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Effects of Monosodium Glutamate on Somatic Development, Obesity and Activity in the Mouse

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PIZZI, W. J. AND J. E. BARNHART. *Effects of monosodium glutamate on somatic development, obesity and activity in the mouse.* PHARMAC. BIOCHEM. BEHAV. 5(5) 551–557, 1976. – Neonatal mice 1 and 5 days of age and older mice 25 days of age were injected with an increasing dose of monosodium glutamate (MSG) for a ten-day period and observed for at least 150 days. Both male and female animals in the 1- and 5-day age group treated with MSG showed large increases in weight over controls along with a shortened body length. The MSG group also showed decreases in locomotor and exploratory behavior. The 25-day animals took much longer to show effects or failed to show any effects, indicating that the MSG-induced changes studied are age dependent. Possible methodological considerations accounting for conflicting reports in the MSG literature are discussed in light of the present findings.

Monosodium glutamate Obesity Activity Developmental neurobiology

RECENT reports have implicated monosodium glutamate (MSG) in neuronal damage of the central nervous system [12, 19, 20, 21, 22, 23, 24]. While these anatomical findings have not gone uncriticized [2, 4, 32], they have more often been supported, with neuronal damage most often being reported to occur in the arcuate nucleus of the hypothalamus [1, 7, 11, 12, 17, 19, 21, 24, 34]. The thorough study by Lemkey-Johnston and Reynolds [12] has demonstrated neuronal damage in other structures including the preoptic region, the tectum, the dorsolateral surface of the thalamus, the dentate gyrus of the hippocampus, the habenular nucleus, the subfornical organ, and the cortex. Along with these CNS structures, the retina has been shown to be susceptible to damage by MSG [8, 14, 20]. Concomitant with this CNS damage there have been reports of a number of developmental, physiological, and behavioral abnormalities including obesity [3, 16, 19], stunted skeletal growth [3, 16, 19], sterility in females [19, 30], disruption of the electroretinogram [26], decreased and increased activity levels [3,19], and learning deficits [5,27].

The present study was designed to examine several of the developmental, physiological, and behavioral complications which have been reported following neonatal administration of MSG; namely, obesity, skeletal abnormalities, and changes in activity levels. There have been a number of contradictory reports regarding MSG-induced obesity and changes in activity levels. Several studies [3, 16, 19] have reported the development of obesity and stunted skeletal structure following administration of MSG to neonatal mice. Failure to find MSG-induced obesity has also been reported by a number of investigators [9, 13, 15, 25, 29, 32, 33]. It should be pointed out that the studies reporting negative results differ in method of administration, age of treatment, and length of the followup period from those yielding positive findings. One investigation [9] which failed to find significant weight gains in animals treated with MSG did report that the carcass fat content was increased to approximately twice that of control animals. A second study in that laboratory [10] found that MSG-treated animals expend significantly less energy than controls as measured by oxygen consumption and carbon dioxide production. This last finding seems consistent with Olney's [19] observation that animals treated with MSG as neonates were lethargic as adults, but inconsistent with Araujo and Mayer's [3] finding of increased activity.

The experiments reported on in this paper will attempt to resolve the inconsistencies mentioned above by using animals at several stages of development and by the use of a battery of activity measures in the adult mouse. Further, these experiments will explore the possibility that MSG-induced abnormalities are an age-dependent phenonmenon. Based on earlier studies in this laboratory with oral administration of MSG in rats, an experimental obesity is predicted if the followup period is of sufficient length.

METHOD

Animals

Animals were 178 albino mouse pups from 19 litters. All animals were born in the laboratory and housed in standard polycarbonate cages with their dams. Weaning and sexing was carried out when the animals reached 29 days of age

which is considered optimal for the health of the mouse [35]. All animals were fed a standard laboratory chow and given water ad lib. Lighting was regualted at 12 hr on an 12 hr off throughout the experiment.

Drug Treatment

Mouse pups 1, 5, and 25 days of age were injected subcutaneously for 10 days with an aqueous solution (10% w/v) of monosodium glutamate according to a modified dose schedule described by Potts, Modrell, and Kingsbury [26]. Animals injected at one day of age began at a dose of 2.2 mg/g body weight with doses of 2.5, 2.8, and 3.2 mg/g body weight on Days 2, 3, and 4 respectively. The dose on Days 5 through 17 started at 3.4 mg/g body weight and increased by 0.2 mg/g body weight to 5.8 mg/g body weight. The 25-day group received ten consecutive doses at 5.8 mg/g body weight. Littermate controls were injected with equal volumes of bacteriostatic water. Following the injections animals were weighed every 5 days on an electronic balance. Animals were placed in a paper cup to reduce movements, and weighed to a hundredth of a gram. Growth curves were followed for a minimum of 150 days and in some cases as long as 250 days.

Activity

Three methods were used to record activity: (1) the standard activity wheel; (2) the open field method; (3) the Boissier and Simon Poke Test [6]. All activity measures were taken between 150 and 200 days of age under ad lib food and water conditions. Each animal was placed in an activity wheel for a 24-hr period with food and water available. No later than five days following this first measure the same animals were tested in the open field situation. This measure consisted of placing each mouse in the center of a 76 × 76 cm box which was lined off to make up 36 squares. The measure of activity was the total number of squares entered in a 3-min period. The criterion for entrance into a square consisted of the animal putting its two front paws into a particular square. The apparatus for the poke test was a modification of that described by Boissier and Simon [6]. It consisted of a black plywood board of 37.5 × 37.5 × 1.3 cm in which were located 16 equidistant holes with a diameter of 4.5 cm. The board was surrounded by walls which suspended it 35.0 cm above the floor. The walls extended 20.5 cm above the poke board surface. Each animal was placed in the center of the board and the number of pokes recorded over a 5-min period. A poke was defined as the insertion of the animal's head, past the level of its eyes, into any hole. At the conclusion of the experiments the animals were anesthetized and body length was calculated as the distance from the tip of the snout to the anal orifice.

RESULTS

Obesity and Somatic Development

Figure 1 shows the mean body weights of the MSG-treated mice versus the controls plotted each 10 days over the test period. The MSG-treated animals showed a greater weight gain in every group with the exception of the 25-day females. While animals were assigned randomly to the MSG and control groups, it should be noted that none of the MSG groups weighed more at 30 days of age. The pattern of weight gain was characterized by being slow and steady, and showed no signs of abating at 200 days of age. An ANOVA was performed on the last 80 days of each group in Fig. 1. The neonatal groups showed highly significant weight differences (1-day males, $F(15,280)=11.692$, $p<0.001$; 1-day females, $F(15,231)=7.305$, $p<0.001$; 5-day males, $F(15,192)=5.190$, $p<0.001$; 5-day females, $F(15,151)=9.337$, $p<0.0001$), while the mature animals showed mixed effects (25-day males, $F(15,128)=1.854$, $p<0.05$; 25-day females, $F(15,120)=1.575$, p = NS). Further analyses utilized a priori contrasts [18]. The dashed vertical lines in Fig. 1 indicate the point at which the MSG-treated animals became significantly heavier. It should be emphasized that every pair of points to the right of the dashed vertical lines show a significant difference as demonstrated by a priori contrast analysis. No line is shown for the 25-day males since only two pairs of points were found to be significantly different – these were at 190 and 210 days of age. Thus, animals injected at an earlier age tended to show obesity sooner than those injected at an older age. The 1-day groups took an average of 110 days following the first injection to display significant differences in weight, while the 5-day groups averaged 135 days, and the male 25-day group showed significance only after 190 days. The 25-day MSG-female group failed to reach significance or even equal the control animals' weights after 250 days. Figure 2 shows a control female mouse from the 1-day group and a representative obese female mouse treated with MSG from day one to day ten.

Table 1 shows the mean body lengths for MSG-treated and control animals in the various age and sex groups. All 1- and 5-day MSG-treated groups, both male and female, were significantly shorter in body length. The 25-day groups failed to show a significant decrease in body length.

Morbidity rates were calculated from the first day of injection to 250 days of age for each of the three age groups of MSG and control animals. Results showed that animals injected with MSG at 1 day of age exhibited a 25.5% morbidity rate. MSG animals injected at 5 days of age showed a morbidity rate of 30.0%, and those administered MSG at 25 days of age showed a 10.5% morbidity rate. The morbidity rate of all three age groups of littermate control animals was relatively homogeneous at approximately 10%.

Activity

Visual inspection of the MSG-treated animals both in their home cages and on a flat surface indicated that they were lethargic. Figure 3 shows the results of three formal measures of activity and exploratory behavior. The 1-day group treated with MSG showed decreases in activity on all three measures; however, only the MSG-treated males showed a decrease on the wheel running test. Inspection of Figure 3 shows the 1- and 5-day groups treated with MSG always show lower mean scores on the wheel running measure, but due to the large variations within groups, these differences were not significant. In the open field test both male and female animals treated with MSG starting at 1-day of age showed decreased activity and exploratory behavior ($p<0.001$, two-tailed). No significant differences were found in animals treated with MSG at 5 or 25 days of age.

All 1-day and 5-day groups treated with MSG showed decreased exploratory behavior on the Boissier Poke Test ($p<0.001$, two-tailed). The 25-day males treated with MSG

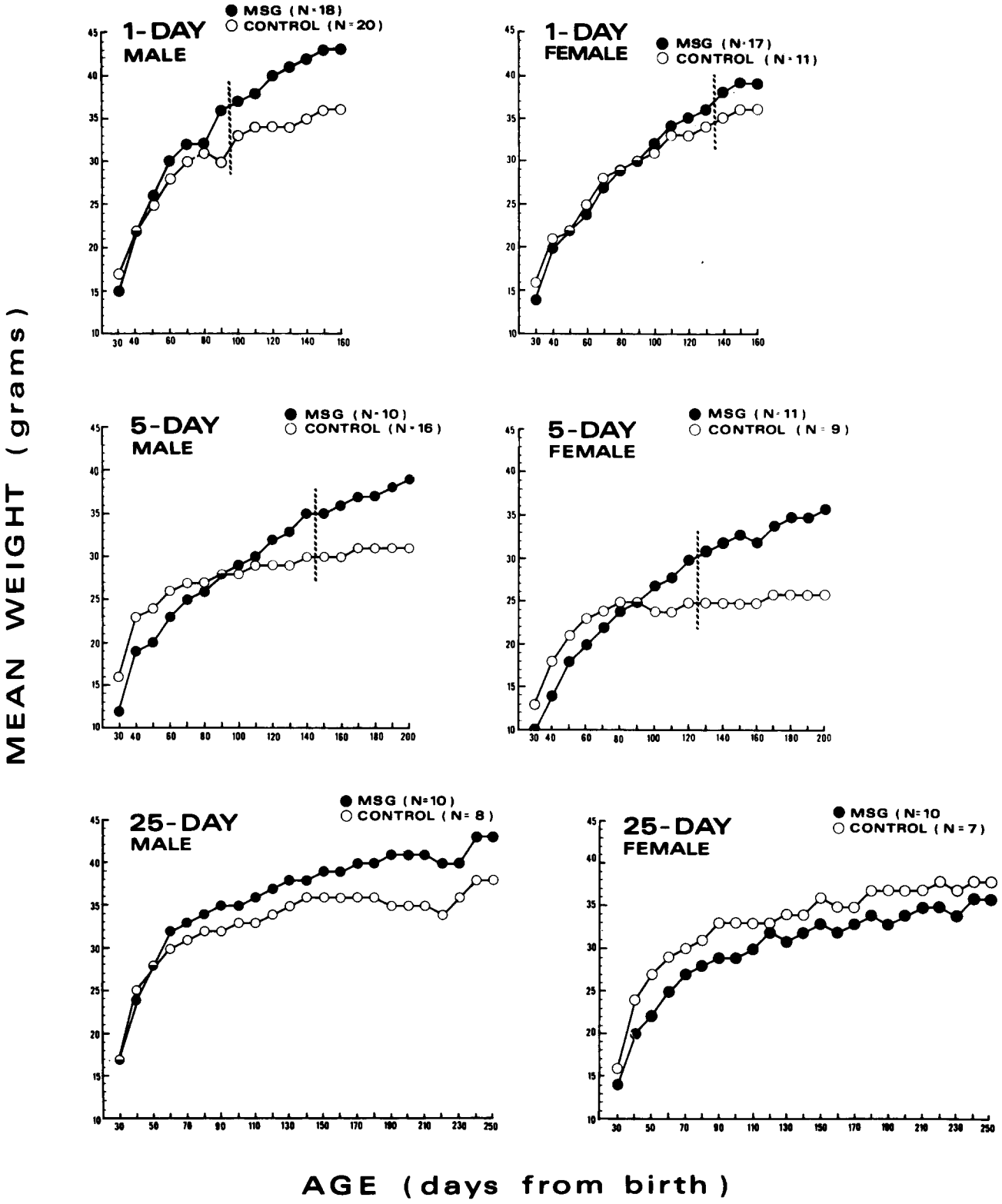

FIG. 1. Mean weights of MSG-treated and control groups plotted in 10-day intervals.

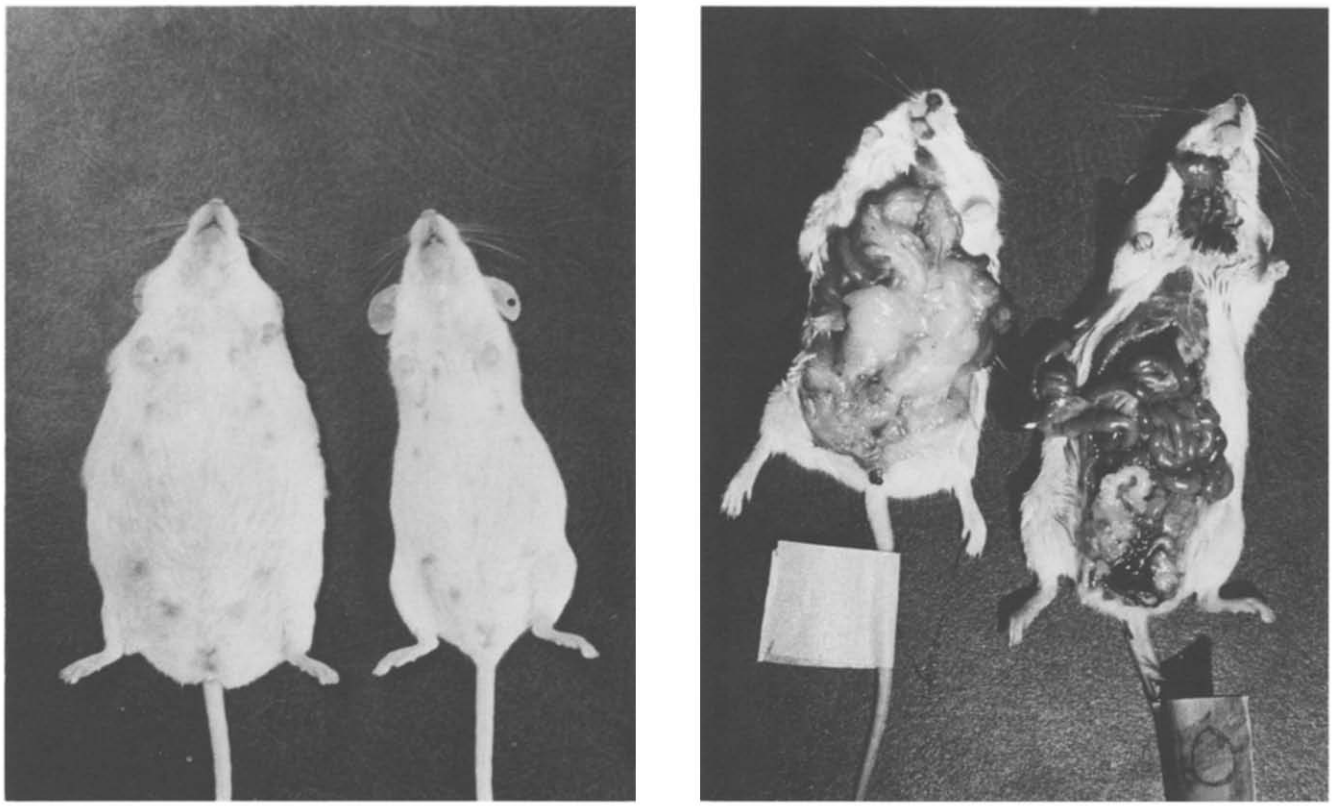

FIG. 2. A. Randomly selected female mice from the one-day age group. The control female mouse (right) was treated with the control vehicle from Days 1–10, while the obese mouse (left) was treated with MSG from Days 1–10. This particular pair of mice do not show the reduced body length referred to in this paper. B. Male mice from the 1-day age group following the treatment described above. The MSG-treated animal (left) shows an extreme accumulation of adipose tissue and reduced body length.

TABLE 1

MEAN BODY LENGTHS (IN MM) OF MSG-TREATED AND CONTROL ANIMALS MEASURED FROM THE TIP OF THE SNOUT TO THE ANUS (ALL MEASURES TAKEN BETWEEN 259 AND 269 DAYS OF AGE)

	Sex	N	Control	N	MSG-treated
1-Day	M	19	106.7 (± 1.05)	17	100.8 (± 1.60)†
	F	11	108.6 (± 0.63)	14	102.0 (± 1.31)‡
5-Day	M	7	103.1 (± 1.40)	7	98.1 (± 1.51)*
	F	7	98.4 (± 1.13)	10	92.9 (± 1.89)*
25-Day	M	8	110.4 (± 1.84)	10	109.2 (± 0.94)
	F	8	111.3 (± 1.09)	7	108.0 (± 2.57)

*$p<0.05$.
†$p<0.01$.
‡$p<0.001$.
Number in parenthesis = SEM.
All probability values are two-tailed.

also showed a significant decrease on this task ($p<0.01$, two-tailed). The pattern of effects in this experiment are similar to those found in the first experiment, with the animals treated at an earlier age more readily showing an effect.

DISCUSSION

The data gathered in this study support the findings of those investigators who have reported increased weight gain and decreased body length following neonatal administration of MSG. All animals treated at 1 or 5 days of age whether male or female showed the effect. Those animals treated later in the course of development showed the weight gains much later or not at all. In those studies failing to report increased weight gains [25, 31, 35] or those in which depressed weights are seen [13, 15, 25], the length of time for which the animals are followed becomes the important factor. During the initial administration of MSG and for a period of time thereafter, the animals lose weight; however, the phenomenon is transient and is followed by a slow steady weight gain leading to obesity. The growth curves must be followed for several months to see this effect. Furthermore, if the phenomenon is age dependent, as these experiments suggest, then method and age of MSG treatment are critical factors. In those studies using rat chow with various amounts of MSG added [15, 31, 33], one would not expect to see an effect since adult animals are beyond the critical age and mouse and rat pups do not begin to eat solid foods until after they have passed the critical age. A number of studies [15, 28, 29] in which physiological and endocrine factors were of primary concern have reported the initial weight loss but then failed to follow the growth curves for a sufficient length of time for

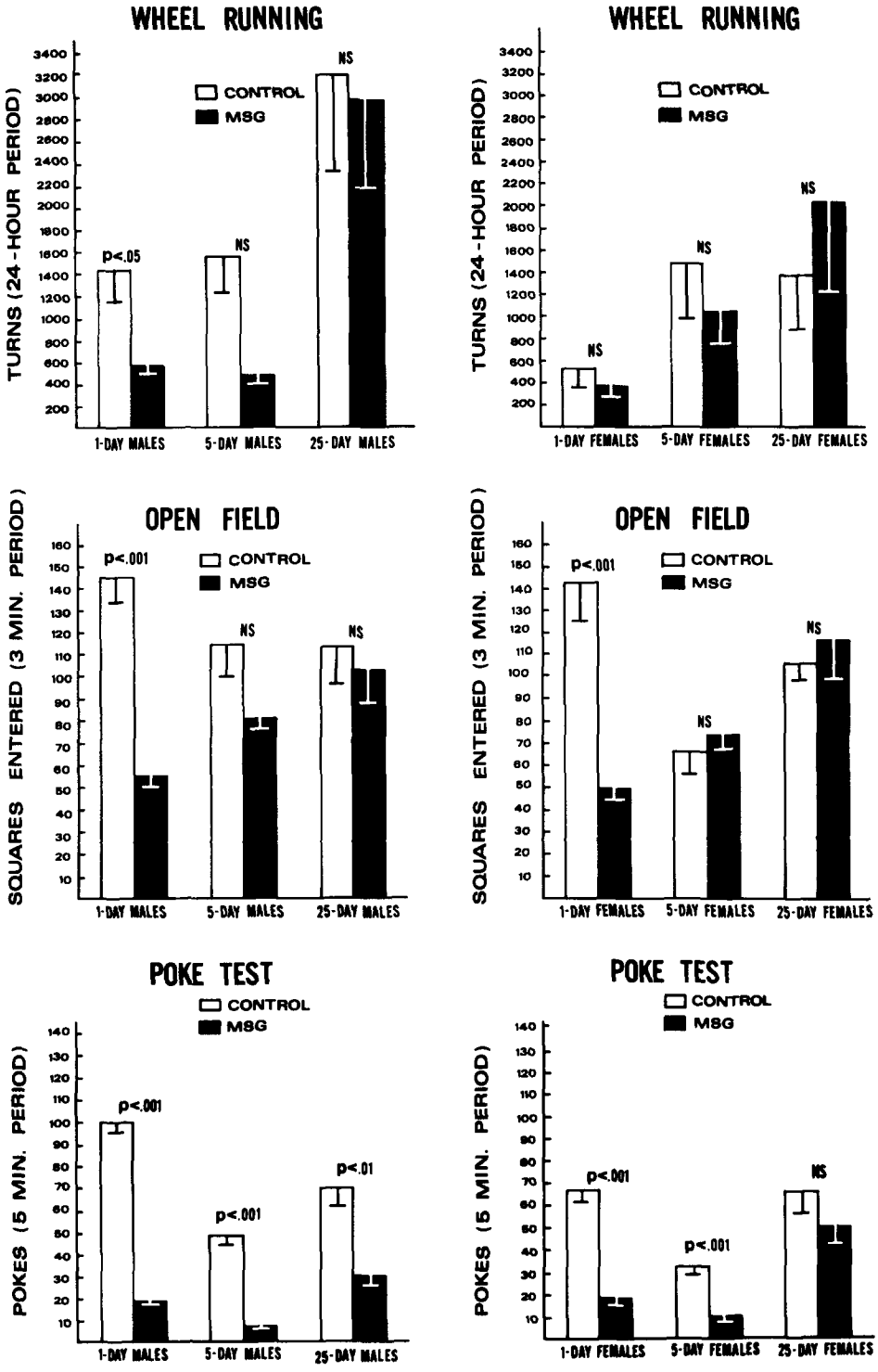

FIG. 3. Mean scores on three measures of activity for male and female mice treated with MSG and a control vehicle at 1, 5, and 25 days of age. The N's are the same as those in Fig. 1, excepting the 1-day control male group which was reduced by one for all three tests.

the obesity to manifest itself. The length of time for which the animals must be followed will probably vary from study to study due to differences in the strain of animal and general laboratory procedures such as location and type of food, lighting schedules, and housing conditions. However, the followup period for investigations of MSG-induced obesity should probably be set at a minimum of 120 days.

The data reported in this study confirm the findings by others [3, 16, 19] of shortened body length in the MSG-treated animals. A cursory inspection of several x-rays of matched experimental and control animals failed to show any severe skeletal deformities.

The increase in activity reported by Araujo and Mayer [3] was not confirmed in this study. Several investigators have reported MSG-treated animals to be lethargic based on informal observations. This observation was indirectly supported by the observations of Djazayery and Miller [10], who found that MSG-treated animals expended significantly less energy as measured by oxygen consumption and carbon dioxide production. The present study utilized a battery of activity measures which showed the MSG-treated animals to have lower levels of activity and exploratory behavior. Again the age at which the animals are treated with MSG seems to be a factor. The animals treated at 1 day of age showed an effect on all three measures of activity with the exception of the females on the wheel running test. The poke test best demonstrated the difference between MSG-treated animals and controls. The animals treated with MSG at 1 and 5 days of age showed highly significant decreases in activity on this test. The 25-day males treated with MSG showed a decrease, but the 25-day females did not. The results of these tests confirm the informal observations of other investigators that MSG-treated animals are lethargic and hypoactive.

Several interesting questions are raised by the MSG literature and the data reported above. The most immediate questions concern other physiological and behavior impairments which may be induced by MSG administered during the neonatal period. There have been brief reports of endocrine dysfunction and sterility in female organisms treated with MSG [19,30] along with several reports of impaired learning [5,27]. The data indicating that MSG disrupts the electroretinogram exemplifies the need for behavioral studies at various dose levels and stages of development. Each of these topics deserves expanded study and quantification.

Finally, there arises the question as to whether MSG-induced obesity represents an example of regulatory or metabolic obesity. The slow steady pattern of weight gain along with a number of reports which failed to show any evidence of hyperphagia lend support for a metabolic interpretation. However, Araujo and Mayer [3] caution that one-quarter of a gram of chow per day could account for a 1-g per week weight increase, and that this might be missed by the food-monitoring systems currently employed. This along with the fact that MSG-induced brain lesions can be found in the arcuate-ventromedial hypothalamic region requires that a careful investigation be made of these alternative explanations.

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