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Does the Rat with Hereditary Hypothalamic Diabetes Insipidus Have Impaired Avoidance Learning and/or Performance?

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MILLER, M., E. G. BARRANDA, M. C. DEAN AND F. R. BRUSH. *Does the rat with hereditary hypothalamic diabetes insipidus have impaired avoidance learning and/or performance?* PHARMAC. BIOCHEM. BEHAV. 5: SUPPL. 1, 35–40, 1976. – Avoidance learning and extinction of rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain) were studied in 2 experiments that differed only in shock intensity. In both experiments rats homozygous for diabetes insipidus were more deficient in both escape and avoidance responding than were their heterozygous or normal controls. Although the heterozygous animals showed improved escape performance at the higher shock intensity, their avoidance behavior was not improved. The superiority of normal and heterozygous animals in extinction performance, relative to the homozygous animals, was eliminated or reversed when the differences in terminal acquisition performance were taken into account by analyses of covariance. Deficiency of ADH, therefore, may not result in faster extinction of avoidance behavior.

Avoidance learning and extinction   Diabetes insipidus   Vasopressin (ADH)
Hereditary hypothalamic diabetes insipidus   Brattleboro strain

ALTHOUGH the role of vasopressin (ADH) in the regulation of water balance is well established, the behavioral effects of ADH are less well established and, indeed, have become controversial. For example, DeWied [5] has reported normal acquisition but faster extinction of a shuttlebox avoidance response in rats with posterior pituitary lobectomy. Similarly, hypophysectomized rats were unable to learn the shuttlebox avoidance response unless supported with hormone replacement therapy, but even with replacement therapy they extinguished faster than normal [4,5]. However, injection of pitressin tannate, an extract of the posterior pituitary lobe, restored extinction performance of these animals to normal [4,8]. Pitressin tannate and lysine vasopressin were also shown to facilitate performance of avoidance responses in extinction in normal animals [6,7].

In the light of this evidence, experimenters have taken advantage of the genetic deficiency of ADH in rats of the Brattleboro strain of hooded (Long-Evans) rats. This strain carries a recessive gene which is expressed as diabetes insipidus (DI) in the double recessive offspring [11, 12, 13]. ADH is absent in the neurohypophysis, blood and urine of rats homozygous for DI [9,10]. Although rats heterozygous for DI have a deficiency in ADH, with neurohypophyseal, blood and urine levels only one half those of normal rats, their water regulation is nearly normal [9,10].

In two studies of avoidance learning and extinction in rats of the Brattleboro strain, conflicting results have been reported. In one of the studies [1] homozygotic (DI) and heterozygotic (HE) male rats of the Brattleboro strain were trained in a two-way shuttlebox avoidance task for 120 trials (10/day for 12 days) followed by 7 days (10 trials/day) of extinction. Although all DI animals reached a performance criterion of 80% by the end of acquisition, their overall avoidance performance was below that of the HE animals (82.6 *vs* 102.9 total avoidance responses in acquisition). In extinction, the DI animals extinguished significantly faster than their HE controls. Since Bohus *et al.* [1] employed normal rats of an albino (Wistar) strain as their controls, we do not here addresss their reported comparisons to those control animals. The experiment appears to offer extinction data consistent with the previous evidence that ADH facilitates performance in extinction since those animals genetically deficient in ADH extinguished faster than those of the same strain with near normal ADH levels.

Different results were obtained in experiments reported by Celestian *et al.* [3] who also studied rats of the Brattleboro strain. In these experiments DI and HE rats were trained in a two-compartment shuttlebox at the rate of 20 trials per day until the rats had reached a performance level of 80% or more avoidance responses for at least 3 consecutive days. HE rats exhibited acquisition and extinction performance which was indistinguishable from that of normal animals. However, DI rats differed in two ways from the normal and HE animals. The DI animals showed a significant decrease in the ability to learn the avoidance respones, with only 30% reaching the criterion in acquisition as compared to 78% for the HE and 64% for the normal rats. In addition, when extinction testing was carried out in those DI rats which did learn the avoidance response, these animals showed significantly greater retention of the avoidance response than did the animals which had endogenous hypothalamic ADH. Thus, these studies were unable to demonstrate an impairment in retention of an acquired avoidance response as a consequence of ADH deficiency. To the contrary, those animals lacking in ADH which were able to learn the avoidance response exhibited a greater than normal ability to maintain the response.

Although the basic nature of the training and testing procedures as well as the kinds of animals used were similar in the Bohus *et al.* [1] and Celestian *et al.* [3] experiments, there were in fact a number of potentially important differences in procedure between the two experiments. For example, Bohus *et al.* employed an auditory warning signal (WS), a weak shock intensity (0.16 mA) and a training regimen of 10 trials/day. Celestian *et al.* on the other hand, employed a visual WS, a higher shock intensity (1.0 mA), and a training regimen of 20 trials/day during acquisition with extinction testing at staggered and variable intervals. Thus, the finding of opposite differences in extinction between DI and HE animals may depend on the combined effects of serveral of these procedural differences. In an attempt to resolve the question of whether the conflicting results could be due to these procedural differences, our first experiment utilized conditions more nearly identical to those of Bohus *et al.* [1].

EXPERIMENT 1

METHOD

Animals

Sixty-nine male and female rats of the Brattleboro strain were categorized as homozygotic (DI) or heterozygotic (HE) for diabetes insipidus on the basis of 3 measurements of urine osmolality, as estimated by refractive index, and 24 hr water intake, expressed as percent body weight [11, 12, 13]. There were 38 DIs – 28 male and 10 female, and 31 HEs – 24 male and 7 female. They were housed individually in a room with light cycle control (lights on at 0700 and off at 1900 hr.) and were maintained on food and water ad lib throughout the experiment. They were between 72 and 225 days of age at the beginning of the experiment.

Apparatus

The experimental training units were 4 automatic shuttleboxes that have been described elsewhere [2]. The warning signal (WS) was a 78-dB (SPL) white noise and an increase in illumination (6 W, 30 V clear glass bulb operated on 28 V through 50Ω) presented at the rear of the compartment occupied by the rat. The primary aversive event was a scrambled ac electric shock of approximately 0.16 mA (estimated current through the rat) delivered through a 750 kΩ resistor in series with the animal. An electromechanical switching circuit controlled the occurrences of WS and shock and recorded response frequency and latency (± 0.1 sec) of the first response on each trial.

Procedure

The animals were assigned nonsystematically, in squads of 4, to one of the shuttleboxes. On the first experimental day 10 pretest trials were given at a fixed 2 min intertrial interval (WS onset – WS onset interval). During these trials the WS was presented alone; it was terminated by a shuttle response or 35 sec after its onset, whichever occurred first.

On the following day avoidance training was begun and continued at the rate of 10 trials/day for 12 days. During avoidance training and subsequent testing, the intertrial interval was reduced to 1 min. During training, the WS-shock interval was 5 sec, and a response during that interval terminated the WS and prevented the shock on that trial (avoidance response). If shock occurred, both WS and shock were terminated by a shuttle response (escape response) or at 35 sec after WS onset (failure to escape). If an animal avoided shock on the first trial of the first day of training, it was counted as an extra pretest trial, and one additional trial was given so that all animals had 10 training trials on the first day of avoidance training.

On the day following the last day of avoidance training, extinction testing was begun and continued at the rate of 10 trials/day for 7 days. During extinction no shock was presented, and the WS was terminated by a response or 5 sec after its onset, whichever occurred first. A response occurring within 5 sec of WS onset was scored as an avoidance response.

RESULTS AND DISCUSSION

As Table 1 indicates, a large proportion of both the DI and HE animals failed to escape shock on one or more trials. Typically, those that did so also failed to avoid, although in both groups some animals that never failed to escape also failed to avoid. Table 1 also indicates that the overall level of avoidance performance in both groups was extremely low and only one HE animal, a female, met a criterion of 80% avoidance responses in 120 trials. The percentage of animals meeting the weaker criterion of 80% or more avoidance responses on any one day are comparable to those reported by Celestian *et al.* [3] who used a 1.0 mA shock, and contrast sharply with the 100% of DI and HE animals that met this criterion in the Bohus *et al.* experiment [1], which used the same shock intensity as in the present experiment (0.16 mA).

Since performance in acquisition, especially toward the end of training, is a good predictor of extinction performance, the gross differences in acquisition between these groups makes it inappropriate to compare the raw extinction data of the two groups.

An attempt was made, therefore, to match animals between the groups on acquisition characteristics that correlate well with extinction performance. When this was done, good matches could be obtained on only 6 pairs of animals; acquisition performance varied between 10 and 88 avoidance responses in the DI subgroup (Median = 19.0) and from 9 to 83 in the HE subgroup (Median = 21.5). The median extinction performances were 23.5 and 9.5 total avoidance responses for the DI and He groups, respectively. This result, although inconclusive because of the few matched pairs, is consistent with the results of Celestian *et*

TABLE 1

PERCENTAGE OF ANIMALS SHOWING INDEXED BEHAVIOR

	0.16 mA DI	0.16 mA HE	0.25 mA DI	0.25 mA HE	N
Failure to escape shock (1 or more times)	55	52	45	7	0
Avoidance learning criteria:					
≥18% of total	29	61	32	50	67
≥80% of total	0	0	0	0	27
≥80% on any day	21	55	18	36	60

al. [3] and the opposite of those of Bohus *et al.* [1].

By using a weaker total acquisition criterion ((18% or 22 avoidance responses in 120 trails of training) more animals of both groups could be included. With this criterion, 11 DI (4 male and 7 female) and 19 HE (14 male and 5 female) animals were selected for further analysis. Of these animals, 1 DI animal failed to escape shock on 8 occasions and 6 HE animals failed to escape, one 4 times, one 3 times and the rest only 1 time. Since the sex difference in both unselected and selected groups were not significant (Mann-Whitney *U* tests), the data from both sexes were pooled. Figure 1 illustrates the mean frequency of avoidance responding of the 2 experimental subgroups during acquisition and extinction.

Even these selected subgroups of relatively well performing animals were poorly matched in acquisition, especially in terminal performance, so an analysis of covariance was employed, using as covariates various indices of acquisition that correlate well with extinction performance. Table 2 presents the unadjusted mean total number of avoidance responses in extinction for the 2 groups and the adjusted mean values when 3 different acquisition covariates were used. In all instances, the apparent slight superiority of the HE group in extinction performance was reduced or reversed by the covariance analyses. In no case were the group differences significant.

In another effort to compare the extinction performance of these groups, a least squares regression line was fitted to each group's extinction curve, avoidance frequency over days, (see Fig. 1) and the slope constants of these regression lines were calculated. They are listed in Table 3 along with the outcome of a t test of the difference between the slopes. Clearly, the rate of extinction of the HE group is greater than that of the DI group, the latter showing little or no decrease in frequency of responding over the course of 7 days of extinction.

Taken together, the results of this experiment indicate that when training conditions and parameters are virtually identical to those used by Bohus *et al.* [1] the following conclusions can be made: 1) failure to escape from shock occurs frequently in both DI and HE groups, 2) avoidance acquisition is poor in both groups, but notably inferior in the DI group, 3) in a small subset of animals from the 2

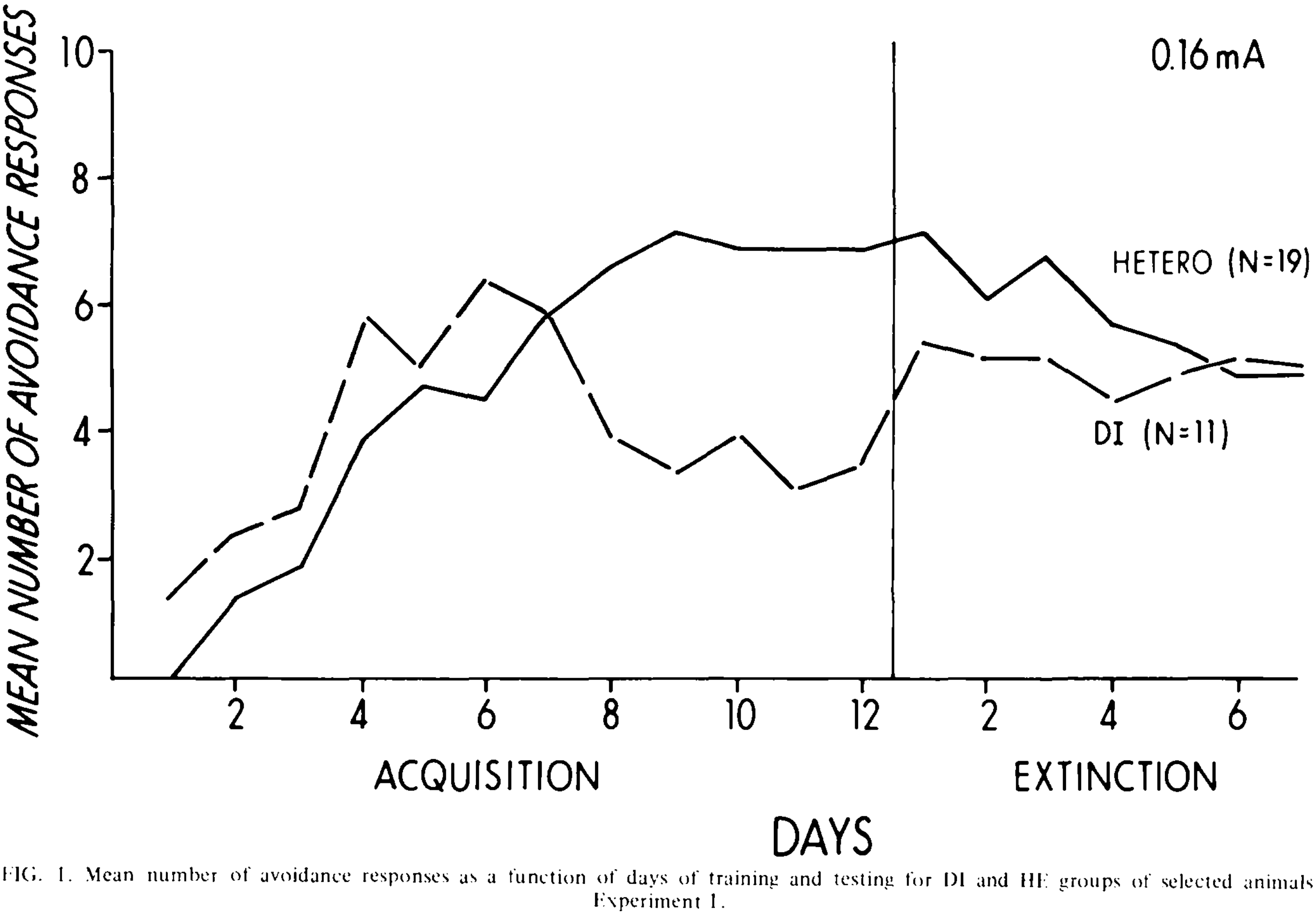

FIG. 1. Mean number of avoidance responses as a function of days of training and testing for DI and HE groups of selected animals Experiment 1.

TABLE 2

MEAN TOTAL NUMBER OF AVOIDANCE RESPONSES IN EXTINCTION

	0.16 mA		0.25 mA		
	DI	HE	DI	HE	N
Unadjusted Means	35.3	40.8	14.0	31.4	50.7
Adjusted Means					
Covariate:					
Total avoidance responses in acquisition*	37.3	39.6	20.7	37.3	41.9
Avoidance responses during last 2 days of acquisition	41.1	37.5	29.0	38.5	35.3
Avoidance responses during last day of acquisition	40.9	37.6	24.6	40.6	36.9

*Violates assumption of homogeneity of regression in 0.25-mA groups.

TABLE 3

MEAN RATE OF EXTINCTION*

Shock Intensity	DI		HE		N
0.16 mA	−0.06		−0.37		
		$p<0.001$			
0.25 mA	−0.01		−0.40		−0.57
		$p<0.001$		$p<0.05$	

*Slope of least squares regression line.

groups that are matched in acquisition, resistance to extinction is greater in the DI than in the HE group, 4) in selected unmatched animals that meet a weak criterion of avoidance learning, small or no differences in extinction performance occur between the DI and HE groups, 5) when differences in acquistion are taken into account in covariance analyses, the differences in extinction between DI and HE groups are reduced or reversed, and 6)when differences in acquisition performance are ignored, the rate of extinction in the HE group is greater than that of the DI group.

These results, therefore, question the conclusion of Bohus *et al.* [1] that animals deficient in ADH extinguish previously learned avoidance behavior more rapidly than those with normal or near normal of ADH.

EXPERIMENT 2

Because such a large proportion of the animals in Experiment 1 failed to escape the 0.16 mA shock, the experiment was replicated using a higher shock intensity (0.25 mA) and a group of normal Long-Evans animals, derived from the parental stock of the Brattleboro line, were added as a control for the genetic deficiencies of the Brattleboro line.

METHOD

Animals

Fifty-two male and female rats of the Brattleboro line (or Brattleboro parental stock) were categorized as homozygotic (DI) or heterozygotic (HE) for diabetes insipidus or as normals (N), on the basis of urine osmolality and 24 hr water intake as well as on the basis of their genetic history. There were 22 DI, 14 HE and 16 N animals with equal numbers of each sex in each group. They ranged in age between 80 and 105 days at the start of the experiment, and they were maintained as in Experiment 1.

Apparatus

The same experimental units used in Experiment 1 were employed.

Procedure

All training and testing procedures were the same as in Experiment 1 with the exception of the shock intensity which was increased to 0.25 mA.

RESULTS AND DISCUSSION

As indicated in Table 1, a significant proportion of the DI animals trained at the 0.25 mA shock intensity of this experiment again failed to escape shock on one or more occasions. In contrast, the higher shock of this experiment reduced the frequency of escape failures in the HE group, and no such failures were observed in the N group. With respect to the various criteria of avoidance learning listed in Table 1, the higher shock intensity of Experiment 2 had little effect on the DI animals and a small deleterious effect on the HE group. Once again, the learning deficit in the DI animals was severe; the performance of the HE animals was intermediate between the DI and N groups.

As in Experiment 1, the 18% total avoidance criterion was used to select animals for further analysis. As in the first experiment, there were no significant sex differences in any of the groups, so the data from both sexes were pooled. In these selected groups there were 7 DIs, 3 male and 4 female; 7 HEs, with the same sex ratio; and 10 Ns, 6 male and 4 female. The mean frequency of avoidance responding on each day of training and extinction testing for each group is illustrated in Fig. 2. Although the selected animals in each group learned at about the same rate and reached approximately 75% avoidance responding by the 10th day of training, during the last two days of training the frequency of avoidance responding declined in the DI and HE groups. Since performance level at the end of acquisition is an excellent predictor of subsequent performance

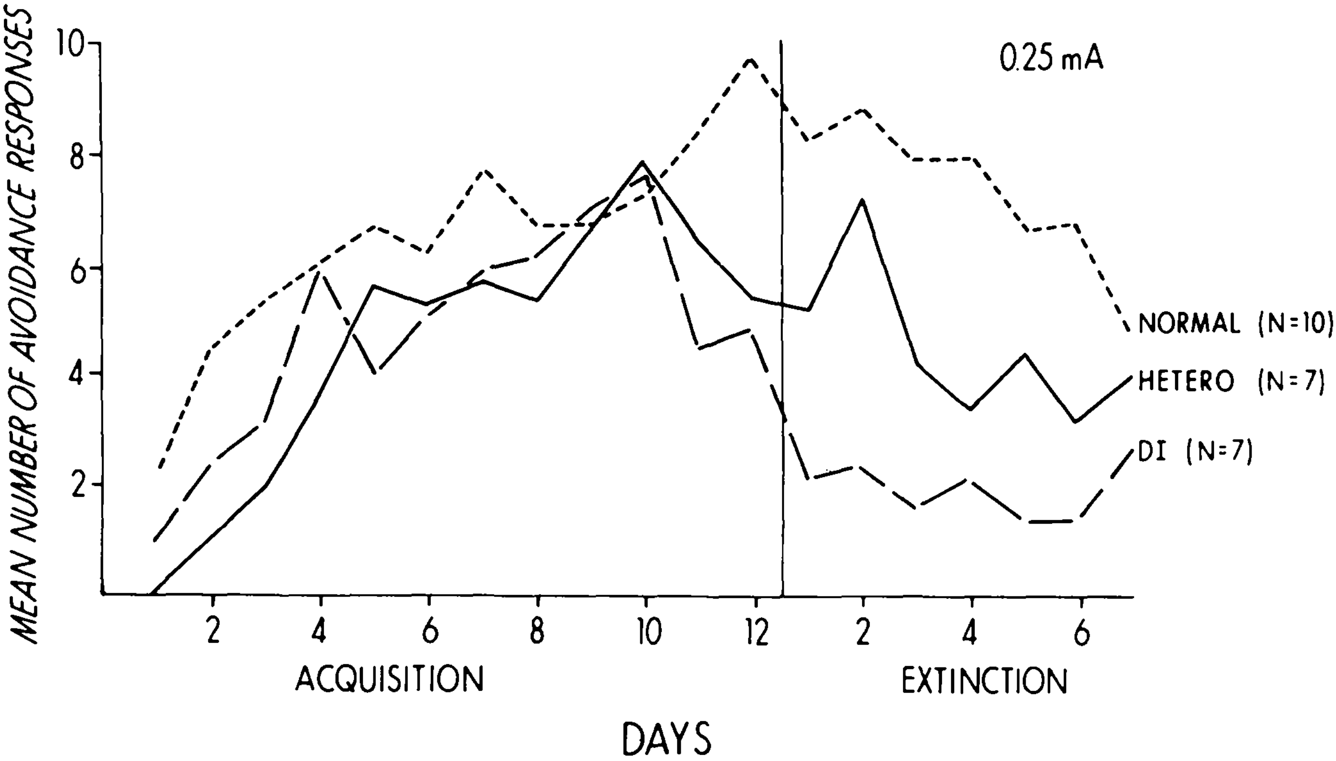

FIG. 2. Mean number of avoidance responses as a function of days of training and testing for DI, HE and N groups of selected animals, Experiment 2.

in extinction, this outcome again precludes direct comparison of extinction performance by the three groups. As before, analyses of covariance were used in an attempt to adjust the extinction data for the obvious differences in terminal acquisition performance among the three groups. The outcome of these analyses is listed in Table 2. A simple analysis of variance, without a covariate, showed a significant group effect ($F2,21) = 6.8$; $p<0.005$), with the DI group having the lowest frequency of avoidance responding in extinction, the N group the highest, and the HE group an intermediate frequency. These results would appear to confirm the conclusion of Bohus *et al.* [1] that deficiency of ADH leads to faster extinction.

Such a conclusion is unwarranted, however, in view of the gross differences in terminal acquisition performance. When the analysis of covariance was used, with total avoidance responses in acquisition as the covariate, the adjusted means showed a pattern similar to the raw extinction data, and the F ratio was again significant. However, as noted in the table, this analysis violates the assumption of homogeneity of regression between groups ($F(2,18) = 4.2$, $p<0.05$). When the covariate was avoidance frequency on either the last day or the last two days of acquisition, the assumption of homogeneity of regression between groups was met ($F<1.0$ in each case), and the adjustment from these covariates eliminated the significant group effect in extinction ($F<1.0$ in each case). We conclude from these analyses, therefore, that when terminal acquisition performance is taken into account, the group differences apparent in the raw extinction data of Fig. 2 do not hold up, i.e., that deficiency in ADH has no significant effect in extinction over and above the obvious effect it has in acquisition.

As in Experiment 1, least squares regression lines were fitted to the three extinction functions of Fig. 2, and the slopes of these lines were compared. Their values are tabulated in Table 3 along with the significance of comparisons of adjacent groups. As before, the conclusion to be drawn from this analysis, which ignores the acquisition difference, is that the DI group extinguishes more slowly than the HE group which in turn extinguishes more slowly than the N group.

GENERAL DISCUSSION

The results of these experiments are consistent in indicating that DI animals are deficient in avoidance acquisition relative to HE or N animals. The deficits observed here are comparable to those reported by Celestian *et al.* [3] who used a 1.0 mA shock and are much greater than the deficit reported by Bohus *et al.* [1] who employed the same 0.16 mA shock of our Experiment 1. Since our training conditions are highly similar to those of Bohus *et al.* it is apparent that other factors must account for the difference in magnitude of the learning deficit.

The results of these experiments are also consistent with respect to extinction performance. The raw extinction data of both experiments indicate that the DI animals make fewer avoidance responses in extinction than either the HE or N animals (a significant effect only in Experiment 2). But do they extinguish faster?

If acquisition differences are ignored and rates of extinction are estimated from the slopes of the least squares regression lines fitted to the extinction curves, then clearly the DI groups extinguished more slowly than the HE and N groups. But this may be an artifact of the performance level at the end of acquisition, the different rates perhaps being another instance of the law of initial values. When terminal performance in acquisition is taken into account by analyses of covariance or matching subjects between groups, then the apparent differences in extinction performance vanish or are reversed. It seems likely that the faster extinction of the DI animals in the Bohus *et al.* experiment might also have vanished if the differences in terminal acquisition performance between their DI and HE animals had been taken into account.

We conclude, therefore, that the ADH deficit in homozygous Brattleboro rats may not result in faster extinction of avoidance behavior, but may either have no effect or, in fact, have the opposite effect of retarding extinction. Further experiments are needed to clarify the role of ADH in the behavior of these animals. Meanwhile, we urge that extreme caution be exercised before strong conclusions are drawn regarding the effect of ADH on extinction of avoidance behavior.

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