Cold Exposure and Heat Reinforced Operant Behavior

Abstract. Six rats, working in a 2°C ambient temperature, were trained to depress a lever to receive a brief period of heat. Four rats were then moved into the 2°C environment to live, while the others continued to live at room temperature. Living at a temperature of 2°C increased the number of heat presentations the animals delivered to themselves.

This report provides a brief description of an experiment (*1*) on the use of a behavioral index of the effects of cold exposure. In a variation of the operant conditioning setup (see *2*), brief periods of heat were delivered to cold-exposed rats, rather than food or water to deprived ones, as reinforcement for the response of depressing a small lever (*3*).

The establishment of stable lever-pressing behavior can be divided into three phases. In the first, the animals (adult, male, Sprague-Dawley rats) were placed in a small response chamber, at an ambient temperature of about 2°C, for 1 hr. Throughout the session 10-sec periods of heat (about a 15°C rise) were presented at the rate of two per minute. The response chamber was heated by the application of voltage to heating coils mounted just below the hardware-cloth floor. A continuous, slow-speed fan below the coils accelerated the passage of heated air into the chamber during the 10-sec "heat time" and cooled it at the end of this period. Throughout each period of heat presentation, a tone of intermediate frequency and moderate intensity was also delivered. The apparatus was housed in a larger refrigerated room maintained at approximately 2°C. The fully automatic control and recording devices were housed in a separate room.

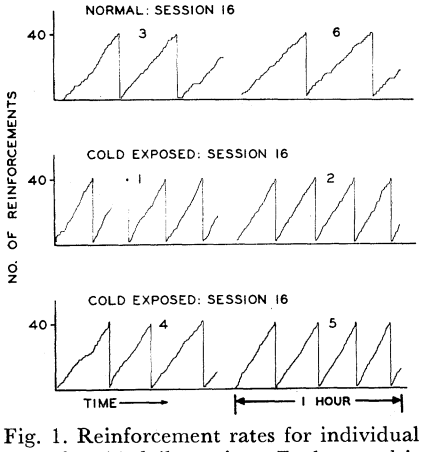

Fig. 1. Reinforcement rates for individual rats after 16 daily sessions. Each record is 1 hr long. The "normal" animals (Nos. 3 and 6) lived at room temperature throughout the experiment; the "cold-exposed" animals (Nos. 1, 2, 4, and 5) lived at a temperature of 2°C from the sixth to the 16th day. Conditions throughout the session were the same for both groups. The gap in the record for rat No. 1 was caused by a faulty pen.

At the end of the 1-hr session, the rat was returned to its cage in the colony room, maintained at about 20°C, where the animal had continuous access to food and water.

The second phase of training began the next day. A T-shaped lever, parallel to the floor and 2 in. above it, had been inserted into the chamber. During this 1-hr session, depressing the lever produced the 10 sec of heat and tone. We found that, unless some such stimulus as the tone was paired with each heat presentation, stable lever pressing could not be established. Depressions made *during* the 10-sec "heat time" had no effect on the duration of the current presentation or on the presentation of subsequent heat periods.

The third daily 1-hr session was divided into two half-hour segments. The conditions of the first half-hour were identical to those of the second session. The speaker that delivered the tone was then disconnected. From this point on (the beginning of the third training phase), each lever depression delivered 10 sec of heat unaccompanied by any other stimulus. The third phase was continued for three additional 1-hr daily sessions. The details of the apparatus and of a similar procedure have been presented elsewhere (*4*).

We have used the behavior so generated as an index of the effects of prolonged exposure to cold. Because we were primarily concerned with changes in the way in which the animal "heated itself," our basic datum was the rate at which reinforcements were delivered rather than the response rate. These two measures are not identical, since the animal could, and did, respond during any 10-sec period of heat presentation—that is, response rate was greater than reinforcement rate. The reinforcements were plotted by a cumulative recorder. The pen reset to the base line after 40 reinforcements.

Following the sixth daily session, four randomly selected animals (Nos. 1, 2, 4, and 5) were moved into an ambient temperature of 2°C, while two others (Nos. 3 and 6) continued to live at room temperature. The daily 1-hr lever-pressing sessions were continued as usual. In session 6, the average reinforcement rate for rats No. 1, 2, 4, and 5 was 0.58 reinforcements per minute; for rats No. 3 and 6 it was 1.03 per minute.

The data shown in Fig. 1 are for session 16, 10 days after the exposed animals began to live in the cold. It will be seen that all animals "deliver" heat to themselves at a roughly constant rate throughout the session; this is apparent in the over-all linearity of the records. It is also clear that the reinforcement rates for the cold-exposed animals are markedly higher, in all cases, than are those for the control animals. The average reinforcement rate for the exposed animals is 2.8 reinforcements per minute; for the control animals it is 1.7. Relative to their performance in session 6, then, the average rate for the exposed animals increased by about 2.2 reinforcements per minute, while that for the controls increased by 0.7 per minute.

The session-to-session course of this rise in reinforcement rate for other rats, as well as the relationships among reinforcement rate, body weight, and food consumption, has been reported elsewhere (*5*).

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References and Notes

1. The experiment was conducted at the U.S. Army Medical Research Laboratory, Fort Knox, Ky. The opinions expressed are entirely ours and do not necessarily reflect those of the Office of the Surgeon General or the Department of the Army.
2. B. F. Skinner, *The Behavior of Organisms* (Appleton-Century-Crofts, New York, 1938).
3. A similar procedure, in which a different means of heat delivery was used, has been described by B. Weiss ["Thermal behavior of the subnourished and pantothenic-acid-deprived rat," *J. Comp. Physiol. Psychol.* 50, 481 (1957)].
4. P. L. Carlton and R. A. Marks, *USAMRL Rept. No. 299, Fort Knox, Ky.* (1957).
5. ———, *USAMRL Rept. No. 325, Fort Knox, Ky.* (1957).

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Influence of Hexane Solubles in Tobacco on a Polycyclic Fraction of Cigarette Smoke

Abstract. No variation in total amounts of those polycyclic hydrocarbons with ultraviolet absorption maxima at 385 mμ was found in the smoke from cigarettes containing varying amounts of hexane solubles. Addition of C^{14}-labeled tobacco paraffins to cigarettes showed that tobacco paraffins are unimportant as precursors of polycyclic hydrocarbons in smoke.

Removal of waxes from tobacco by a "dry cleaning" or extraction with a low-boiling solvent such as hexane has been suggested as a means of reducing the amounts of polycyclic hydrocarbons in cigarette smoke. Lam (*1*) demonstrated the formation of polycyclic hydrocarbons on pyrolysis of tobacco paraffins at temperatures higher than 600°C. Campbell and Lindsey (*2*) reported that extraction of cigarettes with cyclohexane reduced the amounts of polycyclic hydrocarbons by more than 50 percent, but they obtained similar results on extraction of paper cigarettes, and paper contains little or no material soluble in cyclohexane. Gilbert and Lindsey (*3*)

found that polycyclic hydrocarbons are formed on pyrolysis of cellulose, lignin, pectin, starch, sucrose, glucose, and fructose and of citric, malic, and oxalic acids. These compounds, which total up to more than 50 percent of the weight of flue-cured cigarette tobacco, are insoluble in hexane and are not volatile. Compounds which are volatile without decomposition, such as the paraffins and many other compounds present in the hexane extract of tobacco, may be volatilized and transmitted unchanged to the mainstream smoke.

Several experiments were performed (*4*) to obtain some information on the contribution of the hexane-soluble material of tobacco, especially paraffins, to the polycyclic hydrocarbons in cigarette smoke. Estimations were made only on the basis of the height of a peak at 385 mμ in the ultraviolet spectra of fractions obtained by chromatographic separation of the neutral, benzene-soluble portion of the smoke. For each determination 200 cigarettes 70 mm in length were smoked; an automatic smoking machine was used, which took one 35-cm^3 puff of 2-second duration per minute until two-thirds of the cigarette was consumed. The smoke was collected by gravity deposition at room temperature and was extracted with benzene. After removal of bases and acids by extraction with dilute acid and alkali, the solution was concentrated and separated on columns of aluminum oxide, silica gel, and silicic acid. Development of the columns was accomplished by means of cyclohexane containing increasing amounts of benzene. The eluate was collected in small fractions, and the ultraviolet spectra of fractions taken at regular intervals was determined with a Beckman DR recording spectrophotometer. Those fractions showing evidence of peaks at 363 and 385 mμ were combined and evaporated, and the residue was separated on the next column. Estimations were made on the eluate from the last column by a base-line technique, as described by Cooper (*5*).

Variations in the relative size and position of the peaks and in the rate of elution from the last column indicated that a mixture of compounds was present which had absorption peaks at or near 385 mμ. Further separation of this fraction by other methods showed the presence of at least four compounds. The results are therefore a measure of the total polycyclic hydrocarbons of similar ultraviolet spectra which are present in this fraction but are not a true measure of the amounts of any one compound.

Estimations made on the smoke from blended cigarettes gave values of about 0.05 μg per cigarette, with a variation between duplicate determinations of about ±5 percent. The addition of known amounts of 3,4-benzpyrene of this magnitude to the smoke, followed by separation and estimation of the polycyclics in this fraction, resulted in an increase in the estimates of the amounts present of from 85 to 100 percent of the amounts added.

Table 1. Analyses of cigarette tobacco and smoke.

Sample	Tobacco		Smoke	
	Paraffins (%)	Hexane extractives (%)	Total solids (mg per cigarette)	Polycyclics (μg per cigarette)
Control	0.37	5.44	39.3	0.07$_4$
Soxhlet-extracted	0.00	0.00	30.8	0.06$_7$
Percolated	0.06	2.34	32.1	0.06$_7$
Paraffins added	0.57	5.63	41.2	0.07$_1$
Hexane extractives added	0.69	9.06	45.9	0.07$_7$

The results obtained by analysis of the smoke from cigarettes containing varying amounts of paraffins and hexane extractives are shown in Table 1. The control cigarettes were made from a blend of commercial grades of flue-cured (Bright) tobaccos. Cigarettes were made from the same blend after extraction with hexane in a Soxhlet apparatus and after percolation with hexane at room temperature. The other samples show the effect of the addition of more tobacco paraffins and concentrated hexane extract of the same blend of tobacco. Total particulate material in the mainstream smoke, reported as total solids, was determined by drawing the smoke through a tared glass filter disk (Cambridge CM No. 113) and finding the increase in weight. The increase in this value with increasing amounts of hexane-soluble material in the tobacco was quite pronounced and supports the view that many of the hexane-soluble compounds are transferred to the mainstream smoke before they are heated sufficiently to cause extensive decomposition. Variations in the estimated amounts of polycyclic hydrocarbons were so small that they were probably due to experimental errors. Thus, the value found for the cigarettes with paraffins added was intermediate between the values obtained for the controls and those found for the extracted cigarettes, differing from each by about 5 percent. The results indicate that hexane extraction of tobacco would reduce the polycyclics in the smoke by amounts so slight as to be undetectable by these methods.

The use of radioactive tracer techniques offered a method of greater sensitivity for the determination of the contribution of the tobacco paraffins to the formation of polycyclics. Randomly labeled tobacco paraffins were isolated from tobacco grown in an atmosphere of $C^{14}O_2$ at the Nutriculture Laboratory of the Medical College of Virginia. By means of a hypodermic syringe, a hexane solution of 131.2 mg of these paraffins was injected into 200 of the control cigarettes; the solution was injected only into the two-thirds of each cigarette which was to be smoked. After aeration to remove the hexane, the cigarettes were smoked, and both main- and sidestream particulate material was collected. The butts were extracted with cyclohexane, and the same polycyclic fraction was obtained from this solution and from the main- and sidestream smoke by the methods used with the previous samples. The paraffins were also recovered, and the radioactivity was determined in the paraffin and polycyclic fractions. Of the 74.84 μc added, 52.34 μc, or 70 percent, was recovered as unchanged paraffins. The mainstream polycyclic fraction contained only 0.0000127 μc, equivalent to 0.022 μg of the 131.2 mg of radioactive paraffins added to 200 cigarettes. On the assumption that the nonradioactive paraffins in a cigarette behave in the same way, it may be concluded that the 2.2 mg present in two-thirds of a control cigarette would contribute only 0.0004 μg to these polycyclic hydrocarbons. This would be only 1/180 of that derived from other sources and demonstrates that no significant reduction of polycyclic hydrocarbons in cigarette smoke would result from the removal of the paraffins by hexane extraction of the tobacco (*6*).

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References and Notes

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2. J. M. Campbell and A. J. Lindsey, *Brit. J. Cancer* 10, 649 (1956).
3. J. A. S. Gilbert and A. J. Lindsey, *ibid.* 11, 398 (1957).
4. Grateful acknowledgment is extended to Mrs. Thelma Monbarren, R. N. Gladding, and R. C. Bateman for their assistance in various phases of these investigations.
5. R. L. Cooper, *Analyst* 79, 573 (1954).
6. This report was presented at the 133rd national meeting of the American Chemical Society, Agriculture and Food Division, San Francisco, 15 Apr. 1958.

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