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Effect of Age and Enrichment on Certain Brain Dimensions in Brattleboro Rats Deficient in Vasopressin

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As a sequel to our first report in the series of studies on the brains of Brattleboro rats, measurements in caudal diencephalon, subcortical telencephalon, hippocampus, and pyriform cortex were made in three groups each of male heterozygous and homozygous Brattleboro rats. One group raised in standard conditions was killed at 60 days of age, another raised in standard conditions was killed at 90 days of age, and a third group raised in standard conditions for 60 days was killed at 90 days of age after 30 days of enrichment. Comparing 60- and 90-day-old standard condition animals, the heterozygous rats showed a significant increase in height of the caudal diencephalon. Comparing 90-day-old enriched animals with the 90-day-old standard group, significant increases occurred in 8 of 12 comparisons. In comparing the two types of Brattleboro rats, heterozygous rats had significantly greater brain dimensions than homozygous rats in width of the diencephalon in the 60-day-old standard group, and in width of the telencephalon in both the 90-day-old standard and enriched groups. Differences in brain measures between heterozygous and homozygous Brattleboro rats tended to increase with age. Enrichment appeared to prevent this age-related increase in the difference between brain dimensions in the two types of Brattleboro rat. The same pattern was reflected in body weight differences, and it may be due to a greater anabolic response to enrichment in homozygous rats than in heterozygous rats. We suggest that brain abnormalities associated with congenital absence of vasopressin increase with age, and may be ameliorated by repetitive arousal as occurs in enrichment.

INTRODUCTION

In a previous study on Brattleboro rats we reported a decrease in thickness of the cerebral cortex with age and a generalized increase in cortical thickness with exposure to environmental enrichment between 60 and 90

Abbreviations: HE—heterozygous; DI—homozygous; SC, EC—standard, enriched condition; VP—vasopressin.

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days of age (12). We now extend these findings with additional measurements in the brain of the same groups of rats, and add further support for the role of arousal (12, 30) in the enrichment response.

The Brattleboro strain is a mutant of Long–Evans rats with a defect in the synthesis and release of vasopressin (VP) (17, 21). As a result of this defect, the relatively normal heterozygous Brattleboro rat (HE) has abnormalities in the VP content of certain brain regions in which VP has been measured (11). In contrast, VP is completely absent from the brain of the homozygous rat (DI) (3, 11) which manifests diabetes insipidus (20, 22). Both types of Brattleboro rats show open-field activity indicating prolonged arousal (2). The DI rat also shows abnormalities in learning and memory (2, 4, 31) and in the pituitary–adrenal system (2, 16, 27). Retarded growth (10, 21) and reduced pituitary growth hormone in the DI rat (1) are postulated to be due to some factor or mechanism other than the growth hormone releasing factor. Studies of catecholamine concentration and turnover rate in the DI rat reveal abnormalities in specific brain nuclei (25) that have been related to the absence of vasopressin (18, 26). Electrophysiological studies in the hippocampus of the DI rat indicate an abnormality in rhythmic slow activity during paradoxical sleep that is normalized by administration of deglycinamide-8-arginine vasopressin (19).

Developmental studies on normal rat strains (no VP abnormality) indicate that the dorsal cerebral cortex decreased in thickness during aging (6) as did the more ventral pyriform cortex (9). On the other hand, the diencephalon and hippocampus showed an increase in dimensions with aging (6).

Results of enrichment studies on both the dorsal and pyriform cortices indicate that these regions definitely increased in dimensions with environmental stimulation (9). Enrichment studies on the diencephalon and hippocampus were not as consistent, possibly because different regions were measured by separate investigators. Walsh *et al.* (29) reported a decrease in the diencephalon "underlying the frontal" cortex, whereas Diamond *et al.* (5) were unable to find a change in the diencephalon underlying the occipital cortex. Walsh *et al.* (28) reported a 6% ($P < 0.05$) increase in depth of the hippocampus, including both the dorsal and ventral portions, in eight rats, whereas Diamond *et al.* (8) found only a 2% (NS) increase in the dorsal hippocampus in 224 rats. Based on the greater number of rats studied Diamond *et al.*, it appeared that in normal rats the dorsal hippocampus did not change its structure in response to environmental enrichment. It was not clear about the changes in the ventral hippocampus.

In the present study we compared heterozygous with homozygous Brattleboro rats using measurements of the caudal diencephalon, subcortical telencephalon, hippocampus, and left pyriform cortex. We addressed the

following questions: (i) How do these brain dimensions respond to aging and to enrichment between 60 and 90 days of age in the two types of Brattleboro rats? (ii) Are the heterozygous rat brain dimensions greater than those of the homozygous rats, as expected, in the 60-day-old and 90-day-old animals from standard environmental conditions and in the 90-day-old enriched animals? (iii) Is there an effect of age or of enrichment on the percentage difference between heterozygous and homozygous rat brain dimensions? In addressing these questions we hoped to map out general patterns of differences between HE and DI rats to indicate where more detailed morphological studies should be made.

MATERIALS AND METHODS

Animals source, environmental conditions, and histologic procedures were explained in some detail in our first report on these animals (12). Briefly, breeder rats of the Brattleboro strain were obtained from the National Institutes of Health and were bred on three separate occasions to produce three groups of male HE and DI rats. From standard condition cages (40 × 20 × 20 cm; three rats per cage), one group (eight HE and six DI) was anesthetized and decapitated at 60 days of age (60-day SC), and another group (five HE and five DI) was killed at 90 days of age (90-day SC). A third group (12 HE and 12 DI) was decapitated at 90 days of age after living in standard conditions to 60 days of age and then in enriched conditions between 60 and 90 days of age (90-day EC). Enriched rats were housed in two enrichment cages (70 × 70 × 45 cm; 12 rats per cage) with six HE and six DI rats in each cage. They were provided daily with six or more "toys" from a standard pool (15), and had the additional experience of being weighed and transferred between cages at about 30 to 35 days of age. All rats were housed singly in standard cages for 48 h at some time between 33 and 40 days of age during diagnosis of diabetes insipidus.

After decapitation the brains were removed as quickly as possible and processed according to Van der Loos's modification (23) of the Golgi–Cox technique. The brains were cut coronally to provide sections from frontal, parietal, and occipital regions (7, 12). One 50-μm section from each region was selected for brain dimension studies after counterstaining with a modification of the Windle *et al.* (32) thionin stain. Alternate Golgi–Cox sections of 150 μm were used for a separate study of dendritic branching. For this report we used sections from the parietal and occipital regions; the subcortical landmarks were the crossing of the anterior commissure [Fig. 18 in (14)] and the crossing of the posterior commissure [Fig. 42 in (14)], respectively (see Fig. 1).

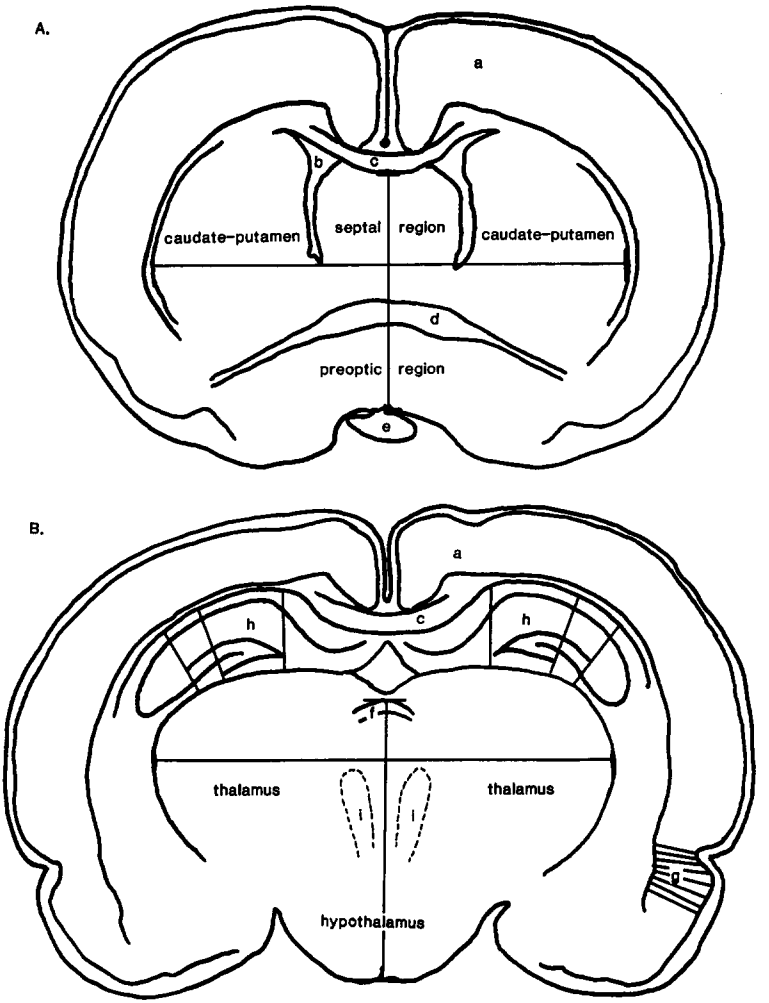

FIG. 1. Drawings representing microslide projected images of coronal sections through the brains of Brattleboro rats. The superimposed lines indicate dimensions measured. A—section through anterior commissure for measurement of subcortical telencephalon. B—section through posterior commissure for measurement of caudal diencephalon, hippocampus, and pyriform cortex. a, Cerebral cortex (measured in a previous study); b, lateral ventricle; c, corpus callosum; d, anterior commissure; e, optic chiasm; f, posterior commissure; g, pyriform cortex; h, hippocampus; i, parafascicular nucleus of thalamus.

Measurement of Brain Regions. Measurements were made on outlines of the brain sections that had been traced from microslide projected images magnified 22.5 times (Bausch and Lomb projector). The diagnosis and

experimental conditions were unknown to the person collecting the data, with the exception that 90-day SC rats were known to have been processed several months after the other two groups. The subcortical telencephalon was measured at the level of the crossing of the anterior commissure as indicated in Fig. 1A: height was measured from the ventral border of the corpus callosum to the ventral brain surface; and width was measured perpendicular to height at the point of maximal dimension. Caudal diencephalon, hippocampus, and pyriform cortex were measured in the section through the crossing of the posterior commissure as indicated in Fig. 1B. Diencephalic height was measured from the dorsal border of the posterior commisure to the ventral brain surface; width was measured as the maximal dimension perpendicular to height (6). The methods for measurement of the hippocampus (6) and the pyriform cortex (9) were also reported previously. In this study we compared only left pyriform cortices because precipitate artifacts, which formed during Golgi–Cox fixation in the 90-day SC group, prevented bilateral measurements on several slides.

Statistical Methods. The data were analyzed by computer programs from the Statistical Package for the Social Sciences. A two-way analysis of variance (subprogram ANOVA) for condition (60-day SC, 90-day SC, 90-day EC) and for genetic (HE, DI) factors was used to assess main effects and interactions. When appropriate the Newman–Keuls (indicated $P < 0.05$ NK) and the Scheffé ($P < 0.05$, 0.01, 0.001) corrections were used for pairwise comparisons (subprogram Oneway).

RESULTS

Changes with Age. Changes occurring with age (60-day SC vs 90-day SC) are indicated in Fig. 2A. A significant increase in brain dimensions occurred in the HE rats in diencephalic height (10.3%). The increases in body weight between 60 and 90 days of age were significant: HE, 44% and DI, 34% (Fig. 2A, broken bars).

Changes with Enrichment. The changes occurring with enrichment (90-day SC vs 90-day EC) are indicated in Fig. 2B. All brain dimensions in both types of Brattleboro rat were greater with enrichment compared with the standard condition group, and the difference attained significance in 8 of the 12 comparisons. In general, the significant increases with enrichment were greater, more frequent, and attained higher significance in the DI rats than in the HE rats. Body weight decreased 4% in the HE rats and increased 4% in the DI rats; neither change was significant.

Changes with Age plus Enrichment. Two significant increases occurred with age plus enrichment (60-day SC vs 90-day EC). Telencephalic width in the HE rats increased with age plus enrichment by 5.6% ($P < 0.001$).

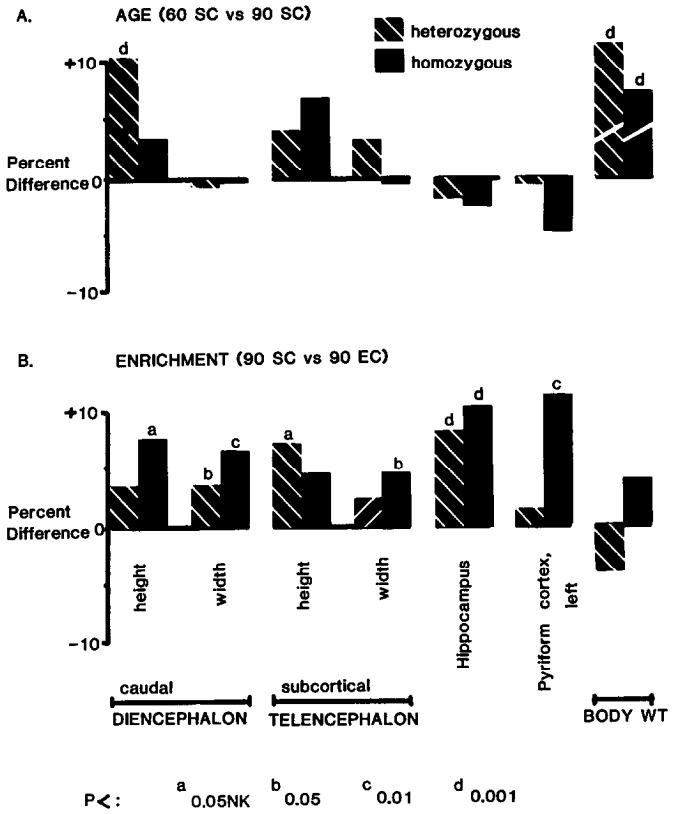

FIG. 2. Percentage difference with age and with enrichment in brain measurements and in body weights in heterozygous and homozygous Brattleboro rats. A—percentage difference with age: (90-day SC/60-day SC × 100) − 100. Broken blocks represent increases in body weight with age: HE = 44%; DI = 34%. B—percentage difference with enrichment (90-day EC/90-day SC × 100) − 100. The labels and *P* values below (B) also apply to (A). For measurements used in calculations see Table 1. [a]*P* < 0.05 NK: Neuman-Keuls correction; other *P* values are Scheffé correction.

Telencephalic height in the DI rats increased with age plus enrichment by 11.8% ($P < 0.01$).

Comparison of Heterozygous with Homozygous Rats. Table 1 shows mean brain measurements and body weights of heterozygous and homozygous Brattleboro rats in the three conditions. The HE rat brain dimensions were greater than those of the DI rats with only three exceptions. The differences in HE vs. DI dimensions were significant in diencephalic width in the 60-day-old SC group (3.7%), telencephalic width in the 90-day-old SC group (5.3%), and telencephalic width in the 90-day-old EC

TABLE 1

Comparison of Heterozygous (HE) and Homozygous (DI) Brattleboro Rats in Brain Measurements and Body Weight

Structure measured		Condition[a]								
		60-day SC			90-day SC			90-day EC		
		Units[b]	%dif.[c]	P[d]	Unit	%dif.	P	Units	%dif.	P
Diencephalon: height	HE	10.58 ± 0.10	−1.4	NS	11.67 ± 0.21	+5.1	NS	12.07 ± 0.14	+0.9	NS
	DI	10.73 ± 0.23			11.10 ± 0.29			11.96 ± 0.20		
Diencephalon: width	HE	17.56 ± 0.08	+3.7	<0.05	17.45 ± 0.13	+3.3	NS	18.08 ± 0.12	+0.6	NS
	DI	16.94 ± 0.28			16.90 ± 0.28			17.97 ± 0.10		
Telencephalon: height	HE	9.43 ± 0.20	+1.4	NS	9.82 ± 0.23	−1.1	NS	10.51 ± 0.17	+1.1	NS
	DI	9.30 ± 0.26			9.93 ± 0.20			10.40 ± 0.18		
Telencephalon: width	HE	19.15 ± 0.22	+1.7	NS	19.75 ± 0.12	+5.3	<0.01	20.22 ± 0.15	+3.0	<0.01
	DI	18.82 ± 0.22			18.76 ± 0.22			19.64 ± 0.14		
Hippocampus:	HE	2.78 ± 0.03	+2.6	NS	2.73 ± 0.03	+3.4	NS	2.95 ± 0.03	+1.4	NS
	DI	2.71 ± 0.04			2.64 ± 0.03			2.91 ± 0.03		
Left pyriform cortex	HE	2.10 ± 0.07	+1.4	NS	2.09 ± 0.12	+6.1[e]	NS	2.12 ± 0.02	−3.2	NS
	DI	2.07 ± 0.06			1.97 ± 0.05			2.19 ± 0.05		
Body weight (in grams)	HE	259 ± 2.5	+15	<0.001	374 ± 11.3	+24	<0.001	359 ± 5.6	+14	<0.001
	DI	225 ± 8.0			302 ± 5.9			314 ± 5.1		

[a] Number of rats in each condition: 60-day SC—HE = 8, DI = 6; 90-day SC—HE = 5, DI = 5; 90-day EC—HE = 12, DI = 12, exception—see footnote *e*.

[b] Units are mean ± SE centimeters after magnification at 22.5; body weight is in grams.

[c] Percentage difference: (HE/DI × 100) − 100.

[d] P value; NS = not significant.

[e] Number of rats measured for left pyriform cortex in 90-day SC group: HE = 3, DI = 4.

group (3.0%). Body weight was significantly greater in HE than in DI rats in all three conditions.

To emphasize an important trend, percentage differences between HE and DI rats in the three condition groups [including data from our recent study (12) on frontal, parietal, and occipital regions of the neocortex] are shown graphically in Fig. 3. The average percentage difference of nine brain measurements in HE and DI rats is greater in the 90-day SC group than in either the 60-day SC or the 90-day EC group. The same trend was reflected in body weight differences.

Summary of Results. (i) In the heterozygous Brattleboro rats, the height of the caudal diencephalon increased with age. (ii) With enrichment, the DI rats showed significant increases in five of the six brain regions measured, and HE rats' brain dimensions increased significantly in only three of the six brain dimensions measured. In general, the increases in the DI rats attained greater significance. (iii) Although brain dimensions of HE rats were not significantly greater than those of DI rats in most comparisons, body weights of HE rats were significantly greater in all three con-

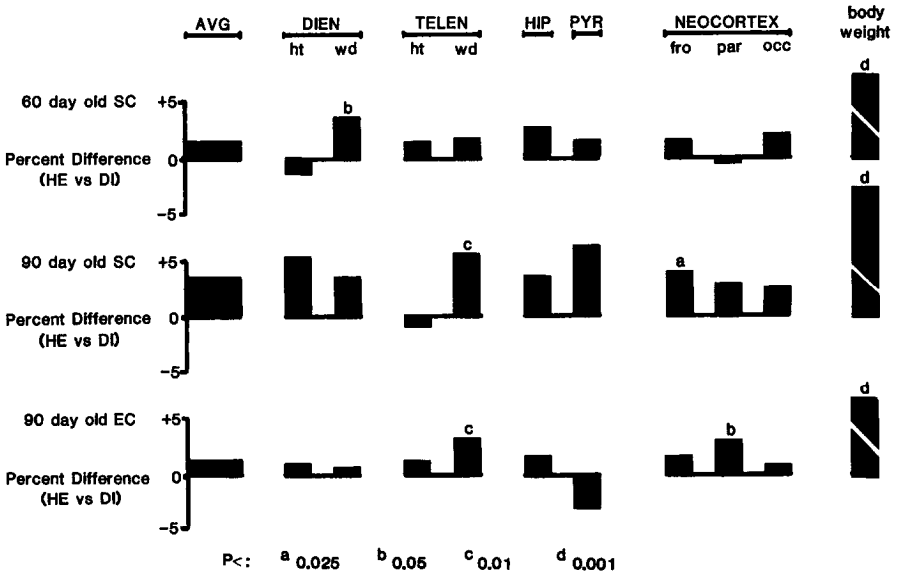

FIG. 3. Percentage difference in brain measures between heterozygous and homozygous Brattleboro rats from each of three conditions. Percentage difference was calculated from units in Table 1: (HE/DI × 100)-100. AVG—average percentage difference between HE and DI brain dimensions, DIEN—caudal diencephalon, TELEN—subcortical telencephalon, HIP—hippocampus, PYR—left pyriform cortex, ht—height, wd—width, NEOCORTEX—information from previous study (12), fro—frontal cortex, par—parietal cortex, occ—occipital cortex.

dition groups. (iv) Average percentage differences between HE and DI brain dimensions were greater in the 90-day SC group than in either the 90-day EC group or the 60-day SC group.

DISCUSSION

Results Compared with Previous Studies. In agreement with our previous findings in the cerebral cortex of the Brattleboros (12), the magnitude of the enrichment response of the DI rats exceeds that of the HE rats. The occurrence of significant increases in subcortical brain dimensions in both types of Brattleboro rats with *enrichment* (Fig. 1B) is in contrast to the conflicting reports in normal (nonvasopressin-deficient) rats (8, 9, 28).

The age trends (Fig. 1A) in the caudal diencephalon, subcortical telencephalon, and pyriform cortex agree with age trends in normal rats (6, 9). However, the failure of hippocampal dimensions to increase with age in the Brattleboro rats is not in accord with age trends in normal (nonvasopressin-deficient) rats (9). This difference in hippocampal response to age may be related to the reduced or absent hippocampal vasopressin in the HE and DI rats (11) reported by Dogterom *et al.* They found no VP in the anterior hippocampus and reduced VP in the posterior hippocampus of HE rats, and VP was completely absent from the brain of DI rats. Because VP accelerates the disappearance of norepinephrine in certain brain regions (18), both types of Brattleboro rat might be expected to have a chronic reduction in nonepinephrine release in the hippocampus under standard environmental conditions. A reduction in nonepinephrine release, considering its possible function as a neurohormone (24), might be expected to correlate with reduced anabolic activity and with the failure of both Brattleboros to increase hippocampal dimensions between 60 and 90 days of age.

Speculations on Findings. A *possible* interpretation of the response of Brattleboro rats to age and to enrichment was suggested by a close inspection of Fig. 2. Note that brain dimensions of DI rats tended to decrease more, or increase less, with age (Fig. 2A), and tended to increase more with enrichment (Fig. 2B) than brain dimensions of HE rats. Figure 3 quantifies these observations as percentage differences between HE and DI rat dimensions. The average difference in brain dimensions and body weight between the two Brattleboro rats was greater after 90 days in standard conditions than after 60 days in standard conditions; the average difference was least at 90 days of age after 30 days of enrichment. It seems then, that the increase in age while in the standard environment widened the gap between homozygous and heterozygous rat brain dimensions, and enrichment prevented this age-related augmentation of differences. We

have speculated (13) that prolonged arousal in the DI rats during enrichment produced metabolic effects which counteract age changes.

An even closer inspection of Fig. 2 shows that certain brain regions responded in a manner which requires comment because their response suggests the need for combined morphologic and biochemical measures in future experiments. These regions are: diencephalic height, which spans the parafascicular region; telencephalic width, which mainly spans the caudate-putamen region; and telencephalic height, which primarily spans the septal region (Fig. 1). In each of those brain regions, certain nuclei in the DI rat were reported to have abnormal catecholamine concentration and/or turnover relative to normal Long–Evans rats (25): a reduced turnover rate of norepinephrine was found in the parafascicular nucleus; a reduced dopamine turnover rate was found in the caudate nucleus; and an increased turnover rate and concentration of norepinephrine was found in the dorsal septal nucleus of DI rats. Although the significance of our findings relative to catecholamine metabolism is highly speculative, we feel that certain possible correlations should be pointed out: (i) Where catecholamine turnover rate was reduced in the DI rats, they failed to increase dimensions with age as much as HE rats (diencephalic height, across the parafascicular region; and telencephalic width, across the caudate-putamen region). (ii) Where catecholamine turnover rate and concentration were increased in the DI rats, their increase with age was greater than that of the HE rats; and their increase with enrichment was less than the HE response (telencephalic height, across the septal region) (Fig. 2). This suggests a ceiling effect with age in the DI rats in the septal region which reduced the relative response to enrichment.

CONCLUSION

The marked and widespread response of Brattleboro rats to environmental enrichment is consistent with the previous suggestion of an arousal-mediated, generalized component in their enrichment response. The results also suggest that brain abnormalities associated with congenital absence of vasopressin increase with age and may be ameliorated by repetitive arousal. This latter point indicates that age and environmental conditions, as well as arousal history, should be considered in any evaluation of behavioral responses of Brattleboro rats.

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