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Reversal of nitrogen narcosis in rats by helium pressure

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Thomas, J.R. 1976. Reversal of nitrogen narcosis in rats by helium pressure. Undersea Biomed. Res. 3(3):249–259.—Changes in operant behavior were obtained for rats breathing nitrogen, helium, and both gases combined. Exposure to 12.9 ATA of nitrogen (O_2 maintained at normobaric value of 0.2 ATA) produced complete cessation of responding on a fixed-ratio reinforcement schedule for 1 hour. The addition of elevated pressures of helium (12.1, 18.2, and 24.2 ATA) to the 12.9 ATA of nitrogen resulted in a reinstatement of responding during 1-hour hyperbaric exposures. The reinstatement of responding indicated a reversal of the nitrogen effects on behavior by increased helium pressures. The functional changes in behavior appeared to parallel the pressure-produced reversal of anesthesia obtained at much higher pressures.

nitrogen narcosis
pressure reversal of narcosis
operant conditioning
nitrogen
hyperbaric performance
rats
helium

Pressure reversal of anesthesia has become an important area of theoretical and interdisciplinary research since its initial demonstration by Johnson and Flagler in 1950 (e.g. Lever, Miller, Paton, and Smith 1971; Miller, Paton, Smith, and Smith 1973; Roth, Smith, and Paton 1973; Stern and Frisch 1973; Trudell, Hubbell, and Cohen 1973; Trudell, Hubbell, Cohen, and Kendig 1973; Miller 1974). Most research studies to date have focused on such concerns as relative gas potencies and molecular models and aspects of such antagonisms. Of particular interest to hyperbaric researchers has been the work demonstrating that anesthetic effects produced by the breathing of high nitrogen partial pressures may be antagonized or reversed by exposure to higher pressures with an additional inert gas such as helium (e.g. Brauer and Way 1970; Lever et al. 1971). Generally, the anesthetic action of nitrogen (usually defined by loss of righting reflex in experimental animals) is obtained at about 20 to 30 atm. Further additions of helium pressures to these nitrogen pressures have reversed, at least partially, the effects of nitrogen.

The reversal of pressure-produced nitrogen anesthesia by the addition of helium has led to speculation about the possible reversal of nitrogen narcosis at much lower pressures and suggests the use of compound-gas breathing mixtures in pressure ranges where nitrogen normally produces behavioral problems. In 1961 Zaltsman observed an antagonism of nitrogen effects in the presence of helium. Recently, a serendipitous finding during decompression

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from a 31-ATA chamber dive indicated the existence of antagonism of nitrogen narcosis by helium pressure (Proctor, Carey, Lee, Schaefer, and van den Ende 1972). During this decompression, subjects were continually exposed to 3.5 ATA of nitrogen; the remainder of the chamber atmosphere consisted of helium with oxygen maintained at 1.0–1.5 ATA. At 19 ATA, narcosis was not observed but it was observed at lower pressures when concurrent helium pressures were reduced. The appearance of nitrogen narcosis at the lower pressures can be interpreted as the lower helium pressures not being capable of reversing the effects of nitrogen as much as the higher helium pressure.

The main objective of the present study was to investigate in behavioral terms whether increased helium pressures can indeed reverse changes that have been produced in the behavior of experimental animals by elevated pressures of nitrogen. In the present study, pressure-effect functions for nitrogen, helium, and both gases combined were obtained for rats reinforced by food on a small fixed-ratio reinforcement schedule.

A fixed-ratio reinforcement schedule requires that an organism emit a fixed number of specific responses to produce an effect on the environment. In addition, such a schedule produces a very specific pattern and high rate of responding (Ferster and Skinner 1957). The behavior generated by this reinforcement schedule has been shown to be sensitive to changes in hyperbaric conditions (Thomas, Walsh, and Bachrach 1971; Jennings 1973; Thomas 1973; Thomas and Bachrach 1974). Fixed-ratio schedules have also been used successfully to investigate the antagonisms of behavioral effects of drugs by other drugs (Brown 1963; Davis 1965). A procedural design similar to that employed in the cited drug studies was used in the present study.

METHOD

Subjects

The subjects were 5 male albino rats (Nmri:O [SD] CV) weighing 300 ± 50 g, maintained at approximately 80% of their free-feeding weights by food presented during experimental sessions and by postsession supplemental feeding. They were housed in individual home cages with continuous access to water.

Apparatus

The subjects performed in a rodent chamber 21.6 cm wide by 24.1 cm long by 20.3 cm high, made of aluminum front and back walls and perforated plexiglass sides and top. Stainless steel grids formed the chamber floor. A response lever that required a minimum force of 0.10 N to make an electrical-switch closure that was recorded as a response was mounted on the front chamber wall. A pellet feeder located behind the front wall dispensed 45-mg food pellets into a food tray beside the response lever. A houselight was mounted on top of the front wall and a pilot light was located above the lever. During most baseline sessions, the rat chamber was placed inside a sound-reducing enclosure with a filtered ventilation system. Programming of experimental contingencies and recording of sessions was accomplished automatically by a system of solid-state digital logic modules connected by cables to the rat chamber.

Pressure sessions and a number of baseline sessions were conducted with the rodent chamber placed inside a hyperbaric chamber with a volume of approximately 170 liters. The hyperbaric chamber was provided with several pressure-fitted penetrations to permit connections to the gas supply, programming equipment, and monitoring instruments.

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The three gases used in the study (nitrogen, helium, and oxygen) were supplied from an externally located gas bank to the chamber through pressure-reducing valves and regulators. A gas recirculator and scrubber unit assured rapid mixing of the chamber gas and forced the chamber atmosphere through a carbon dioxide absorbent to control carbon dioxide accumulation. Carbon dioxide level was monitored by an infrared analyzer provided with a 350 cc/min flow of chamber gas through a flow gauge. Oxygen was monitored by a paramagnetic analyzer, which was also provided with a 350 cc/min flow of chamber gas. A system of heating and cooling coils inside the hyperbaric chamber thermostatically maintained the chamber temperature at 25°C ± 2°. Chamber pressure was monitored with a high-pressure gauge connected to the chamber.

Procedure

Each subject performed five daily 1-hour sessions per week. Sessions were preceded and followed by a blackout condition of variable length during which the lights in the rat chamber were off. During an experimental session, the houselight and the pilot light above the response lever remained on.

Baseline session The subjects, after being reduced to and maintained at 80% of their free-feeding weights, were trained by the method of successive approximation to depress the response lever. Closure of the switch activated a circuit that instantly dispensed a food pellet into the food tray beside the lever. After several sessions during which only a single-lever response was required to produce a food pellet, the response requirement was gradually increased over a number of sessions until 25 responses were required to produce a single pellet. The five subjects were then exposed to the fixed-ratio requirement of 25 responses for 3 months to insure stable baseline rates of responding before exposure to hyperbaric manipulations.

Pressure sessions The subjects were first adapted to the hyperbaric chamber and conditions. They were initially exposed in the hyperbaric chamber to an elevated pressure of 2 ATA (atmospheres absolute) breathing nitrogen (oxygen partial pressure maintained at 0.2 ATA) for a number of sessions to allow stabilization of responding under hyperbaric conditions. When response rates became stable during the 1-hour exposures to 2 ATA and were within nonpressure baseline ranges, the study proper was begun.

Each subject was exposed to a series of pressure sessions during which the hyperbaric chamber atmosphere was a nitrogen–oxygen mixture. The desired mixture was obtained by controlling flow rates of each of the pure components while monitoring the exact gas composition with the oxygen analyzer. The oxygen component of all gas mixtures during the entire study was kept constant at 0.2 ATA. The subjects were exposed to four different nitrogen partial pressures: 6.9, 8.4, 9.9, and 12.9 ATA in ascending order to find the minimal effective pressure (MEP) of nitrogen (rat 5 was not exposed to 8.4 ATA). The MEP was defined as the lowest nitrogen pressure which produced complete cessation of responding for an entire 1-hour hyperbaric exposure.

Compression rate for all manipulations in this study was 1 ATA/min. The highest nitrogen (N_2) pressure explored, 12.9 ATA, produced the desired response cessation in all five subjects and was used as the MEP for the rest of the study. The subjects were exposed to the 12.9 ATA N_2 three or four times (once each week) preceded by and followed by regular baseline sessions, before exposure to helium (He) sessions. We exposed them to this pressure occasion-

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ally throughout later manipulations of the study to assure that the MEP nitrogen value alone always produced complete response cessation for 1 hour.

To assess whether raised helium pressures might reverse the cessation of responding induced by nitrogen, we exposed the subjects to a number of nitrogen–oxygen–helium pressure sessions. The subjects were first compressed on nitrogen to the MEP value; then helium was added to the chamber atmosphere to increase the pressure to a higher value. Three helium partial pressures were added to the existing 12.9 ATA of nitrogen: 12.1, 18.2, and 24.2 ATA. These helium pressures are approximately the same as, one and one-half times, and double the nitrogen MEP values, respectively.

After reaching the desired total pressure, the subjects were observed for 1 hour. Reversal of cessation in responding produced by nitrogen was defined as reinstatement of lever responding during the 1-hour hyperbaric exposure. The subjects were exposed to each nitrogen–helium combination for 2 to 4 times each, with a minimum of 10 baseline sessions between hyperbaric exposures. Three of the subjects were further exposed to 48.5 ATA helium, added to 12.9 ATA N_2. Several additional probe sessions were run during which the subjects were observed at 12.9 ATA N_2 for about 20 min first, then observed during the same session with 24.2 ATA helium added.

The effects of raised pressures of helium alone on the fixed-ratio requirement baseline were also determined. Four of the subjects were exposed to three helium pressures that approximated the helium pressures previously added to the 12.9 ATA N_2. The three helium pressures—12.9, 19.0, and 25.0 ATA—were each investigated for one or two 1-hour exposures.

RESULTS AND DISCUSSION

Exposure of the subjects to the highest nitrogen pressure, 12.9 ATA, produced complete cessation of responding on the fixed-ratio baseline for 1 hour. The main finding of the present study is that additional helium pressures added to the 12.9 ATA N_2 resulted in reinstatement of responding, indicating a reversal of the nitrogen-produced cessation in responding.

Rate of responding (responses/second) on the fixed-ratio schedule of food reinforcement as a function of exposure to different gas mixtures and partial pressures is shown for all five subjects in Fig. 1. The points plotted at the far left of each section (0.8 ATA N_2) are the inidividual response-rate means of all baseline sessions preceding hyperbaric exposures. The brackets indicate the range of response rates. The isolated triangles plotted next to the baseline means in Fig. 1 represent the average response-rate values during the last three adaptation exposures to 2 ATA and indicate that the hyperbaric chamber, compression, or noise of gas flow produced no changes in rate of responding outside of baseline ranges.

The top sections of Figs. 2 and 3 show cumulative response records of entire baseline sessions for two subjects. The records show the high (over 1 response/second) and constant overall rate of responding generated by the fixed-ratio reinforcement schedule. Careful inspection of the baseline cumulative records indicates that the pattern of responding consisted of a brief pause followed by a high running rate until food reinforcement was obtained. The biphasic pattern of responding is quite typical of the behavior generated by this reinforcement schedule (Ferster and Skinner 1957).

Breathing increased pressures of 6.9 to 12.9 ATA N_2 produced a decline in response rates, with decrements in responding directly related to increases in the partial pressure of nitrogen. Previous studies have shown that decreases in response rates on small fixed-ratio schedules

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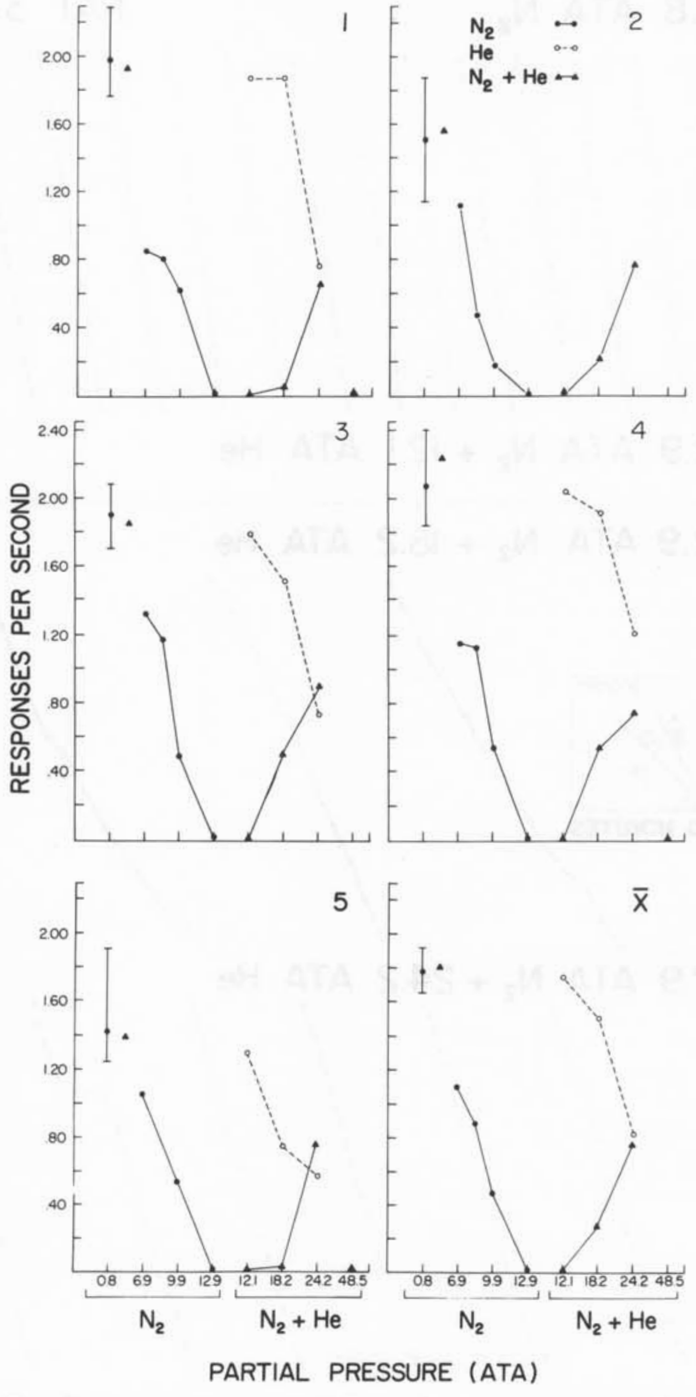

Fig. 1. Rate of responding on fixed-ratio schedule of reinforcement as a function of exposure to nitrogen, helium, and combined gas mixtures. Data for five individual subjects and a group mean are presented. Isolated points with brackets indicate the response-rate means and ranges of baseline sessions. Isolated triangles indicate mean response rates for adaptation exposures.

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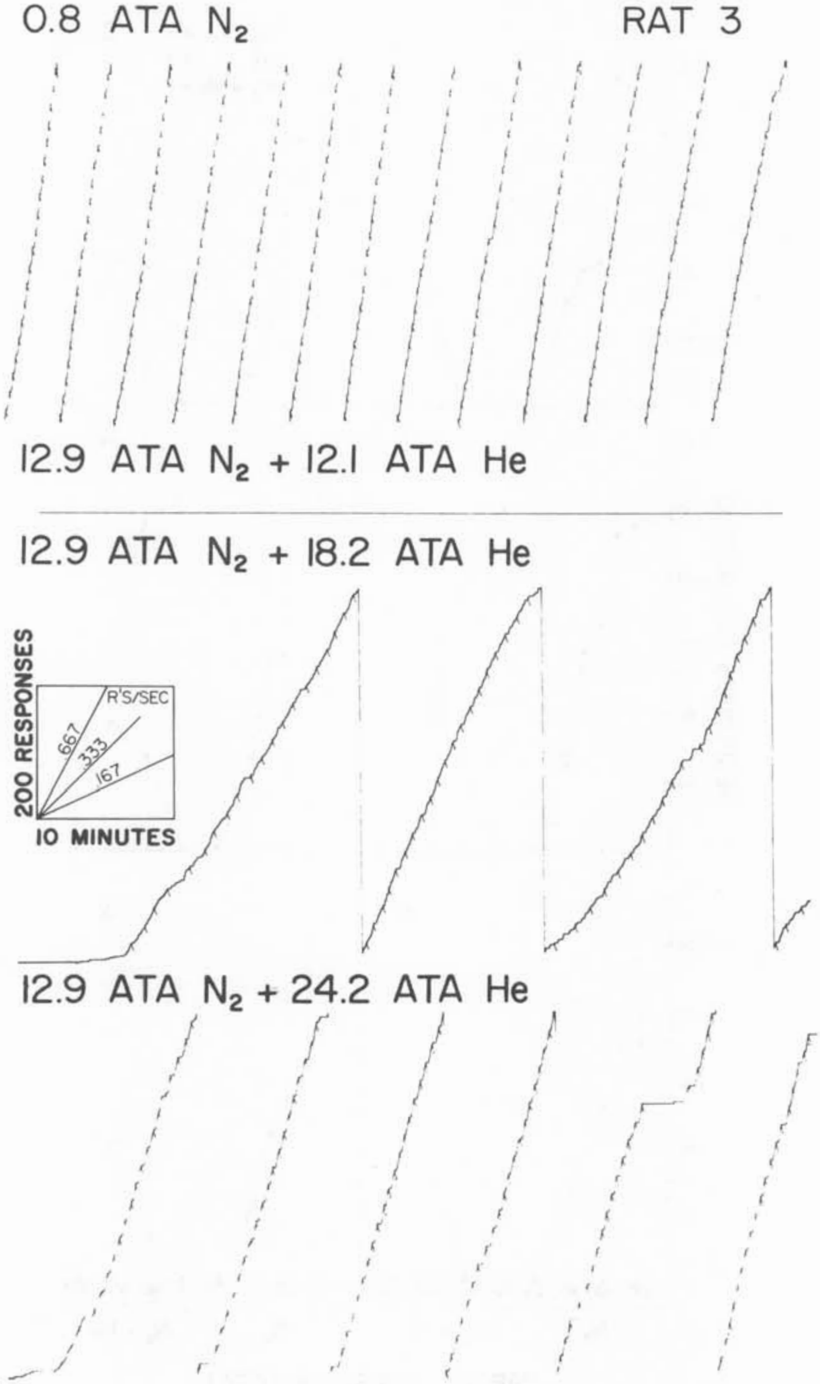

Fig. 2. Cumulative response records of one subject for a baseline session (0.8 ATA N_2) and three hyperbaric sessions when three different helium partial pressures (12.1, 18.2, and 24.2 ATA) were added to 12.9 ATA N_2. Each recorded response stepped the recording pen upward and pips indicate food reinforcements.

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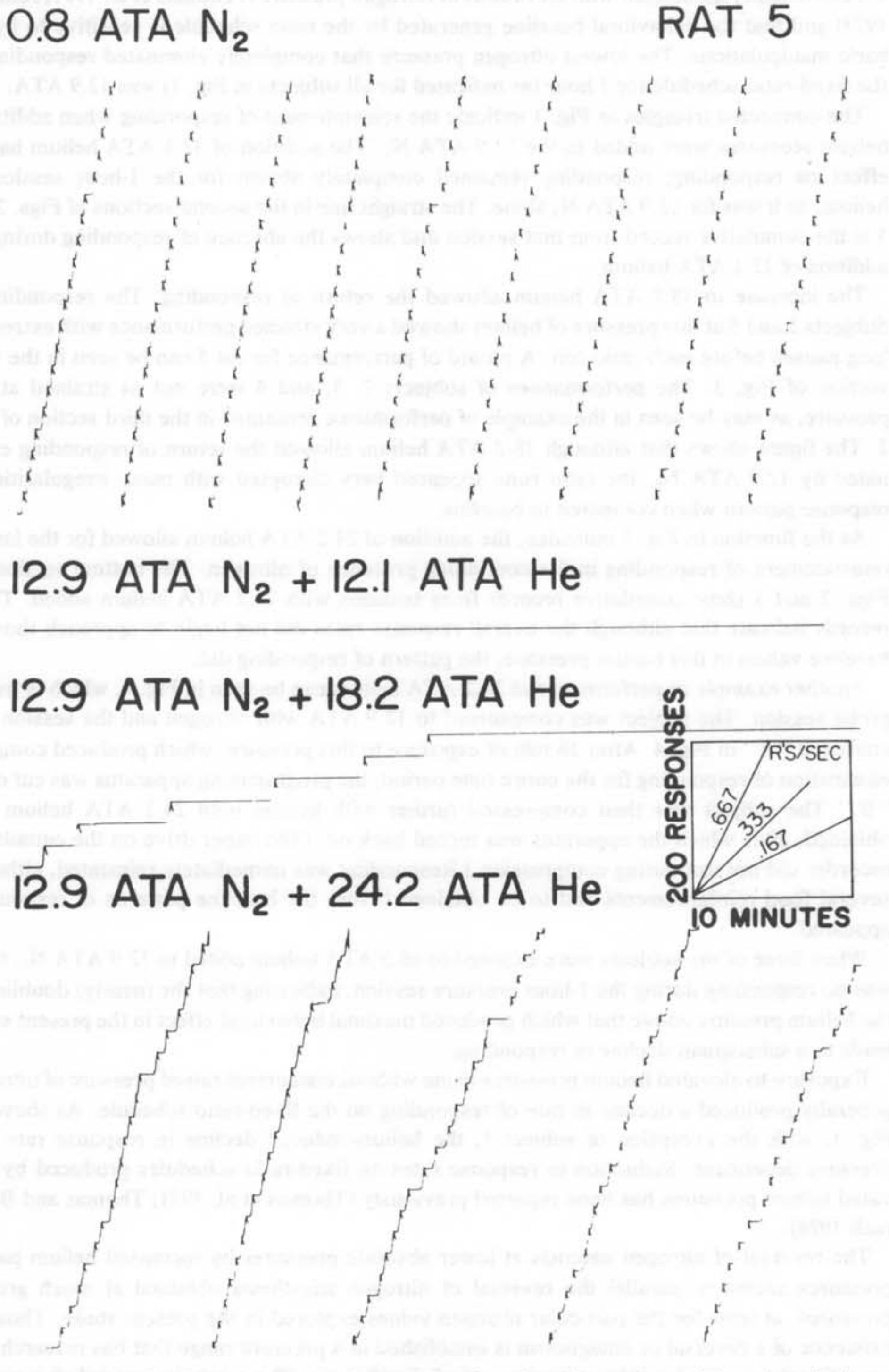

Fig. 3. Cumulative response records of one subject for a baseline session and three hyperbaric sessions. Record details are the same as in Fig. 2.

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are consistently associated with elevations of nitrogen pressures (Thomas et al. 1971; Jennings 1973) and that the behavioral baseline generated by the ratio schedule is sensitive to hyperbaric manipulations. The lowest nitrogen pressure that completely eliminated responding on the fixed-ratio schedule for 1 hour (as indicated for all subjects in Fig. 1) was 12.9 ATA.

The connected triangles in Fig. 1 indicate the reinstatement of responding when additional helium pressures were added to the 12.9 ATA N_2. The addition of 12.1 ATA helium had no effect on responding; responding remained completely absent for the 1-hour session on helium, as it was for 12.9 ATA N_2 alone. The straight line in the second sections of Figs. 2 and 3 is the cumulative record from that session and shows the absence of responding during the addition of 12.1 ATA helium.

The increase to 18.2 ATA helium allowed the return of responding. The responding of Subjects 1 and 5 at this pressure of helium showed a very strained performance with extremely long pauses before each ratio run. A record of performance for rat 5 can be seen in the third section of Fig. 3. The performances of subjects 2, 3, and 4 were not as strained at this pressure, as may be seen in the example of performance presented in the third section of Fig. 2. The figure shows that although 18.2 ATA helium allowed the return of responding eliminated by 12.9 ATA N_2, the ratio runs appeared very disrupted with many irregularities in response pattern when compared to baseline.

As the function in Fig. 1 indicates, the addition of 24.2 ATA helium allowed for the largest reinstatement of responding in the continuing presence of nitrogen. The bottom sections of Figs. 2 and 3 show cumulative records from sessions with 24.2 ATA helium added. These records indicate that although the overall response rates did not begin to approach those of baseline values at this helium pressure, the pattern of responding did.

Another example of performance at 24.2 ATA helium can be seen in Fig. 4, which is from a probe session. The subject was compressed to 12.9 ATA with nitrogen and the session was started at "A" in Fig. 4. After 18 min of exposure to this pressure, which produced complete elimination of responding for the entire time period, the programming apparatus was cut off at "B." The subject was then compressed further with helium until 24.2 ATA helium was obtained, after which the apparatus was turned back on. (The paper drive on the cumulative recorder did not run during compression.) Responding was immediately reinstated, although several food reinforcements had to be obtained before the baseline patterns of responding appeared.

When three of the subjects were exposed to 48.5 ATA helium added to 12.9 ATA N_2, there was no responding during the 1-hour pressure session, indicating that the (nearly) doubling of the helium pressure above that which produced maximal behavioral effect in the present study leads to a subsequent decline in responding.

Exposure to elevated helium pressures alone without concurrent raised pressure of nitrogen generally produced a decline in rate of responding on the fixed-ratio schedule. As shown in Fig. 1, with the exception of subject 1, the helium-induced decline in response rate was pressure dependent. Reduction in response rates on fixed-ratio schedules produced by elevated helium pressures has been reported previously (Thomas et al. 1971; Thomas and Bachrach 1974).

The reversal of nitrogen narcosis at lower absolute pressures by increased helium partial pressures seems to parallel the reversal of nitrogen anesthesia obtained at much greater pressures, at least for the particular nitrogen values explored in the present study. Thus the existence of a reversal or antagonism is established in a pressure range that has research and operational meaning in addition to theoretical significance. The narcosis reversal observed is dependent upon the particular concurrent helium pressure, with more reversal occurring with

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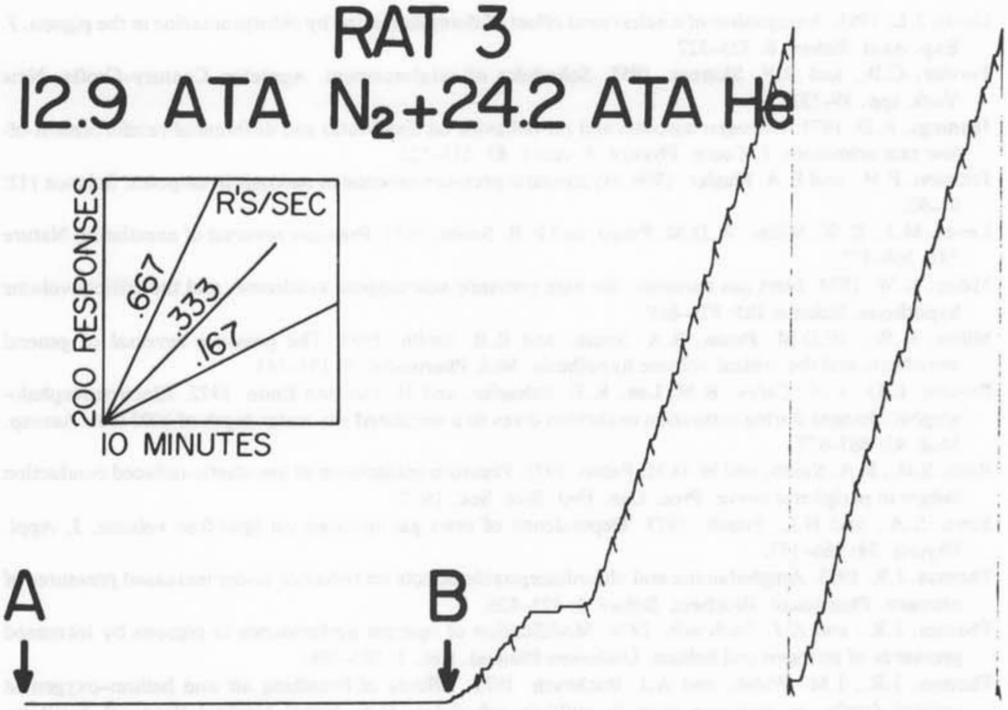

Fig. 4. Cumulative response record from a probe session. The session was begun at "A" with the subject exposed to 12.9 ATA N_2. At "B," 24.2 ATA He was added to the existing nitrogen pressure. The recorder was off during the compression period.

greater helium pressure, which is in accord with the helium pressure-dependent reversal of nitrogen anesthesia (Lever et al. 1971).

Further increases in helium pressures beyond that producing the maximum nitrogen-anesthesia reversal apparently lead to a reduction in the nitrogen-reversing effectiveness of helium (Lever et al. 1971). In the present study, 24 ATA helium allowed responding to be reinstated; however, at 48 ATA helium there was no reinstatement of responding, which suggests that behavioral effects of nitrogen may be antagonized by increases in helium pressures only up to a point, with further increases leading to a decline in response output. This reveals an additional parallel between nitrogen-anesthesia and nitrogen-narcosis reversal. The demonstration of the reversal of nitrogen-produced effects on behavior by helium pressure and its comparability with pressure reversal of nitrogen anesthesia at great pressures establishes a functional continuity of pressure-dependent modification of biological actions and may allow extension of existing models to phenomena at much lower pressures, a possibility already recognized by Miller (1974).

REFERENCES

Brauer, R.W., and R.O. Way. 1970. Relative narcotic potencies of hydrogen, helium, nitrogen, and their mixtures. J. Appl. Physiol. 29: 23–31.

Brown, H. 1963. d-amphetamine-chlorpromazine antagonism in a food reinforced operant. J. Exp. Anal. Behav. 6: 395–398.

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in cooperation with Global Underwater Explorers. (http://rubicon-foundation.org)

Davis, J.L. 1965. Antagonism of a behavioral effect of d-amphetamine by chlorpromazine in the pigeon. J. Exp. Anal. Behav. 8: 325–327.

Ferster, C.B., and B.F. Skinner. 1957. Schedules of reinforcement. Appleton–Century–Crofts, New York. (pp. 39–132)

Jennings, R.D. 1973. Nitrogen narcosis and rat behavior on fixed-ratio and differential-reinforcement-of-low rate schedules. J. Comp. Physiol. Psychol. 83: 515–522.

Johnson, F.H., and E.A. Flagler. 1950. Hydrostatic pressure reversal of narcosis in tadpoles. Science 112: 91–92.

Lever, M.J., K.W. Miller, W.D.M. Paton, and E.B. Smith. 1971. Pressure reversal of anesthesia. Nature 231: 368–371.

Miller, K.W. 1974. Inert gas narcosis, the high pressure neurological syndrome, and the critical volume hypothesis. Science 185: 876–869.

Miller, K.W., W.D.M. Paton, R.A. Smith, and E.B. Smith. 1973. The pressure reversal of general anesthesia and the critical volume hypothesis. Mol. Pharmacol. 9: 131–143.

Proctor, L.D., C.R. Carey, R.M. Lee, K.E. Schaefer, and H. van den Ende. 1972. Electroencephalographic changes during saturation excursion dives to a simulated sea water depth of 1000 feet. Aerosp. Med. 43: 867–877.

Roth, S.H., R.A. Smith, and W.D.M. Paton. 1973. Pressure antagonism of anesthetic-induced conduction failure in peripheral nerve. Proc. Can. Fed. Biol. Soc. 16: 7.

Stern, S.A., and H.L. Frisch. 1973. Dependence of inert gas narcosis on lipid-free volume. J. Appl. Physiol. 34: 366–373.

Thomas, J.R. 1973. Amphetamine and chlordiazepoxide effects on behavior under increased pressures of nitrogen. Pharmacol. Biochem. Behav. 1: 421–426.

Thomas, J.R., and A.J. Bachrach. 1974. Modification of operant performance in pigeons by increased pressures of nitrogen and helium. Undersea Biomed. Res. 1: 181–188.

Thomas, J.R., J.M. Walsh, and A.J. Bachrach. 1971. Effects of breathing air and helium–oxygen at several depths on response rates in multiple schedules. U.S. Naval Medical Research Institute, Bethesda, Md.

Trudell, J.R., W.L. Hubbell, and E.N. Cohen. 1973. Pressure reversal of inhalation anesthetic-induced disorder in spin-labeled phospholipid vesicles. Biochim. Biophys. Acta 291: 328–334.

Trudell, J.R., W.L. Hubbell, E.N. Cohen, and J.J. Kendig. 1973. Pressure reversal of anesthesia: the extent of small-molecule exclusion from spin-labeled phospholipid model membranes. Anesthesiology 38: 207–211.

Zaltsman, G.L. 1961. Physiological principles of a sojourn of a human in conditions of raised pressure of the gaseous medium. English translation: Foreign Technology Division, Wright–Patterson AFB, Ohio, 1967. (AD655360)[1]

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The animals used in this study were handled in accordance with the provisions of Public Law 89-44 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.—*Received March 1976; revised manuscript received April 1976.*

Thomas, J.R. 1976. Renversement de l'intoxication à l'azote chez le rat par, la pression de l'hélium. Undersea Biomed. Res. 3(3):249–259.—Des changements de comportement opérant ont été observé chez des rats respirant de l'azote, de l'hélium, et les deux gaz en combinaison. L'exposition à 12,9 ATA d'azote (O_2 maintenu à la valeur normobarique de 0,2 ATA) a amené la cessation complète des réspones à un schéma de renforcement à rapport fixe pendant 1 heur. L'addition de pressions élevées de l'helium (12,1; 18,2; et 24,2 ATA) à l'azote (12,9 ATA) a déterminé une reprise de répose pendant les expositions hyperbares d'une heure, ce qui indique le renversement des effets de l'azote sur le comportement par les pressions augmentées de l'hélium.

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Les altérations functionnelles comportementaux correspondent sans doute au renversement de l'anesthésie induit par des pressions beaucoup plus élevées.

intoxication à l'azote
renversement de l'intoxication par pression
conditionnment opérant
azote
performance sous hyperbarie
rats
hélium

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