to diazepam in all experimental circumstances, but rather were behaviorally more sensitive in only one particular circumstance. These results are similar to those sometimes obtained when external stimuli associated with availability of reinforcers are switched for those associated with the absence of reinforcers. This ''discrimination reversal'' procedure usually results in a rapid transition during which responding quickly adjusts and occurs only in the presence of the new reinforcer-related stimuli. However, subjects sometimes do not readily adjust to the new stimulus conditions and do not respond in the presence of stimuli that are newly associated with reinforcers. When this happens, of course, reinforcers are not obtained, and although the stimulus is nominally related to reinforcers, it remains functionally related to the absence of reinforcers. In circumstance where the two external stimuli are a bright and a dim light, for example, and an experimental subject remains suppressed in presence of a bright light that is newly-associated with availability of reinforcers, experimenters sometimes temporarily reduce the intensity of the light so that it is more similar to the previous dim light associated with reinforcers. After responding occurs and reinforcers are delivered, experimenters gradually increase brightness until the experimental subject completes the transition and responding finally occurs in the presence of the bright light. Effects of ''dose-fading'' for diazepam in the Experiment 2 were similar to effects of ''brightness-fading'' for a light in discrimination-reversal experiments, suggesting that protracted suppressive effects of diazepam did not result from pharmacologic action alone, but were influenced as well by behavioral processes described as stimulus discrimination.

A third notable feature of the present findings was effects in Phase G of Experiment 1 when probe administrations of diazepam suppressed operant responding in experimental sessions even though diazepam continued to be administered each day *after* all three daily sessions (Fig. 1, upper panel, arrow at ''upper B and G''). For responding in Sessions 2 and 3 particularly (Fig. 2, upper and middle panel, respectively), these results showed that ''tolerance'' to suppressive effects of diazepam was lost even during continued daily drug administration. These effects are not readily explained in terms of pharmacological action, but they are readily explained in terms of behavioral processes. Specifically, just as diazepam became selectively associated with the particular physical arrangements of experimental sessions during earlier phases of the experiment, requiring ''learning transitions'' at each change in stimulus complex, diazepam also became selectively associated with physical arrangements of the colony-room at the end of the day, and interoceptive effects of drug were no longer part of the stimulus complex associated with reinforced responding. Consequently, when diazepam was once again administered acutely *before* sessions, it functioned as a disruptive stimulus and suppressed responding just as it had during earlier phases of the experiment.

In summary, the remarkably contrasting effects of chronically-administered diazepam in the present experiments are not readily understood in terms of either age-related or pharmacology-related processes, but they are readily explained in terms of behavioral processes. It is generally agreed that pharmacologic effects of drugs can produce subjective experience, and that these subjective properties can function as behavioral discriminative stimuli in reinforcement contingencies controlling behavior. It is also generally agreed that behavior can be influenced by properties of stimuli that may be only adventitiously associated with reinforcement contingencies. Results of the present experiments suggest that chronically-administered diazepam functioned primarily as a ''behaviorally-active'' stimulus long after direct sedative effects diminished, and it seems parsimonious to describe these results in terms of ''learning'' and not in terms of ''tolerance''.

Acknowledgements

Supported by USPHS Grant DA01987. I thank T. Smalls for indispensable technical help and A.A. Ogonowski for helpful comments on the manuscript.

References

Advokat, C., McInnis, C., 1992. Environmental modulation of behavioral tolerance in spinal rats. Brain Research 581, 46–52.

Bacotti, A.V., 1976. Home cage feeding time controls responding under multiple schedules. Animal Learn. Behav. 4, 41–44.

Bronson, M.E., 1993. Tolerance/cross-tolerance to the discriminative stimulus effects of chlordiazepoxide and bretazenil. Mol. Chem. Neuropathol. 18, 85–98.

Caggiula, A.R., Epstein, L.H., Antelman, S.M., Saylor, S.S. *et al.*, 1991. Conditioned tolerance to the anorectic and corticosterone-elevating effects of nicotine. Pharmacol. Biochem. Behav. 40, 53–59.

Cole, S.O., 1992. Reversal of diazepam-induced impairment in successive discrimination performance by flumazenil. Drug Dev. Res. 27, 161–168.

Colpaert, F.C., 1996. System theory of pain and of opiate analgesia: no tolerance to opates. Pharmacol. Rev. 48, 355–402.

Colpaert, F.C., Koek, W., 1996. Behavioral specificity of chlordiazepoxide-produced StD. Behav. Pharmacol. 7, 49–55.

Colpaert, F.C., Niemegeers, C.J.E., Janssen, P.A.J., 1980. Factors regulating drug cue sensitivity: limits of discriminability and the role of a progressively decreasing training dose in fentanyl-saline discrimination. J. Pharmacol. Exp. Ther. 212, 474–480.

Costello, N.L., Carlson, J.N., Glick, S.D., 1991. Acute administration of diazepam and buspirone in rats trained on conflict schedules having different degrees of predictability. Pharmacol. Biochem. Behav. 40, 787–794.

Ehrman, R.N., Ternes, J., O'Brien, C.P., McLellan, A.T., 1992. Conditioned tolerance in human opiate addicts. Psychopharmacology (Berl.) 108, 218–224.

Epstein, L.H., Caggiula, A.R., Stiller, R., 1989. Environment-specific tolerance to nicotine. Psychopharmacology (Berl.) 97, 235–237.

Ferster, C.B., Skinner, B.F., 1957. Schedules of Reinforcement. Appleton-Century-Crofts, New York.

Fowler, S.C., Bowen, S.E., Kallman, M.J., 1993. Practice-augmented tolerance to triazolam: evidence from an analysis of operant response durations and interresponse times. Behav. Pharmacol. 4, 147–157.

Holt, R.A., Martin, I.L., Bateson, A.N., 1997. Chronic diazepam exposure decreases transcription of the rat $GABA_A$ receptor $gamma_2$-subunit gene. Mol. Brain Res. 48, 164–166.

Hunt, T., Poulos, C.X., Cappell, H., 1990. A contingent, conditioned suppression of eating following chronic benzodiazepine-induced hyperphagia. Pharmacol. Biochem. Behav. 35, 373–378.

Löscher, W., Hönack, D., 1992. Withdrawal precipitation by benzodiazepine receptor antagonists in dogs chronically treated with diazepam or the novel anxiolytic and anticonvulsant β-carboline abecarnil. Naunyn-Schmiedeberg's Arch. Pharmacol. 345, 452–460.

Löscher, W., Rundfeldt, C., Hönack, D., 1991. Tolerance to anticonvulsant effects of the partial benzodiazepine receptor agonist abecarnil in kindled rats involves learning. Eur. J. Pharmacol. 202, 303–310.

Mackintosh, N.J., 1983. Conditioning and Associative Learning. Oxford Univ. Press, New York.

Mana, M.J., Kim, C.K., Pinel, J.P.J., Jones, C.H., 1992. Contingent tolerance to the anticonvulsant effects of carbamazepine, diazepam, and sodium valproate in kindled rats. Pharmacol. Biochem. Behav. 41, 121–126.

McMillan, D.E., 1992. Effects of drugs on behavior before and during chronic diazepam administration. Eur. J. Pharmacol. 215, 145–152.

Melchior, C.L., 1990. Conditioned tolerance provides protection against ethanol lethality. Pharmacol. Biochem. Behav. 37, 205–206.

Morse, W.H., Skinner, B.F., 1957. A second type of superstition in the pigeon. Am. J. Psychol. 70, 308–311.

Nierenberg, J., Ator, N.A., 1990. Drug discrimination in rats successively trained to discriminate diazepam and pentobarbital. Pharmacol. Biochem. Behav. 35, 405–412.

Perrine, D.M., 1996. The Chemistry of Mind-Altering Drugs: History, Pharmacology, and Cultural Context. American Chemical Society, Washington D.C.

Rocha, B.A., Ward, A.S., Egilmez, Y., Lytle, D.A., Emmett-Oglesby, M.W., 1996. Tolerance to the discriminative stimulus and reinforcing effects of ketamine. Behav. Pharmacol. 7, 160–168.

Schuster, C.R., Dockens, W.S., Woods, J.H., 1966. Behavioral variables affecting the develoment of amphetamine tolerance. Psychopharmacology (Berl.) 9, 170–182.

Sidman, M., 1956. Time discrimination and behavioral interaction in a free operant situation. J. Comp. Physiol. Psychol. 49, 469–473.

Siegel, S., 1976. Morphine analgesic tolerance: its situation specificity supports a Pavlovian conditioning model. Science 193, 323–325.

Siegel, S., 1989. Morphine tolerance acquisition as an associative process. Journal of Experimental Psychology 1977, 3–13.

Smith, J.B., 1986. Effects of chronically administered *d*-amphetamine on spaced responding maintained under multiple and single-component schedules. Psychopharmacology (Berl.) 88, 296–300.

Smith, J.B., 1990. Situational specificity of tolerance to decreased operant responding by cocaine. Pharm. Biochem. Behav. 36, 473–477.

Smith, J.B., 1991a. Situational specificity of tolerance to effects of phencyclidine on responding of rats under fixed-ratio and spaced-responding schedules. Psychopharmacology (Berl.) 103, 121–128.

Smith, J.B., 1991b. Situational specificity of tolerance to decreased operant responding by morphine and *l*-nantradol. Psychopharmacology (Berl.) 103, 115–120.

Taiwo, Y.O., Coderre, T.J., Levine, J.D., 1989. The contribution of training to sensitivity in the nociceptive paw-withdrawal test. Brain Research 487, 148–151.

Tang, A.H., Franklin, S.R., 1991. The discriminative stimulus effects of diazepam in rats at two training doses. J. Pharmacol. Exp. Ther. 258, 926–931.

Thompson, T., Pickens, R., 1971. Stimulus Properties of Drugs. Appleton-Century-Crofts, New York.

Tietz, E.I., 1992. Repeated anticonvulsant testing: contingent tolerance to diazepam and clobazam in kindled rats. Epilepsy Res. 11, 89–101.

Tiffany, S.T., Drobes, D.J., Cepeda-Benito, A., 1992. Contribution of associative and nonassociative processes to the development of morphine tolerance. Psychopharmacology (Berl.) 109, 185–190.

Tsibulsky, V.L., Amit, Z., 1993. Role of environmental cues as Pavlovian-conditioned stimuli in enhancement of tolerance to ethanol effects: 1. Lethal effects in mice and rats. Pharmacol. Biochem. Behav. 45, 473–479.

Wolgin, D.L., Benson, H.D., 1991. Role of associative and nonassociative mechanisms in tolerance to morphine ''anorexia''. Pharmacol. Biochem. Behav. 39, 279–286.

Woods, J.H., Winger, G., 1995. Current benzodiazepine issues. Psychopharmacology (Berl.) 118, 107–115.

Woods, J.H., Katz, J.L., Winger, G., 1992. Benzodiazepines: use, abuse, and consequences. Pharmacol. Rev. 44, 151–347.

Young, A.M., Kapitsopoulos, G., Makhay, M.M., 1991. Tolerance to morphine-like stimulus effects of *mu* opioid agonists. J. Pharmacol. Exp. Ther. 257, 795–805.