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Multiple Within-Day Conflict Testing to Define the Time Course of Anxiolytic Drug Effects

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COMMISSARIS, R. L., Z. XIE, P. J. NINICHUK AND V. L. MARKOVSKA. *Multiple within-day conflict testing to define the time course of anxiolytic drug effects.* PHARMACOL BIOCHEM BEHAV **53**(2) 369–377, 1996. – The present article describes a method for multiple within-day conflict testing to conduct drug treatment time course studies more efficiently. Groups of female Sprague–Dawley rats were trained for conflict testing in a standard one-session/day procedure [conditioned suppression of drinking (CSD)]. In this task, thirsty rats (24 h water-restricted) drink from a tube that is electrified only when a tone is on (approximately 20% of the 10-min session time). In Experiment 1 it was found that there was no significant variation in CSD conflict behavior when subjects were tested at 0600, 1200, or 1800 h using the traditional procedure of one test/day. In Experiment 2, subjects were assigned to treatment groups such that there were three 5-min test sessions per day and the test-retest interval was either 2, 4, or 6 h (centered around 1200 h). Test-retest intervals of 6 h (i.e., tests at 0600, 1200, and 1800 h) resulted in comparable levels of punished responding across the repeated within-day tests, whereas test-retest intervals of 2 h and, to a lesser extent 4 h, resulted in unequal within-day conflict behavior characterized by a greater number of shocks accepted and a greater volume of water consumed during the earliest test periods each day. In another group of rats, it was determined that conflict behavior sampled five times/day in 3-min sessions separated by a 3-h test-retest interval (i.e., tests at 0600, 0900, 1200, 1500, and 1800 h) also resulted in stable conflict behavior across the various within-day test periods. In Experiment 3, it was found that acute IP challenges with anticonflict treatments that exhibit either a long duration of action (phenobarbital: 40 mg/kg) or a significant delay to onset in addition to a long duration (MK-801: 0.20 mg/kg) yielded time course data comparable to those obtained using the traditional one test/day procedure. These findings indicate that the use of multiple within-day conflict testing can greatly increase the efficiency of these procedures, particularly when drug treatment timecourse information is desired.

Anxiolytics Conflict behavior Time course Phenobarbital MK-801 Anxiety Repeated testing procedures

CONFLICT paradigms have been used extensively as animal models for the study of anxiety and antianxiety agents. In these paradigms, animals are both rewarded and punished for the same behavior. For example, a lever press results in a food pellet and also a brief electric shock. Control (i.e., nondrug) conflict behavior is usually intermediate between that which would be produced by the reward alone and that which would be produced by the punisher alone. The validity of conflict procedures as models for the study of anxiety arises largely from the effectiveness of a variety of anxiolytic treatments to reduce this behavioral suppression and the ineffectiveness of a variety of nonanxiolytic treatments to affect this behavior [see (3)].

Many conflict tasks utilize electric shocks as the punisher to suppress behavioral responding. The Geller-Seifter procedure is a repeated measures operant conflict task (5). Although the Vogel punished drinking procedure (12) is most frequently used as a one-trial procedure, a modification of this procedure, the Conditioned Suppression of Drinking (CSD), is a repeated-measures punished drinking conflict procedure (4,8,9). Typically, subjects in the Geller-Seifter or CSD conflict tasks are tested once/day for many test days. Drug treatment test days usually are interspersed (usually one drug treatment/week) with vehicle test days and drug effects are determined using within-subjects statistical procedures.

The above-mentioned conflict testing procedures have proven to be effective for examining a range of doses of one

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or several agents following a brief pretreatment interval. However, these procedures are relatively cumbersome for time course determinations, requiring either multiple groups of subjects to examine different pretreatment intervals or multiple weeks of testing (at various pretreatment intervals) in the same subjects.

Following procedures originally described by Hanson et al. (6) and, more recently Wenger (13), the use of repeated within-day behavioral testing in combination with cumulative dosing has been used to expedite dose–response determinations in behavioral procedures. In these procedures, a test day consists of several test sessions of a relatively brief duration, usually 5–15 min; these test sessions are separated by a brief time out. Subjects are administered escalating doses of a test agent over the course of these multiple within-day test sessions. Thus, on each test day, a full dose–response curve for the effects of a particular drug can be determined in each subject. This modification has greatly increased the rate of acquisition of dose–response data in studies involving free operant behavior (1,13) as well as drug discrimination testing (15).

If the test-retest intervals were of the appropriate duration, multiple within-day behavioral testing procedures also could be used to examine the time course for the influence of a particular single treatment. Such a procedure would greatly increase the efficiency of data collection when the time course is unknown or when the time course is anticipated to be quite long. To date, there are no detailed reports on the use of multiple within-day testing with conflict paradigms. Therefore, the present studies were designed to examine the utility of multiple within-day conflict testing using the CSD conflict paradigm. Specifically, two types of studies were conducted: 1) studies examining baseline (i.e., nondrug) conflict behavior as a function of various multiple within-day test schedules (e.g., one, three, or five tests/day at various test-retest intervals), and 2) studies comparing the time course for the effects of two anticonflict treatments (phenobarbital, MK-801) determined using a traditional one test/day procedure and multiple tests/day procedures.

GENERAL METHOD

Subjects

The subjects were female Sprague–Dawley rats from Charles River Farms (Portage, MI) and weighed 200–225 g at the start of the experiment. They were housed two to four per cage in a vivarium with a regulated light cycle (lights on 0700–1900 h) and constant temperature and humidity. All subjects received food ad lib; access to water was restricted (see the Procedure section below).

Apparatus

The apparatus was a standard rodent behavioral test chamber (Coulbourn Instruments, Inc., Model #E10-10) equipped with a drinking tube and a grid floor. The drinking tube was calibrated to allow for measurement of water intake to the nearest 0.5 ml. The rods of the grid floor were made electrically continuous and were connected to one lead of a two-pole shocker (Coulbourn Instruments Inc., Model E13-02); the second lead of the two-pole shocker was connected to the metal drinking tube.

General Procedure—Conditioned Suppression of Drinking (CSD) Conflict Task

Acclimation. For the first five sessions, water-restricted (24 h without water) subjects were placed in the test apparatus and were allowed to drink freely from the tube. These 10-min sessions occurred at the same time of day (1300–1500 h) Mon–Fri. The purpose of these NO SHOCK-NO TONE sessions was to acclimate the subjects to the test apparatus and the schedule of restricted access to water.

CSD conflict sessions. After the acclimation period, the conflict schedule was introduced and was maintained for the duration of the experiment. As with the acclimation period, initially the test sessions were 10-min in duration at the same time of day (1300–1500 h) Mon–Fri. These conflict sessions were characterized by periods in which licking of the drinking tube either 1) was rewarded by water only or 2) was simultaneously rewarded by water and punished by electric shock. The presence or absence of a TONE signaled the schedule that was in effect. Licking during the NO TONE condition resulted in water only (no shock); these NO TONE periods accounted for 80% of the total test session time. Periods where licking was punished were signaled by the presence of a tone. During the first 2 s of these TONE ON periods, licking behavior was not punished (warning period); during the latter 5 s of the TONE ON periods, tube contact resulted in the delivery of an electric shock (0.5 mA) to the mouth of the rat. The duration of the shock was equal to the duration of tube contact (less than 150 ms). A total of 20 cycles of the TONE ON (7 s)-NO TONE (23 s) alternations occurred during each 10-min test session.

Hughes et al. [7] recently reported that a minimum deprivation of 21 h from the last ad lib access to water was required for the acquisition and maintenance of stable conflict behavior in a modified Geller-Seifter paradigm. Based on these results and to be consistent with previous studies using the CSD conflict paradigm (4,8,9), subjects in the present studies received NO ad lib access to water during the week, i.e., all water consumed on Mon–Fri occurred during CSD test sessions. All subjects were given ad lib access to water from Friday posttest until Sunday noon.

Specific Experiments Conducted

Experiment 1: Baseline CSD conflict behavior: Influence of time of day. Studies in Experiment 1 were designed to examine whether there existed a difference in CSD conflict baselines when animals were tested at different times during the day. Separate groups (n = 8/group) of subjects were trained and tested in the CSD conflict paradigm. These 10-min sessions were conducted Mon–Fri at either 0600, 1200, or 1800 h. Control CSD conflict data were gathered for 2 weeks; the data from the last week was used for analysis.

Experiment 2: Baseline CSD conflict behavior using multiple within-day testing. Following 3–5 weeks of control CSD conflict testing using the traditional one 10-min test/day, subjects were divided into groups for the purpose of examining baseline CSD conflict behavior with multiple within-day test schedules. All groups of subjects were tested in the CSD paradigm for a total of 15 min each day. The shock intensity and the TONE : NO TONE cycling were not altered. Three groups of rats were used to examine CSD conflict behavior during three 5-min tests each day; the test-retest interval within each day for these groups was 2, 4, or 6 h. Testing was centered around 1200 h for all groups. A fourth group of subjects was used to examine baseline CSD conflict behavior during five, 3-min tests each day; the test-retest interval for this group of subjects was 3 h and was centered around 1200 h. Subjects were tested in these multiple within-day sessions for 2 weeks; the data for the last 4 days of the last week were used for statistical analysis.

Experiment 3: Time course for anticonflict drug treatment effects using multiple within-day conflict testing. Following 3–5 weeks of control CSD conflict testing using the traditional one 10-min test/day, additional subjects were divided into groups for the purpose of examining the time course for the effects on conflict behavior of either 40 mg/kg phenobarbital (an agent with a long duration of action) or 0.2 mg/kg MK 801 (an agent with a long time to onset for maximal anticonflict effect).

Subjects in the phenobarbital time course experiment were tested in 3-min sessions, five times/day using a 3-h test-retest interval. Ten additional groups of subjects were tested using a more traditional one test/day 15-min session (testing at 0600, 0900, 1200, 1500, or 1800 h) and served as the reference controls. All subjects received IP injections of either saline or 40 mg/kg phenobarbital 30 min before 0900 h on Wednesday. This 0830 h injection served as a 30-min pretreatment for the 0900 h test, a 3.5-h pretreatment for the 1200 h test, a 6.5-h pretreatment for the 1500 h test, and a 9.5-h pretreatment for the 1800 h test. Data for the effects of phenobarbital or saline at pretreatment intervals from 21–33 h were obtained on the Thursday test sessions in the same subjects.

Two groups of subjects in the MK-801 time course study were tested in 5-min sessions, four times/day using a 6-h test-retest interval; the fourth test session (2400 h) was included in this study to obtain data following the 18-h and 42-h pretreatments. Eight additional groups of subjects, tested using a single daily 15-min test session (testing at 0600, 1200, 1800, or 2400 h) served as controls. Subjects were injected IP with 0.2 mg/kg MK-801 or saline, 30 min before 0600 h on Wednesday. This 0530 h injection served as a 30-min pretreatment for the 0600 h test, a 6.5-h pretreatment for the 1200 h test, a 12.5-h pretreatment for the 1800 h test, and an 18.5-h pretreatment for the 2400 h test). Data for the effects MK-801 or saline at pretreatment intervals from 24.5–42.5 h were obtained on the Thursday test sessions in the same subjects.

Statistical Analyses

The number of shocks received/session is the measure of punished responding in the CSD conflict task; the volume of water consumed/session is the measure of unpunished responding. The data regarding these two dependent variables were analyzed separately. For the data from Experiment 1, the data were analyzed by 3 × 4 factorial ANOVAs with repeated measures; the main effects were time of testing (0600, 1200, and 1800 h) and test day (Tue, Wed, Thur, and Fri). The data from Experiment 2 were analyzed by separate factorial ANOVAs for each group of rats (i.e., each test-retest interval); for example, the data from the study examining CSD conflict behavior using three 5-min tests/day using a 2-h test-retest interval were analyzed using a 3 × 4 factorial ANOVA with main effects of time of testing (1000, 1200, and 1400 h) and test day (Tue, Wed, Thur, and Fri).

For the data on the effects of phenobarbital or MK-801 treatment on conflict behavior, the data from the multiple within-day test procedures and the single test/day procedures were analyzed separately. Pretreatment baselines (Tuesday data) in the phenobarbital study were evaluated using a 2 × 5 factorial ANOVA with main effects of time of testing (five levels) and treatment (two levels). The effects of phenobarbital or saline treatment on shocks received were compared using a 2 × 2 × 5 factorial ANOVA with main effects of test day (two levels; Wednesday or Thursday), treatment (two levels), and time of testing (five levels). Pretreatment baselines (Tuesday data) in the MK-801 study were evaluated using 2 × 4 factorial ANOVA with main effects of time of testing (four levels) and treatment (two levels). The effects of MK-801 or saline treatment on shocks received were compared using a 2 × 2 × 4 factorial ANOVA with main effects of test day (two levels; Wednesday or Thursday), treatment (two levels), and time of testing (four levels). Post hoc comparisons following ANOVAs were conducted using the Student–Newman–Keuls (SNK) test. In all statistical comparisons, $p < 0.05$ was used as the criterion for statistical significance (11).

Drugs

Phenobarbital sodium was purchased from Sigma Chemical (St. Louis, MO); (+) MK-801 hydrogen maleate was purchased from Research Biochemicals Incorporated (RBI; Natick, MA). All doses refer to the salts; both drug and vehicle treatments were dissolved in saline and were injected intraperitoneally (IP) in a volume of 1 ml/kg body weight.

RESULTS

Experiment 1: Baseline CSD Conflict Behavior: Influence of Time of Day

Historically, subjects accept approximately 30–50 punished responses and consume approximately 10–12 ml water in control (i.e., nondrug) CSD conflict test sessions using the standard 10-min test session and a 0.5 mA shock intensity (4,9,14). Consistent with these previous reports, Fig. 1 illustrates that subjects in the present studies accepted approximately three to four shocks/min and consumed 1.0–1.5 ml/min during standard CSD conflict test sessions. The values for punished and unpunished responding are presented using rates for the purpose of comparing across the test conditions of unequal duration. Figure 1 also illustrates that there was no difference in these parameters for subjects tested at 0600, 1200, or 1800 h. Statistically, for both shocks received and water intake, the main effects for time of testing [shocks: $F(2, 14) < 1.0$, NS; water: $F(2, 14) = 1.21$, NS], the main effects for test day [shocks: $F(3, 21) = 1.36$, NS; water: $F(3, 21) < 1.0$, NS], and the time of testing × test day interactions [shocks: $F(6, 42) < 1.0$, NS; water: $F(6, 42) < 1.0$, NS] were not significant. It should be noted that, when compared to unpunished licks (i.e., during the NO TONE periods—approximately 200–300/min), licks during the TONE ON condition was an insignificant contribution to water intake in the CSD test sessions; thus, water intake accurately reflects unpunished responding.

Experiment 2: Baseline CSD Conflict Behavior During Multiple Within-Day Testing

Figure 2 illustrates baseline conflict behavior obtained using the multiple within-day test procedure of three 5-min tests/day when the test-retest interval was 2, 4, or 6 h. The left panels illustrate the data obtained using this three tests/day procedure and a 2-h test-retest interval. As can be seen, subjects accepted considerably more shocks and drank considerably more water in the earliest test period and far less during the last test period. Statistically, these observations were supported by significant main effects for time of testing [shocks: $F(2, 77) = 12.21$, $p < 0.05$; water: $F(2, 77) = 6.13$, $p < 0.05$]; the main effects for test day [shocks: $F(3, 77) = 1.16$, NS; water: $F(3, 77) < 1.0$, NS], and the time of testing × test day interactions [shocks: $F(6, 77) < 1.0$, NS; water: $F(6, 77) = 1.36$, NS] were not significant. Post hoc Student–Newman–Keuls tests revealed that, compared to the values ob-

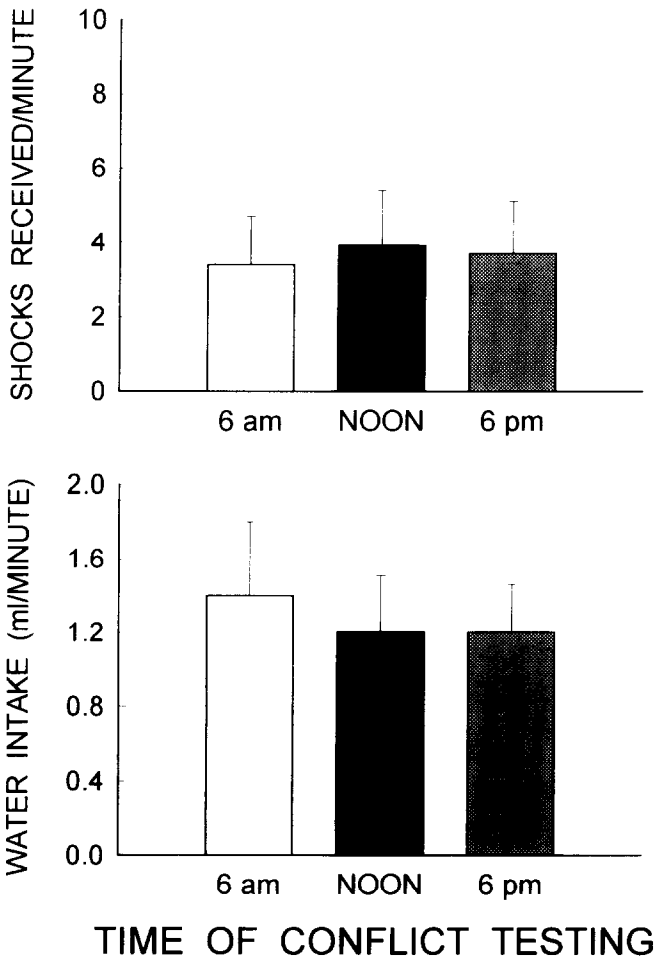

FIG. 1. Lack of influence of time of testing on control conflict behavior. Plotted are the values for shocks received (punished responding) and water intake (unpunished responding) in the conditioned suppression of drinking conflict task when subjects are tested in 10-min sessions at 0600 h, 1200 h, and 1800 h. Values represent the mean ± SEM obtained from eight subjects/group. No significant differences were found.

tained at 1200 h, shocks accepted and water intake were greater at 1000 h; water intake at 1400 h was significantly less than at 1200 h.

The middle panels of Fig. 2 illustrate baseline CSD conflict behavior when three 5-min tests/day were used and the test-retest interval was 4 h. As can be seen, subjects accepted more shocks and drank more water in the earliest test period and less during the last test period. The magnitude of these differences was not as great as was observed at the 2-h test-retest interval. Statistically, these observations were supported by significant main effects for time of testing [shocks: $F(2, 77) = 2.84$, $p < 0.05$; water: $F(2, 77) = 3.63$, $p < 0.05$]; the main effects for test day [shocks: $F(3, 77) < 1.0$, NS; water: $F(3, 77) < 1.0$, NS], and the time of testing × test day interactions [shocks: $F(6, 77) = 1.55$, NS; water: $F(6, 77) < 1.0$, NS] were not significant. Post hoc Student-Newman-Keuls tests revealed that, compared to the values obtained at 1200 h, shocks accepted and water intake were greater at 0800 h; water intake at 1600 h was significantly less than at 1200 h.

The right panels of Fig. 2 illustrate baseline CSD conflict behavior when three 5-min tests/day are used and the test-retest interval was 6 h. As can be seen, the use of three 5-min tests/day at a 6-h test-retest interval resulted in relatively stable levels of conflict behavior across the three test sessions within each day. Statistically, for both shocks received and water intake, the main effects for time of testing [shocks: $F(2, 77) = 1.25$, NS; water: $F(2, 77) = 1.55$, NS], the main effects for test day [shocks: $F(3, 77) < 1.0$, NS; water: $F(3, 77) = 1.48$, NS], and the time of testing × test day interactions [shocks: $F(6, 77) < 1.0$, NS; water: $F(6, 77) < 1.0$, NS] were not significant.

Figure 3 depicts baseline CSD conflict behavior when testing was conducted using five 3-min tests/day and a 3-h test-retest interval. As can be seen, baseline conflict behavior was stable across the five test sessions within the day. Statistically, for both shocks received and water intake, the main effects for time of testing [shocks: $F(4, 28) = 1.41$, NS; water: $F(4, 28) = 1.20$, NS], the main effects for test day [shocks: $F(3, 21) < 1.0$, NS; water: $F(3, 21) = 1.45$, NS], and the time of day × test day interactions [shocks: $F(12, 81) < 1.0$, NS; water: $F(12, 81) = 1.61$, NS] were not significant.

Experiment 3: Time Course for Anticonflict Drug Treatment Effects Using Multiple Within-Day Conflict Testing

Figure 4 illustrates the time course for the effects of 40 mg/kg phenobarbital or saline on CSD conflict behavior when conflict testing was conducted using the traditional single daily test (left panel; 10 squads of rats were used) or five 3-min test sessions/day using a 3-h test-retest interval (right panel; two squads of rats). In the experiment using the traditional single daily test session, control conflict behavior was comparable in the various groups prior to the administration of phenobarbital or saline [TUE data; main effects for treatment, $F(1, 70) = 2.59$, time of testing, $F(4, 70) < 1.0$, and the treatment × time of testing interaction, $F(4, 70) < 1.0$, were not statistically significant]. Saline treatment did not affect punished responding over the course of the 33 h of conflict testing after treatment. Phenobarbital administration resulted in a dramatic increase in punished responding as early as 30 min post-administration. The duration of the anticonflict effect of phenobarbital was approximately 24 h. Statistically, the main effects for treatment, $F(1, 70) = 67.22$, $p < 0.05$, time of testing, $F(4, 70) = 8.08$, $p < 0.05$, and test day, $F(1, 70) = 25.90$, $p < 0.05$, were significant, as were the treatment × time of testing, $F(4, 70) = 2.85$, $p < 0.05$, the treatment × test day, $F(1, 70) = 37.24$, $p < 0.05$, the time of testing × test day, $F(4, 70) = 7.11$, $p < 0.05$, and the treatment × time of testing × test day, $F(4, 70) = 7.49$, $p < 0.05$, interactions. Post hoc Student-Newman-Keuls tests revealed that subjects treated with phenobarbital accepted significantly more shocks when compared to saline-treated controls at 0.5, 3.5, 6.5, 9.5, and 21.5 h posttreatment in this one test/day procedure.

The right panel of Fig. 4 illustrates the effects of this phenobarbital challenge at the same pretreatment intervals using the within-day repeated measures conflict testing (five tests/day at 3-h intervals). Control conflict behavior was comparable in the two groups prior to the administration of phenobarbital or saline [TUE data; main effects for treatment, $F(1, 14) < 1.0$, time of testing, $F(4, 56) < 1.0$, and the treatment ×

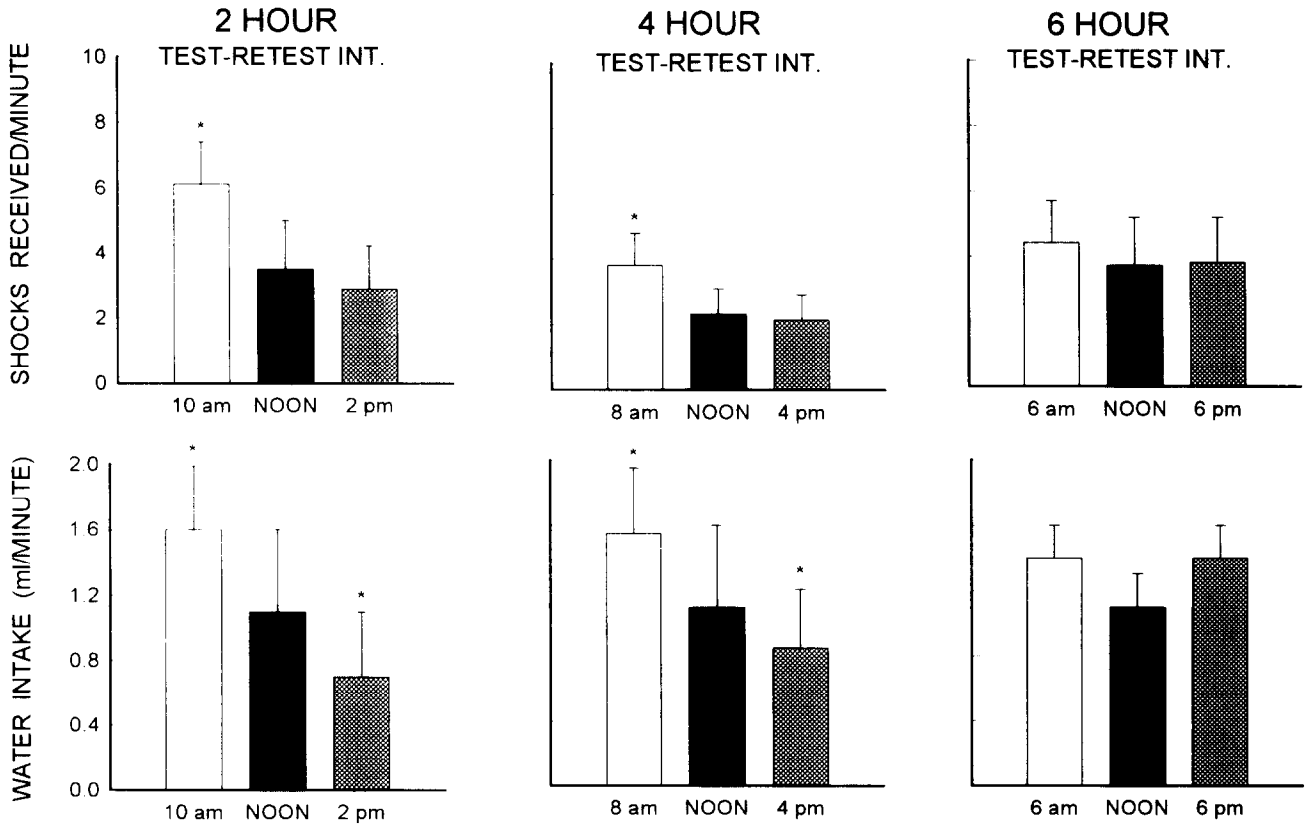

FIG. 2. Multiple within-day conflict testing baselines—influence of test-retest interval. Plotted are the values for shocks received and water intake in the conditioned suppression of drinking conflict task when subjects are tested three times/day using a 2-h (left panels), 4-h (middle panels), or 6-h (right panels) test-retest interval. Values represent the mean ± SEM obtained from eight subjects/group. Each test session was 5 min in duration. See Fig. 1 legend for further details. *The indicated value is significantly different from that obtained at 1200 h for the particular test-retest interval, $p < 0.05$, Student-Newman-Keuls post hoc test following 3 × 4 factorial ANOVA.

time of testing interaction, $F(4, 56) = 1.25$, were not statistically significant]. Saline treatment did not affect punished responding over the course of the 33 h of conflict testing after treatment. Phenobarbital administration resulted in a dramatic increase in punished responding as soon as 30 min postadministration. The duration of the phenobarbital anticonflict effect was approximately 24 h. Statistically, the main effects for treatment, $F(1, 14) = 111.72$, $p < 0.05$, time of testing, $F(4, 56) = 8.79$, $p < 0.05$, and test day, $F(1, 14) = 11.12$, $p < 0.05$, were significant, as were the treatment × time of testing, $F(4, 56) = 8.58$, $p < 0.05$, the treatment × test day, $F(1, 14) = 12.59$, $p < 0.05$, the time of testing × test day, $F(4, 56) = 3.49$, $p < 0.05$, and the treatment × time of testing × test day, $F(4, 56) = 5.28$, $p < 0.05$, interactions. Post hoc Student-Newman-Keuls tests revealed that subjects treated with phenobarbital accepted significantly more shocks when compared to saline- treated controls at 0.5, 3.5, 6.5, 9.5, 21.5, and 24.5 h posttreatment in this five tests/day procedure. In both test conditions, phenobarbital treatment produced a modest increase in water intake (i.e., 1.0–1.5 ml) relative to pretreatment baseline; this effect persisted for approximately 6–9 h postadministration for both test conditions (data not shown).

Figure 5 illustrates the time course for the effects of 0.20 mg/kg MK-801 or saline on CSD conflict behavior when conflict testing was conducted using the traditional single daily test (left panel; eight squads of rats were used) or four 5-min test sessions/day using a 6-h test-retest interval (right panel; two squads of rats). In the experiment using the traditional single daily test session, control conflict behavior was comparable in the various groups prior to the administration of MK-801 or saline [TUE data; main effects for treatment, $F(1, 56) = 1.68$, time of testing, $F(3, 56) < 1.0$, and the treatment × time of testing interaction, $F(3, 56) < 1.0$, were not statistically significant]. Saline treatment did not affect punished responding over the course of the 42.5 h of conflict testing after treatment. MK-801 treatment produced dramatic biphasic effects. At 0.5 and 6.5 h postadministration, MK-801 treatment dramatically reduced shocks received; this effect was associated with dramatic ataxia and significant decreases in water intake (data not shown). At pretreatment intervals from 12.5–36.5 h, MK-801 treatment increased punished respond-

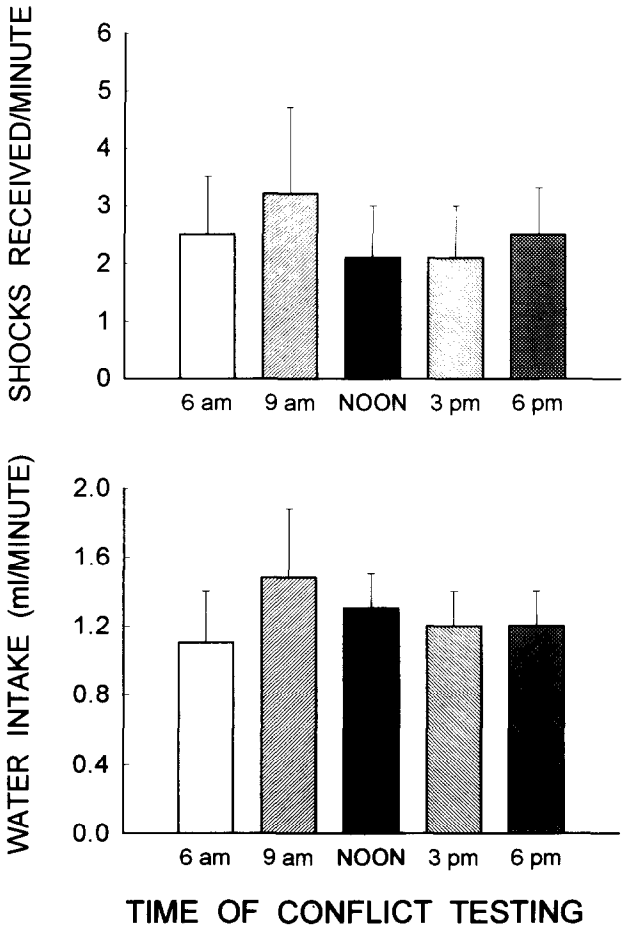

FIG. 3. Multiple within-day conflict testing baselines—five tests/day. Plotted are the values for shocks received and water intake in the conditioned suppression of drinking conflict task when subjects are tested five times/day using a 3-h test-retest interval. Values represent the mean ± SEM obtained from eight subjects/group. Each test session was three min in duration. See Fig. 1 legend for further details. Factorial ANOVA with repeated measures revealed no significant differences for either time of testing or test days.

ing; the time to peak effect was approximately 24 h. Statistically, the main effects for time of testing, $F(3, 56) = 5.70$, $p < 0.05$, and test day, $F(1, 56) = 24.17$, $p < 0.05$, were significant, whereas the main effect for treatment, $F(1, 56) = 1.43$, NS, was not. The treatment × test day, $F(1, 56) = 18.88$, $p < 0.05$, the time of testing × test day, $F(3, 56) = 20.50$, $p < 0.05$, and the treatment × time of testing × test day, $F(3, 56) = 15.86$, $p < 0.05$, interactions were significant, whereas the treatment × time of testing interaction, $F(3, 56) = 2.28$, NS, was not. Post hoc Student–Newman–Keuls tests revealed that subjects treated with MK-801 accepted significantly fewer shocks when compared to saline controls at the 0.5 and 6.5 h pretreatment intervals and significantly more shocks when compared to controls at 24.5, 30.5, and 36.5 h posttreatment.

The right panel of Fig. 5 illustrates the time course for the effects of 0.20 mg/kg MK-801 or saline on CSD conflict behavior when conflict testing was conducted using a within-day repeated measures conflict testing procedure characterized by four 5-min test sessions/day using a 6-h test-retest interval. Pretreatment conflict behavior was comparable in the various groups prior to the administration of MK-801 or saline [TUE data; main effects for treatment, $F(1, 14) < 1.0$, time of testing, $F(3, 42) < 1.0$, and the treatment × time of testing interaction, $F(3, 42) < 1.0$, were not statistically significant]. Saline treatment did not affect punished responding over the course of the 42.5 h of conflict testing after treatment. MK-801 treatment again produced a dramatic and biphasic effect; at 0.5 and 6.5 h postadministration, MK-801 treatment dramatically reduced shocks received; this effect was associated with dramatic ataxia and significant decreases in water intake (data not shown). At pretreatment intervals from 12.5–36.5 h, MK-801 treatment increased punished responding; the time-to-peak effect was approximately 24 h. Statistically, the main effects for treatment, $F(1, 14) = 92.87$, time of testing, $F(3, 42) = 4.03$, $p < 0.05$, and test day, $F(1, 14) = 54.75$, $p < 0.05$, were significant. The treatment × test day, $F(1, 14) = 38.39$, $p < 0.05$, the time of testing × test day, $F(3, 42) = 39.02$, $p < 0.05$, and the treatment × time of testing × test day, $F(3, 42) = 37.58$, $p < 0.05$, interactions were significant, whereas the treatment × time of testing interaction, $F(3, 42) = 2.46$, NS, was not. Post hoc Student–Newman–Keuls tests revealed that subjects treated with MK-801 accepted significantly fewer shocks when compared to saline controls at the 0.5 and 6.5 h pretreatment intervals and significantly more shocks when compared to controls at 18.5, 24.5, 30.5,and 36.5 h posttreatment. In both test conditions, MK-801 treatment produced a biphasic effect on water intake relative to controls, with a dramatic reduction (approximately 10–12 ml) observed at the 0.5- and 6.5-h pretreatment intervals and a modest increase (approximately 1.0–1.5 ml) in water intake relative to baseline at the 12.5–24.5-h pretreatment intervals (data not shown).

DISCUSSION

Traditional repeated-measures conflict tests utilize a one test/day approach across many days. Although highly effective in examining a range of agents and doses when only a single pretreatment dose and interval are used, this one test/day technique can be somewhat inefficient with respect to conducting dose–response and/or time course studies. As described in the introductory paragraphs, the use of multiple within-day test sessions in combination with cumulative dosing has greatly increased the efficiency of dose–response data collection (1,6,13,15). The present studies demonstrate that stable levels of conflict behavior can be obtained using repeated within-day test procedures, and that such repeated within-day conflict test procedures can be used to more efficiently collect drug time course information.

With respect to the situation in which three 5-min test sessions were conducted each day, test-retest intervals of 2 h and, to a lesser extent 4 h, did not result in consistent levels of punished responding across the three test sessions each day. Under these conditions, subjects accepted more shocks during the first test session each day, an intermediate number of shocks during the noon-time test session, and the fewest shocks during the last test session of the day. Because there is no significant circadian variation in CSD conflict behavior that might account for this observation, these test conditions are likely to be undesireable for conducting time course stud-

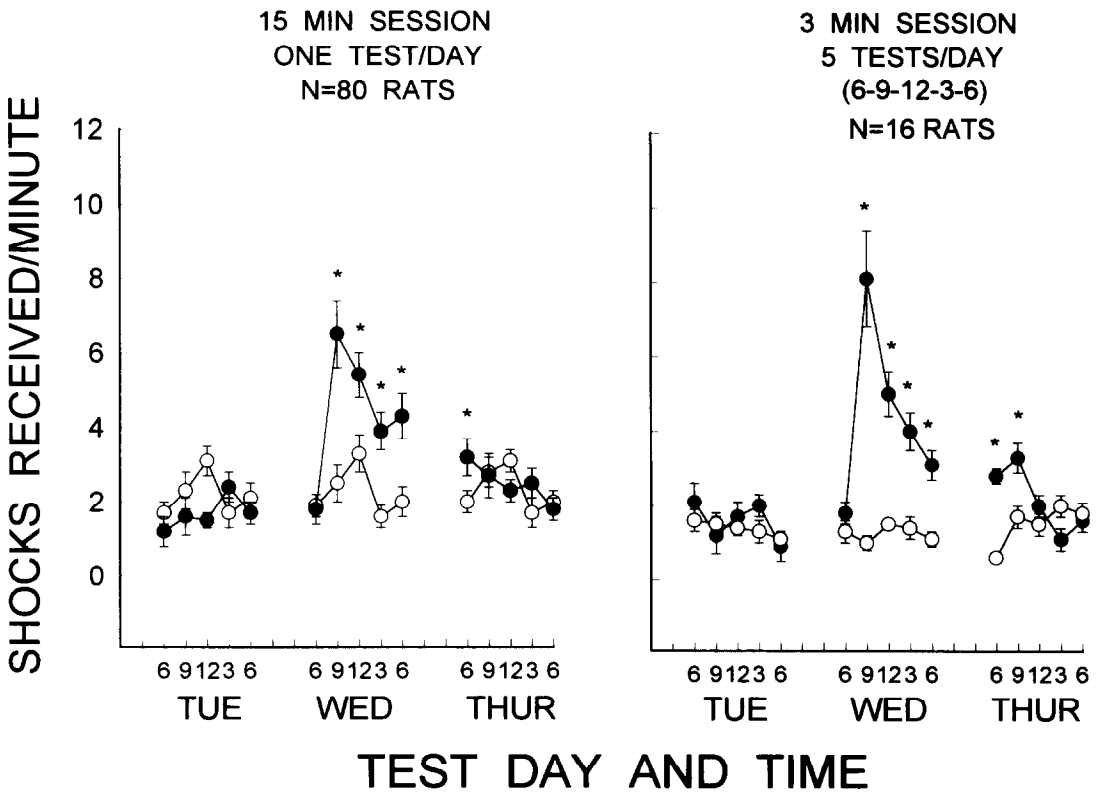

FIG. 4. Time course for the effects of phenobarbital on conflict behavior—traditional one test/day vs. multiple within-day test procedures. Plotted are the values for shocks received in the conditioned suppression of drinking conflict paradigm in subjects tested once/day at various times of day (left panel) vs. subjects tested five times/day at a 3-h test-retest interval (right panel). No drugs were administered on Tuesday (TUE); for all subjects, saline (open symbols) or 40 mg/kg phenobarbital (filled symbols) was administered IP 0830 h Wednesday. Values represent the mean ± SEM obtained from eight subjects/group. See Fig. 1 legend for further details. *Data from phenobarbital-treated subjects are significantly different from saline controls at the indicated pretreatment interval in the indicated test condition, $p < 0.05$, Student-Newman-Keuls post hoc test following 2 × 2 × 5 factorial ANOVA.

ies. When 15 min of CSD conflict testing was distributed evenly over the course of 12 h each day (either three times/day using a 5-min session and a 6-h test-retest interval or five times/day using a 3-min test session and a 3-h test-retest interval), relatively consistent levels of punished responding were observed across the various sessions conducted within each day.

The reduction in shocks received and water consumed at the second and third test sessions when testing was conducted using the 2-h test-retest interval may reflect relative satiety at the 1200 h and 1400 h test sessions following the 5-min session at 1000 h. Whether a 2-h test-retest interval could be effectively used in conjunction with shorter, more frequent test sessions (e.g., seven sessions/day at 2 min/session) remains to be determined.

The apparent lack of a circadian variation in this behavior is somewhat surprising. It should be noted, however, that subjects are exposed to conflict testing repeatedly over the course of these experiments. It is possible that circadian variation may be more pronounced in an acute (i.e., nonlearned) conflict task such as the Vogel acute conflict task (12).

Acute challenges with phenobarbital and MK-801 produced relatively long- lasting but considerably different effects on conflict behavior. Consistent with its long duration of action in humans (10), phenobarbital treatment was characterized by an increase in shocks received shortly after administration, followed by a gradual reduction in this effect over time. Both the initial effect and the decline in anticonflict effect over time were comparable for subjects tested using the traditional one test/day procedure and a multiple within-day test procedure.

The time course for the effects of MK-801 on conflict behavior was quite different from that exhibited by phenobarbital. MK-801 administration produced a dramatic reduction in punished responding and water intake at the earliest test intervals, followed by an increase in shocks received at later intervals. This dramatically delayed onset to maximal anticonflict effect is similar to previous reports on the effects of this agent on conflict behavior (2,14) and was comparable for the traditional one test/day and the multiple within-day test procedures.

The use of multiple within-day conflict test procedures such as those described above could result in considerable savings in terms of the number of animals used, animal housing/care costs, and also personnel hours. Using the phenobarbital time course determination as an example, the multiple

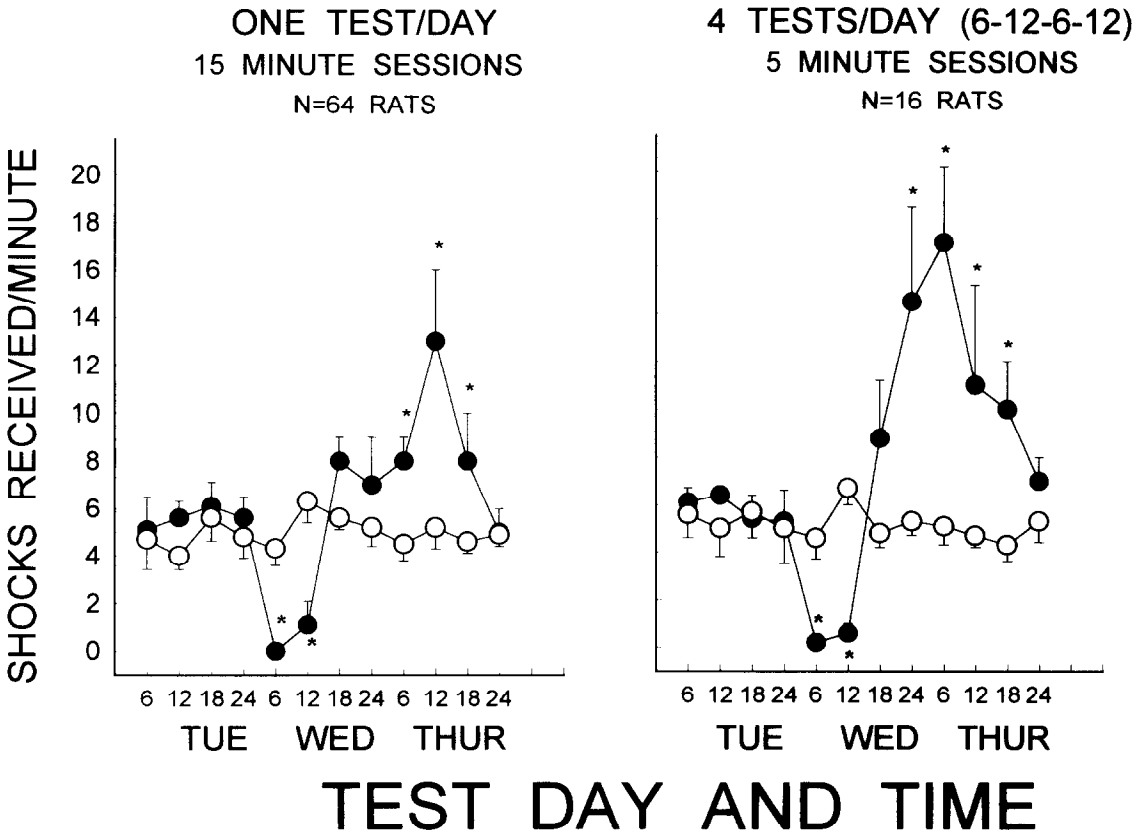

FIG. 5. Time course for the effects of MK-801 on conflict behavior—traditional one test/day vs. multiple within-day test procedures. Plotted are the values for shocks received in the conditioned suppression of drinking conflict paradigm in subjects tested once/day at various times of day (left panel) vs. subjects tested four times/day at a 6-h test-retest interval (right panel). No drugs were administered on Tuesday (TUE); for all subjects, saline (open symbols) or 0.20 mg/kg (+) MK-801 (filled symbols) was administered IP at 0530 h Wednesday. Values represent the mean ± SEM obtained from eight subjects/group. See Fig. 1 legend for further details. *Data from MK-801-treated subjects are significantly different from saline controls at the indicated pretreatment interval in the indicated test condition, $p < 0.05$, Student-Newman-Keuls post hoc test following 2 × 2 × 4 factorial ANOVA.

within-day conflict procedure utilized one-fifth the total number of subjects than did the single test/day procedure. Animal housing and maintenance costs also were reduced by this factor in the multiple within-day conflict procedure. Personnel hours also were dramatically reduced, although to a somewhat lesser extent because of the increased time for planning and logistical issues associated with the multiple within-day conflict procedure. We are currently experimenting with a procedure in which the test cage can also serve as the home cage and multiple within-day conflict sessions can be conducted in a fully automated manner. Such a modification would further reduce personnel costs.

In summary, both baseline conflict behavior and drug effect time course data obtained using the multiple within-day test procedures were comparable to those obtained using multiple squads of rats in the more traditional one test/day conflict procedure. Thus, the present findings indicate that the use of multiple within-day conflict testing can greatly increase the efficiency of these procedures, particularly when drug treatment time course information is desired.

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