Water Deprivation and Trichloroethylene

Effect on Hypothalamic Self-Stimulation

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Rats with implanted hypothalamic electrodes were trained to press a lever for electrical brain stimulation during a daily 30 minute test period. In rats continuously deprived of water for three days, the rate of lever-pressing declined slightly on the first day but remained at that level during the next two days. Exposure of nondehydrated rats to high concentrations of trichloroethylene during the text periods markedly depressed the rate of pressing on three consecutive days. In rats deprived of water for three days and exposed to trichloroethylene daily, the onset of depression was delayed during each test period, and greater tolerance to trichloroethylene developed than in nondehydrated rats. When these rats were later deprived of water for ten days, the rate decreased progressively from days 5 to 10, which was not characteristic of control rats.

PREVIOUS experiments in this laboratory demonstrated that general body dehydration produced by deprivation of water for 48 hours increased susceptibility of rats and mice to the acute toxic effects of lead and antimony.[1-3] To determine whether this effect was specific for lead and antimony or was a general physiological property applicable to all types of toxic chemicals, a series of experiments was undertaken with chemicals of different toxic properties. Trichloroethylene was chosen as a test chemical because, unlike the other compounds studied, it is insoluble in water, soluble in lipids, and, in acute doses, exerts its effects on the central nervous system as a narcotizing agent. The operant behavior technique of hypothalamic self-stimulation was selected as the measure of central nervous system function.

Although no experiments on the effects of trichloroethylene on self-stimulation have been found in the literature, the role of this compound in other behavioral responses of rats has been studied with varied results. Exposure of rats for three hours to 200 and 800 ppm of trichloroethylene did not modify a conditioned food-motivated, rope-climbing response but stimulated spontaneous climbing.[4] Subjection to 2,500 to 5,500 ppm increased the latent period and finally inhibited a food-motivated conditioned response.[5] Spontaneous running was lowered during five hours' exposure to 1,600 ppm and swimming endurance decreased after six hours of exposure at 800 ppm.[6] Exploratory behavior in a maze was enhanced after eight hours of exposure at 600 ppm but decreased at 200 and 1,600 ppm.[7] At higher concentrations of trichloroethylene, impairment usually predominated.

Studies on hypothalamic self-stimulation during water deprivation have been reported. Twenty-three hours of water deprivation was without effect in two groups of rats tested at different lever-pressing rates.[8] A

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Table 1.—*Effect of Dehydration and Trichloroethylene on Hypothalamic Self-Stimulation in Rats*

Group	Not Exposed to Trichloroethylene		Exposed to Trichloroethylene			
			High Concentration		Moderate Concentration	
	Non-dehydrated (Controls)	De-hydrated*	Non-dehydrated	De-hydrated*	Non-dehydrated	De-hydrated*
No. of rats	27	36	14	13	19	22
	Mean No. of Lever-Presses per 30-Minute Test Period					
Mean of 3 normal preexposure non-dehydration days	1,867	2,020	1,955	1 979	1,984	2,119
Exposure or dehydration* days or both						
1	1,869	1,833	759	1,038	1,298	1,538
2	1,968	1,782	891	1,040	1,704	2,069
3	1.889	1,857	948	993	1,873	2,172
Recovery days						
1	...	...	1,734	1,744	1,631	2,011
2	...	...	1,868	1,871	1,765	1,943
3	...	...	1,904	1,859	1,881	1,997

* Continuous water deprivation during three days, thus corresponding to 24, 48, and 72 hours' dehydration by first, second, and third exposure day, respectively.

few cats and rats increased their lever-pressing rate after 48 hours of water and food deprivation,[9] probably as a result of food deprivation, which leads to an increase in the rate of self-stimulation.[8] Rats deprived of water for from 23 to 71 hours, when given a choice of water or self-stimulation as a reward for pressing a lever, preferred water at low-current levels and self-stimulation at higher current levels.[10] Dehydration, even for nine days, was without any measurable effect on the activity of the rats.[11,12]

Since no reports in the literature have been found on the effects of dehydration on the reaction to a narcotizing agent such as trichloroethylene, the present studies were undertaken using hypothalamic self-stimulation in rats as a behavioral measure of these effects.

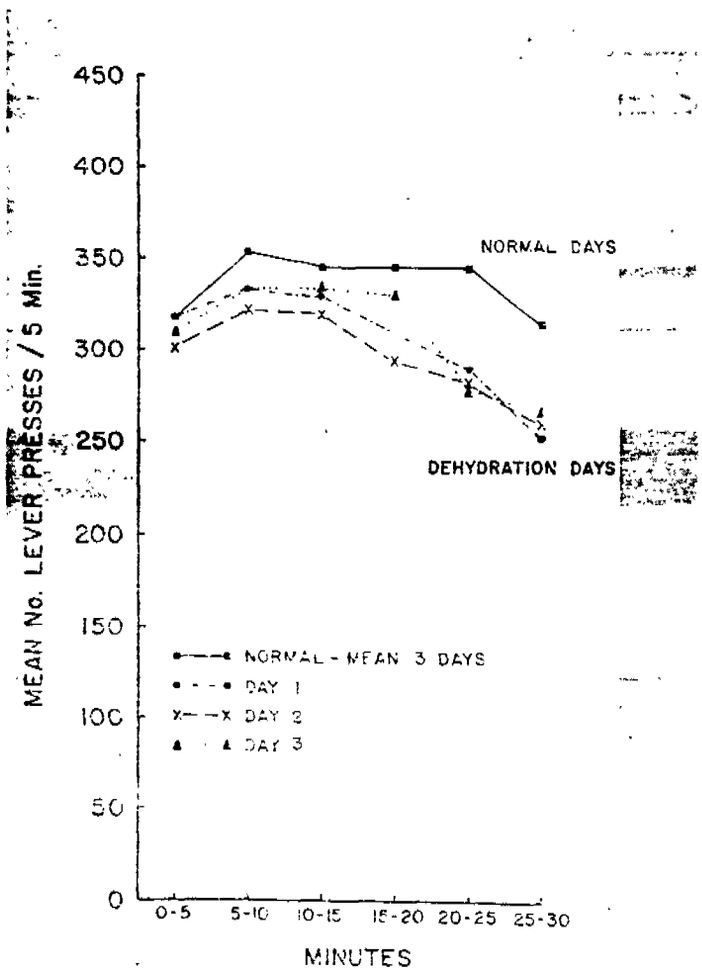


Fig 1.—Effect of water deprivation for one, two, and three days, respectively, on hypothalamic self-stimulation.

Methods

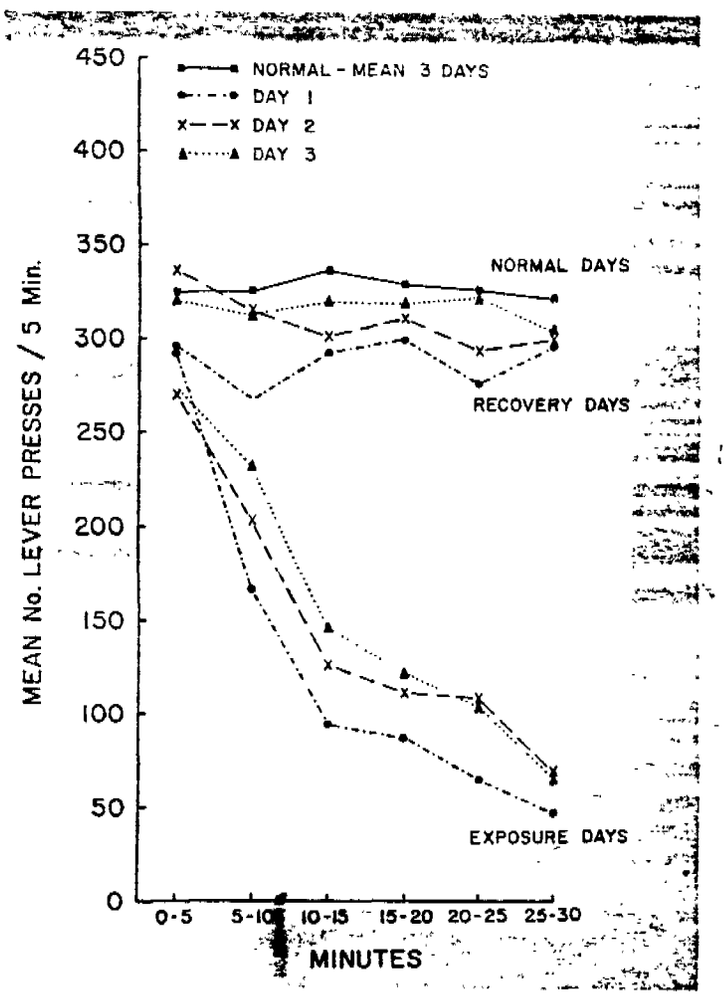
Operant Behavior Tests.—Bipolar electrodes were implanted in the lateral hypothalamic area of male rats receiving pentobarbital sodium. Following recovery, the rats were trained in a modified Skinner box to "self-stimulate," ie, to press a lever which, by closing a circuit, delivered a one-fourth second, 60-cps pulse from a constant current stimulator to the hypothalamic area. The rate of lever-pressing could be controlled within limits by adjusting the strength of the current and remained fairly constant under stable conditions. A normal rate of approximately 70 lever-presses per minute over a 30-minute test period was used except in one experiment in which the rate was 85 lever-presses per minute. The number of lever-presses was recorded by five-minute intervals.

For the trichloroethylene exposures, the solvent vapor was added to the air supplied to the test chamber at a rate calculated to produce a concentration of approximately 3,000 ppm for a heavy exposure and 2,500 ppm for a more moderate exposure. These levels represent the most severe single exposures which do not cause death or detectable organic injury in rats in less than five and ten hours, respectively.[13] To minimize the effect of any possible variation in the concentration of vapor or temperature in the test chamber, dehydrated and nondehydrated rats were tested simultaneously in duplicate chambers supplied by the same air-vapor mixture or were alternated when using the same test chamber. The air flow into the test chambers was the same during the normal preexposure and recovery periods as during the trichloroethylene exposure periods. The chamber temperature could not be controlled but was constant on any one day and averaged 83 ± 3 F (28.3 ± 1.6 C) for all experiments.

Dehydration was produced in the rats by continuous deprivation of water. The rats were fed rat chow pellets and allowed water ad lib except during the test periods and in the dehydration experiments. Hematocrit values were determined in a group of normal rats dehydrated for 48 hours. The rats were weighed daily.

The same procedure was followed in each experiment. Two groups of rats were tested in the self-stimulation chamber for 30 minutes daily for a period of nine days. During the first three days, the normal rate of lever-pressing was obtained so that each rat could serve as its own control. During the next three days, one group of rats was continuously deprived of water, thus corresponding to 24, 48, and 72 hours of dehydration. On each of these three days this dehydrated group and the second or nondehydrated group were exposed to trichlo-

Fig 2.—Effect of high concentration of trichloroethylene vapor on hypothalamic self-stimulation in nondehydrated rats.



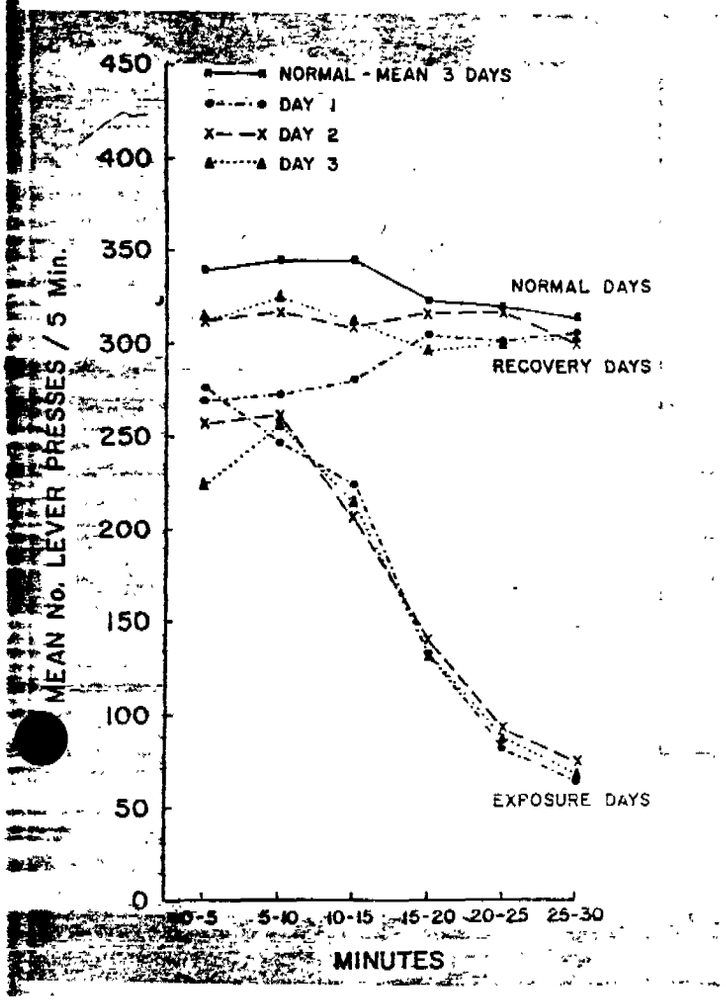


Fig 3.—Effect of high concentration of trichloroethylene vapor on hypothalamic self-stimulation in dehydrated rats.

roethylene vapor during the test period. Water was then restored to the dehydrated group. Tests were repeated on each group during the last three days of the experiment to observe the rate of recovery without trichloroethylene. To determine the effect of dehydration alone, a third group of rats was tested daily on three normal days followed by three days of dehydration. A fourth or control group was tested daily for six days without any exposure or dehydration.

Several months after the end of these experiments, all of the rats which survived the trichloroethylene experiments were retrained and tested daily during ten days of continuous water deprivation without trichloroethylene exposure.

Results

Dehydration by continuous water deprivation for 24, 48, or 72 hours produced a body-weight loss of 7%, 12%, and 16.5%, respectively. Forty-eight hours of water deprivation in a group of normal rats produced a change in the hematocrit value from 49.7% to 57.6%.

The mean number of lever-presses for the 30-minute test period is given for each group in Table 1 for the normal, dehydration, exposure, and recovery days, respectively. The number of lever-presses in the rats subject to dehydration alone fell significantly below normal on the first dehydration day ($P = < 0.01$) but did not continue to fall as dehydration was continued during the next two days. The decrease in the rate of lever-pressing on the first day occurred chiefly during the last half of the test period (Fig 1). Exposure to a high concentration of trichloroethylene in both nondehydrated and dehydrated rats markedly reduced the total number of lever-presses below their preexposure levels on each of the three exposure days ($P = < 0.001$). The decrease in the rate was not as great in the dehydrated as in the nondehydrated rats on all three exposure days, but this difference was significant only on the first day ($P = 0.05$). The rate of both the nondehydrated and dehydrated rats exposed to trichloroethylene was markedly lower on all three days than was the rate with dehydration alone ($P = < 0.005$). With the more moderate concentration of trichloroethylene, the results were similar, although the overall decrement was less. The lever-pressing rate was significantly below the preexposure level on the first and second exposure days with the toxic exposure alone ($P = < 0.005$ and

$P < 0.05$) and on the first exposure day when toxic exposure and dehydration were combined ($P = 0.005$). Again the depression in the rate of lever-pressing was less in the dehydrated rats than in the nondehydrated rats, but the difference was not significant.

The rats exposed to the moderate concentration of vapor developed a tolerance to the trichloroethylene during the three days of exposure, ie, the total number of lever-presses was significantly higher on the third than on the first exposure day for the nondehydrated rats ($P = < 0.05$) and the dehydrated rats ($P = < 0.01$).

Rates on the first recovery day were still significantly below preexposure levels in all groups except the nondehydrated group exposed to the moderate concentration of trichloroethylene ($P = < 0.025$). Thus, the effect of exposures persisted at least 24 hours after the exposure ended.

Since the rate of lever-pressing varied during the 30 minutes of the test, the mean number of lever-presses for each five-minute interval was compared in the dehydrated and nondehydrated groups. The rate of lever-pressing of control rats either remained at a constant level or decreased slightly during a 30-minute test period. Rats not deprived of water when exposed to a high concentration of trichloroethylene vapor exhibited an immediate and continuous decline in the rate of pressing during the 30-minute test period on all three exposure days (Fig 2). Dehydrated rats similarly exposed showed a decrease below normal in lever-pressing rate during the first five minutes of the test, but, in general, an increase during the second five-minute period, after which their pressing rate declined sharply (Fig 3). Comparison of the change in pressing rate during this five- to ten-minute period in the dehydrated and nondehydrated rats showed that dehydration significantly delayed the depressing effect of the trichloroethylene during the early part of the test period ($P = < 0.01$). In the rats exposed to a lower concentration of trichloroethylene, dehydration again delayed, but to a lesser extent, the depressing effect of the trichloroethylene on the pressing rate (Fig 4 and 5).

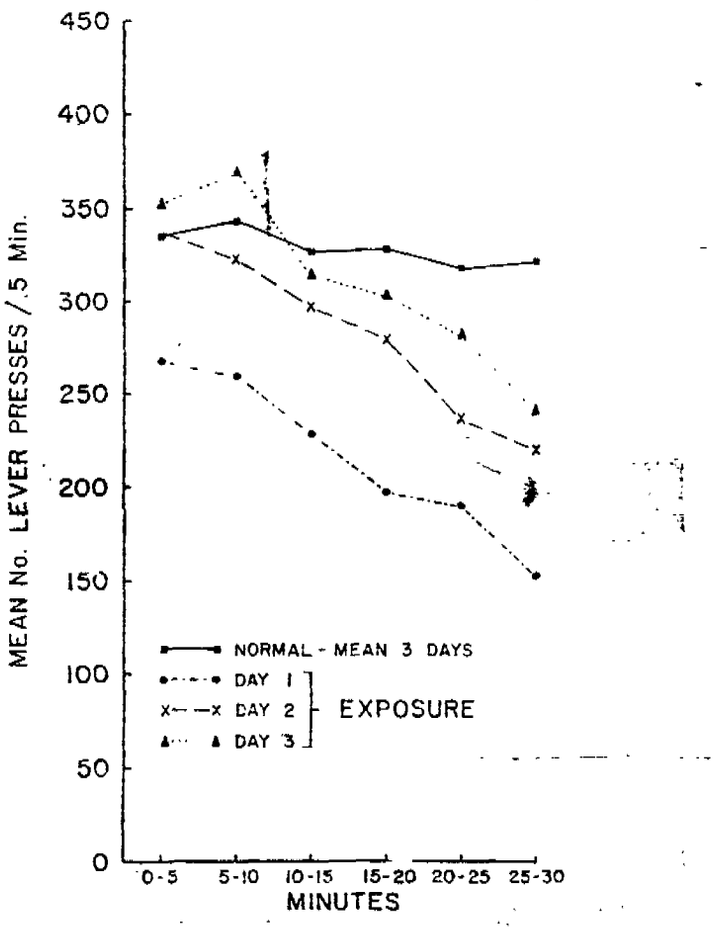


Fig 4.—Effect of moderate concentration of trichloroethylene vapor on hypothalamic self-stimulation in nondehydrated rats.

The effect of ten days' continuous water deprivation on daily hypothalamic self-stimulation without exposure to trichloroethylene was studied in 24 rats which had been used previously in two or three of the

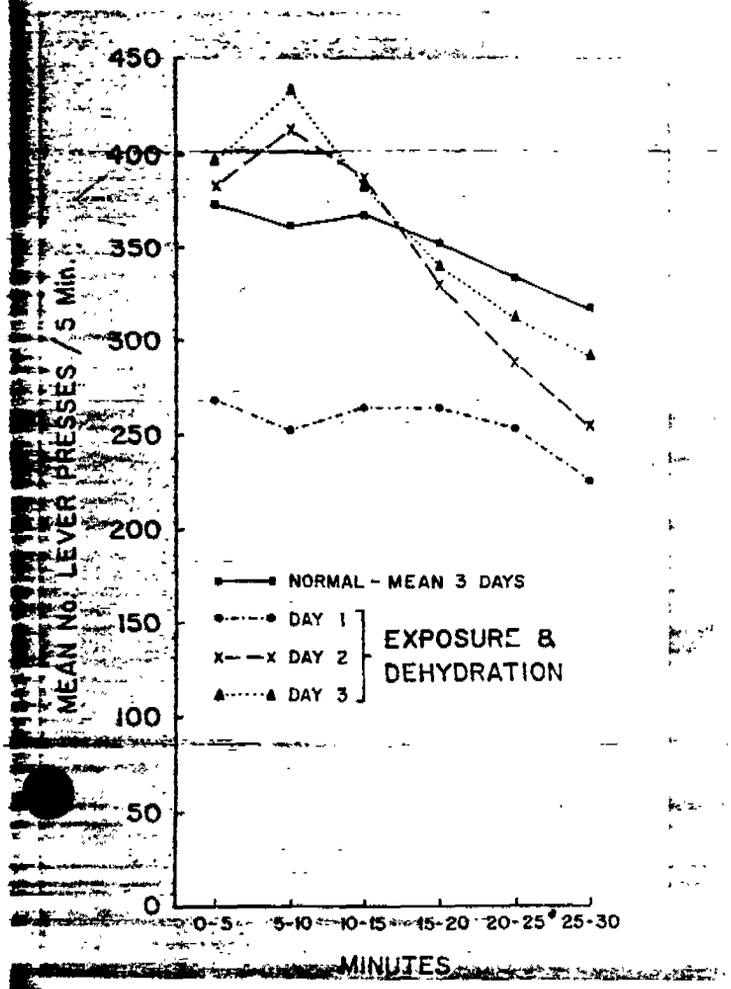


Fig 5.—Effect of moderate concentration of trichloroethylene vapor on hypothalamic self-stimulation in dehydrated rats.

above experiments. Thus, these were older rats in which the electrodes had been implanted for some months. During this ten-day period, they lost approximately 35% of their body weight. These rats showed a decline in lever-pressing rate during the 30-minute test period on each of the three predehydration days and on each of the ten dehydration days. The total number of lever-presses per day did not decrease significantly during the first three days of dehydration, as compared with the rate on predehydration days, but declined sharply from the fifth to the tenth day of dehydration (Table 2, experiment 1). The decrease in the lever-pressing rate correlated closely with the loss in weight ($r = 0.934$, $P = 0.01$).

One rat died on the seventh and one on the tenth day of dehydration. In order to determine if this deterioration with dehydration could be overcome by an increase in the intensity of the brain-stimulating current, eight of the surviving rats were retrained several months later at a rate of 85 lever-presses per minute. They were then subjected to dehydration for ten days. No rats died during this period. The lever-pressing rate again decreased sharply from the fifth to the tenth day of dehydration (Table 2, experiment 2).

Comment

These experiments demonstrated that deprivation of water for 24 hours slightly depressed the rate of self-stimulation in rats, but continued dehydration to 72 hours did not further affect this activity. However, this initial decrease in the rate was statistically significant only in the first experiment, in which the rates of self-stimulation from a large number of rats (36) were averaged. This decrease in the rate during the first 24 hours of dehydration, although present, was not significant in the smaller group of 24 rats dehydrated during the ten-day period. No decrement in hypothalamic self-stimulation rate was reported by Mogenson[8] during 23 hours of water deprivation for a group of eight rats in one experiment and 6 of 12 rats in another experiment, although some decrease was evident in the other six rats of the latter group. Thus, it appears that dehydration exerts a minor effect on hypothalamic self-stimulation which is evident only when a large number of rats is used.

Since rats consume less food when deprived of water and since deprivation or reduction of food intake has been reported

Table 2.—*Effect of Ten Days' Water Deprivation on Hypothalamic Self-Stimulation*

	Mean No. of Lever Presses per 30-Minute Test Period per Day	
	Experiment 1	Experiment 2
No. of Rats	24	8
Normal		
Day 1	...	2,783
2	...	2,950
3	1,931	3,007
Dehydration		
Day 1	1,842	2,845
2	1,888	2,773
3	1,933	2,889
4*		
5	1,759	2,731
6	1,733	2,495
7	1,620	2,654
8	1,631	2,238
9	1,492	2,343
10	1,504	2,185

* Tests were omitted on Sundays.

to increase the lever-pressing rate,[8] an increase in rate would have been expected.

No measurements were made to relate these behavioral results to the content of water in the brain. However, Jelsma and McQueen[14] reported that water deprivation in dogs on a dry diet caused a slight but significant decline in the water content of the brain during the first 24 hours of dehydration, but no further alteration when continued up to 72 hours. If rats resemble dogs in this respect, the slight depression in the lever-pressing rate after 24 hours' dehydration could be explained on this basis.

Acute exposure of rats to trichloroethylene vapor, a powerful narcotizing agent, markedly depressed hypothalamic self-stimulation during a 30-minute test period. In dehydrated rats, the onset of this depressing effect was delayed or preceded by a brief period of increased self-stimulation. This finding suggests either that the concentration of the solvent in the brain cells built up less rapidly in the dehydrated rats or that changes in the brain cells with dehydration made them more resistant to the early low concentration of the solvent or both. As the exposure progressed, these differences in responses were no longer apparent. Dehydrated rats also developed greater tolerance to trichloroethylene vapor than did the nondehydrated rats, which indicates that the brains of these animals were more resistant to the chemical.

The concentration of trichloroethylene studied in these experiments was much higher but the duration of exposure was much shorter than that used by most previous investigators with other types of conditioned behavioral responses. The results, however, are comparable.

The hypothalamic self-stimulation test used in these experiments has an advantage over the food reinforcement behavioral techniques used in some of the previous studies.[4,5] When food reinforcement is used in conjunction with narcotizing agents, the possibility exists that decrements in response are due to depression of the appetitive neural mechanism by the narcotizing agent, thus complicating interpretation of the behavioral effects. This factor may be responsible for some of the variations previously reported. The activation of the neural "reward" circuits, as in self-stimulation, bypasses this problem.

Prolonged (ten days) water deprivation produced a progressive decrease in the rate of self-stimulation from the fifth to the tenth day of dehydration in the rats which had been subjected several times to trichloroethylene and dehydration. This result is in contrast to that obtained in other unpublished studies in this laboratory with rats which had not been subjected to any previous stresses. When these rats were deprived of water for ten days, the lever-pressing rate decreased after 24 hours of dehydration but thereafter remained constant. Only in those rats which became too weak to press the lever did the rate decrease. The difference in these results suggests the possi-

bility that repeated exposure to high concentrations of trichloroethylene combined with dehydration may have produced some residual damage. However, the difference could have been due to the prolonged implantation of the electrodes. Further experiments are required to determine the cause of the decrement in performance of these rats.

These experiments demonstrated that susceptibility of rats to trichloroethylene as measured by the hypothalamic self-stimulation technique is not increased by dehydration. Other unpublished studies in this laboratory have shown that deprivation of water for 48 hours did not affect either the mortality or average survival time of rats. Thus, the effect of dehydration on susceptibility to this organic vapor differs, in general, from its effect on acute lead and antimony poisoning.[1-3] This result may be due to a difference in the chemical and physical properties of these materials, such as water-lipid solubility. On the other hand, the brain may be more adequately protected against dehydration than the heart, which is the principal site of acute antimony damage, and the various tissues which are affected by lead.

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MORALITY

Mankind's interest in moral values, particularly as they affect his relationship to his fellow man, his society, and his God, stretches back at least as far as historical records can carry us. The great minds of every age have sought answers about the nature, universality, and permanence of moral principles. Yet it often seems that we are no closer to definitive answers than was Socrates when he admitted to Meno that he did not know how virtue is acquired, whether it can be taught, or even what it really is.—*The Acquisition and Development of Values, Perspective on Research*. Report of a Conference, May 15-17, 1968, Washington, DC, National Institute of Child Health and Human Development, Bethesda, Md.