

EFFECTS OF GABA_A MODULATORS ON THE REPEATED ACQUISITION OF RESPONSE SEQUENCES IN SQUIRREL MONKEYS

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The present study investigated the effects of positive and negative GABA_A modulators under three different baselines of repeated acquisition in squirrel monkeys in which the monkeys acquired a three-response sequence on three keys under a second-order fixed-ratio (FR) schedule of food reinforcement. In two of these baselines, the second-order FR schedule and the discriminative stimuli for the response sequence were manipulated (“chain-strained” and “tandem-strained”). In the third baseline condition, response-independent tail shock was presented during acquisition of the response sequence. All of these baselines maintained high error levels and produced low rates of acquisition. Under both the chain-strained and tandem-strained conditions, the positive GABA_A modulator triazolam (0.0032–0.1 mg/kg) and the negative GABA_A modulators β-CCE (ethyl-β-carboline-3-carboxylate; 0.01–1 mg/kg), β-CCM (methyl-β-carboline-3-carboxylate; 0.0032–0.1 mg/kg), and FG-7142 (methyl-β-carboline-3-carboxamide; 0.18–10 mg/kg) dose-dependently decreased overall response rate compared to administration of saline (control). Under the same two conditions, triazolam and the negative GABA_A modulators also increased the percentage of errors; however, the effects on accuracy frequently depended on the baseline condition and the particular modulator. In contrast, triazolam only decreased errors and enhanced acquisition in the presence of concurrent response-independent tail shock when compared to saline administration under this condition. The neutral GABA_A modulator, flumazenil (1 mg/kg), had no effect on rate or accuracy of responding when administered alone, but antagonized the rate-decreasing and error-increasing effects produced by the negative GABA_A modulators. Together, these data suggest that the effects of both the positive and negative GABA_A modulators on acquisition can be similar in squirrel monkeys (i.e., both types of modulator may produce rate-decreasing and error-increasing effects) and that their effects on acquisition depend, in part, on the environmental conditions maintaining acquisition.

Key words: GABA_A modulators, benzodiazepines, learning, repeated acquisition, key press, squirrel monkeys

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system, and it binds to a distinct site on the GABA_A receptor complex, which consists of five protein subunits that form a transmembrane chloride channel (for reviews see Barnard et al., 1998; Mehta & Ticku, 1999). When GABA binds to its site on the complex, the channel opens allowing nega-

tively charged chloride ions to enter the cell. There are also a number of other subunit-dependent binding sites on the GABA_A receptor complex that are capable of modulating the flux of chloride ions through the channel, and these include the benzodiazepine, barbiturate and neuroactive steroid sites. One interesting feature of the benzodiazepine site is that the effects can be bidirectional. Drugs that bind to this site and facilitate the actions of GABA, thereby increasing chloride flux, are called positive modulators. Drugs that bind to this site and inhibit the actions of GABA, thereby decreasing chloride flux, are called negative modulators.

Positive GABA_A modulators have been shown to disrupt the acquisition of behavior in numerous animal species, including humans (Auta et al., 1995; Bickel, Hughes, & Higgins, 1990; Thiebot, 1985). The effects of negative GABA_A modulators on acquisition behavior are less well characterized and, hy-

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pothetically, should enhance acquisition as the negative modulators generally produce effects that are opposite from those produced by the positive modulators. For example, positive GABA_A modulators produce sedation and are anticonvulsant whereas negative GABA_A modulators produce insomnia and are convulsant (Venault *et al.*, 1986). Electrophysiological studies have shown that negative GABA_A modulators facilitate long-term potentiation (LTP) at low doses. For example, ethyl- β -carboline-3-carboxylate (β -CCE) has been reported to increase the magnitude of LTP in two areas of the hippocampus in guinea pigs (Yasui, Kawasaki, Matsushita, & Satoh, 1993). LTP is thought to model some of the processes involved in learning and memory. Consistent with these observations, studies involving rodents have reported that negative GABA_A modulators enhanced acquisition in passive avoidance procedures (File & Pellow, 1988; Izquierdo, Pereira, & Medina, 1990; Venault *et al.*, 1986) and improved retention of information pertaining to a novel environment (Venault *et al.*, 1986). Using a two-trial recognition paradigm, Mayo *et al.* (1992) also found that injections of β -CCM directly into the brain (the nucleus basalis magnocellularis) of rats improved recognition of a novel arm in a Y-maze when it was injected either before or after an exploration trial. In at least two of these studies, the direct involvement of the benzodiazepine receptor site was demonstrated by the administration of the neutral GABA_A modulator, flumazenil, which competitively antagonized the effects of the negative GABA_A modulators (Izquierdo *et al.*, 1990; Mayo *et al.*, 1992).

The purpose of the present study was to examine the effects of GABA_A modulators on acquisition under different baseline conditions in squirrel monkeys. More specifically, three negative GABA_A modulators (β -CCM, β -CCE and FG-7142) with varying efficacy at the benzodiazepine receptor site were selected in order to determine if they might improve the acquisition of appetitively reinforced response sequences that changed with each daily session (repeated acquisition). To establish conditions under which an improvement in acquisition behavior could be seen easily, the baseline of repeated-acquisition behavior was degraded by manipulating the stimuli associated with the response sequence

(chain vs. tandem stimuli) and increasing the value of the fixed ratio (FR) under the second-order FR schedule in order to produce ratio strain. The "tandem-strained" condition also served as a control for the conditioned reinforcing and discriminative stimulus properties of the stimuli in the "chain-strained" condition (Thompson, 1970; Williams, 1997). Both of these conditions maintained high error levels and produced slow within-session acquisition under control conditions.

For comparison, the effects of a positive GABA_A modulator, triazolam, were also investigated under these baseline conditions and administered under a third condition used to degrade acquisition; namely, the presentation of response-independent tail shock. Under this condition, levels of shock were carefully selected to disrupt acquisition, but not to produce response suppression. This is an important point as positive modulators have been shown to increase rates of responding that have been suppressed by response-contingent shock (Barrett, 1976; Barrett & Katz, 1981; Paronis & Bergman, 1999; Sepinwall & Cook, 1980). Because this baseline condition degraded acquisition without producing substantial response suppression, it served as an important means of determining whether or not a positive modulator could improve acquisition behavior when degraded by response-independent aversive stimuli. Although animal and human studies have shown that positive GABA_A modulators disrupt learning and memory, several reports suggest that the positive modulators, in particular, can improve responding in learning and memory tasks in "anxious" subjects (Desai, Taylor-Davies, & Barnett, 1983; Hartley, Spencer, & Williamson, 1982; Nakano, Gillespie, & Hollister, 1978; Parrott & Hindmarch, 1978).

METHOD

Subjects

Nine adult female squirrel monkeys (*Saimiri sciureus*) served as subjects. Eight of the subjects had a history of behavioral testing under a variety of schedules of reinforcement, whereas 1 subject (SQ Ro) was experimentally naive at the beginning of behavioral

testing. Each subject was maintained at 85% of its free-feeding body weight on a diet consisting of banana-flavored food pellets, Purina[®] Monkey Chow, Zu/Preem[®] Marmoset or Primate Diet, fresh fruit, peanuts, and vitamins. The pellets were earned during the experimental session whereas the remainder of the diet was fed to each subject several hours after each daily session. All subjects were individually housed in a temperature- and humidity-controlled room that was maintained on a 12:12 hr light/dark cycle (7:00 a.m. to 7:00 p.m.). Water was available during the experimental sessions and in the home cages. For behavioral testing, which occurred during the light cycle, each subject was removed from the colony-room cage and transported to another room with experimental chambers. In all situations, experiments were performed in accordance with the declaration of Helsinki and with the Guide for the Care and Use of Laboratory Animals as adopted and promulgated by the National Institutes of Health. The Institutional Animal Care and Use Committee at Louisiana State University Health Sciences Center approved the experimental protocols.

Apparatus

During each session, the subjects were seated in a clear, smooth Plexiglas restrainer or "chair" (STC-300, BRS/LVE, Inc., Laurel, MD). A water bottle was mounted on the left side of the chair in a position such that the subject could easily drink from it. The chair was placed in front of a response panel inside a ventilated sound-attenuating chamber (Model MEC-004, BRS/LVE). The panel was equipped with three recessed response keys (Model 21-17, Coulbourn Instruments, Lehigh Valley, PA) that were mounted 5 cm apart in a triangular configuration with the left and right keys at 30° angles from the center key. The response keys on the panel were equipped with tricolor cue lights. The colors used throughout testing were green, white and amber. An incandescent light ("house-light") (Model 1819, GTE Products Corp., Hillsboro, NH) was mounted at the top of the response panel directly above the center key. A food aperture that measured 4.7 cm by 4.7 cm was located 5 cm below the center key. Food pellets were delivered into this aperture by a pellet dispenser (Model D-1, Gerbrands

Corp., Arlington, MA), which was mounted behind the panel. Each time the dispenser was operated, it delivered one pellet. Behavioral events were scheduled and recorded with a personal computer (IBM[®], Armonk, NY), a printer (Epson LX-80[®], Torrance, CA) and cumulative recorders (Model C-4, Gerbrands Corp., Arlington, MA). For the baseline involving response-independent tail shock, the tail of each monkey was shaved completely and a conductive adhesive electrode (#7561, Graphic Controls Corp., Buffalo, NY) applied in order to deliver a brief (250 ms) electric shock from a shock generator (model ENV-410A, MED Associates Inc., St. Albans, VT) located within the interface cabinet.

Repeated-Acquisition Procedures

Subjects responded under three different behavioral baselines: chain-strained, tandem-strained, and chain with concurrent response-independent tail shock. Each baseline used a repeated-acquisition technique (Boren, 1963; Boren & Devine, 1968) in which subjects had to acquire a three-response sequence. Reinforcement occurred under a second-order schedule such that completions of the three-response sequence were reinforced under an FR schedule.

Chain-strained condition. Under this condition, each subject acquired a different three-response sequence each session in the presence of three different stimuli. Each of the three response keys was illuminated at the same time by one of three colors, either green, white, or amber. In the second link of the three-response sequence, the houselight served as an additional discriminative stimulus with the white keys. The subject's task was to press the correct key in the presence of each color. For example, keys green-left (L) key correct; keys white + houselight-center (C) key correct; keys amber-right (R) key correct. This type of sequential responding is procedurally defined as a "chain" because each response except the last produces a discriminative stimulus controlling the response that follows (Kelleher, 1966). After the completion of the third correct response, there was a 0.5-s flash of light in the feeder aperture. Following this "feeder flash" and a 1-s delay, the chain was reset. The same response chain (in this case, Left-Center-Right or LCR)

was repeated throughout a session. An example of a set of six sequences (for six different sessions) was as follows: LCR, CRL, RLC, LRC, CLR, and RCL, with the order of colors always green, white and amber. Each set of sequences were selected to be equivalent in several ways and there were restrictions on their ordering across sessions (Thompson, 1970). For example, each of the six sequences used was scheduled with equal frequency, and adjacent positions within a sequence for a given session were different. This eliminated sequence presentations such as right-right-center (RRC) or center-left-left (CLL).

Food reinforcements occurred under a second-order FR schedule. Under this second-order schedule, the FR values were 75, 150, 200, or 350, depending on the subject. Thus, if responding was reinforced under a FR 75, the subject had to complete the three-response chain 75 times in order to obtain a food pellet (i.e., FR 75 [chain FR 3(FR 1): S]). In general, increasing the second-order FR value increased the percentage of errors and decreased response rate. Different FR values were necessary for each subject in order to produce comparable error levels across subjects. When subjects pressed an incorrect key (e.g., pressing L or R when C was correct), the error was followed by a 5-s timeout. During timeouts, the keylights were off and responses had no programmed consequence. An error did not reset the chain; that is, the stimuli and sequential position were the same before and after the timeout. Each session terminated after 60 reinforcers or 60 min, whichever occurred first. Sessions were generally conducted 5 days a week (Monday through Friday).

Tandem-strained condition. Under this baseline condition, the stimuli for each response in the three-response sequence were identical. More specifically, for each response in the sequence the keys were illuminated only with white light and the houselight was not illuminated. For example, keys white-left (L) key correct; keys white-center (C) key correct; keys white-right (R) key correct. In order to produce comparable error levels and response rates under the tandem second-order FR schedule, the FR values were 15, 20, 35, or 45, depending on the subject. These values were considerably lower than those

used under the chain schedule because of the increased task difficulty due to the lack of discriminative stimuli (cf. Thompson, 1975, 1978). All other aspects of the two schedules (timeouts, etc.) were identical.

Response-independent tail-shock condition. This baseline was similar to the chain-strained condition except the second-order FR value for food presentation was five for all subjects. In addition, under this baseline condition, 10 response-independent tail shocks were randomly presented during the session (i.e., on average, one shock every 6 min). The intensity of the 250 ms shock was varied for each subject in order to produce comparable levels of degraded acquisition. More specifically, the shock intensity was 2.5 mA for SQ J, 5 mA for SQ G, 1 mA for SQ A, and 3.5 mA for SQ D.

Drugs

Ethyl- β -carboline-3-carboxylate (β -CCE), methyl- β -carboline-3-carboxylate (β -CCM), N-methyl- β -carboline-3-carboxamide (FG-7142) (RBI, Natick, MA), flumazenil (Hoffman-La Roche, Nutley, NJ), and triazolam (Upjohn, Kalamazoo, MI) were dissolved in 10% DMSO, then diluted to the appropriate concentration with a vehicle consisting of 50% propylene glycol, 11% polyethylene glycol, 2% benzyl alcohol, and 37% sterile water. Lorazepam (Wyeth Laboratories, Philadelphia, PA) was administered in the event of a convulsion and was dissolved in 80% propylene glycol, 18% polyethylene glycol, and 2% benzyl alcohol. The volume of both drug and vehicle (control) injections was 0.5 ml/kg of body weight and these injections were administered in the gluteus muscle before the start of the experimental session. The dose range for each drug was determined from published literature (e.g., Auta, Winsauer, Faust, Lambert, & Moerschbaecher, 1997; Winsauer, Delatte, Stevenson, & Moerschbaecher, 2002) or by preliminary determinations (not shown), and the time of injection was selected to ensure the onset of the effects of each drug prior to the beginning of the session. The time of injection prior to the session was 20 min for β -CCE, β -CCM and FG-7142, 5 min for flumazenil, and 30 min for triazolam. The respective control injections of either saline or vehicle were also administered at these times. Dosages of each drug were given in a mixed order. The largest doses of each

drug were never given within the same week in order to minimize the development of tolerance or supersensitivity. At least 1 week of baseline (nondrug or vehicle) sessions intervened between the end of a series of injections with one drug and the start of a series with another. Generally, baseline sessions were conducted on Mondays and Wednesdays, drug sessions on Tuesdays and Fridays, and vehicle control sessions on Thursdays.

Data Analysis

The data for each session were analyzed in terms of the overall response rate (total responses per minute, excluding timeouts) and the overall accuracy or percentage of errors, (errors)/(errors + corrects) × 100. The data for each monkey were analyzed by comparing the range of variability for drug sessions with the range of variability for vehicle or baseline sessions. A drug was considered to have an effect to the extent that the mean data for each dose fell outside of the baseline or vehicle control ranges of variability. The percentage of errors was not included in the data analysis when response rate was less than five responses per minute because of the small number of responses involved. In addition, the within-session changes in responding were monitored by a cumulative recorder.

RESULTS

Chain-Strained Condition

Triazolam. The top panels in Figure 1 show the effects of triazolam on overall response rate and the percentage of errors for 3 monkeys responding under the chain-strained condition. Triazolam produced dose-dependent decreases in response rate and small increases in the percentage of errors. Note that the largest increases in the percentage of errors also occurred at doses of triazolam that substantially decreased overall response rate. In SQ Ra, for example, only the 0.032-mg/kg dose increased the percentage of errors and this dose decreased the rate of responding to less than 10 responses per minute.

β-CCE (Ethyl-β-carboline-3-carboxylate). The effects of β-CCE on overall response rate and percentage of errors in each monkey responding under the chain-strained condition are shown in the bottom panels of Figure 1. Dose-dependent decreases in response rate

and increases in percentage of errors were produced by β-CCE in each of the subjects, although small differences in the lowest effective dose of β-CCE were apparent among the 3 subjects (SQ H, 0.32 mg/kg; SQ Ro, 0.56 mg/kg; SQ V, 0.1 mg/kg). SQ V convulsed at the highest dose of β-CCE (i.e., 0.56 mg/kg); therefore, a response rate of zero was recorded for this session. The effects of a 1-mg/kg dose of flumazenil, both alone and in combination with an effective dose of β-CCE, are also shown in Figure 1. In each subject, this dose of flumazenil alone had little or no effect on rate or accuracy of responding, but it antagonized both the rate-decreasing and error-increasing effects of β-CCE.

β-CCM (Methyl-β-carboline-3-carboxylate). The top panels in Figure 2 show the effects of β-CCM on overall response rate and percentage of errors for each monkey responding under the chain-strained condition. Although β-CCM was tenfold more potent on a mg/kg basis than β-CCE, the effects on overall response rate and accuracy were similar to those for β-CCE. The only notable difference was that the dose-effect curves for response rate and percentage of errors were somewhat steeper in all 3 subjects, which reduced the number of doses that produced rate-decreasing and error-increasing effects prior to eliminating responding. Convulsions also occurred in SQ H at 0.1 mg/kg of β-CCM.

FG-7142 (N-methyl-β-carboline-3-carboxamide). In the bottom panel of Figure 2, the effects of FG-7142 on overall response rate and percentage of errors are shown for each monkey responding under the chain-strained condition. Similar to β-CCE and β-CCM, FG-7142 dose-dependently decreased response rate and increased errors in all 3 subjects, although a decrease in rate was evident only at the highest dose in SQ H and it was quite small. Larger doses were not tested in this subject due to the limited solubility of the drug and the possibility of producing convulsions at doses of FG-7142 greater than 10 mg/kg. The effects of FG-7142 on accuracy of responding were also somewhat different from those of β-CCE and β-CCM in that specific doses of FG-7142 increased the percentage of errors without decreasing overall response rate. This was particularly evident in subjects SQ H and Ra, where a twofold increase in the percentage of errors was evident

CHAIN STRAINED-RATIO

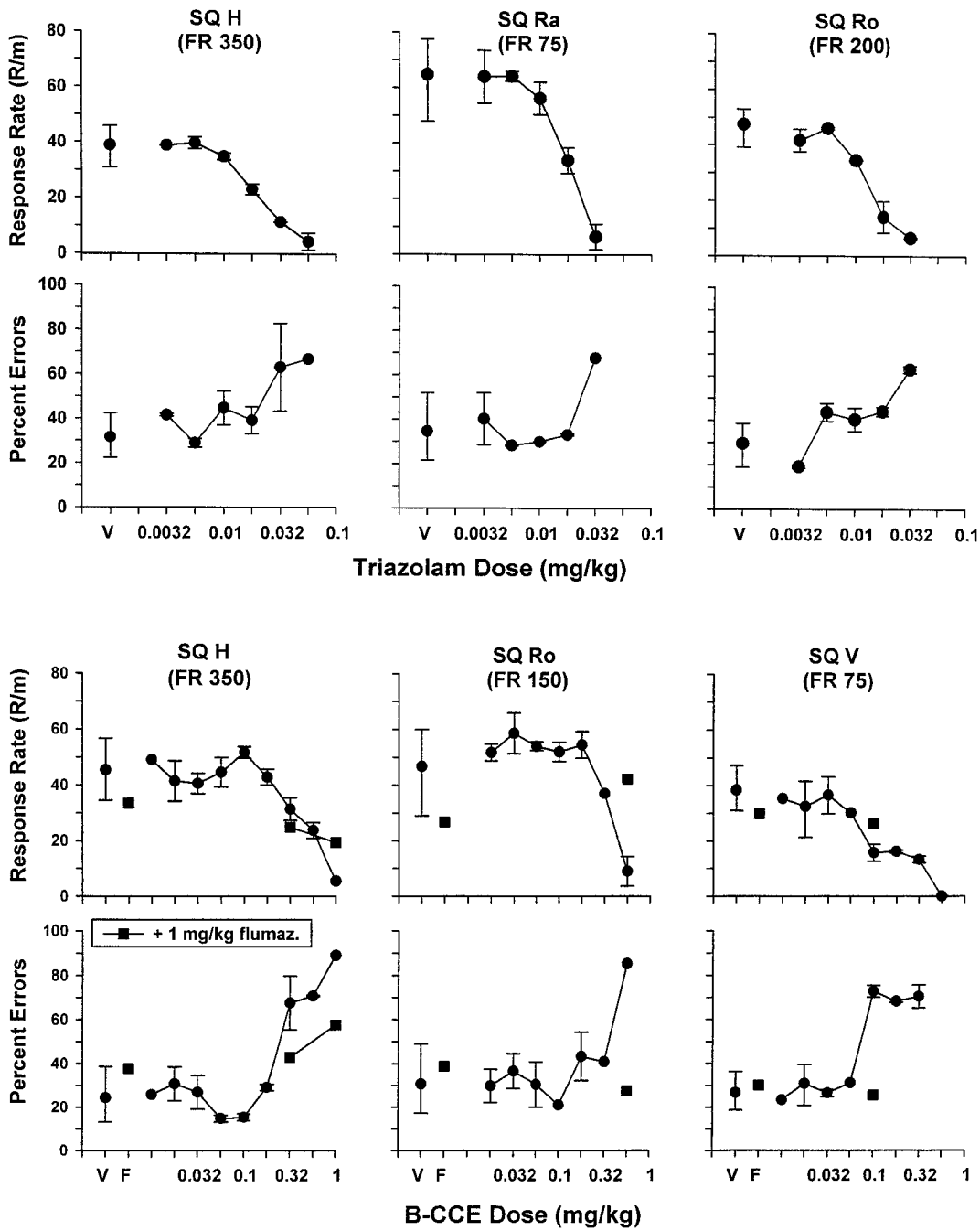


Fig. 1. Effects of triazolam (top panels) and β -CCE (bottom panels) on overall response rate and percentage of errors in 3 squirrel monkeys responding under the chain-strained baseline condition. (Filled squares represent the effects of 1 mg/kg of flumazenil alone [points above F] or in combination with β -CCE.) Points above V indicate the mean and range of 8 to 20 sessions that were preceded by a vehicle injection. The points with vertical lines in the dose-effect curve indicate the mean and range for two determinations. The points without vertical lines indicate either a single determination or an instance in which the range is encompassed by the point. When the response rate for a particular dose was less than five responses per minute, no data point was shown for the percentage of errors at that dose because of the small number of responses emitted.

CHAIN STRAINED-RATIO

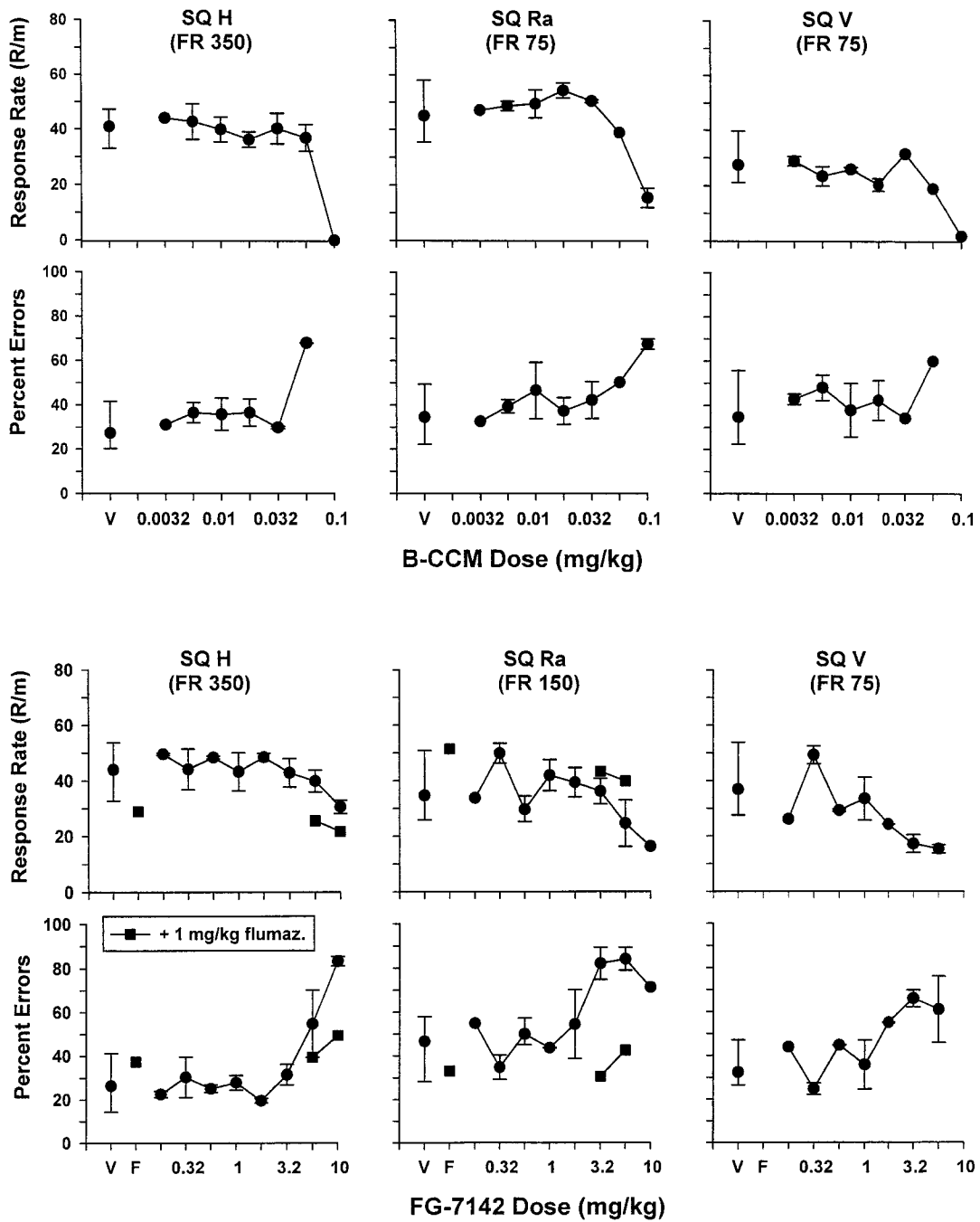


Fig. 2. Effects of β -CCM (top panels) and FG-7142 (bottom panels) on overall response rate and percentage of errors in 3 squirrel monkeys responding under the chain-strained baseline condition. The effects of FG-7142 in combination with 1 mg/kg of flumazenil are also shown in the bottom panels. Other details are the same as in Figure 1.

following 5.6 mg/kg and 3.2 mg/kg, respectively.

The effects of a 1-mg/kg dose of flumazenil, both alone and in combination with selected doses of FG-7142, are also shown for Monkeys H and Ra in Figure 2. In each of these subjects, flumazenil alone had little or no effect on rate or accuracy of responding, but it antagonized the error-increasing effects of FG-7142. Because the doses of FG-7142 that increased errors did not necessarily decrease rate, flumazenil only antagonized the rate-decreasing effects of FG-7142 at the 5.6-mg/kg dose in SQ Ra.

Cumulative records in Figure 3 depict the within-session effects of β -CCE, β -CCM, and FG-7142 when administered to Monkey V. The records in this figure represent the effects of doses of each drug that produced comparable rate-decreasing effects (approximately 20 responses per minute) under the chain-strained condition. The control record at the top of the figure shows the characteristic pattern of responding for Monkey V when vehicle was administered before the start of the session. As indicated by the records, all three drugs disrupted the within-session patterns of acquisition at each of the doses shown. In addition, the overall effects of the drugs on acquisition were somewhat similar. In terms of relative potency for producing a comparable rate-decreasing effect, the ranking from highest to lowest potency was as follows: β -CCM > β -CCE > FG-7142. More important, none of these drugs enhanced acquisition under this baseline of repeated acquisition.

Tandem-Strained Condition

Triazolam. The effects of triazolam in 2 monkeys responding under the tandem-strained condition are shown in Figure 4 (top panels). Under this condition, the effects of triazolam were similar to those obtained under the chain strained-ratio schedule; namely, triazolam produced dose-dependent decreases in response rate and small increases in the percentage of errors.

β -CCE. The bottom panels of Figure 4 show the effects of β -CCE on overall response rate and the percentage of errors in 3 monkeys responding under the tandem-strained condition. Under this baseline condition, β -CCE produced dose-dependent decreases in re-

sponse rate in all 3 monkeys and increases in the percentage of errors in 2 of 3 monkeys. The effects of a 1-mg/kg dose of flumazenil, both alone and in combination with selected doses of β -CCE, are also shown in Figure 4. In the 2 subjects receiving flumazenil (SQ D and B), 1 mg/kg alone had little or no effect on rate or accuracy of responding. When administered in combination with β -CCE, however, this same dose antagonized the rate-decreasing and error-increasing effects of β -CCE.

β -CCM. The top panels of Figure 5 show the effects of β -CCM in 2 monkeys responding under the tandem-strained condition. Similar to β -CCE under this condition, β -CCM produced dose-dependent decreases in response rate in each of the subjects and error-increasing effects that varied between subjects. For example, β -CCM produced a four to fivefold increase in the percentage of errors in SQ D, but it had little or no effect on errors in SQ B despite comparable decreases in response rate between the 2 monkeys. Figure 5 also shows that an ineffective dose of flumazenil antagonized both the rate-decreasing and error-increasing effects of two doses of β -CCM in SQ D.

FG-7142. The effects of FG-7142 in 3 monkeys responding under the tandem-strained condition are shown in Figure 5 (bottom panels). Similar to β -CCE and β -CCM, the higher doses of this negative GABA_A modulator produced decreases in response rate. FG-7142 was also similar to β -CCE and β -CCM in that it did not reliably increase errors across all of the subjects; that is, it increased the percentage of errors substantially in Monkey D and had little or no effect on the percentage of errors in subjects SQ B and A. Note that Monkey D was more sensitive to the error-increasing effects and less sensitive to the rate-decreasing effects of all three negative modulators. The effects of flumazenil (1 mg/kg) in combination with selected doses of FG-7142 are also shown in this figure. In subjects SQ D and B, 1 mg/kg of flumazenil antagonized the rate-decreasing and error-increasing effects of FG-7142.

Cumulative records in Figure 6 depict the within-session pattern of responding under the tandem-strained condition when β -CCE, β -CCM and FG-7142 were administered to Monkey B. The records in this figure repre-

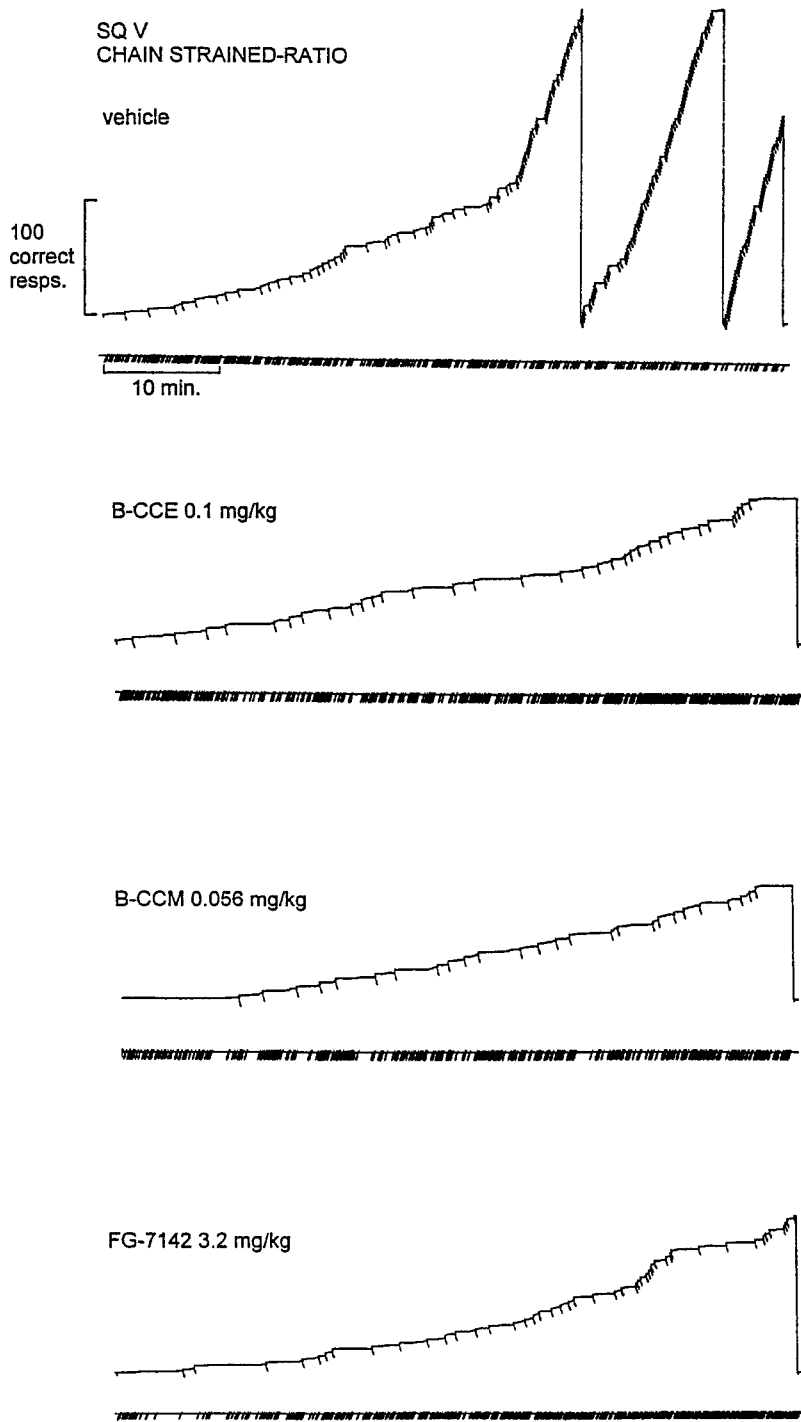


Fig. 3. Cumulative response records showing the within-session pattern of responding for Monkey V during a vehicle (control) session and sessions preceded by doses of β -CCE, β -CCM, and FG-7142. The doses depicted for each drug produced comparable rate-decreasing effects under the chain-strained condition. The record for the vehicle session approximated the mean values for both overall response rate and percentage of errors of all vehicle sessions. The response pen stepped up with each correct response and was deflected downward with each completion of the three-response chain. Every 75th deflection of the response pen indicates a completion of the chain and the delivery of a reinforcer. Errors are indicated by deflections of the event (lower) pen. Each session terminated after 60 reinforcers or 60 min, whichever occurred first.

TANDEM STRAINED-RATIO

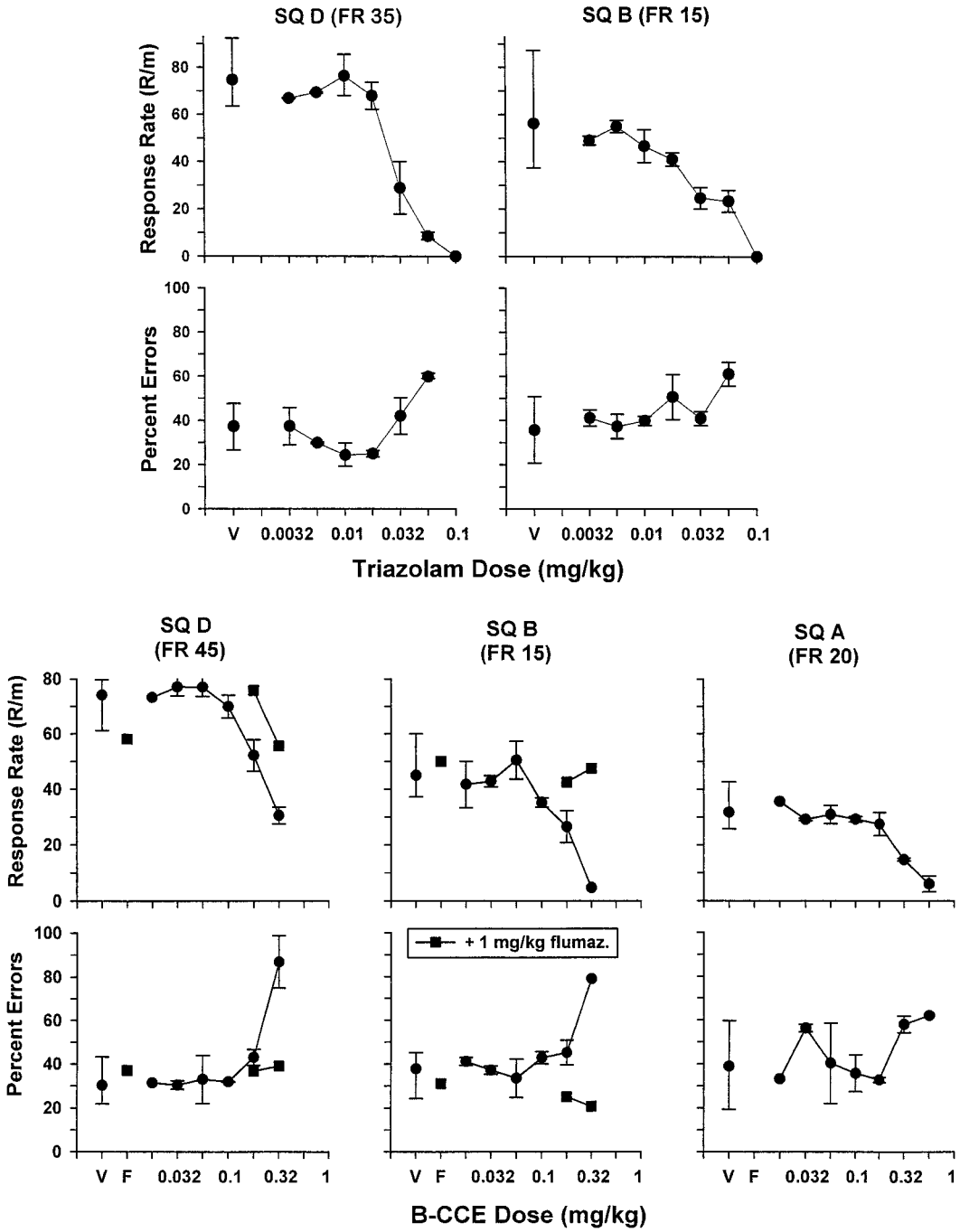


Fig. 4. Effects of triazolam (top panels) and β -CCE (bottom panels) on overall response rate and percentage of errors in squirrel monkeys responding under the tandem-strained baseline condition. The effects of 1 mg/kg of flumazenil in combination with β -CCE are also shown above F (bottom panel). Other details are the same as in Figure 1.

TANDEM STRAINED-RATIO

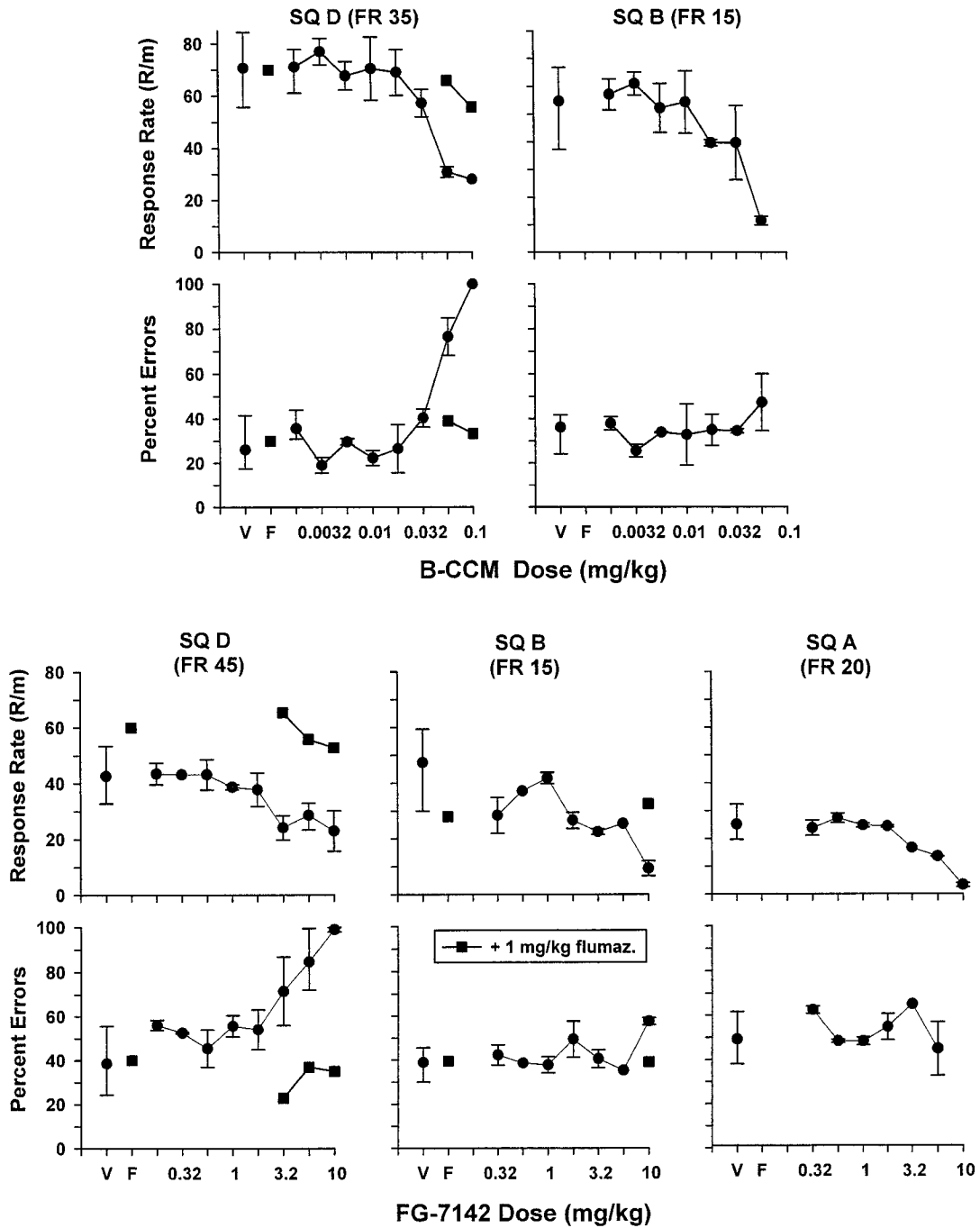


Fig. 5. Effects of β -CCM (top panels) and FG-7142 (bottom panels) on overall response rate and percentage of errors in squirrel monkeys responding under the tandem-strained baseline condition. The effects of both drugs in combination with 1 mg/kg of flumazenil are also shown (filled squares). Other details are the same as in Figure 1.

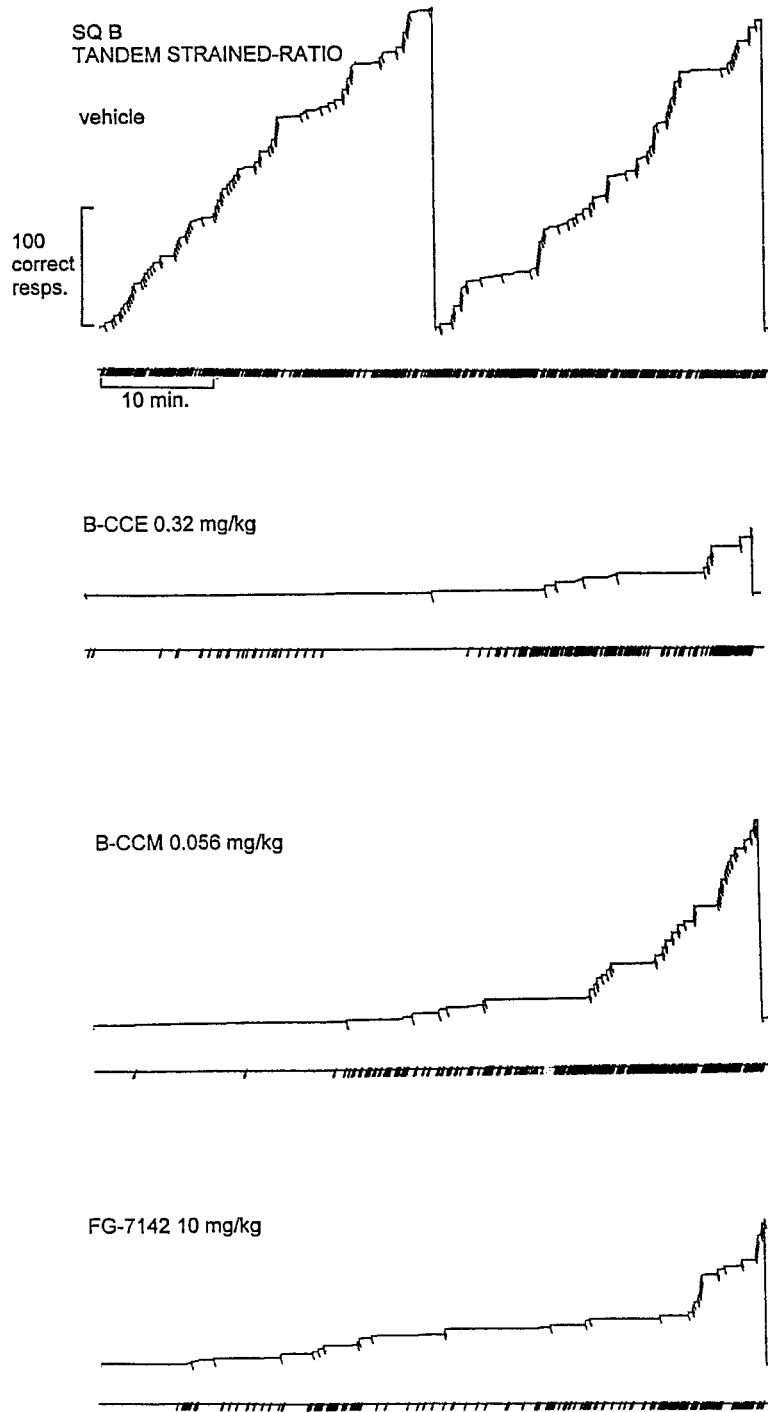


Fig. 6. Cumulative response records showing the within-session pattern of responding for Monkey B during a vehicle (control) session and sessions preceded by doses of β -CCE, β -CCM, and FG-7142. The doses depicted for each drug produced comparable rate-decreasing effects under the tandem-strained condition. For this subject, every 15th deflection of the response pen indicates a completion of the tandem sequence and the delivery of a reinforcer. Other details are the same as in Figure 3.

sent the effects of doses of each drug that decreased response rate to approximately 10 to 15 responses per minute. The control record at the top of the figure shows the characteristic pattern of responding for Monkey B when vehicle was administered before the start of the session. As indicated by this record, the tandem-strained condition generated higher error levels and fewer sequence completions than the chain-strained condition (cf. Figure 3). Nevertheless, similar to their effects under the chain-strained condition, all three negative modulators disrupted acquisition at each of the doses shown. In addition, the overall within-session effects of the drugs on acquisition were somewhat alike. Note that more pausing occurred under the tandem-strained condition than under the chain-strained condition after drug administration. Again, none of the negative modulators enhanced acquisition by decreasing the number of errors or increasing the rate of sequence completions under this baseline condition.

Response-Independent Tail-Shock Condition

Triazolam. Figure 7 shows the effects of triazolam in 4 monkeys responding on a repeated acquisition of behavioral chains procedure with concurrent response-independent tail shock. Under these conditions, triazolam had little or no effect on response rate except at the highest dose, which eliminated responding in 3 of 4 subjects (SQ G, J, and A). In addition, triazolam decreased errors in acquisition in every subject at one or more doses, which is in contrast to the error-increasing effects of triazolam obtained under the chain-strained and tandem-strained conditions.

The within-session effects of response-independent tail shock on the acquisition of response chains are shown in Figure 8 along with the effects of a single dose of triazolam (0.056 mg/kg) on responding under this baseline condition. As indicated by the records on the left side of the figure, response-independent tail shock reduced the number of consecutive correct responses and increased the density of errors compared to sessions when shock was not presented. Therefore, shock degraded acquisition, which can be characterized in terms of within-session error reduction. Note, for example, that in the absence of shock (upper left record) the

slope for the second excursion of the stepping pen is much greater than that for the first excursion, indicating that acquisition has occurred. In the presence of response-independent shock, however, the slope for the first and second excursions of the stepping pen are nearly identical, indicating little or no acquisition of the response sequence.

The records on the right side of Figure 8 depict how the within-session effects of the same dose of triazolam were dependent on the presence or absence of response-independent tail shock. In the absence of tail shock, 0.056 mg/kg of triazolam markedly slowed acquisition of the response sequence by increasing errors (top right record). Compared with the session where vehicle was administered, the error rate was high throughout most of the session, and the number of consecutive correct responses did not increase until almost 40 min after the start of the session. This effect was in direct contrast to the effects obtained with the same dose of triazolam when response-independent tail shock was presented. Under this baseline condition, the same dose of triazolam actually reduced the number of errors and increased the number of consecutive correct responses to the point where the within-session pattern of responding was similar to that observed in the absence of both shock and drug (compare record on the upper left with the record on the lower right).

Figure 9 shows the marked decrease in the percentage of errors that occurred over the course of the session when 0.01 or 0.056 mg/kg of triazolam was administered to 3 of the 4 monkeys responding under the chain schedule with response-independent tail shock. As shown, the 0.01-mg/kg dose decreased the percentage of errors in all 3 subjects by the second bin of sequence completions, whereas the 0.056-mg/kg dose only decreased the percentage of errors in subject SQ G as compared to the percentage of errors following vehicle administration. Similar to baseline sessions (not shown), the percentage of errors following vehicle administration showed little or no decline over the first six bins (120 sequence completions), which represented almost the entire session for subjects SQ G and J. Error levels did decrease in subject SQ D following vehicle administration, but this did not occur until the

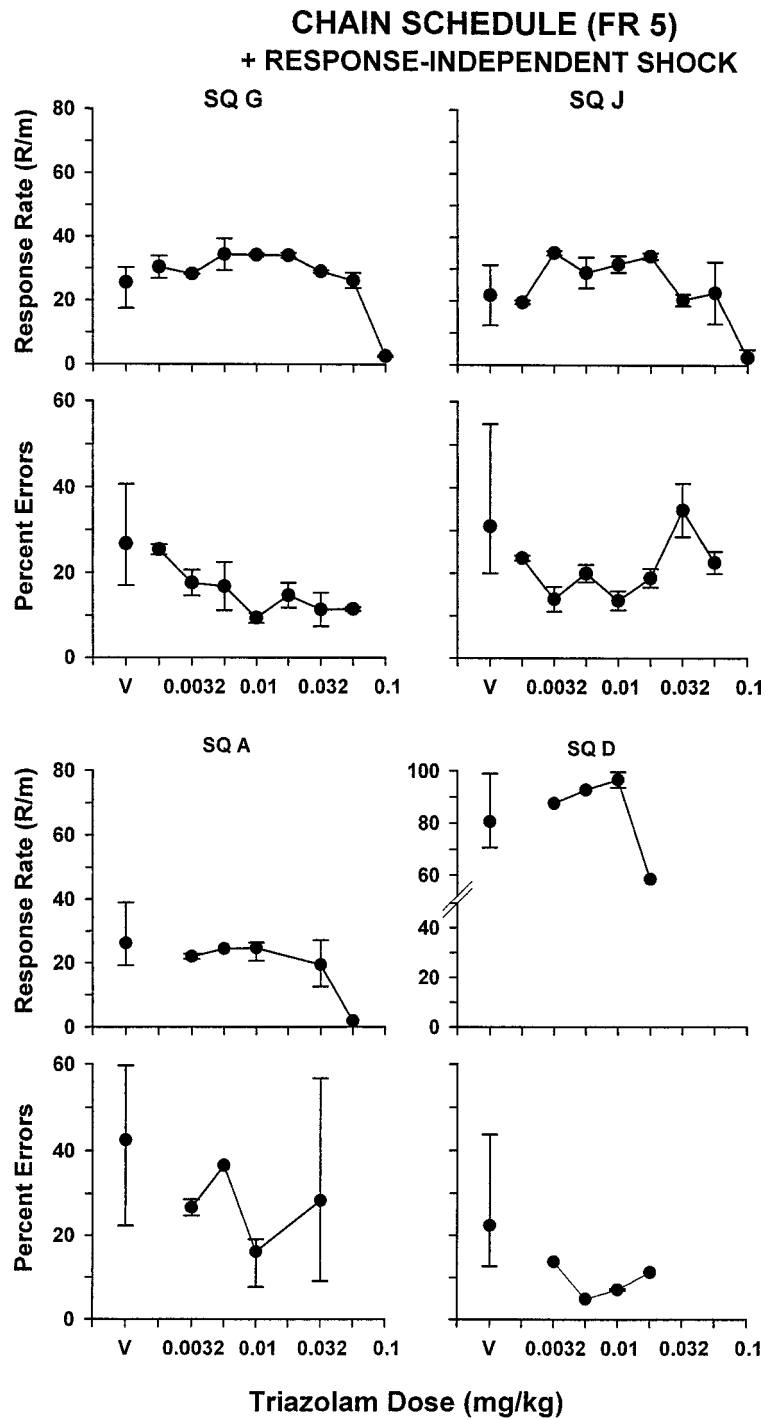


Fig. 7. Effects of triazolam on overall response rate and percentage of errors in 4 squirrel monkeys responding under the chain schedule with response-independent tail shock. Other details are the same as in Figure 1.

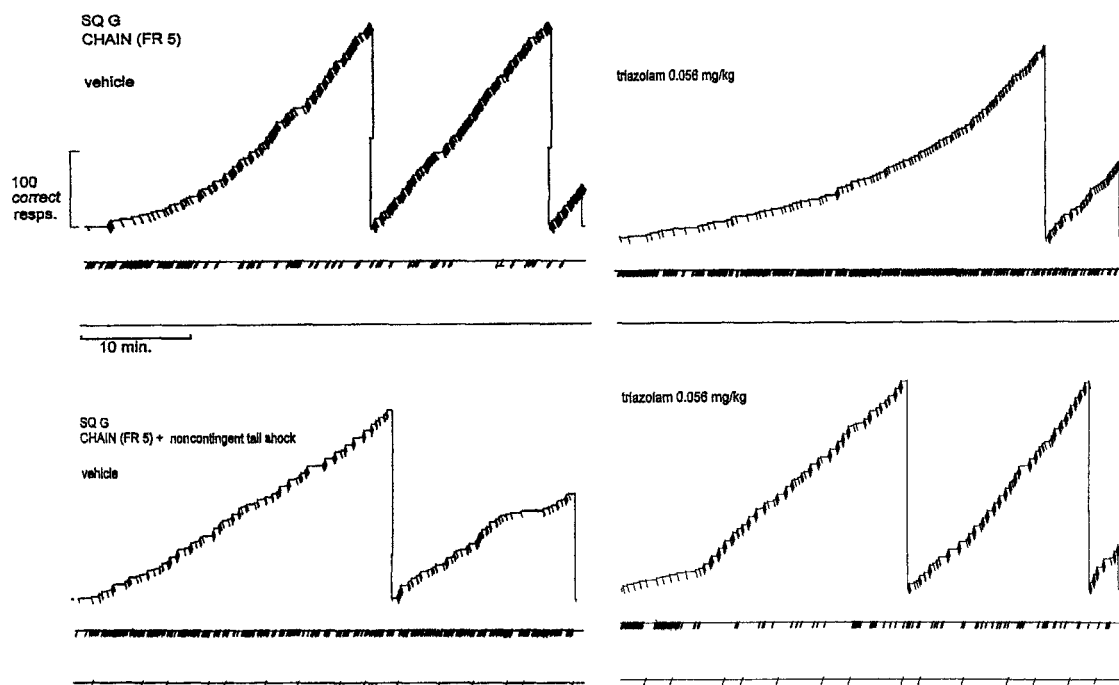


Fig. 8. Cumulative response records for Monkey G showing the within-session pattern of responding during a vehicle (control) session under a second-order FR 5 schedule, with and without response-independent shock (left panels), and sessions under both baselines that were preceded by an administration of 0.056 mg/kg of triazolam (right panels). For all the records in this figure, the stepping pen indicates correct responding and sequence completions, the pen below the stepping pen indicates errors, and the bottom pen indicates tail-shock presentations. Other details are the same as in Figure 3.

seventh bin, indicating that acquisition was only slowed by the response-independent shock during this session.

DISCUSSION

The present study examined the effects of both positive and negative GABA_A modulators on acquisition behavior (learning) using the technique of repeated acquisition of response sequences with squirrel monkeys. When subjects responded under either a chain-strained or tandem-strained condition, triazolam was found to produce rate-decreasing and small error-increasing effects. The disruptive effects of triazolam were comparable to those reported previously in squirrel monkeys (Winsauer et al., 2002), patas monkeys (Brocklehurst, Devia, Faust, & Moerschbaecher, 1987), and humans (Bickel et al., 1990) responding under similar repeated-acquisition procedures. For example, in patas monkeys responding under a multiple schedule of acquisition and performance of con-

ditional discriminations, triazolam produced dose-related decreases in response rates in both components of the multiple schedule. Furthermore, triazolam was found to disrupt accuracy in the learning component at doses lower than those required to disrupt performance (Brocklehurst et al., 1987).

Whereas triazolam decreased response rate and increased errors under the chain- and tandem-strained conditions, it had little or no effect on response rate prior to eliminating responding and decreased errors under the second-order chain schedule with response-independent tail shock. Given that acquisition was degraded under all three of the baseline conditions, the effects of triazolam under the response-independent shock condition did not appear to be dependent on the variability in the baseline or the relative density of reinforcement. In addition, the effects of triazolam on the shock baseline did not appear to result solely from the drug's ability to produce rate-dependent effects (e.g., Sanger & Blackman, 1981) or to increase punished

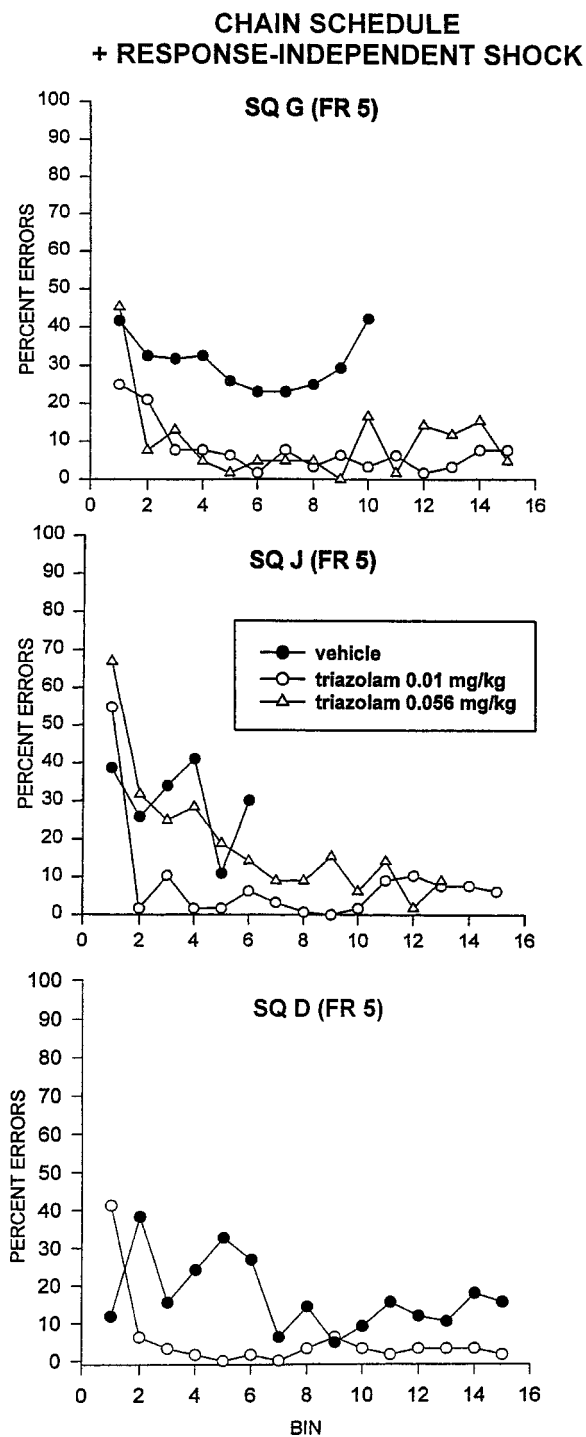


Fig. 9. Within-session distribution of errors for 3 subjects responding under a chain schedule with response-independent shock during a vehicle session or sessions preceded by 0.01 or 0.056 mg/kg of triazolam. Depicted in each panel is the percentage of errors plotted as a function of successive bins of 20 sequence completions. The second-order schedule of reinforcement was the same for each subject (i.e., FR 5).

responding (Barrett, 1976; Barrett & Katz, 1981; Paronis & Bergman, 1999; Sepinwall & Cook, 1980), because an increase in rate would not necessarily decrease the percentage of errors. Rather, these data suggest that the effects of triazolam (a positive GABA_A modulator) on acquisition behavior were determined, in part, by its interaction with other variables. Because triazolam is a benzodiazepine, there is also the possibility that the enhancement of responding in acquisition under the response-independent shock condition was due to its "anxiolytic" effects. Although establishing the reduction in anxiety as the predominant effect of triazolam is difficult under the present conditions, there are data with the positive modulators indicating that they can improve responding in learning and memory tasks in "anxious" humans (Desai et al., 1983; Hartley et al., 1982; Nakano et al., 1978; Parrott & Hindmarch, 1978).

Under the chain- and tandem-strained conditions, the negative GABA_A modulators, β -CCE, β -CCM, and FG-7142 disrupted the acquisition of the response sequences. In general, as the dose of each negative modulator increased, overall rates of responding decreased. The fact that β -CCE, β -CCM, and FG-7142 decreased response rate in all subjects is consistent with previous reports, which also have shown these drugs can produce rate-decreasing effects under FR schedules of food presentation, both in monkeys (Wettstein, Teeple, & Morse, 1993) and rats (Sannerud et al., 1993). For example, in rats responding under a FR-30 schedule of food presentation, FG-7142 was found to decrease response rate in a dose-dependent manner (Sannerud et al., 1993). Similar to FG-7142 in rats, β -CCE has also been reported to produce dose-dependent decreases in response rate in rhesus monkeys responding under a FR-30 schedule (Wettstein et al., 1993). The relative potency for producing rate-decreasing effects in the present study was β -CCM > β -CCE > FG-7142, the same rank order as observed for these compounds under other experimental conditions (Braestrup, Schmiechen, Neef, Nielsen, & Petersen, 1982; Corda, Blaker, Mendelson, Guidotti, & Costa, 1983).

Of specific interest in this study were the effects of β -CCE, β -CCM, and FG-7142 on accuracy of responding. Under the chain-strained condition, the percentage of errors

generally increased and there was less within-session error reduction; however, several differences were apparent among the drugs studied. For example, β -CCE produced dose-dependent error-increasing effects and these effects tended to occur at larger doses, which also produced rate-decreasing effects. FG-7142 was similar to β -CCE in that it also produced dose-dependent error-increasing effects. However, unlike β -CCE, these error-increasing effects occurred at doses that had little or no effect on rate of responding in 2 of the 3 monkeys. Interestingly, the effects of β -CCM also differed somewhat from both those of β -CCE and FG-7142, as β -CCM produced error-increasing and rate-decreasing effects only at the highest dose. In addition, the magnitudes of the error-increasing effects were smaller than those produced with β -CCE and FG-7142 in all 3 monkeys. These data would indicate that under the chain-strained condition, β -CCE and FG-7142 were more disruptive than β -CCM.

Under the tandem-strained condition, the effects of the negative modulators on accuracy were more variable among the subjects and fewer differences were apparent among the drugs. For example, FG-7142, which increased errors in all 3 monkeys under the chain-strained condition, only increased the percentage of errors in 1 monkey and had little or no effect in 2 other monkeys under the tandem-strained condition. Similarly, β -CCE appeared to be less disruptive to accuracy of responding under the tandem-strained condition because only the largest dose administered to each subject increased errors. That the tandem-strained condition was less sensitive than the chain-strained condition to the disruptive effects of the negative GABA_A modulators might, at first glance, seem inconsistent with the concept that difficult tasks are more sensitive to the disruptive effects of drugs than simple tasks (Dews, 1955; Thompson, 1974; Winsauer, Thompson, & Moerschbaecher, 1985). However, under the tandem-strained condition, the discriminative stimuli associated with each response in the sequence were removed and the keys were illuminated only with white light, which maintained higher error levels, reduced the number of correct sequence completions and produced less within-session acquisition than the chain-strained condition.

Given that there was minimal within-session acquisition under these conditions, the disruptive effects of the negative GABA_A modulators may have been obscured.

That the behavioral effects of many drugs can be modified by the presence or absence of external discriminative stimuli has been demonstrated previously (Katz, 1983; Laties, 1972, 1975). For example, two phenothiazines (chlorpromazine and trifluoperazine) were shown to have smaller effects on response rate in pigeons responding under a tandem FR schedule than under a chain FR schedule (Thomas, 1966). In another study, Thompson (1975) found acquisition of tandem response sequences to be less sensitive to the error-increasing effects of chlordiazepoxide and phenobarbital than acquisition of response chains in pigeons. In these studies, the results were attributed to differential stimulus control between the two conditions. For instance, in the Thompson (1975) study, error levels were lower and there was less baseline variability under the "chain-learning" condition than under the "tandem-learning" condition. According to the law of initial value (Wilder, 1967), an error-increasing effect of a drug is more readily observed when the error levels under control conditions are low. Furthermore, the disruptive effect of a drug is more likely to be detected as the control variability decreases. This may explain why the chain-strained baseline condition was more sensitive than the tandem-strained condition to the error-increasing effects of the negative GABA_A modulators.

The disruptive effects obtained with the negative modulators under these two baseline conditions of repeated acquisition differ markedly from some previous studies that have reported enhanced learning after their administration (File & Pellow, 1988; Venault *et al.*, 1986). Two prominent differences, however, are that none of the previous studies characterized the effects of the negative GABA_A modulators on acquisition behavior using monkeys as subjects, and the majority of these studies did not generate complete dose-effect curves. Although a large number of techniques are used to study drug effects on the acquisition and retention of behavior (e.g., passive avoidance, active avoidance, mazes), the results from these behavioral tasks are sometimes difficult to interpret.

Changes in performance may occur for reasons other than those related to the drug's effect on acquisition. For example, in a passive-avoidance paradigm, the effects of the drug may be due to changes in locomotor activity, sensory ability, or physiological responses to stress rather than a selective effect on acquisition. Indeed, any of these factors may be responsible for the apparent discrepancy between the results obtained in this study and the previous studies. Given that negative GABA_A modulators, such as DMCM and FG-7142, have been shown to produce dose-dependent decreases in food consumption in rats (Cooper, 1987; Cooper, Barber, Gilbert, & Moores, 1985), one might also speculate that the anorectic effects of these drugs are responsible for the observed disruptions. However, decreases in deprivation level do not generally result in error-increasing effects, as Thompson and Moerschbaecher (1979) have shown that prefeeding patas monkeys prior to the repeated acquisition of response chains decreased rates of responding but did not increase errors.

Not surprisingly, the benzodiazepine receptor antagonist flumazenil, which is considered to be a neutral GABA_A modulator, antagonized both the rate-decreasing and error-increasing effects of selected doses of all three negative GABA_A modulators. Although flumazenil was not administered to all of the subjects under both conditions, these data indicate that the benzodiazepine site on the GABA_A receptor complex mediated the disruptive effects of the negative modulators. These results are consistent with numerous studies that examined the interaction of flumazenil and negative GABA_A modulators. In rhesus monkeys, for example, flumazenil has been shown to attenuate both the respiratory stimulant effects of β -CCE under both air and 5% CO₂, and the rate-decreasing effects of β -CCE on FR responding (Wettstein *et al.*, 1993). In a separate study (Wettstein, 1989), flumazenil also was shown to antagonize the rate-decreasing effects of FG-7142 in squirrel monkeys responding under a 3-min fixed-interval (FI) schedule of food presentation. Additionally, flumazenil has been shown to antagonize the suppressive effects of β -CCE in squirrel monkeys responding under a punishment procedure (Barrett, Brady, Witkin, Cook, & Larscheid, 1985), and antagonize

the discriminative stimulus effects of β -CCE in rhesus monkeys (Takada, Winger, Cook, Larscheid, & Woods, 1986).

In conclusion, whereas many of the effects of positive and negative GABA_A modulators have been found to be opposite in nature, the present results involving the chain-strained and tandem-strained conditions suggest that both types of modulator can disrupt acquisition behavior similarly in squirrel monkeys. These results conflict with several previous studies in rodents suggesting that the negative modulators are capable of enhancing learning. More research will be needed to determine if the differences reported between studies are due to species differences, the behavioral procedures used, or some combination of both. Regardless, the present data replicate and extend the principle that the stimulus conditions that maintain responding, along with other environmental events such as response-independent stimuli, are important determinants of a drug's effect on learning.

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