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FR Discrimination Training Effects in SHR and Microencephalic Rats

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LOUPE, P. S., S. R. SCHROEDER AND R. E. TESSEL. *FR discrimination training effects in SHR and microencephalic rats.* PHARMACOL BIOCHEM BEHAV 51(4) 869-876, 1995.—Fixed-ratio (FR) discrimination learning in adult male spontaneously hypertensive rats (SHR), methylazoxymethanol-induced microencephalic Sprague-Dawley (MAM), and Sprague-Dawley control rats was examined. SHR and MAM rats had little problem learning incrementally more difficult FR discriminations (FR1 vs. FR16, FR4 vs. FR16, and FR8 vs. FR16) that resulted in parallel increases in errors in all animals, and displayed only modest learning deficits during a subsequent FR4 vs. FR16 position reversal. When training involved nonincremental changes in difficulty (FR8 vs. FR16, FR4 vs. FR16, FR8 vs. FR16, FR12 vs. FR16, and FR14 vs. FR16), SHR and MAM rats evidenced relatively large learning deficits during the initial FR8 vs. FR16 discrimination but had no difficulty with the last two discriminations. Furthermore, training selectively and significantly elevated hippocampal weight in MAM rats. These findings: a) question prior suggestions that MAM and SHR model separate human developmental disabilities; b) indicate that manifestation of learning deficits in even markedly brain-damaged organisms depends on initial task difficulty and can be overcome by experience; and c) are the first indicating that training-induced antagonism of prenatally induced hippocampal hypoplasia and its consequences is possible.

Fixed-ratio discrimination learning Methylazoxymethanol Spontaneously hypertensive rats
Hippocampal plasticity Microencephaly

ADMINISTRATION of the antimitotic drug methylazoxymethanol (MAM) to pregnant rats during gestation results in offspring with central anatomical and behavioral characteristics reminiscent of those found in individuals with severe mental retardation. For example, such offspring (MAM rats) are microencephalic [e.g., (3)], an anatomical sign commonly associated with mental retardation, microencephaly (9), and manifest learning deficits relative to controls when exposed to difficult (complex) but not simple maze tasks (4,25). MAM rats are also hyperactive (15,25) and have sometimes been reported to be hyporesponsive to the locomotor activating effects of psychomotor stimulants [(4), but see (1,2)]. In addition, MAM rats respond to stress by emitting aberrant behaviors [e.g., stereotypies (33)] like those displayed by many mentally retarded individuals (19,28).

In contrast, spontaneously hypertensive rats (SHR) have been proposed to model a less debilitating form of developmental disability, attention deficit disorder with hyperkinesis (ADDH) (24). Consistent with this proposal, SHR have difficulty learning both radial-arm maze tasks that require repetitive arm entry and tasks that require immobility (18,39).

However, ADDH and mental retardation are not mutually exclusive diagnoses (19), and behavioral similarities in MAM and SHR rats suggest the possibility that SHR may also model aspects of mental retardation as well. For example, like MAM rats (see above), SHR are hyperactive in several situations (15,35) and may be hyporesponsive to the locomotor effects of psychomotor stimulants [(21,40), but see (12,35)]. In addition, although the topographies manifested by SHR differ from those displayed by MAM rats, like MAM rats, SHR exposed to stressful environmental situations emit aberrant behaviors [aggression and stereotypies (15,33)] reminiscent of those "spontaneously" emitted by many mentally retarded individuals (19,28). However, despite these similarities, and to the best of our knowledge, the learning abilities of SHR and MAM rats have not previously been directly compared.

Learning in individuals with mental retardation is presumably more susceptible to variations in the task difficulty than

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in those without retardation. Contrary to this contention, however, adult rats neonatally depleted of catecholamines by intracranial injection of 6-hydroxydopamine (6HD), another putative rat model of individuals with mental retardation (3,6), did not manifest learning deficits relative to control animals when required to learn incrementally more difficult fixed ratio (FR) discriminations (FR1, FR4, and FR8 vs. FR16 discriminations) although the 6HD-treated animals tended to learn a subsequent (FR4–FR16) position reversal more slowly (41). The absence of learning deficits was not attributable to the lack of effectiveness of 6HD treatment per se or, apparently, to the use of insufficiently difficult discriminations, but could reflect a relative insensitivity of the incremental FR discrimination training procedure used earlier or the mental retardation model examined.

The purpose of the present study was to investigate these latter possibilities as well as to compare the learning abilities of SHR and MAM rats with those of control animals. This was accomplished through the use of two different forms of FR discrimination training. In the first experiment, the same incremental training procedure indicated before with adult, neonatal 6HD-treated rats (i.e., FR1 vs. FR16, FR4 vs. FR16, FR8 vs. FR16, reestablishment of an FR4 vs. FR16 baseline, FR4 vs. FR16 position reversal) was used. In addition, the magnitude of MAM-induced microencephaly was determined by comparing control and MAM rat whole-forebrain weights [e.g., (34)]. In the second experiment, a nonincremental procedure was used. In this procedure other animals were required to learn a more difficult initial FR discrimination (FR8 vs. FR16) followed by the learning of FR4 vs. FR16, FR8 vs. FR16, FR12 vs. FR16, and FR14 vs. FR16 FR discriminations. Furthermore, the forebrain was divided into several regions and their weights were determined in age-matched, food-deprived MAM, SHR, and control animals that had or had not undergone FR discrimination training.

METHOD

Animals and Drug Treatment

Twenty timed-pregnant female Sprague–Dawley (SD) and adult male SDs and SHRs were purchased from Harlan Sprague–Dawley, Inc. (Indianapolis, IN). On gestation day 15, the timed-pregnant females were given an intraperitoneal (IP) injection (1 ml/kg) of either 25 mg/kg methylazoxymethanol acetate (MAM) (n = 10) or vehicle (saline; n = 10) with an injection volume of 1 ml/kg body wt. Litter sizes were reduced to 10 pups per dam, and the pups remained with their birth mothers until weaning on postnatal day 21. After weaning, the pups were placed in group housing (three per cage) until they were 3 mo of age; water and Purina Rat Chow (Ralston Purina Co., St. Louis, MO) were available ad lib. To ensure that microencephaly had been induced, 30-day-old female pups were sacrificed and the weights of the forebrain and the cerebellum tissues were determined. Only MAM-treated litters in which the females showed at least a 40% reduction in forebrain tissue weights as compared to saline-treated litters were used in the experiment. The mean forebrain tissue weight of the MAM female littermates used in the present study was 639 ± 25 mg as compared to a forebrain tissue weight of 1070 ± 20 mg in the saline female littermates.

The 90-day-old male offspring from the MAM- or saline-treated litters, untreated adult male SDs, and male SHRs were individually housed and reduced to 85% of their free-feeding weights (free-feeding weights ranged between 350 and 375 g). To ensure that the SHRs were hypertensive, mean arterial blood pressures (MAP) were determined directly in adult, food-deprived, conscious, and unrestrained SHRs (n = 3) and SDs (n = 3) by means of indwelling femoral artery catheters using procedure described by (16). The MAP in SHRs was 144 ± 5 mm Hg, whereas that in the SDs was 98 ± 4 mm Hg ($p < 0.001$). In addition, two male offspring per MAM (n = 9) or saline litter (n = 7) and half of the adult male SDs (n = 5) and SHRs (n = 5) were maintained at their reduced body weights but allowed to remain undisturbed in their home cages throughout the experiment except for biweekly weighing (untrained animals). Approximately two other male offspring per MAM or saline litter (total n = 32) and the rest of the adult SDs (n = 11) and SHRs (n = 11) were retained for behavioral training. All animals were housed in clear plastic cages and maintained on a 12L : 12D cycle in an American Association for Accreditation of Laboratory Animal Care approved animal colony.

Apparatus

Six 29 × 25.3 × 28-cm experimental operant chambers housed in larger insulated shells with ventilation fans (Coulbourn Instruments Co., Allentown, PA) were used. A center retractable lever and two side levers were located 2 cm from the grid floor on one side of the chamber. A three-diode cue-light panel was located above each lever. A food trough was located above the center retractable lever and cue light, approximately 9.5 cm from the floor. A houselight was located 26.5 cm from the floor to the right of the food trough on the same side of the chamber.

Training

The weight-reduced animals were initially trained to complete FRs of gradually increasing magnitude (FR1, FR2, FR4, FR6, FR8, and FR16) on the center lever when cue lights above it were illuminated; responding was reinforced by presentations of a 45-mg food pellet (P.J. Noyes, Lancaster, NH). Upon completion of the ratio, the center lever retracted and its cue lights were extinguished before the food pellet was dropped into the food trough. Training sessions during this and all subsequent steps were conducted 5 days/week and lasted until 30 min had passed or 60 reinforcements has been presented (whichever occurred first).

After one to two sessions at FR16, a single nonretractable lever, located on the left side of the retractable lever, was added to the chamber for one session. Completion of the FR16 now extinguished the cue lights over the retractable lever, caused the lever to retract, and illuminated cue lights over the side lever, and a single side-lever press was required for food presentation. During the next session, a second nonretractable lever located to the right of the retractable lever was added, and cue lights above it also illuminated after lever retraction. However, a press on the left-side lever was still required for food presentation; right-side lever presses had no programmed consequences.

This two-session sequence was then repeated, except that during the first of these sessions only the right-side lever was present, and the center lever retracted after one of two lower FR values. For animals to be subjected to incremental training, the lower FR value was 1 (FR1); for those to be subjected to nonincremental training, the lower FR value was 8 (FR8). During subsequent sessions both FR requirements were made available such that if the ratio was 16 on a given trial, a response on the left lever was reinforced by food presentation, whereas if the ratio was 1 (or 8), a response on the right lever

was reinforced. The intertrial interval was 0.5 s. Errors (i.e., responding on an incorrect side lever) resulted in a 5-s timeout during which the house- and all stimulus lights were extinguished, and the center lever was retracted. Furthermore, a ratio associated with an error continued to be represented following the timeout until a correct response was made. However, once performance stabilized, this correction procedure was eliminated and the probability that the lever retracted after completion of an FR1 or an FR16 on any particular trial was 0.5.

Incrementally trained animals (MAM-treated, $n = 8$; saline-treated, $n = 6$; untreated SDs, $n = 5$; and SHRs, $n = 6$) were required to learn and perform incrementally more difficult discriminations, the first one being an FR1 vs. FR16 discrimination. The lower FR value was the increased to 4 and then 8 to make the task incrementally more difficult; performance on each discrimination was allowed to stabilize between increases. Animals were then reexposed to the FR4 vs. FR16 discrimination and, after performance stabilized, the identities of the correct side levers were reversed [i.e., the right-side lever was now paired with the higher ratio (16) and the left-side lever was paired with the lower ratio (4)].

For technical reasons, nonincremental training was conducted in two separate replicates in additional animals. Training of one replicate was begun 4 mo before that of the second but at the same time relative to the animals' birth dates. The first replicate consisted of five MAM-treated and four saline-treated SDs; the second consisted of four MAM-treated and five saline-treated SDs, and five untreated SDs and five SHRs. Under this procedure, discrimination training began with an FR8 vs. FR16 discrimination and continued until performance stabilized. The lower ratio was then sequentially changed to 4, 8, 12, and finally 14, with performance allowed to stabilize between each alteration in task difficulty (approximately seven to 10 sessions for each of these FR values). After training was completed, the animals were allowed to remain at their reduced weights and, except for biweekly weighing, in their home cages until these and the age-matched untrained animals were sacrificed (within 1 mo of the end of training).

Brain Weight Determinations

Rats were rendered unconscious by exposure to a carbon dioxide-saturated environment, and then decapitated. In the experiment involving incremental training wet weights of whole forebrains and cerebella of trained MAM-treated and saline-treated animals were compared. In the study involving nonincremental training, the hippocampi, cortices, striata, and cerebellum in trained animals and age-matched, weight-reduced, untrained animals were isolated (11) and their wet weights determined. In the second experiment of the nonincremental training procedure, the brain dissections were conducted with the experimenter blinded as to which animal groups the brains came from. All of these brain regions were fixed in formaldehyde and some were analyzed for protein concentration (31).

Statistical Analysis

Statistical analyses were conducted using organic insult (MAM-treated vs. saline-treated SDs) or genetic risk factor (untreated SDs vs. SHRs) as the between-subject factors, and the varying levels of task difficulty and sessions as within-subject factors in a repeated measures ANOVA (BMDP4V). Performance in the discrimination procedures was assessed in terms of the percent error (ratio of incorrect to total side lever selections) and response rate (number of responses on the center lever per minute of center lever presentation) for each session per animal. As there were no statistically significant differences between saline-treated and untreated SD controls in the percent error, data from these groups were combined except where indicated. One-sample t-tests using Bonferroni's correction for experimentwise error rates were used to assess whether the percent error rates of the animal groups differed significantly from chance error rates (50%) at varying task difficulties. Two-way ANOVA followed by unpaired t-tests were also used to assess the effects of training, organic insult, or genetic risk factor on tissue weight.

RESULTS

Incremental Training

Changes in task difficulty following removal of the correction procedure were generally associated with significant changes in mean percent error (error rates) in each group (Figs. 1 and 2); for MAM, SHR, and controls, respectively, FR1 vs. FR16 × FR4 vs. FR16: [$F(1, 7) = 19.45, p < 0.005$; $F(1, 3) = 16.78, p < 0.05$; and $F(1, 10) = 6.34, p < 0.05$]; FR4 vs. FR16 × FR8 vs. FR16: [$F(1, 7) = 10.11, p < 0.05$; $F(1, 3) = 5.77, p < 0.09$; and $F(1, 10) = 26.57, p < 0.001$]; FR8 vs. FR16 × FR4 vs. FR16: [$F(1, 7) = 41.34, p < 0.001$; $F(1, 3) = 399, p < 0.001$; $F(1, 10) = 210, p < 0.0001$]; and FR4 vs. FR16 × FR4 vs. FR16 reversal: [$F(1, 7) = 224$, $p < 0.0001$; $F(1, 3) = 29.42, p < 0.05$; $F(1, 10) = 171, p < 0.0001$]. Nevertheless, SHRs manifested a small (although significant) increase in mean percent error relative to that of control animals only during the initial FR1 vs. FR16 discrimination with correction [$F(1, 15) = 12.62$; $p < 0.005$] (Fig. 1), and, after the correction procedure was removed, quickly developed error rates comparable to those in control rats during this and the subsequent two (FR4 vs. FR16 and FR8 vs. FR16) discriminations. In addition, error rates in SHRs during the reversal portion of this experiment only tended to be higher than those in controls ($p < 0.14$). In contrast, error rates in MAM animals were essentially identical to those in

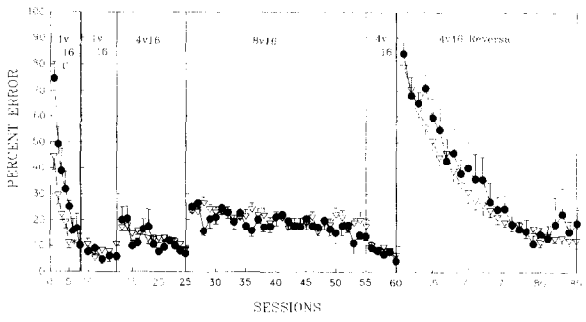

FIG. 1. Food-maintained incremental fixed-ratio (FR) discrimination learning in adult male spontaneously hypertensive (SHR) and control Sprague-Dawley (SD) rats. Training began when the animals were 3 mo of age using once-daily sessions conducted 5 days/week. The ordinate, percent error, indicates the ratio of incorrect side lever presses to total side lever presses per session; the abscissa indicates the number of sequential training sessions. ∇ and ● (and brackets), means (± SEM) of observations in 11 SD and six SHR rats. The numbers above each panel (e.g., FR1 vs. FR16) indicate the FR discrimination being learned. C, A correction procedure (see Methods) was used during this portion of the experiment.

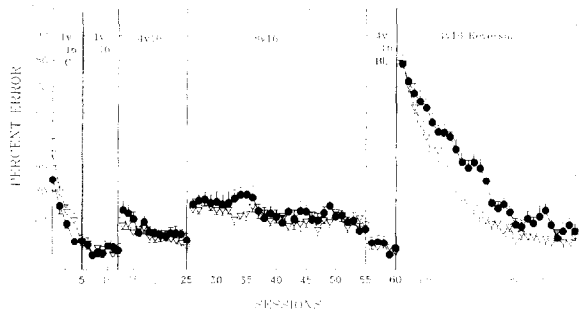

FIG. 2. Food-maintained incremental fixed-ratio (FR) discrimination learning in adult male methylazoxymethanol-exposed (MAM) and control Sprague-Dawley (SD) rats. Training began when the animals were 3 mo of age using once-daily sessions conducted 5 days/week. The ordinate, percent error, indicates the ratio of incorrect side lever presses to total side lever presses per session; the abscissa indicates the number of sequential training sessions. ∇ and ● (and brackets), means (± SEM) of observations in 11 SD and eight MAM rats. The numbers above each panel (e.g., FR1 vs. FR16) indicate the FR discrimination being learned. C, A correction procedure (see Methods) was used during this portion of the experiment.

controls during the FR1 vs. FR16, FR4 vs. FR16, and FR8 vs. FR16 discriminations and differed from those in controls only during the reversal portion of the procedure (Fig. 2). In this portion, MAM error rates were modestly although significantly [$F(1, 17) = 15.76$; $p < 0.001$] higher than controls (Fig. 2); these rates did not differ significantly from those in SHR rats.

Despite these differences in the responsiveness of MAM and SHR rats to variations in task difficulty, both organic insult and genetic risk factor influenced response rates in a qualitatively similar manner. Thus, mean response rates of MAM animals were significantly lower than those of controls during discriminations: FR1 vs. FR16, [$F(1, 18) = 5.47$, $p < 0.05$]; and FR4 vs. FR16 [$F(1, 18) = 9.12$, $p < 0.01$]; and those of the SHRs were significantly lower than the controls during discriminations: FR1 vs. FR16 [$F(1, 15) = 10.56$, $p < 0.01$]; FR4 vs. FR16 [$F(1, 15) = 19.27$, $p < 0.001$); and FR8 vs. FR16 [$F(1, 15) = 4.75$, $p < 0.05$] (Table 1).

Nonincremental Training

As in incremental training, changes in discrimination difficulty were associated with monotonic changes in errors in all groups (Figs. 3 and 4); for MAM, SHR and controls, respectively: FR8 vs. FR16 × FR4 vs. FR16: [$F(1, 8) = 59.95$, $p < 0.0001$; $F(1, 4) = 152.17$, $p < 0.001$; $F(1, 6) = 12.11$, $p < 0.05$]; FR4 vs. FR16 × second FR8 vs. FR16: [$F(1, 8) = 34.20$, $p < 0.0005$; $F(1, 4) = 57.04$, $p < 0.005$; $F(1, 6) = 14.89$, $p < 0.01$]; FR8 vs. FR16 × FR12 vs. FR16: [$F(1, 8) = 309$, $p < 0.0001$; $F(1, 4) = 212$, $p < 0.0001$; $F(1, 6) = 105$, $p < 0.001$]; FR12 vs. FR16 × FR14 vs. FR16: [$F(1, 8) = 59.45$, $p < 0.0001$; $F(1, 4) = 62.97$, $p < 0.005$; $F(1, 6) = 18.74$, $p < 0.005$]. However, contrary to the generally modest effects obtained during incremental training as well as the absence of effect of organic insult and genetic risk factor on FR8 vs. FR16 discrimination during such training, mean percent error was significantly greater in both MAMs and SHRs than in controls during the FR8 vs. FR16 discrimination with correction portion of nonincremental procedure [$F(1, 11) = 12.30$, $p < 0.005$; $F(1, 6) = 23.69$, $p < 0.05$, respectively). These differences were maintained when the correction was removed: controls vs. MAMs: [$F(1, 10) = 16.93$; $p < 0.005$]; controls vs. SHRs: [$F(1, 6) = 4.29$; $p < 0.05$]. Nevertheless, error rates in the more difficult discrimination task of FR12 vs. FR16 and FR14 vs. FR16 were similar among all groups. Furthermore, all groups performed these latter discrimina-

TABLE 1

CENTER-LEVER RESPONSE RATES IN VEHICLE-, MAM-, AND UNTREATED SPRAGUE-DAWLEY (SD) RATS AND SPONTANEOUSLY HYPERTENSIVE RATS (SHR) DURING INCREMENTAL AND NONINCREMENTAL FIXED-RATIO (FR) DISCRIMINATION TRAINING

FR Training	Vehicle-treated SD	MAM-treated SD	Untreated SD	SHR
Incremental				
1 vs. 16C	70 ± 5	60 ± 4	66 ± 3	40 ± 4*
1 vs. 16	80 ± 4	65 ± 4†	81 ± 4	60 ± 4*
4 vs. 16	114 ± 9	87 ± 5†	113 ± 5	75 ± 6*
8 vs. 16	126 ± 11	110 ± 7	128 ± 6	106 ± 7*
4 vs. 16	105 ± 10	95 ± 4	109 ± 6	97 ± 9
4 vs. 16R	125 ± 9	108 ± 8	122 ± 6	97 ± 10
Nonincremental				
8 vs. 16C	113 ± 17	115 ± 16	108 ± 22	78 ± 6*
8 vs. 16	104 ± 30	103 ± 11	109 ± 26	112 ± 22
4 vs. 16	114 ± 36	116 ± 10	94 ± 22	78 ± 4
8 vs. 16	100 ± 31	115 ± 8	106 ± 23	96 ± 8
12 vs. 16	116 ± 30	130 ± 16	117 ± 17	130 ± 8
14 vs. 16	126 ± 20	138 ± 18	129 ± 20	155 ± 15

Numbers indicate the mean (± SEM) of center-lever response rates (responses per minute) averaged across the first and last session at each level of discrimination difficulty in nine vehicle-treated SD rats, nine MAM rats, five untreated SD rats and five SHR. C, Presence of correction procedure; R, use of the reversal procedure.

*$p < 0.05$ compared to untreated SD rats.

†$p < 0.05$ compared to vehicle-treated SD rats.

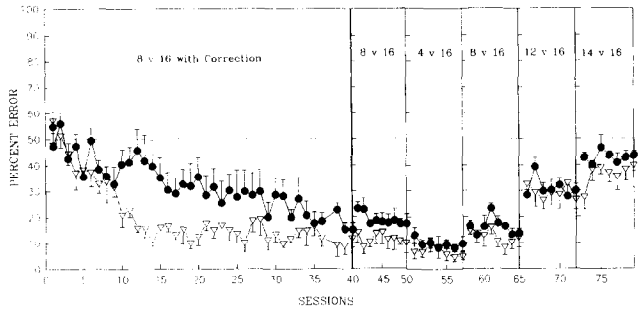

FIG. 3. Food-maintained nonincremental fixed-ratio (FR) discrimination learning in adult male spontaneously hypertensive (SHR) and control Sprague-Dawley (SD) rats. Training began when the animals were 3 mo of age using once-daily sessions conducted 5 days/week. The ordinate, percent error, indicates the ratio of incorrect side lever presses to total side lever presses per session; the abscissa indicates the number of sequential training sessions. ∇ and ● (and brackets), means (± SEM) of observations in 14 SD and five SHR rats. The numbers above each panel (e.g., FR8 vs. FR16) indicate the FR discrimination being learned. C, A correction procedure (see Methods) was used during this portion of the experiment.

tions with mean percent error rates that were significantly better than 50% chance levels (all $p < 0.005$).

Response rates during this portion of training were generally unaffected by organic insult, genetic risk factor, or task difficulty (Table 1). The only exception was an slight decrease in SHR mean response rate relative to controls during the FR8 vs. FR16 with correction portion [$F(1, 14) = 4.29, p < 0.05$].

Brain Tissue Weights

As expected, prenatal exposure to MAM at the dose and time used in the present study resulted in a marked reduction in forebrain weight of incrementally trained animals (1241 ± 38 mg for controls and 648 ± 4 mg for MAM rats; 48% decrease; $p < 0.0001$). Likewise, cortical and striatal wet tissue weights of nonincrementally trained and age-matched, weight-reduced, but untrained MAM animals were similar as were those in trained and untrained controls (Fig. 5). However, MAM treatment significantly reduced these weights relative to those of controls (each $p < 0.05$), whereas those of the cerebellum, as in previous studies [e.g., (25)], were not (Fig. 5). Hippocampal wet weights were also reduced by MAM treatment compared to those of controls, and training had no effect on hippocampal wet weight in control animals ($p < 0.05$) (Fig. 6). Nevertheless, in contrast to those of the other brain regions examined, training increased the wet weight of the hippocampus in MAM rats when the replicates were combined [67 ± 4 mg in trained and 51 ± 2 mg in untrained MAM rats; 35% increase; $F(1, 25) = 6.25, p < 0.05$] as well as in the individual replicates ($p < 0.05$) (Fig. 6). In addition, these increases were associated with a 15% increase in protein concentration (data not shown). However, despite similarities in the effects of nonincremental FR discrimination training on learning in MAM and SHR rats (Figs. 3 and 4), tissue weights in SHRs did not differ from those of controls and were unaffected by training (Table 2).

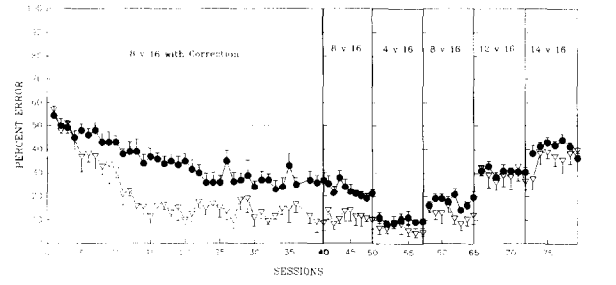

FIG. 4. Food-maintained nonincremental fixed-ratio (FR) discrimination learning in adult male methylazoxymethanol-exposed (MAM) and control Sprague-Dawley (SD) rats. Training began when the animals were 3 mo of age using once-daily sessions conducted 5 days/week. The ordinate, percent error, indicates the ratio of incorrect side lever presses to total side lever presses per session; the abscissa indicates the number of sequential training sessions. ∇ and ● (and brackets), means (± SEM) of observations in 14 SD and nine MAM rats. The numbers above each panel (e.g., FR8 vs. FR16) indicate the FR discrimination being learned. C, A correction procedure (see Methods) was used during this portion of the experiment.

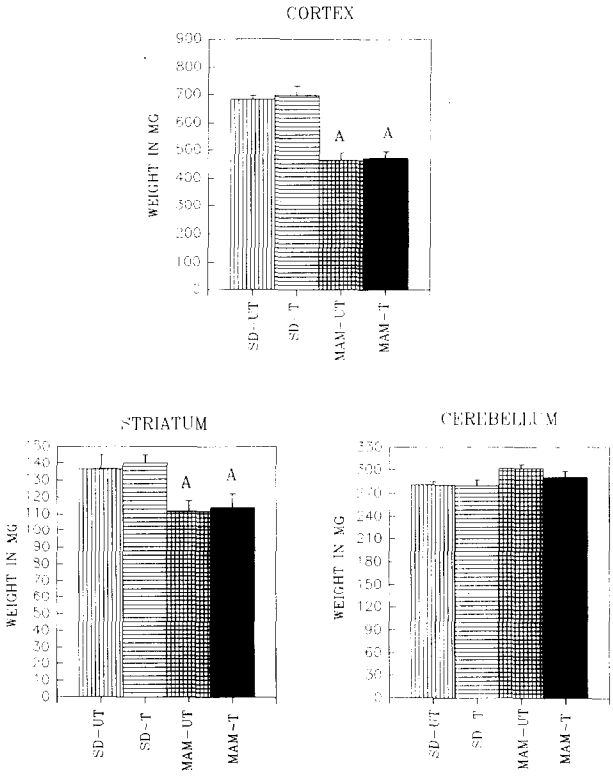

FIG. 5. Effects of prenatal methylazoxymethanol (MAM) exposure and postnatal nonincremental fixed-ratio (FR) discrimination training on cortical, basal ganglia, and cerebellar weights in adult male rats. All rats were reduced to approximately 85% of free-feeding weights beginning at 3 mo of age, but untrained animals were allowed to remain in their home cages throughout the experiment. Animals were sacrificed for brain tissue weight determinations within 1 mo after the trained animals had completed training. Bars (and brackets) represent the mean (± SEM) of tissue weights in nine trained (MAM-T) and untrained (MAM-UT) MAM rats, and nine trained vehicle-injected Sprague-Dawley (SD; SD-T) and even untrained vehicle-injected SD (SD-UT) rats. A, A significant difference ($p < 0.05$) in tissue weight due to prenatal MAM exposure.

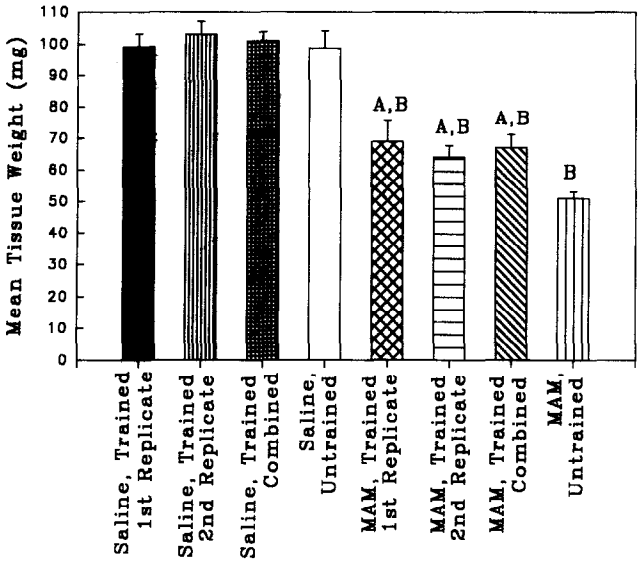

FIG. 6. Effects of nonincremental fixed-ratio (FR) discrimination training and prenatal methylazoxymethanol (MAM) exposure on hippocampal weight in adult male rats. All rats were reduced to approximately 85% of free-feeding weights beginning at 3 mo of age, but untrained animals were allowed to remain in their home cages throughout the experiment. Animals were sacrificed for brain tissue weight determinations within 1 mo after the trained animals had completed training. Bars (and brackets) represent the mean (± SEM) of tissue weights in four (first replicate), five (second replicate), and nine (replicates combined) trained and nine untrained Sprague-Dawley (SD) rats prenatally exposed to vehicle (saline), and in five (first replicate), four (second replicate), and nine (replicates combined) trained and nine untrained SD rats prenatally exposed to MAM. A and B, Significant hippocampal weight differences ($p < 0.05$) between untrained and trained MAM rats, and between MAM- and saline-treated rats, respectively.

DISCUSSION

The results of the present and companion studies support several conclusions. First, the likelihood that deficits will occur during trial-and-error learning of conditional (e.g., FR) discriminations in rats, as in humans (26,27,38), does not depend on the difficulty of the task per se. Rather, it appears to be positively related to the initial difficulty of the task and, even in rats with severe brain dysfunction (e.g., MAM rats), negatively related to the amount of experience with comparable but less difficult tasks. Second, despite proposals that SHR and MAM rats model different forms of developmental disability (4,24), the patterns of learning competence and deficit displayed in the present study by these animals are remarkably similar. Last, exposure to at least a nonincremental FR discrimination training procedure has the capacity to reverse the anatomical and, possibly, the functional consequences of prenatally (MAM)-induced hippocampal hypoplasia in an adult organism.

FR Discrimination Learning

With the possible exception of SHR during the FR1–FR16, the rat mental retardation models examined in the present (and companion) study had no problem learning incrementally more difficult FR discriminations (including an FR8 vs. FR16 discrimination) even though the increases in difficulty incrementally increased errors in all animals. In addition, exposure to a subsequent position reversal task only resulted in modest learning deficits in these models, and these deficits were only statistically significant in MAM rats. In contrast, SHR and MAM animals manifested relatively large learning deficits when training was initiated with an FR8 vs. FR16 during nonincremental training. However, although the animals' performance of this discrimination began at chance levels (percent errors of approximately 50%), their performance even during the first sessions of subsequent FR12–FR16 and FR14–FR16 discriminations occurred at levels that were better than chance and equivalent to those of controls. These results not only illustrate the capacity of prior experience to reduce the extent to which subsequent learning deficits will occur in even severely microencephalic organisms; they also argue that SHR and MAM rats do not markedly differ in learning ability, and therefore that these animals do not necessarily model separate human developmental disabilities.

The training procedures used in the present study also differed in their effects on response rate. Incremental training was associated with progressive increases in response rates in all animals. In contrast, nonincremental training was generally associated with relatively stable response rates across levels of discrimination difficulty. These findings are consistent with

TABLE 2

BRAIN REGIONAL WEIGHTS IN NONINCREMENTAL FR-DISCRIMINATION TRAINED AND UNTRAINED SPONTANEOUSLY HYPERTENSIVE (SHR) AND UNTREATED SPRAGUE-DAWLEY (SD) RATS

	Cortex	Hippocampus	Striatum	Cerebellum
SHR				
Trained	719 ± 2	95 ± 3	121 ± 10	269 ± 4
Untrained	680 ± 2	87 ± 8	118 ± 3	263 ± 6
Untreated SD				
Trained	698 ± 11	99 ± 3	140 ± 5	274 ± 7
Untrained	683 ± 14	99 ± 5	136 ± 4	280 ± 4

Numbers indicate the mean (± SEM) of brain tissue weights in milligrams from five animals. All rats were reduced to approximately 85% of free-feeding weights beginning at 3 mo of age, but untrained animals were allowed to remain in their home cages throughout the experiment. Animals were sacrificed for brain tissue weight determinations within 1 mo after the trained animals had completed training.

the typical positive relationship between the magnitudes of FR requirements and response rate (29). However, as in adult, neonatal 6HD-treated rats (see companion study), response rates early during incremental training were significantly lower in both SHR and MAM than in controls, yet only SHR manifested such deficits during nonincremental training; the reasons for these differences are unclear.

Experiential Factors and Brain Anatomy

The capacity of experiential factors to induce significant central anatomical effects in normal organisms has been repeatedly demonstrated. Thus, environmental enrichment rearing in normal rats results in small though significant increases in brain weight and changes gross and microscopic cortical and hippocampal morphology that are thought to facilitate the subsequent ability of these animals to learn additional tasks [e.g., (5,10,37). Comparable effects may also occur in humans as the amount of prior educational experience has been reported to be positively associated with the amount of dendritic branching in human brain (13) and negatively correlated with the occurrence of human dementias such as those associated with Alzheimer's disease (14).

That the response-contingent presentation of reinforcement may be associated with qualitatively similar, but perhaps quantitatively larger, anatomical and functional consequences is suggested by results presented several years ago by Cramer and colleagues (7,8) and more recently by Rao et al. (23). The former investigators found that hippocampal weights in juvenile rats were greatly reduced (by approximately 40%) in a brain-regionally selective manner by restrictions in the opportunity for spatial learning during suckling (7). In addition, this reduction is likely to be functionally significant, because similarly restricted rats were learning impaired during later eight-arm radial-maze training (8). Rao et al. (23) observed that electrical brain self-stimulation, but not experimenter-applied stimulation, was associated with increases in dendritic branching in pyramidal neurons of the motor cortex and hippocampus in normal rats, and that the magnitude of these increases were larger than those typically associated with environmental enrichment.

However, with the exception of neonatal 6HD-induced catecholamine depletion (see companion study; 41), evidence supporting the possibility that experiential factors might be capable of reversing preexisting anatomical or biochemical perinatal brain insults, particularly in adult animals, is not available. For example, environmental enrichment, at least to the extent that it has been evaluated, has had little or no effect on the hypoplastic or neurotransmitter depleting effects of other perinatal interventions including maternal X-irradiation, lead or alcohol exposure, or norepinephrine depletion induced by neonatal administration of 6-hydroxydopamine (20,22,30,36). It was therefore surprising to find, based on comparisons of brain-regional weights in two replicates of age-matched, food-deprived trained and untrained animals, that nonincremental FR discrimination training had caused a marked, selective amelioration of the reduction in hippocampal weight (and protein content) induced by prenatal MAM exposure. In contrast, weights of brain regions in SHR or control animals, and those of other MAM brain regions, were not significantly affected by training.

The relative contributions of the various aspects of the present study (e.g., order with which FR discriminations of various difficulty were presented, the prolonged occurrence of learning, reinforcement presentation, operant response requirements, food deprivation, handling, etc.) to the occurrence and magnitude of this amelioration are not known at the present time. The mechanisms responsible also remain to be determined. However, they could involve the similarly unidentified but hippocampally selective mechanisms whose effects are inhibited by suckling restriction (7,8) and/or experientially elicited increases in the synthesis and use of neurotrophic factors (e.g., nerve growth factor) that are larger than those that may occur within the hippocampus of normal rats (10,17); research concerning these issues is ongoing in our laboratory.

Nevertheless, the hippocampus is thought to be of critical importance in the type of learning associated with an FR discrimination task [spatial learning (32)]. It is therefore tempting to speculate that the eventual ability of MAM animals of the present study to perform the difficult FR12–FR16 and FR14–FR16 discriminations with competence equivalent to that of controls is mediated by the training-associated increase in MAM hippocampal weight. However, a comparable anatomical change did not occur in SHR rats even though they, like MAM rats, manifested initial deficits in FR8–FR16 discrimination learning in the nonincremental procedure, and were eventually capable of performing these difficult discriminations. Thus, such weight increases do not appear to be necessary for the occurrence of training-associated improvement in learning in all organisms.

Regardless of the exact importance of hippocampal weight changes as indices of improved learning capacity, the present findings and those of the companion study strongly suggest that FR discrimination training can have profound, long-lasting, and probably beneficial neurobiologic effects in rat models of human mental retardation. They also provide, to the best of our knowledge, the first evidence suggesting that prenatally induced brain hypoplasia, an anatomical anomaly likely to at least partially mediate learning deficits present in the mentally retarded, can be antagonized by operant training (or, indeed, any experiential variable) during adulthood. To the extent that similar effects are attainable in humans, this finding could also have important therapeutic ramifications. It could also help explain why microencephaly is not always associated with clear evidence of mental retardation (9): The functional and hypoplastic effects of this anatomical anomaly on learning can be mitigated by experience in a brain-regionally selective manner.

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