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Hyperactivity Induced by Prenatal Administration of Methylazoxymethanol: Association with Altered Performance on Conditioning Tasks in Rats

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CANNON-SPOOR, H. E. AND W. J. FREED. *Hyperactivity induced by prenatal administration of Methylazoxymethanol: Association with altered performance on conditioning tasks in rats.* PHARMACOL BIOCHEM BEHAV 20(2) 189–193, 1984.—Methylazoxymethanol (MAM), an alkylating agent which kills dividing cells, produces microcephaly when administered to rats at 15 days gestation. Rats treated prenatally with MAM were tested on a variety of behavioral tests. The MAM-treated animals performed better than controls in the acquisition of a food-reinforced operant response, but poorer than controls on a passive avoidance procedure. When required to reverse the passive avoidance procedure by actively avoiding the portion of a chamber that was associated with shock, MAM-treated rats performed better than controls. The MAM-treated rats were microcephalic and were also hyperactive compared to controls. It was postulated that the behavioral changes observed in the conditioning tasks may be attributable to hyperactivity. A possible neurochemical basis for this hyperactivity is discussed.

Methylazoxymethanol Microcephaly Hyperactivity Conditioning

METHYLAZOXYMETHANOL acetate (MAM) is an alkylating agent which kills dividing cells by methylating purine bases of nucleic acids [2,17]. When administered to pregnant rats in late pregnancy (15th day of gestation), while the cortical neurons of the fetal brain are developing, MAM produces microcephaly [22]. The amount of deficit, as measured by reduction in size of the cerebral hemispheres, is dose dependent and has been reported to be as much as 60% of the forebrain mass [7]. It would be expected that such a massive loss of brain tissue would be reflected in obvious abnormalities; however, observations of behavior and physiology show no gross defects. Although animals treated with MAM and similar agents are hyperactive [8, 13, 21], studies of conditioning and learning have obtained inconsistent results. For instance, MAM-treated rats perform comparably to normal animals on some operant conditioning tasks and on discrimination learning [18]. On the other hand, rats and mice treated with MAM or similar agents are significantly impaired in terms of their performance on a variety of maze tasks [6,18]. The purpose of the present study is to suggest a unifying conceptual framework for the discrepant results of these behavioral measures.

METHOD

Animals and Drug Treatment

Three pregnant Sprague Dawley rats weighing between 270–340 grams were injected intraperitoneally with 20 mg/kg methylazoxymethanol acetate (diluted to a concentration of 20 mg/ml in 0.9% saline) on day 15 of gestation. Two additional pregnant rats were injected IP on day 15 of gestation with saline. Twenty-one days after birth the offspring were weaned from the mothers. One litter of MAM-exposed rats did not survive. Eight males and seven females made up the control group, 10 males and 11 females were in the experimental group. Rats were housed in groups of three to four per cage and were maintained on a 12 hour light/dark cycle. The rats were given rat chow and water ad lib except where noted otherwise.

Operant Conditioning

Apparatus. The rats were trained and tested, essentially as previously described, in four chambers constructed from camping coolers which were modified so that the space in which the rat was placed measured 27×27×22 cm [5]. Masking noise was provided by exhaust fans. The chambers were dimly lighted by light entering through ventilation outlets. Two bars, with a pilot light above each bar, and a food magazine were mounted on one wall. Reinforcement contingencies were programmed electronically.

Procedure. Rats were food deprived for two days prior to magazine training. For magazine training 45 mg Noyes food pellets were delivered non-contingently at one-minute inter-

vals for 60 minutes with the bars disconnected. The rats were observed to determine whether they had learned to eat the pellets when delivered. Any rats which did not become magazine trained were not tested further. To measure conditioning, on the next day one of the bars was activated so that each bar press caused delivery of a single food pellet. For the first 60 minutes one food pellet was also delivered non-contingently every five minutes. After 60 minutes the pilot light over the active (correct) bar was illuminated, and the rats were tested for 40 minutes more. Numbers of correct and incorrect bar presses were recorded every 10 minutes. Rats were tested for four more days for 30 minutes per day (without non-contingent pellets and with the pilot light on) and were given sufficient food supplements to maintain approximately 85% of their free-feeding weights.

Spontaneous Activity

Apparatus and procedure. Rats were individually placed in one of eight 34×21×32 cm Motron-Produktor Co. Activity Meter units. The apparatus was illuminated from above by 50 watt incandescent bulbs covered by Kodak No. 1A red Safelight filters. Only the horizontal photocell banks were used. Cumulative activity was recorded after 5, 10, 15, 30, 45, and 60 min.

Passive Avoidance

Apparatus. The testing apparatus consisted of two compartments, a large one (24×21×22 cm) with a grid floor and a small compartment (14×11×29 cm) illuminated from above by two 15 watt fluourescent lights.

Procedure. The rat was placed in the small compartment and the door between compartments removed. The time until the rat moved to the large compartment was recorded. When the rat had placed all four feet on the grid of the large compartment, a 2 mA constant-current shock was delivered for five seconds. Once the rat escaped back to the small compartment, it was returned to its home cage for one minute and then retested. This procedure was repeated until the rat remained in the small compartment for a criterion of two minutes. Rats were tested to criterion for two consecutive days.

Reversal of Passive Avoidance

Procedure. On the second day of passive avoidance testing, following the last trial, the rats were placed in the large compartment rather than in the small one. Time until the rat entered the small compartment was recorded. Each rat received three trials of the reversal procedure. No shock was delivered.

Histology

Animals were sacrificed and perfused with phosphate-buffered formalin. Frozen 40 micron coronal sections were stained with cresyl violet. The effect of MAM was quantified in terms of linear measurements (as described by Rodier [19]) taken from projected images of the stained sections. Selected matched sections were measured from four male and five female control rats and from five male and nine female MAM-treated brains at Rodier's Level II, which is at the level of the anterior commissure. The linear measurements included measures of cortical thickness (at four sites), width of caudate-putamen (one site), and width of septum (one site). Each measure except septal width was made bilaterally.

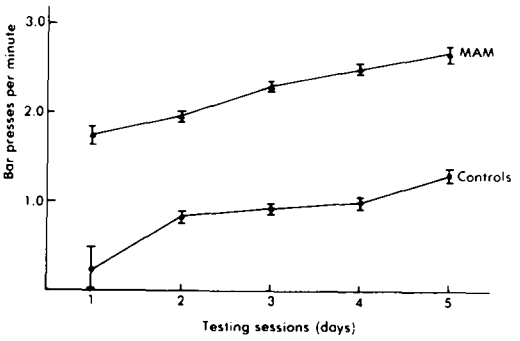

FIG. 1. The results of operant conditioning expressed as mean±SEM number of bar presses per minute (correct plus incorrect) for the MAM (triangles) (n=19) and the control (circles) (n=13) groups for five testing sessions (one per day).

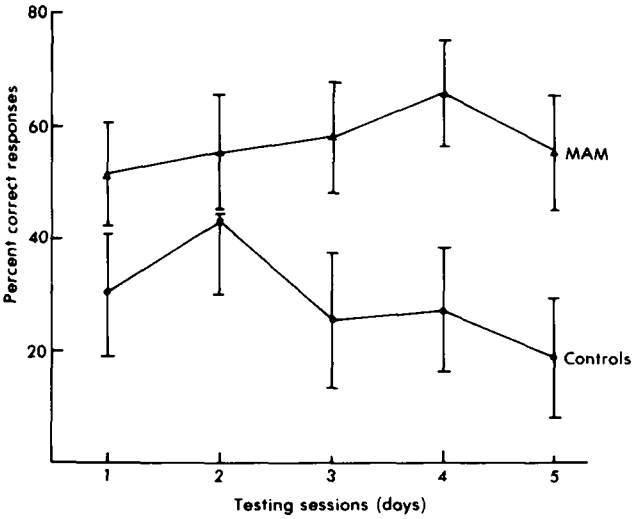

FIG. 2. The results of operant conditioning expressed as mean±SEM percent correct bar presses for MAM (triangles) (n=19) and control (circles) (n=13) groups for five testing sessions (one per day).

RESULTS

Animal Weights

Mean (±SEM) weights at time of weaning were 37±1.2 grams (n=21) and 52±1.1 grams (n=18) for the MAM rats and the controls, respectively, Student's t (37)=9.05, $p=0.001$. Two months later, when behavioral testing was begun, the weights were 306±10 (n=21) and 356±10 grams (n=15) for the MAM rats and controls, $t(33)=3.73$, $p=0.001$.

Operant Conditioning

Number of Responses. The data are shown in Fig. 1. No significant differences were found between males and females in either treatment group (MAM males n=10, females n=9, control males n=8, females n=5). Data for the sexes was therefore combined. There was a tendency for the MAM rats to bar press more than the controls, although this trend did not reach statistical significance (treatment main effect $F(1,30)=3.35$, $p=0.07$; two-way ANOVA with one repeated measure).

Percent correct responses. The results are shown in Fig.

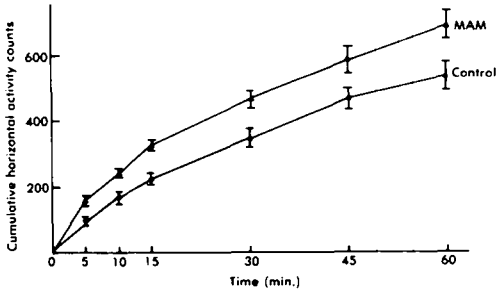

FIG. 3. Spontaneous activity for the MAM rats (triangles) and controls (circles) is illustrated as mean±SEM cumulative horizontal movement counts plotted at various time points during the 60 min recording period.

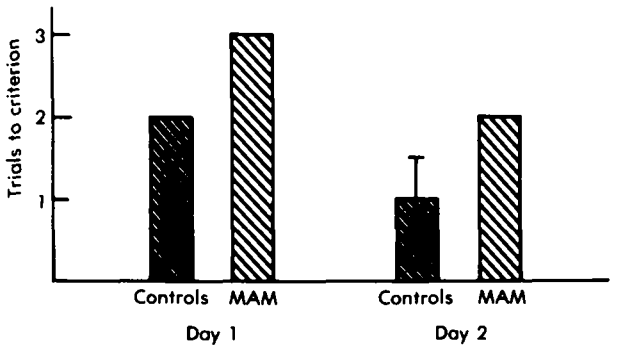

FIG. 4. The passive avoidance data expressed as median number of trials to criterion±S.I.Q.R. (semi interquartile range) for MAM rats (open bars) and controls (darkened bars) on Days 1 and 2 of testing. Value of S.I.Q.R. is zero when not shown on graph.

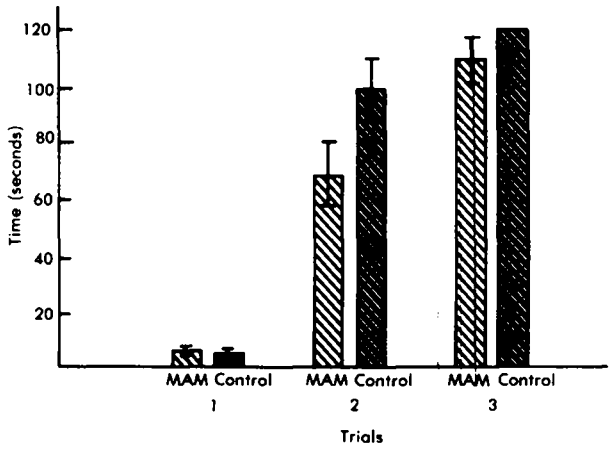

FIG. 5. Passive avoidance data expressed as mean±SEM response latencies (in seconds) for MAM rats (clear bars) and controls (darkened bars) on three trials of testing for Day 1. Value of SEM is zero when not shown on bar.

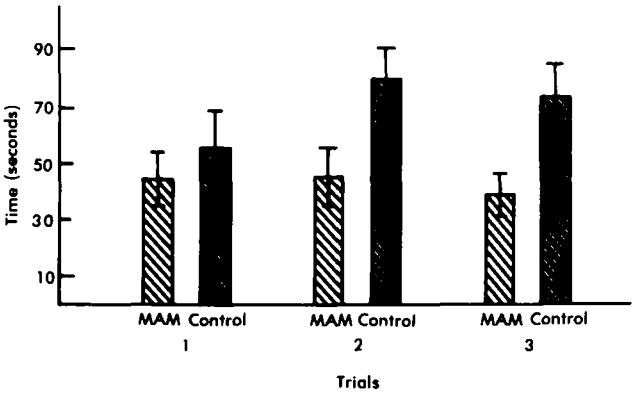

FIG. 6. Reversal of passive avoidance conditioning expressed as mean±SEM response latency (in seconds) for MAM rats (open bars) and controls (darkened bars) on three trials.

2. No significant differences were found between sexes, and scores for males and females were therefore combined. A two-way ANOVA with one repeated measure showed the main effect of treatment to be statistically significant, $F(1,30)=4.35$, $p=0.04$, with the MAM rats outperforming the controls.

Spontaneous Activity

The data are shown in Fig. 3. The two-way ANOVA was applied to the cumulative spontaneous activity scores. The MAM treated rats were significantly more active than the controls, $F(1,32)=7.42$, $p=0.01$ for treatment main effect. There were no significant sex differences.

Passive Avoidance

Trials to criterion. No significant differences were found between sexes in either treatment group. Consequently, the sexes were combined. The MAM rats required significantly more trials to reach criterion than did the controls ($n_1=15$, $n_2=21$, $U=105.5$, $p=0.047$ on Day 1 and $U=105.0$, $p=0.046$ on Day 2; two-tailed Mann-Whitney U tests). See Fig. 4.

Response latency. The results are shown in Fig. 5. No significant differences were found in latency of response between the MAM treated rats and the controls. A two-way analysis of variance showed the treatment main effect for Day 1 to be non-significant, $F(1,34)=3.43$, $p=0.07$. All the rats, except two in the MAM group, had reached criterion after three trials. On Day 2 all rats reached criterion after the first trial. A t-test for independent groups showed that the difference in trial 1 and latencies was not significant, $t(34)=1.63$, $p=0.11$, two-tailed.

Reversal of Passive Avoidance

The results are shown in Fig. 6. A three way ANOVA with repeated measures demonstrated a significant treatment main effect. The MAM rats had a significantly shorter response latency than the controls, $F(1,34)=5.21$, $p=0.02$. There were no significant sex differences.

Histology

No significant differences in linear measurements were found between the left and right sides of the sections, so the mean of the two measures was used. Within each treatment group, no significant sex differences were found and the data for the sexes was therefore combined. The two treatment groups were compared using a two-way ANOVA with re-

peated measures. The CNS structures at Rodier's Level II were significantly reduced in the MAM rats (treatment main effect $F(1,21)=33.73$, $p=0.001$). Individual comparisons (Newman-Keuls) for each measure showed reductions ($p<0.05$) for all areas except "B", which is a measure of cortical thickness adjacent to the olfactory tuberculum (see Fig. 7). Mean percent reductions for each measurement were as follows: "A" 15%, "B" 3%, "C" 20%, "D" 21%, "E" 10%, and "F" 18%. Brain weights or structure volumes were not determined; however, for an estimation of how these linear measurements would translate to volumes, the percentage reductions in the linear measurements to the third power can be examined. The mean reductions in these "volumetric" measurements are shown in Fig. 7. The reductions in brain measurements and behavioral measures within the MAM group were not correlated.

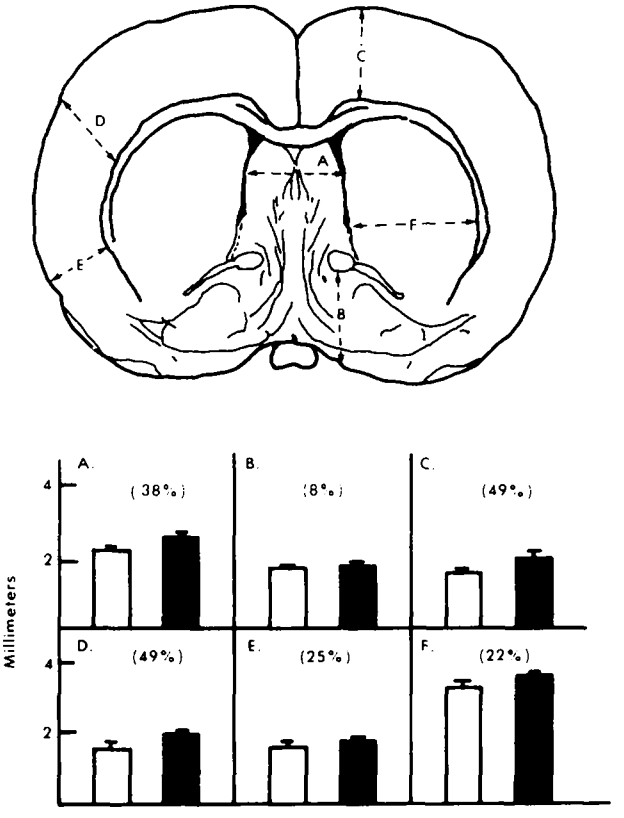

FIG. 7. Rodier's linear measurement sites (upper diagram). Letters designate sites referred to in bar graphs (below). Each set of bars represents a linear measurement (indicated in upper left-hand corner) in millimeters (means±SEM). The figures in parentheses represent the reductions in the cubed linear measurements (see text). MAM animals are represented by open bars and the controls by dark bars.

DISCUSSION

The effect of prenatal exposure to MAM on morphological development of the rat brain was analyzed by comparing measurements of matched sections from experimental and control brains. All experimental rats showed reductions in linear measurements in the structures measured. The average reduction in the linear measurements to the third power was 44%. This is similar to reductions in brain weight found in the literature. Johnstone and Coyle weighed cortical slabs and found a reduction of 30% at two days post-birth and 60% or more by the eighth day in rats exposed prenatally to 20 mg/kg MAM on the 15th day of gestation [12]. Hanada and colleagues [8] state that on microscopic inspection MAM rats' cortical cytoarchitecture was abnormal, and cerebral weights were reduced by 50%. Other estimations of reductions in forebrain weights vary between 24% and 50% [18].

While microcephaly is the most obvious morphological result of prenatal exposure to MAM, the most consistent behavioral finding has been hyperactivity [8, 13, 21]. Our data support the suggestion that MAM treated rats are hyperactive. Exposure to MAM at one to four days after birth, rather than prenatally, also results in reductions in brain size in the rat; however, postnatally exposed rats are hypoactive compared to normal controls, suggesting that microcephaly per se is not sufficient to produce hyperactivity [14]. Microcephaly resulting from exposure to MAM on the 15th day of gestation is chiefly evident in the forebrain and lateral neocortex, because the neurons of the neocortex are undergoing mitosis at this time [12].

Coyle and Johnstone have reported a marked increase in presynaptic markers for cholinergic and noradrenergic terminals in the atrophied cortex of MAM-treated rats [11]. Matsutani *et al.* [16] also measured concentrations of monoamines in the cerebral hemispheres of rats exposed to MAM on day 15 of gestation and reported elevations of 1.6, 2.0 and 2.8 times those of control values for 5-hydroxytryptophan (5-HT), norepinephrine (NE) and dopamine (DA), respectively. Serotonergic and noradrenergic neurons appeared to be intact and present in normal amounts, but compressed into a smaller brain volume, while dopaminergic synapses appeared to be overproduced. Brain catecholamines are associated with the regulation of motor function and increased concentrations of these amines at central receptor sites leads to arousal or increased motor activity [10,20]. Release of catecholamines is thought to be the mechanism by which amphetamine administration results in hyperactivity [9]. Relative catecholaminergic hyperinnervation of the atrophic MAM cortex could account for the hyperactivity observed in the MAM rat. This notion is consistent with the finding that in the postnatally treated rats which are microcephalic, but not hyperactive, amounts of NE, DA and 5-HT present in the cerebellum or in the rest of the brain do not differ from amounts present in control brains [14].

Previous studies on behavioral impairment in MAM-exposed animals have dealt with the data by means of a variety of hypotheses, for example: a possible visual deficit resulting from the cytotoxic effect of MAM on retinal neuroblasts, impairment of spatial learning abilities, or generalized inferior learning as a result of large reductions in cortical mass [6,18]. It appears to us that the results of our learning tests may be explained most parsimoniously in terms of an effect of hyperactivity on the MAM rats' ability to perform the behavioral tasks. For example, the passive avoidance paradigm required that the rat suppress activity to avoid shock. It would be expected that hyperactive animals might therefore have greater difficulty with this task. Our data indicate that this was the case for the MAM rats, which took longer than the controls to learn to remain in place. Conversely, when the correct response demanded movement on the part of the rat, the MAM rats had significantly shorter response latencies than normal rats. Increased behavioral arousal might be expected to be reflected also in improved

performance on an operant conditioning task. In moderate doses, for example, amphetamine facilitates performance on various types of operant conditioning paradigms [23]. The experimental rats in the present study showed a trend towards higher rates of responding in an operant conditioning paradigm and had significantly more correct responses than the control rats. Rabe and Haddad tested MAM rats on several operant conditioning schedules [18]. While the authors found the rats' performance comparable to that of the controls, during extinction of a "discriminated time-out" schedule, the severely microcephalic rats made significantly more responses during the previous "reinforcement -on" period than did the controls. Amphetamine also increases rates of responding on various operant conditioning paradigms when baseline response rates are low [15].

The performance of MAM-treated rats on maze learning has generally been reported to be poorer than that of untreated controls [8,18]. The effects of amphetamine on maze performance are not clear-cut. In some instances, amphetamine appears to increase rate of radial maze arm-entry without affecting choice accuracy [3]. In a Y-maze avoidance task, amphetamine facilitated the acquisition of a response. Although more errors (incorrect choice of arm to avoid shock) were made initially in training compared to controls, this difference disappeared within a short time (five days) [1]. Others have reported no improvement in maze learning as a result of amphetamine treatment [4]. Thus, the effects of hyperactivity, as induced by amphetamine, are inconclusive with regard to maze learning, and it is unclear whether the hyperactivity model can account for effects of MAM on maze learning performance.

Thus, exposure of pregnant rats to MAM on day 15 of gestation results in offspring which demonstrate microcephaly and a probable concomitant catecholaminergic hyperinnervation of the cerebral cortex. In the present study, a number of behavioral alterations were seen in the MAM-treated rats. All of these changes would be expected if the animals were hyperactive and, in fact, hyperactivity was found to be present. The behavioral changes seen in the present study were in many respects similar to the activating effect of low to moderate dosages of amphetamine. It is, therefore, possible that enriched telencephalic catecholamines induced by prenatal MAM treatment resulted in hyperactivity as well as the other behavioral alterations that were observed here.

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