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Amphetamine and Chlordiazepoxide Effects on Behavior under Increased Pressures of Nitrogen[1,2]

JOHN R. THOMAS

Behavioral Sciences Department, Naval Medical Research Institute, Bethesda, Maryland 20014

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THOMAS, J. R. *Amphetamine and chlordiazepoxide effects on behavior under increased pressures of nitrogen.* PHARMAC. BIOCHEM. BEHAV. 1(4) 421–426, 1973.–Rats trained on a multiple fixed-ratio (FR) spaced-responding (DRL) schedule for food reinforcement were exposed to hyperbaric pressures (equivalent to 100, 200, 250, and 300 feet of sea water) breathing compressed air. High rates of responding on the FR schedule decreased under pressure and low rates on the DRL schedule increased. Amphetamine and chlordiazepoxide produced dose-related accentuation of some rate change effects at depth as well as changes in performance at depth that were not predictable from the effects of the drugs at ambient pressure.

Hyperbaric pressure Inert gas narcosis Amphetamine Chlordiazepoxide Reinforcement schedules Rate-dependency

NITROGEN narcosis refers to the general behavioral and physiological changes which occur when an organism is exposed to raised pressures of air [2,7]. Such changes are observed when organisms breathe compressed air at elevated atmospheric pressures in a hyperbaric chamber or while diving to increased depths in the open water. Effects usually occur at pressures equal to depths deeper than 100–150 feet and show similarity to changes due to alcohol, hypoxia, and early stages of anaesthesia [1]. The term, nitrogen narcosis, has been used under the assumption that the narcotic or intoxicating-like effects on behavior are due to the high partial pressures of nitrogen. More recently the terms, compressed air narcosis [1] and inert gas narcosis [2], have been used. Measurement of the specific effects of increased pressures of air on behavior has generally been inconsistent and unclear, and the necessity for a behavioral analysis of the effects of nitrogen narcosis has been indicated [5]. A number of studies have approached the effects of hyperbaric conditions on behavior from a behavioral analysis viewpoint with rather high degrees of experimental control and fine-grain analysis of behavioral changes [4, 12, 13, 14, 15].

The present study is concerned with the use of such behavioral baselines to assess the effects of two pharmacological compounds on behavior at increased pressures. Knowledge of the behavioral effects of pharmacological agents under hyperbaric conditions is at present practically unknown. Although there have been a number of studies which have investigated pharmacological and chemical agents under increased pressures [e.g., 3,11], none of these studies has focused upon behavioral aspects. In the present study a multiple behavioral baseline containing both a fixed ratio (FR) or counting schedule and a spaced-responding (DRL) schedule was used to evaluate the effects of a range of raised pressures on the ongoing performance of an organism breathing compressed air and to evaluate the effects of two prototype drugs, amphetamine and chlordiazepoxide, on behavior under increased pressure.

METHOD

Animals

Two experimentally naive male albino rats (NMRI: 0[SD], Sprague-Dawley derived), approximately 60 days old at the start of the study, were maintained at 80% of their free-feeding weights.

Apparatus

The experimental chamber was a Harvard Instrument

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[2] The animals used in this study were handled in accordance with the provisions of Public Law 89-544 as amended by Public Law 91-579, the 'Animal Welfare Act of 1970' and the principles outlined in the 'Guide for the Care and Use of Laboratory Animals,' U.S. Department of Health, Education and Welfare Publication No. (NIH) 73-23.

Company rat cage, 9.5 in. long by 8.5 in. wide by 8 in. high. Two Lehigh Valley Electronics levers (LVE 121-03) were mounted on the front wall, 1 in. above the grid floor and 1 in. from either of the side walls. A brass food hopper (Scientific Prototype Co.) was mounted 0.5 in. above the grid floor and in the center of the front wall equidistant from each of the levers. The hopper was connected by a short tube to a Gerbrands pellet feeder which could dispense 45 mg Noyes pellets. A 12 VDC pilot light with a red lens cover was mounted 4.5 in. above the right lever and a pilot light with a blue lens cover was mounted the same distance above the left lever. A third pilot light with a yellow lens cover was mounted in the center of the front wall 1 in. from the top. During training and most baseline sessions the rat cage was mounted on slides inside a BRS-Foringer rat housing (RCH-001) which served as a ventilated sound-reducing enclosure.

All of the pressure or dive sessions and noise control sessions were conducted with the rat cage mounted on a set of slides inside a Bethlehem hyperbaric chamber. The chamber can withstand internal pressures of 1,000 pounds per square in. (psi), which is comparable to a simulated depth of 2,245 feet of sea water. The chamber is penetrated with several threaded openings for pressure-fitted connectors to the gas supply and the scheduling equipment. Across the upper interior surface of the hyperbaric chamber is a metal plate with heating and cooling coils which are thermostatically controlled to maintain a temperature of 23–27° C.

Scheduling and recording of sessions were accomplished by a system of solid-state digital logic modules. The gas mixture used during dive sessions was compressed air (i.e., nitrogen 78.1%, oxygen 20.9%, argon 0.9%, carbon dioxide 0.03%, other rare gases 0.003%).

Procedure

Scheduling and training. The animals performed in the experiment seven days a week over a nine-month period. The rats were trained by the method of successive approximation to press both of the levers to produce a food pellet. The rats were then exposed to a multiple fixed ratio, differential reinforcement of low rate schedule (mult FR DRL). The multiple schedule was such that when the pilot light above the right lever was illuminated and the pilot light on the top of the front wall was off, a fixed ratio (FR) schedule was in effect. On this schedule the rat was required to press the right response lever twenty times (FR 20) to produce a pellet. When the pilot light above the left lever and the light on the top front wall were illuminated, a spaced responding or differential reinforcement of low rate schedule (DRL) was in effect. On the DRL schedule a pellet was produced by a single response on the left lever which followed a preceding lever press on that lever by at least 18 sec. A limited hold (LH) contingency was added to the DRL such that a response could not produce a pellet if it followed a preceding response by more than 24 sec (limited hold of 6 sec).

The FR 20 and DRL 18 LH 6 schedules alternated with each other. Each schedule was in effect for 3 min. A blackout period of 30 sec during which all lights were off in the cage occurred between the termination of one schedule and the beginning of the other. A response on either lever during the blackout extended the blackout period by 30 sec. The two animals performed on the multiple baseline for 100 sessions before exposure to experimental manipulations.

Auditory control session. After stable baselines were obtained on the multiple schedule, each animal was exposed to a number of auditory control sessions with the rat cage mounted in the hyperbaric chamber to allow the animals to adapt to the noise of gas flow. Compressed air was allowed to flow into the chamber for a duration equivalent to that of an experimental dive. All chamber valves were left open so that ambient pressure was maintained and only noise level was manipulated.

Hyperbaric exposures. Both subjects were exposed to hyperbaric pressures equivalent to depths in feet of sea water while breathing compressed air. The four depths were 100 feet (44.5 pounds per square in. gauge pressure, psig), 200 feet (89 psig), 250 feet (111.3 psig), and 300 feet (133.5 psig). The animals were exposed to the four depths in different orders and in a semirandom sequence. At least 12 control baseline sessions occurred between successive dives. Compression rate to depth was 10 feet per min. Time at depth was one hour. Decompression rate from depth was 10 psi per min with 2-to-4 min stops at 80, 60, 30, 15, and 5 psi as appropriate for particular depths.

Drugs. After the above hyperbaric exposures, dose–response curves were obtained for two drugs, *d*-amphetamine sulfate and chlordiazepoxide hydrochloride, at ambient pressure and at a depth of 250 feet. The doses of amphetamine investigated were: 0.25 mg/kg, 0.5 mg/kg, 1.0 mg/kg, 2.0 mg/kg, and 2.5 mg/kg. The doses of chlordiazepoxide investigated were 1.0 mg/kg, 5 mg/kg, 10 mg/kg, and 20 mg/kg. The animals were exposed to the doses of each drug at surface and at depth in a semirandom sequence. Both drugs were dissolved in saline and injected intraperitoneally. During dive sessions drugs were administered right before the start of descent and a session was begun when the depth of 250 feet was reached. On drug only sessions the drugs were given 25 min before the start of a session and a drug session was one hour in duration. At least six baseline sessions occurred between any two drug sessions, and dive sessions with drugs were always separated by at least 14 baseline sessions. The volume of each injection was 0.1 m/100 g body weight. Occasional control saline injections were given.

RESULTS

Baseline

Performance on the mult FR 20 DRL 18 LH 6 became fairly stable for both animals as indicated by visual inspection of daily records in approximately 60 sessions. The mean response rates (resp/min) on the schedules for the two rats during the next 30 sessions were (ranges given in parentheses): Rat A: FR-53.6 (45.9–61.2), DRL-4.3 (3.0–5.4); Rat B: FR-119.1 (105.2–127.0), DRL-5.1 (3.8–5.8). Figure 3A shows a portion of a cumulative record from a baseline control session for Rat B. The auditory control sessions indicated that after several exposures the noise associated with gas flow in the hyperbaric chamber had no effect on baseline performance.

Hyperbaric exposures. Figure 1 shows changes on the mult FR DRL schedule as a function of the four different simulated depths. The data points are based on the response rate obtained at each depth expressed as a percent of the mean response rate obtained during control sessions. The mean control response rate of Rat A during this part of the

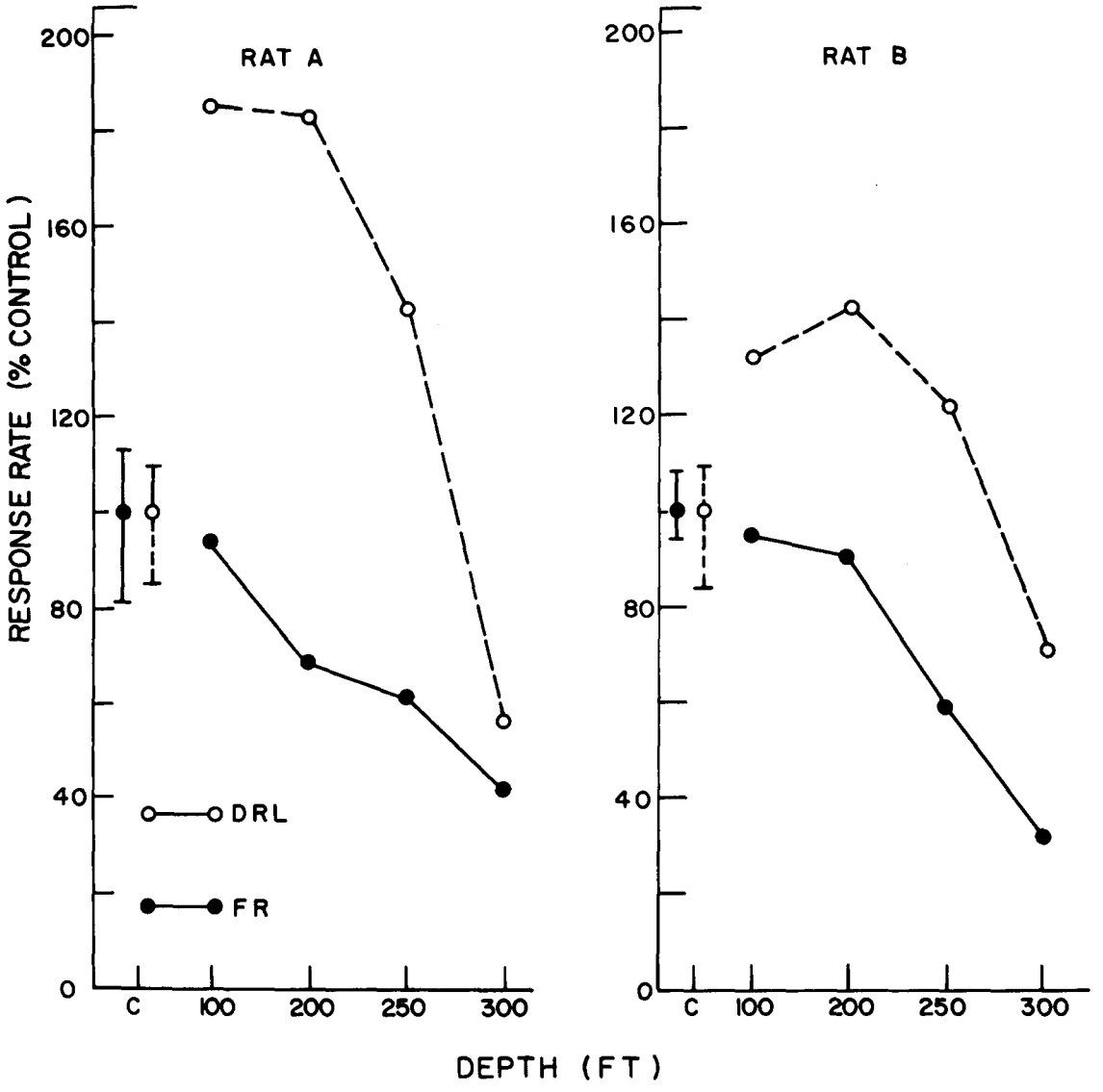

FIG. 1. Changes in response rate on multiple FR DRL schedule as a function of four simulated depths. Range of response rates during control sessions are indicated as brackets.

study was 52.0 resp/min for the FR and 4.8 for the DRL. The mean control response rate for Rat B was 117.7 for the FR and 5.1 for the DRL. Ranges for both schedules for all control sessions obtained between hyperbaric exposures are shown as brackets in the figure.

Both animals showed changes in response rates as a result of exposure to hyperbaric conditions. The direction of change from control rates depended on the schedule of reinforcement. Response rates on the FR schedule were generally decreased compared to control, whereas response rates on the DRL schedule were increased above control response rates (except at the 300-foot depth). Both animals generally showed a declining function for both schedules with increases in depth. Figure 3B shows a portion of a cumulative record of Rat B from the exposure to 250 feet with air.

Drug and Depth Effects

Figure 2 show changes on the multiple schedule as a function of drug dose for the two drugs at ambient pressure and at a depth of 250 feet. The data points are based on percent change from the mean control response rates obtained during control sessions between drug sessions or drug and hyperbaric sessions. Ranges of control rates for this part of the study are shown as brackets.

Amphetamine. As shown in the upper left of Fig. 2 the two lowest doses of amphetamine produced an increase in DRL response rate for Rat A above control values with other doses of amphetamine producing changes that were not outside control ranges. The same doses of amphetamine at 250 feet produced much higher rates of responding on the DRL schedule for Rat A than amphetamine at surface. The highest dose (2.5 mg/kg) under pressure produced a

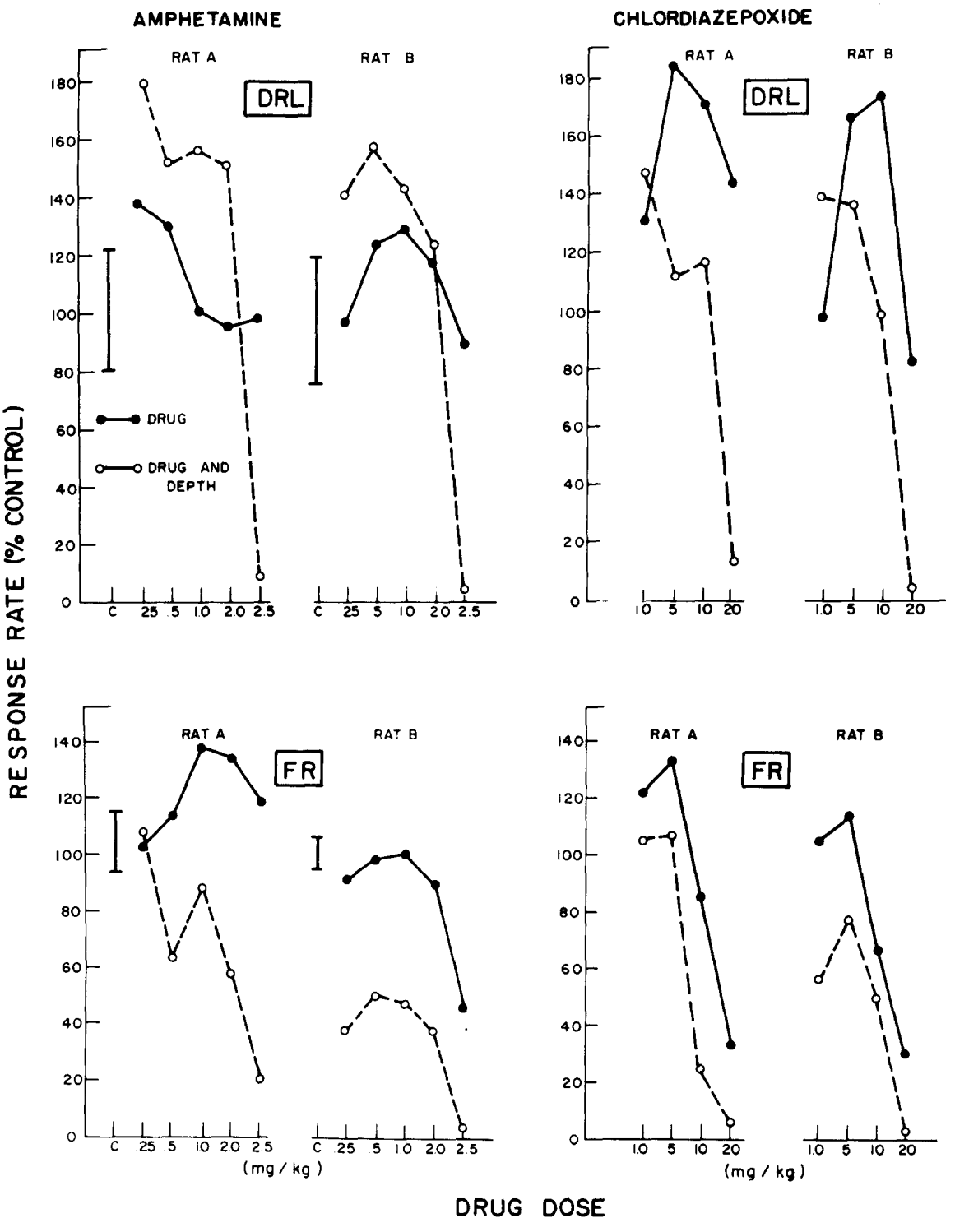

FIG. 2. Changes in response rate on multiple FR DRL schedule as a function of several doses of amphetamine (left portion of figure) and chlordiazepoxide (right portion) at surface and at a depth of 250 feet. Ranges of response rates during control sessions are indicated as brackets.

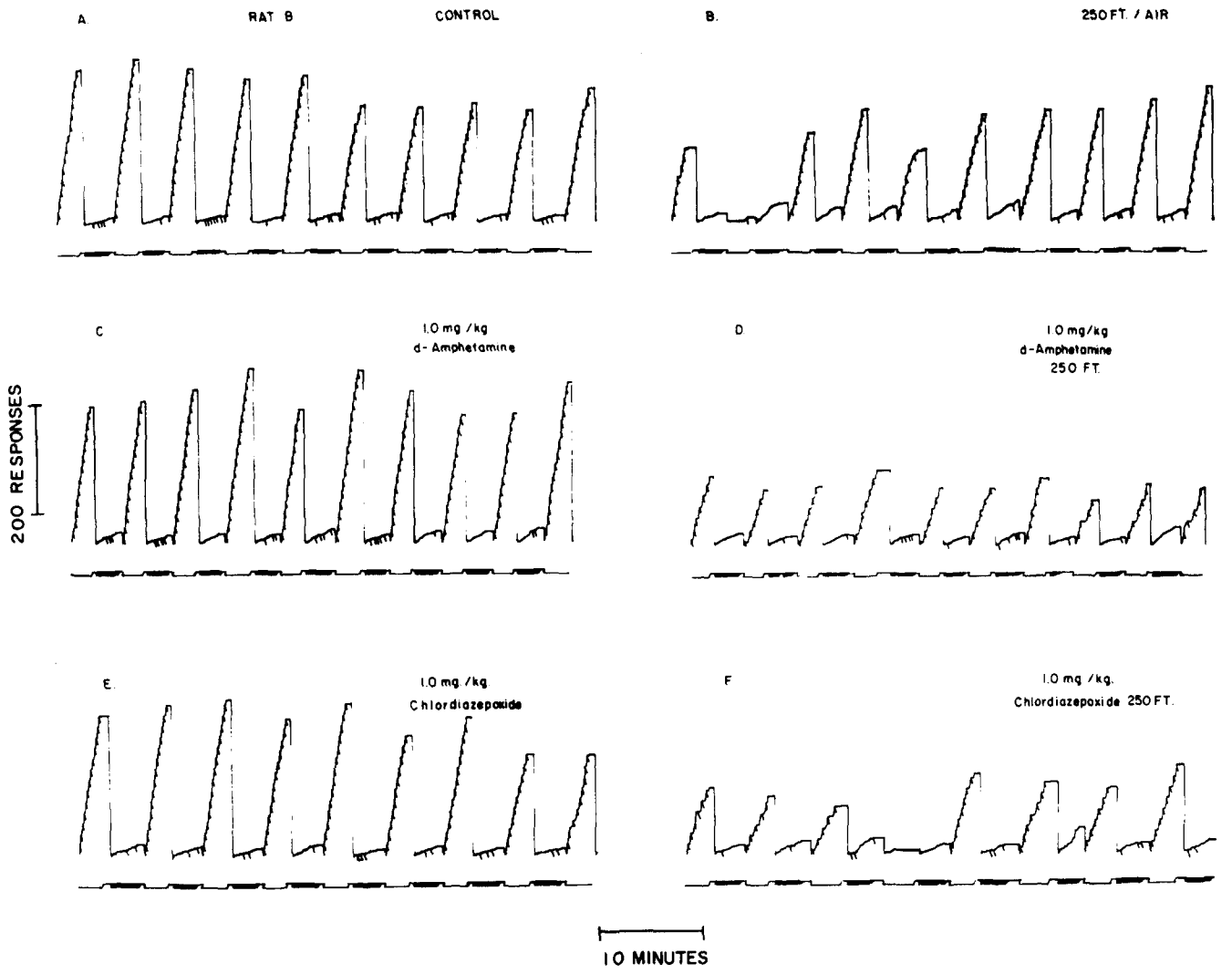

FIG. 3. Cumulative response records of Rat B from control session (A), at 250 feet (B), with amphetamine at surface (C), and at depth (D); with chlordiazepoxide at surface (E), and at depth (F). Each recorded response steps the recording pen upwards and pips indicate reinforcements. The recording pen resets at the end of the interval for each schedule. The event pen is down during the FR schedule and up with a dark heavy bar during the DRL schedule.

marked decline in DRL response rates. Similar effects were shown in rate changes on the DRL schedule for the other animal, although the pattern of the dose–response curve for the lower doses was somewhat different.

As may be seen in the lower left of Fig. 2, Rat A showed increases in FR response rates with amphetamine. However, the same doses at 250 feet produced a decline in FR response rates for this animal (except 0.25 mg/kg). The other animal, who had a much higher control response rate on the FR, generally showed a decline in FR rates as a function of amphetamine dosage. The same doses under depth produced even greater declines in FR response rate for this animal. Cumulative response records of Rat B with 1.0 mg/kg amphetamine at surface and at depth are shown in Fig. 3C and 3D.

Chlordiazepoxide. Increasing doses of chlordiazepoxide produced an increase followed by a decrease in DRL response rates for Rat A as shown in the upper right of Fig. 2. All four doses produced an increase above control values, however. Except for the 1.0 mg/kg dose DRL response rates were not increased for this subject by chlordiazepoxide at 250 feet. The 1.0 mg/kg dose produced an increase in DRL response rates at depth greater than at surface, however, all other doses produced lower DRL response rates at depth than at surface with the highest dose (20 mg/kg) producing the greatest decrement in behavior. Similar effects were shown by the other animal's DRL rates with both chlordiazepoxide and chloridazepoxide and depth.

The FR response rates of both rats showed an increase above control values followed by a decrease in rates as a function of dosage. The changes are presented in the lower right portion of Fig. 2. Chlordiazepoxide at depth produced lower rates on the FR schedule than chlordiazepoxide at surface for both animals. The dose–response pattern was very similar at both depth and ambient pressure. Cumulative response records showing performance on the multiple schedule with 1.0 mg/kg chlordiazepoxide both at sur-

face and at depth for Rat B are shown in Fig. 3E and 3F.

DISCUSSION

The present research indicated several aspects of performance under hyperbaric conditions. Over the range of depths from 100–250 feet lever responding was generally well maintained. At 300 feet responding was less well maintained. This indicates that the general motor coordination necessary to lever press was not seriously impaired by the large increases in absolute pressure or by the increases in the partial pressures of nitrogen.

The specific effects of increased pressure on behavior, however, apparently depend critically on the contingencies of reinforcement maintaining the behavior. Rate of responding under pressure changed differentially depending on the control baseline rates. High rates of responding generated by the FR schedule generally decreased under hyperbaric exposure, while low rate behavior generated by the DRL schedule generally increased. This type of rate-dependency or schedule-dependency changes in behavior under hyperbaric conditions is similar to previously reported effects [14,15].

Enhanced DRL response rates above control values found for several doses of amphetamine is consistent with earlier reports of the effects of amphetamine on temporally spaced responding [9,10]. Under increased pressures four out of five of the amphetamine doses investigated produced higher response rates on the DRL schedule than the same doses at ambient pressure. The largest amphetamine dose produced a decrement in DRL responding, markedly lower than control rates. It appears that the rate-increasing effects produced by lower doses of amphetamine on behavior maintained on DRL schedules are enhanced under increased pressure. The enhancement in DRL response rates for both subjects at the lower doses of amphetamine at depth was greater than the effects of depth alone (See Fig. 1) which suggests a synergism between amphetamine and increased pressures of nitrogen.

At ambient pressure one animal showed an increase in FR response rate over a range of amphetamine doses and the other animal showed a general decrement in FR response rate for the same dose range. Rat A, who had a rather low control FR response rate, showed the increase in rates as a function of drug dose and Rat B, who had a much higher rate, showed the decrease. Previous research has shown that the same dose of the same drug may produce either increases or decreases in response rate depending on the particular baseline rate [e.g., 6]. At increased pressure, amphetamine produced lower rates of responding on the FR schedule than the same doses at ambient pressures. This suggests that amphetamine under pressure produces a reduction in rate of responding on FR schedules independent of whether the drug produces increases or decreases in rate at ambient pressure.

Chlordiazepoxide produced increases in DRL rates above control values followed by decreases in rate as a function of dose. Similar increases in rate of responding on DRL schedules due to chlordiazepoxide have been reported previously [8]. All doses of chlordiazepoxide under pressure except the smallest produced lower DRL response rates than the same doses at ambient pressure. Rate of responding on the FR schedule also increased and then declined with chlordiazepoxide with increasing dosage. All doses of chlordiazepoxide at depth produced lower FR rates than the same doses at surface. Generally, the rate change effects of chlordiazepoxide at ambient pressure and at depth were similar for both reinforcement schedules.

The specific behavioral effects of hyperbaric conditions were found to depend on the particular baseline employed. Either increases or decreases in response rates may occur under increased pressures, depending on the baseline response rate. The differential behavioral effects at depth as a function of control rates suggest a conceptual relation to similar rate-dependency effects found for many pharmacological agents. The similarity in such rate-dependency effects, in addition to the demonstrated interactions between drug and pressure effects on behavior, suggests that performance changes under hyperbaric conditions may be viewed within a behavioral pharmacology framework. The present research also indicates that some drug effects on behavior are accentuated under increased pressures, whereas other drug effects on behavior under increased pressures are not predictable from behavioral effects of the same drug doses at ambient pressure.

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