Bulletin of the Psychonomic Society
1989, 27 (2), 153-156

Morphological and behavioral effects of perinatal exposure to aspartame (Nutrasweet®) on rat pups

RAZ YIRMIYA, EDWARD D. LEVIN, CLINTON D. CHAPMAN, and JOHN GARCIA
University of California, Los Angeles, California

Possible side effects of perinatal exposure to L-aspartyl-L-phenylalanine methyl ester (aspartame) were studied by providing aspartame-containing water to female rats from 30 days before conception until the pups were 30 days of age. Compared with rat pups of mothers who drank plain water, aspartame-exposed pups were not different in morphological (i.e., pinnae detachment, eye opening, incisor eruption, and body weight) and reflex (i.e., surface righting and negative geotaxis) development. Additionally, no difference was found in the time taken by mothers to retrieve their litters. At 30 days of age, performance in a radial-arm maze of aspartame-exposed rats differed from that of rats not exposed to aspartame. Entries prior to repeat—that is, the number of arms chosen until an arm was reentered—was higher for the aspartame-exposed rats. Maze performance of rats exposed to aspartame for 30 days as adults did not differ from that of rats exposed only to water. These results suggest that aspartame may either facilitate the development of components involved in this procedure or reduce factors that interfere with this task.

L-aspartyl-L-phenylalanine methyl ester (aspartame) has been approved by the Food and Drug Administration, but several questions regarding the safety of this nonnutritive sweetener still exist. Central to these questions is the effect of aspartame on fetal and child development.

Aspartame is quickly metabolized into phenylalanine and aspartic acid, which make up roughly 50% and 40% of aspartame by weight, respectively (Opperman, 1984). The risk of aspartame may be greater in the fetus because the placenta can concentrate amino acids in fetal plasma (Ranney, Mares, Schroeder, Hustsell, & Radzialowski, 1975). Children may also be at an increased risk to potential side-effects of aspartame because they have much lower body mass than adults, and thus dose levels in children, relative to body weight, would be greater than in adults consuming equal amounts of aspartame-containing foods (Partridge, 1986).

Several studies of the effects of aspartame and its constituent amino acids on infant development have been reported. Increased levels of phenylalanine have been shown to reduce protein synthesis in the brains of neonate rats (Siegel, Aoki, & Colwell, 1971) and mice (Taub & Johnson, 1975), but not in older animals (Taub & Johnson, 1975). Moreover, during brain development, hyperphenylalanemia can inhibit myelin formation and reduce overall brain weight (Johnson & Shah, 1980). Aspartate also can have deleterious effects on the developing brain; for example, gavaged aspartate can produce neuronal damage in infant mice (Olney & Ho, 1970). Aspartame, ingested voluntarily together with monosodium glutamate, has been reported to cause hypothalamic damage in weanling rats (Olney, Labruyere, & Gubareff, 1980); however, when large doses of aspartame or aspartame plus glutamate were intubated into infant monkeys, no such damage was observed (Reynolds, Bauman, Stegink, Filer, & Naidu, 1984).

We presented a plain water solution or an aspartame-containing solution to female rats 30 days before conception and throughout the entire preweaning period. Morphological, reflex, and radial-arm maze exploratory behavior were all measured in the rat pups. The radial-arm maze was used in the present study because it has been shown to be sensitive to early exposure to environmental toxins and drugs. For example, poorer performance resulted from prenatal administration of low levels of lead (Alfano & Petit, 1981) and the anesthetic halothane (Levin & Bowman, 1986), and better performance followed prenatal administration of diazepam (Benton, Dalrymple, Brain, & Grimm, 1985).

METHOD

Subjects were 14 female Sprague-Dawley rats (Simonsen, Gilroy, CA), weighing 261-310 g. Rats were housed individually and given ad-lib access to lab chow pellets. Each female rat was given a 45-ml Nalgene tube filled with either distilled water containing 8 g/liter aspartame (Group Asp; $n=8$) or plain distilled water (Group Water; $n=6$). After 30 days of exposure to aspartame or plain water, a male mate was placed into each cage for 4.5 days. During this period, plain water was continuously available for all rats. After the males were removed, each female was given the 45-ml tube filled with water each day, with aspar-

This research was supported by National Institutes of Health Grants NS-11618 (to John Garcia) and NS-07628 (to John C. Liebeskind). We thank G. D. Searle and Co. for generously supplying us with aspartame. We also thank David Chang and Alma Lopez for their help in testing the rats. Raz Yirmiya is with the Departments of Psychiatry and Anatomy, and Edward Levin, Clinton Chapman, and John Garcia are with the Department of Psychology. Address all correspondence to Raz Yirmiya, Department of Psychology, University of California, Los Angeles, CA 90024-1563.

tame added to Group Asp's water. Five of the 8 rats in Group Asp and 3 of the 6 rats from Group Water became pregnant. When the pups were 2-4 days old, they were crossfostered within groups so that 7 pups remained per litter. The rat pups were weaned at 21 days of age and placed in group cages (3-4 rats with their litter mates per cage), and those in Group Asp continued to receive aspartame added to their water.

A separate group of 30 Long Evans adult male rats, weighing 280-327 g, were housed individually and exposed to 30 days of either water, a low dose of aspartame (4 g/liter), or a high dose of aspartame (8 g/liter).

All tests were conducted during the first half of the light phase of a 14:10-h light:dark cycle. Seven measurements were made prior to weaning: (1) Body weight was measured at ages 5, 7, and 9 days; (2) bilateral pinnae detachment was measured starting on the day of birth; (3) bilateral eye opening was measured starting on the day of birth; (4) upper incisor eruption was recorded starting when each pup reached 5 days of age; (5) surface righting reflex was tested when the pups were 5, 6, and 7 days of age, with 2 trials (60-sec limit) per day. Mean average latencies from the supine to the prone position were recorded each day for each pup; (6) negative geotaxis was tested when the pups were 5, 6, and 7 days of age. Each pup was individually placed head down on a 40° incline covered with fine sandpaper; latency to turn around and initiate movements upward was measured for each rat on each day (45-sec limit); (7) maternal retrieval was tested on Days 2, 3, and 4 when the mothers were with their own pups, and on Day 7 after the pups were redistributed to equate litter sizes. Each mother was removed from her home cage, one pup was placed in each corner of the cage, and the remaining pups were randomly placed throughout the cage. The mother's latency to retrieve all pups to a single common area was measured (10-min limit).

At 30 days of age, each rat pup was tested in a radial-arm maze. The 30 adult males exposed to water, or low or high dose of aspartame were also tested on the maze. The maze consisted of eight arms arranged like spokes on a wheel without the rim. The maze was made of plywood painted flat black. The arms radiated out at equal angles from a central platform 35 cm in diameter. Each arm was 80 cm long and 10 cm wide. The maze was elevated 30 cm from the floor. The testing room was filled with a variety of extramaze visual cues, according to which the rats could navigate about the maze. The maze was wiped off with water before each rat was placed on it. A session on the maze began with a single rat confined to the center platform with a Plexiglas ring for 10 sec. The ring was then raised, and the rat was allowed to explore the maze until it either entered all eight arms, took 300 sec, or fell off the maze. An entry was defined as the rat's placing all four feet on an arm. Two measurements of performance were taken: entries to repeat and latency. Entries to repeat was defined as the number of arms chosen until an arm was reentered. Latency was defined as the number of seconds it took the rat to enter each arm.

Data were averaged for each litter, and analyses of variance (ANOVAs) and *t* tests were done on these averages. Litter was used as the error term. Latency data from the developmental tests are reported as means and standard errors of the mean (presented in parentheses). These latencies were converted to reciprocals before each analysis, in order to help meet the assumptions of homogeneity of variance.

RESULTS

The mothers consumed an average of 360 mg of aspartame each day. This consumption converted to an average daily dose of 1,188 mg/kg.

Morphological Measurements

Overall, morphological development was similar between groups. There were no differences between Group Asp and Group Water in their latencies for pinnae detachment [Group Asp = 3.9 (.4) days; Group Water = 3.8 (.4) days], incisor eruption [Group Asp = 12.9 (.7); Group Water = 12.3 (1.8) days], and eye opening [Group Asp = 14.4 (.6) days; Group Water = 13.9 (.4) days]. Similarly, body weights did not differ between groups [$F(2,5) < 1$], and the group × day interaction was not significant [$F(2,5) < 1$]. Body weights increased over days in both groups [$F(2,5) = 27$, $p < .005$] (see Figure 1, top panel).

Reflex Measurement

There were no differences over days between Group Asp and Group Water in either the righting reflex test [$F(1,6) < 1$] or the negative geotaxis test [$F(1,6) = 2.34$, $p > .1$] (see Figure 1, bottom two panels). Similarly, the group × days interactions for the righting reflex [$F(2,5) = 1.77$, $p > .25$] and negative geotaxis [$F(2,5)$ 3.47, $p > .1$] were not significant.

Mother's Retrieval

The time to retrieve all pups to a common area was the same for each group on Days 2, 3, and 4, and also on Day 6, after the rat pups were redistributed [$F(2,3) < 1$].

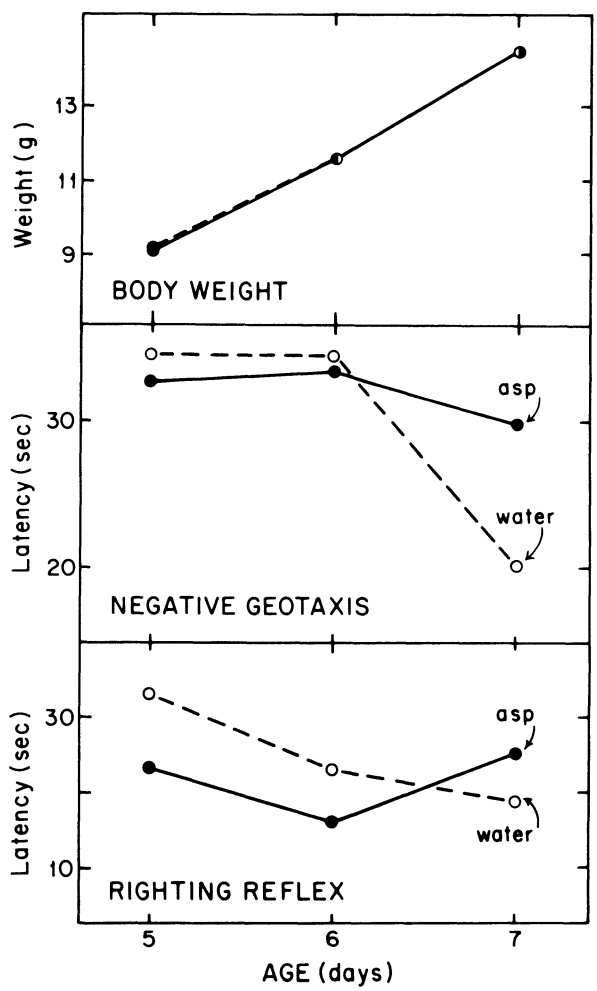

Figure 1. Mean body weights (top panel), and latencies during the negative geotaxis (middle panel) and surface righting (bottom panel) tests. All measures were quite similar for both the group exposed to aspartame perinatally (asp) and the group given plain water (water).

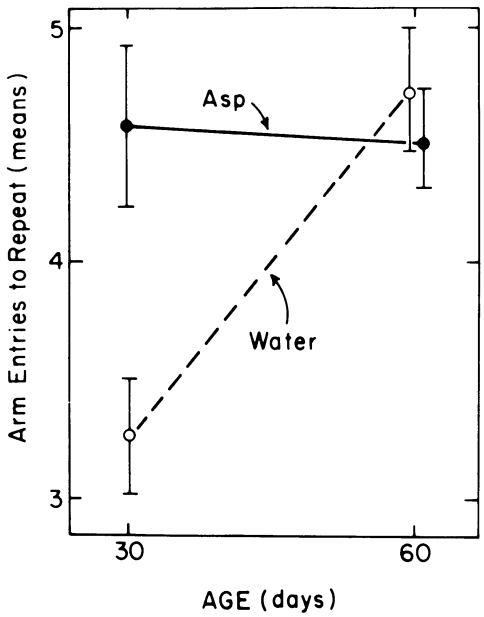

Figure 2. Mean number of entries to repeat an arm of a radial eight-arm maze. When the rats were first tested at 30 days of age, the number of entries to repeat was significantly larger for the rats perinatally exposed to aspartame (Asp) than for the rats exposed only to water (Water). By 60 days of age, the number of entries to repeat was equally above chance for both groups.

Radial Arm Maze

At 30 days of age, the control rats (3.27 ± 0.24; *M* ± SEM) showed a nearly random (3.25) number of entries to repeat, whereas the aspartame-treated rats averaged well above chance (4.58 ± 0.34) (Figure 2). At 60 days of age, the control group improved to a level of 4.75 (±0.27), whereas the aspartame-treated group stayed about the same at 4.52 (±0.21). The interaction of group × day of testing was significant [$F(1,5) = 21.59$, $p < .01$]. Post hoc analysis of the simple main effects showed that at Day 30 the aspartame-treated rats were significantly better than controls ($p < .05$), and that at Day 60 the groups were not significantly different. Choice latency, as measured by seconds taken for each arm entry, did not show any significant aspartame-related effects. In the experiment on the effects of aspartame on adult rats, there were no differences between the groups in entries to repeat or choice latency. Entries to repeat for the control group was 3.90 (±.50), for the low-aspartame group 3.71 (± .52), and for the high-aspartame group 4.40 (±.61).

DISCUSSION

As in an earlier study (Brunner, Vorhees, Kinney, & Butcher, 1979), reflex and morphological development of rat pups was not affected by perinatal exposure to aspartame. Consistent with this conclusion is the finding that infant macaque monkeys, which voluntarily drank aspartame at dose levels from 1 to 3 g/kg, were normal with respect to body weight gain, body length, blood and urine composition, and EEG activity (Reynolds et al., 1984).

Conclusions in toxicological work are limited by the reasonableness of the doses used relative to human consumption. It is, therefore, important to determine comparable dose levels of aspartame for humans based on the lowest effective levels observed in the studies reported here with rats. Some estimates of dose levels involving essential amino acids are that one needs five to six times more in rats, relative to body weight, to achieve a comparable metabolic effect to that found in humans (Wurtman & Maher, 1984). Based on this 11:2 ratio, an aspartame dose of 1,188 mg/kg in rats (the dose level used in the present study) has a similar metabolic effect to 216 mg/kg in humans. An alternative way of comparing dose levels in rats to levels in humans is based on total body surface area. Using this method, a dose of 1,200 mg/kg in a 250-g rat is roughly equivalent to 150 mg/kg in a 70-kg human. Projections of the daily intake of aspartame vary over a wide range. An upper limit widely accepted by the food science industry has been set at 34 mg/kg (Krause et al., 1985) for adults and as high as 77 mg/kg for children who, of course, have much lower body weights (Olney et al., 1980). However, even this higher estimate is less than the levels tested in the present study, which had no effects on morphological and reflex development.

Performance on the radial-arm maze differed between rat pups exposed to aspartame and those not exposed. This improvement in performance after exposure to a compound during development is not unprecedented. For example, administration of diazepam to pregnant mice resulted in improved radial-arm maze performance in the offspring (Benton et al., 1985). It is important not to interpret improved maze performance as necessarily implying improved spatial memory. Aspartame exposure may have improved performance not by enhancing memory, but by attenuating processes that interfere with maze performance, such as fear. However, the latency data did not support this particular interpretation. Perinatal exposure to aspartame may have accelerated the development of processes that contribute to this behavior, or the elevated levels of the components of aspartame may have acute effects. However, because 30 days of aspartame consumption by adult rats had no effect on radial-arm maze performance, it seems that early exposure to aspartame is needed to produce the change in maze performance. Further work, using only a single component of aspartame, might reveal the role of specific amino acids in maze performance.

REFERENCES

Alfano, D. P., & Petit, T. L. (1981). Behavioral effects of postnatal lead exposure: Possible relationship to hippocampal dysfunction. *Behavioral & Neural Biology*, **32**, 319-333.

Benton, D., Dalrymple, J. C., Brain, K. L., & Grimm, V. (1985). Prenatal administration of diazepam improves radial maze learning in mice. *Comparative Biochemistry & Physiology*, **80C**, 273-275.

Brunner, R. L., Vorhees, C. V., Kinney, L., & Butcher, R. E. (1979). Aspartame: Assessment of developmental psychotoxicity of a new artificial sweetener. *Neurobehavioral Toxicology*, **1**, 79-86.

Johnson, R. C., & Shah, S. N. (1980). Effects of a-methylphenylalanine plus phenylalanine treatment during development on myelin in rat brain. *Neurochemical Research*, **5**, 709-718.

Krause, W., Halminski, M., McDonald, L., Dembure, P., Salvo, R., Freides, D., & Elsas, L. (1985). Biochemical and neuropsychological effects of elevated plasma phenylalanine in patients with treated phenylketonuria. *Journal of Clinical Investigation*, **75**, 40-48.

Levin, E. D., & Bowman, R. E. (1986). Behavioral effects of chronic exposure to low concentrations of halothane during development in rats. *Anesthesia & Analgesia*, **65**, 653-659.

Olney, J. W., & Ho, O. L. (1970). Brain damage in infant mice following oral intake of glutamate, aspartate or cysteine. *Nature*, **227**, 609-610.

Olney, J. W., Labruyere, J., & Gubareff, T. (1980). Brain damage in mice from voluntary ingestion of glutamate and aspartate. *Neurobehavioral Toxicology*, **2**, 125-129.

Opperman, J. A. (1984). Aspartame metabolism in animals. In L. D. Stegink & L. J. Filler, Jr. (Eds.), *Aspartame: Physiology and biochemistry* (pp. 141-159). New York: Marcel Dekker.

PARTRIDGE, W. M. (1986). Potential effects of the dipeptide. In R. J. Wurtman & J. J. Wurtman (Eds.), *Nutrition and the brain* (pp. 199-241). New York: Raven Press.

RANNEY, R. E., MARES, S. E., SCHROEDER, R. E., HUSTSELL, T. C., & RADZIALOWSKI, F. M. (1975). The phenylalanine and tyrosine content of maternal and fetal body fluids from rabbits fed aspartame. *Toxicology & Applied Pharmacology*, **32**, 339-346.

REYNOLDS, W. A., BAUMAN, A. F., STEGINK, L. D., FILER, L. J., & NAIDU, S. (1984). Developmental assessment of infant macaques receiving dietary aspartame or phenylalanine. In L. D. Stegink & L. J. Filler, Jr. (Eds.), *Aspartame: Physiology and biochemistry* (pp. 405-423). New York: Marcel Dekker.

REYNOLDS, W. A., STEGINK, L. D., FILER, L. J., & RENN, E. (1980). Aspartame administration to the infant monkey: hypothalamic morphology and plasma amino acid levels. *Anatomical Record*, **198**, 73-85.

SIEGEL, F. L., AOKI, K., & COLWELL, R. E. (1971). Polyribosome disaggregation and cell-free protein synthesis in preparations from cerebral cortex of hyperphenylalaninemic rats. *Journal of Neurochemistry*, **18**, 537-547.

TAUB, F., & JOHNSON, T. C. (1975). The mechanism of polyribosome disaggregation in brain tissue by phenylalanine. *Biochemical Journal*, **151**, 173-180.

WURTMAN, R. J., & MAHER, T. J. (1984). Strategies for assessing the effects of food additives on the brain and behavior. *Fundamental & Applied Toxicology*, **4**, 318-322.

(Manuscript received August 22, 1988.)