

Neurobehavioral evaluation of rhesus monkey infants fed cow's milk formula, soy formula, or soy formula with added manganese

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Abstract

The possible neurobehavioral effects of excess manganese in soy formula were studied. Male rhesus monkeys ($n=8/\text{group}$) were fed a commercial cow's milk based formula (Control), a commercial soy protein based formula (Soy), or the soy formula with added manganese (Soy+Mn) from birth to 4 months of age. Soy formulas naturally have higher manganese (Mn) content than cow's milk formulas. Monkeys received behavioral evaluations, growth measurements, and cerebrospinal fluid (CSF) sampling from birth to 18 months of age. Soy and Soy+Mn groups engaged in less play behavior and more affiliative clinging in social dyadic interactions. These groups also had shorter wake cycles and shorter periods of daytime inactivity than controls. An impulsivity test was sensitive to the Soy group diet. The Soy+Mn group also had a blunted response to the dopamine agonist apomorphine. Groups did not differ significantly in CSF dopamine and serotonin metabolite concentrations, but these concentrations were correlated with several tasks affected by experimental formula. This experiment suggests that components of soy formula, including Mn, may influence brain development as reflected in behavioral measures.

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1. Introduction

Soy-based infant formulas were first introduced for infants with cow's milk allergy but have since become more widely used. A 1998 report [1] found that 25% of formula sales were soy formula and 40% of infants in the United States received some soy formula in their first year.

There has been concern about possible adverse effects of constituents of soy formulas, which are based on a plant (soy) source of protein. Phytoestrogens, soy constituents with estrogenic properties, have the potential to adversely affect reproductive tract development and sexual differ-

entiation of the brain [6]. Phytates, soy constituents with metal chelating properties, have the potential for interfering with essential trace element absorption [26,6]. Phytoestrogens and phytates are plant products and are not present in breast milk or cow's milk formula but are present in soy formula. Also, two neurotoxic agents, aluminum and manganese, are present in breast milk and cow's milk based formulas as well as in soy formula, but much higher concentrations are found in soy formula [22,25]. The actual adverse effects of these soy formula constituents in infants based on the amounts in formula, their toxicity and the stages of development when exposure occurs cannot be determined from existing literature.

The safety of soy formula for infant development has received some scientific attention and is scheduled for

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review by the National Toxicology Program's Center for Evaluation of Risks to Human Reproduction [33]. No neurobehavioral effects have been found in the small number of human studies reported in the literature thus far. Two retrospective studies looked at IQ in children and educational attainment in adults who were fed soy formula as infants [41,31]. Neither study found effects. However, sample size, confounder control, retrospective ascertainment of exposure and sensitivity of the measures are all limitations of those studies. A 6-year prospective study comparing breast feeding, cow's milk formula and soy formula, and including neurobehavioral development endpoints is currently underway under sponsorship of the US Department of Food and Agriculture (<http://www.ars.usda.gov/is/AR/archive/Jan04/soy0104.pdf>). Animal studies with soy formula using neurobehavioral endpoints have not been conducted.

The present study was conducted in rhesus monkey infants. Nonhuman primates are a particularly valuable model for infant formula studies utilizing neurobehavioral endpoints because postnatal brain development that occurs in nursing primates differs considerably from that of laboratory rodents. Specifically, primates, human and nonhuman, are at a more mature stage of brain development when they begin nursing than rats and mice. Thus different developmental processes may be influenced by formula components and contaminants. Another valuable aspect of nonhuman primate models is that complete early nutrition of monkey infants can be achieved with commercial human infant formulas, which are used routinely in nonhuman primate nurseries. A number of studies in infant monkeys have been conducted looking at absorption, growth and development with formulas of different nutritional content [5,7,21,34,38], including soy formula [28,27,30].

Methods for the assessment of neurobehavioral development in monkey infants have been modeled on those used in human infants and have been used in connection with the evaluation of a number of nutritional deficiencies [15,36,39], and potentially toxic exposures such as methyl mercury [19], methanol [3], cocaine [20], alcohol [40], and lead [23]. A recent study of growth and neurobehavioral development in monkeys fed formulas supplemented with docosahexaenoic acid (DHA), thought to promote brain development, has been conducted [4].

A focus of the present investigation is the manganese content of formula. Manganese neurotoxicity is well known from studies of humans exposed in the workplace [35] and has also been seen in nonhuman primate studies [11,13,12]. Manganese *developmental* neurotoxicity has not been explored in humans, but some information is available from rodent studies. Recently, studies in rats that used oral administration during the period of rapid brain development [46,45] have demonstrated Mn accumulation in brain, as well as decreases in dopamine concentrations in striatum after the rats mature. Striatal dopamine concentrations

correlated with behavioral assessments (burrowing detour and passive avoidance tests) conducted in adult rats.

To further focus on the potential contribution of manganese to soy formula effects, we analyzed metabolites of serotonin and dopamine in CSF. Damage to the dopamine system has long been associated with Mn neurotoxicity because a Parkinson's like syndrome is induced in exposed workers [24]. In addition, Mn can contribute to the formation of toxic reaction products generated by auto-oxidation of dopamine and other catecholamines [8]. Mn is also known to have effects on enzymes involved in catecholamine metabolism such as dopamine beta-hydroxylase, monoamine oxidase [42,43], and catechol-*o*-methyl transferase [10]. In addition, Mn is linked to impacts on serotonin (5HT) through its action on monoamine oxidase, which is an enzyme in the degradative pathway of both dopamine and 5HT [42]. Both dopamine and 5HT are known to be important in major childhood behavior disorder syndromes [44], putting an added emphasis on the potential ability of Mn to disrupt this pathway.

Our study addressed the possible neurobehavioral effects of excess Mn in soy formula by comparing monkey infants fed a commercial formula based on cow's milk to those fed commercial soy formula, with approximately 10× greater Mn content, and to infants fed soy formula with added Mn (30× cow's milk formula content). This provided three Mn doses. However, the cow's milk and soy formula differ in other ways in addition to their Mn content. While both formulas meet the recommendations of the Food and Nutrition Board of the National Academy of Sciences for infant nutrition, they differ in several nutritional dimensions related to protein, carbohydrate, and fat composition as well as other known and unknown components.

2. Materials and methods

2.1. Assurance of compliance with animal codes

All protocols were approved prior to use by the University of California, Davis Animal Care and Use Committee and followed the requirements of the Animal Welfare Act and the Guidelines for the Use and Care of Laboratory Animals. Monkeys were housed at the California National Primate Research Center (CNPRC), an AAALAC accredited vivarium.

2.2. Subjects

Male newborn rhesus monkeys (*Macaca mulatta*) from uncomplicated pregnancies were transferred the morning of birth to the primate nursery and assigned to one of three diet groups: commercial cow's milk-based infant formula [Control]; commercial soy formula [Soy]; or commercial soy formula with added Mn (as chloride) [Soy+Mn]. There were eight subjects in each formula group. Infants were born

Table 1
Characteristics of dams and newborns

	Control	Soy	Soy+Mn
Dam age (years)	8.0±0.7*	8.7±0.5	9.2±1.0
Dam weight (kg)	7.5±0.5	8.0±0.8	7.6±0.5
Dam parity (#)	4.0±0.6	4.7±0.9	3.7±0.9
Infant birthweight (g)	558±25	514±20	538±20

* Mean±S.E.M., there were no statistically significant differences between formula groups.

during a 6-week period in one breeding season at the CNPRC. Fourteen of the animals were born in mixed-age groups in outdoor field cages, five were born in outdoor harem groups, and five were born in individual indoor caging. These pregnancy housing conditions were evenly distributed across treatment groups. Three fathers contributed two infants each to the cohort; all other infants had different fathers. Dam characteristics and infant birthweight are shown in Table 1. Monkeys were not time mated so that gestational age at birth is not known. At the conclusion of testing (18 months of age) the monkey infants were released from the project and returned to the colony.

2.3. Formulas, manganese intake and diets

The control formula was Similac with Iron (Ross Products, Columbus, OH) (containing 50 µg Mn/L as measured in our laboratory) and the soy formula was Albertson's Baby Basics (originally marketed by Bright Beginnings, Gordonsville, VA) containing 300 µg Mn/L. The Soy+Mn formula consisted of the Albertson's Baby Basics formula to which Mn chloride was added to a final concentration of 1000 µg Mn/L. Mn doses, based on formula intake, are shown in Table 2.

Formula was fed by animal care staff according to an animal husbandry protocol that started with hand feeding at 2 h intervals from a 65 mL nursing bottle with a nipple and progressed to self-feeding from a sipper tube on a hanging bottle at cage side. Decisions on amount and interval of feedings and progression to self-feeding were made by animal care staff based on their experience. The experimental formulas were fed exclusively through 4 months of age, at which time monkeys were transitioned to commercial nonhuman primate diet (Lab Diet #5047, PMI Nutrition International, St. Louis, MO) supplemented with enrichment foods (forage boards with grains and twice weekly fresh vegetables and fruits). Lab Diet #5047 contains 44 mg Mn/kg dry matter, which is the estimated daily dietary requirement for Mn for macaques [32].

2.4. Housing

After transfer to the nursery, neonates were housed individually in temperature controlled (82–84 °C) incubators (30.5 × 30.5 × 30.5 cm modified tank, Nalgene, Twinsburg, OH) containing a stuffed terrycloth duck, an infant pacifier

and a cloth diaper. After 3 weeks, the infants were gradually adapted to metal cages (Lab Product Inc., Maywood NJ) and socialized with 1–2 additional animals of similar age. These cages were grouped in racks of 4 (“quad cages”) and had perches, swings, and additional toys. Pair socialization with like-age infants from the same formula group was conducted for increasing periods of time during the day and, at 5 weeks of age, two infants determined to be compatible were permanently housed together in a double cage. An insert could be placed between the cages to separate the infants when needed for transfer to the test room. Between 4 and 5 months of age, the infants were transferred to adult double cages that accommodated groups of four infants.

The behavioral test room was located on the same corridor as the nursery. Infants were transported in cages identical to their incubators. The test room contained a variety of apparatuses used for different tests. Each animal was tested individually, except for Wisconsin General Test Apparatus (WGTA) testing, for which two infants could be tested in separate apparatuses at the same time.

2.5. Behavioral test schedule

In order to characterize the behavior and behavioral development of the different formula groups, an extensive test battery was administered over an 18-month period (Table 3). The battery included measures of motor, cognitive and social domains, as well as specific tests related to the dopamine system (reward delay, fixed interval (FI) dopamine drug response). In order to assess a variety of behaviors in the rapidly maturing infants, the period of time devoted to each test was limited to a predetermined number of sessions. Not all infants generated enough data in each test to evaluate the quality of their performance. Thus a participation criterion, described below for each test, was used to exclude individuals from the data analysis when needed.

2.6. Growth and health

Body weights were obtained daily during the first 2 weeks of life as part of the nursery care protocol. Additionally, body

Table 2
Manganese dose estimates for formula groups

	Control	Soy	Soy+Mn
<i>Postnatal day 16</i>			
Body weight (g)	673±105	610±81	696±69
Formula intake (mL) ^a	231±47	222±44	232±35
Mn (mg/kg/day)	17	109	333
<i>Months 1–4</i>			
Body weight (kg) ^b	1.09±.04	1.02±.04	1.12±.08
Formula intake(mL) ^c	389±19	361±14	362±3
Mn (mg/kg/day)	18	106	323

^a Average of eight infants per group.

^b Average of weights at 1, 2, 3, and 4 months of age.

^c Average of four cages housing two monkeys each per group.

Table 3

Assessment schedule	
Test	Age
Motor development	1–14 weeks
Dyadic social interaction	1–5.5 months
Automated activity monitoring	4, 8 months
Iron absorption studies	4, 8 months
CSF sampling	4, 10, 12 months
<i>WGTA tests</i>	
Object discrimination learning	5–8 month
Object discrimination reversal	8–9 months
Delayed nonmatch to sample training	9–10.5 months
Delayed nonmatch to sample test	10.5–11.5 months
Position learning and reversal	11.5–12.5 months
Post-session temperament score	8–12.5 months
Reward delay	12.5 months
<i>CANTAB tests</i>	
Fixed interval training	13–16 months
Dopamine drug challenge	16 months
Continuous performance test	17–18 months
Stereotypy observations	18 months

weights were obtained weekly for the first 14 weeks and monthly thereafter to the conclusion of testing. Crown rump length, a measure of linear growth, was also obtained monthly. Animal health was evaluated daily by the animal care staff. Medical records of symptom reports and veterinary intervention were summarized.

2.7. CSF catecholamines (4, 10, and 12 months of age)

CSF samples were collected from the cisterna magna (cerebellomedullary cistern) of anesthetized infants with a 25-gauge, 5/8-in. needle at a maximum volume of 0.5 mL/kg under Telazol (5–8 mg/kg i.m.) anesthesia. The average sample volume was 0.5 mL. Catecholamine analysis was conducted using HPLC with electrochemical detection in the laboratory of Dr. Beard. Briefly, CSF was combined with ultrapure 50 mM perchloric acid (1:1) with dihydroxybenzylamine (DHBA) added as an external standard. Samples were immediately centrifuged at 15,000×g for 5 min at 4 °C, filtered through a 0.2 µm filter, and 10 µL injected. Efficiency of recovery was corrected for the DHBA external standard and all data expressed as pmol/µL. Identification and quantification were obtained in all samples for homovanilic acid (HVA) and 5-hydroxy-indoleacetic acid (5HIAA). Other metabolites were below detection or quantification limits in some samples so that complete data sets were not available for statistical analysis.

2.8. Gross motor maturation (1–14 weeks of age)

Infants were observed weekly from 1 to 14 weeks of age for a 10-min session in a specially designed cage that allowed vertical climbing [15]. Weekly sessions were omitted (no more than two for any infant) if they occurred

shortly after an iron absorption test. The iron absorption test required relocation to metabolic cages and isotope injection and was considered disruptive to normal behavior of the infants. The 7-min scoring period was preceded by a 3-min adaptation period. The method uses a complete and mutually exclusive set of motor and postural behaviors that are categorized for mature and immature versions of each behavior (walking, sitting, climbing, clinging, manual exploration, visual surveillance). It was based on interval sampling using 30-s sampling periods and a paper-and-pencil checklist. Details of the method have been presented [14]. Observer reliabilities established prior to observation were 83.5%.

2.9. Round-robin social dyadic observations (1–5.5 months)

Infants were observed for two 10-min periods once a week in the metal quad cages. Two infants who had not previously interacted socially were paired for each session. The formula group of each partner was rotated so as to be balanced over the series of sessions for each animal. Infants were paired with their play partner in a separate cage, identical to the home cages. This cage was located in the home cage room and was reserved for round-robin observation. The observation cage contained the same attachment objects (towel, stuffed terrycloth duck) and toys as were available in the home cage. After novel pairings were exhausted the infants continued another round of one pairing with each available partner. Round-robin observations were discontinued when infants were able to move from the nursery to the adult caging area, as determined by colony managers, resulting in different total number of sessions for different infants. The largest number of sessions completed by all monkeys with a strange play partner was used as the database for statistical analysis. Behaviors recorded on mechanical counters by a familiar observer stationed about 0.6 m from the cage were chase play, rough-and-tumble play, and cling. Infants were considered to be engaged in chase play if both animals were running, jumping, or grabbing for each other either behind or in front of the partner. Rough-and-tumble play consisted of many types of behaviors such as: infants rolling together, biting each other, or trying to disengage from clinging; rough sexual behavior (mounting); and pouncing on the partner multiple times within a short time frame. Cling was defined as ventral–ventral or dorsal–ventral contact of >1 s with one or both of the infants having their arms wrapped around the partner. The mechanical counters allowed recording of frequency and duration for two behaviors (chase play and rough-and-tumble play); the additional behavior (cling) was recorded only as a frequency. Since these behaviors involved both dyad members the values for each session were entered twice in the data set, once for each of the participating infants as the focal animal. One individual conducted all the dyadic observations.

2.10. Spontaneous activity/wake–sleep cycle (4 and 8 months of age)

An actimeter designed for assessing activity in humans (PAM2, Individual Monitoring Systems, Baltimore, MD) has been adapted for use in monkeys [16]. The actimeter, which weighs 29 g, is placed in a pouch attached to a harness so that it is located in the middle of the infant's back where it cannot be reached. During monitoring, monkeys are individually housed by inserting a separator in the two-cage area normally occupied by a social pair. Forty-eight-hour monitoring periods were conducted on weekends when the cage room is largely undisturbed apart from routine feeding and cleaning. The data from the actimeter was transferred to a software program for summary and analysis. The monitoring provides data on the rest–activity cycle, hyper/hypo activity, and abnormal patterning of “naps”, as well as printouts of activity patterns [18].

2.11. Cognitive testing in the WGTA (5–13 months of age)

In the WGTA test environment, the infant reaches out from a small, familiar transfer cage to a test board. The problem to be solved by the infant is set up on the board by the tester who observes and records the infant's behavior from behind a one-way mirror. The board has food wells that can be covered by various stimulus objects. The infant indicates its choice by displacing the stimulus objects, and, if the choice is correct, a food item can be retrieved. Food items used in the initial WGTA training included marshmallows, raisins, and pieces of apple. By the end of initial training, raisins were used almost exclusively. The infants were trained to displace objects and retrieve food rewards from 5 to 8 months of age, prior to training on individual tasks. Each of the subsequent WGTA tests was conducted for a fixed number of sessions so that the infants would begin each new test at the same age.

The initial task (8 months of age) was a *visual object discrimination* (OD) in which one of two objects (opaque plastic box or green paper box) was baited (food placed in the well under the object) out of sight of the infant. Twenty sessions were conducted consisting of twenty 30-s trials with 5–10 s intervals between each trial. The numbers of correct choices, incorrect choices, the response latencies, and the number of balk trials (no choice) were recorded. If a discrimination criterion of 18/20 correct trials was reached before the conclusion of the 20 session series, a reversal was conducted in which the previously incorrect object was designated as correct. If criterion was met for the first reversal prior to the 20 session maximum, further reversals were conducted. If the discrimination criterion was not met, no reversals were conducted.

The second task, *delayed nonmatch to sample* (DNMS) (10.5 months of age), was preceded by training to displace an object, which consisted of a clear plastic box (10 × 10 × 12.5

cm) containing a small toy, to obtain food rewards. For the DNMS task, the box containing a sample stimulus (toy) was first presented alone for 30 s and the infant was allowed to displace it and retrieve a food reward. After a 10-s interval, the test board was again presented with the sample stimulus and a new stimulus. The infant was rewarded for selecting the new stimulus. If no choice was made in 30 s, the trial was terminated. Each trial used a unique pair of stimuli. Ten sessions of 20 trials each were conducted, with the sample stimulus and the sample/novel stimulus pair presented for 30 s and a 30-s intertrial interval. Testing was discontinued if the infant reached a criterion of 18/20 correct trials in a given session.

For the third task, *position learning and reversal learning* (11.5 months of age), two identical objects (white plastic blocks with black trim) were placed on the test board. Either the right or left side (randomly assigned) was correct (well baited with food reward) for each infant. Twenty sessions were conducted consisting of twenty 30-s trials with 10-s intervals between each trial. Each time the infant reached a criterion of 16/20 correct trials in a session, the correct position was switched to the opposite side (reversal). The number of reversals completed, and the number of correct and incorrect choices and of balks (no response) were recorded for each reversal.

2.12. Temperament ratings

Following each testing session in the WGTA, the animal's temperament and behavior during the session were rated using eight categories (adapted from Schneider et al. [40]): object orientation, distractibility, goal directedness, irritability, activity, impulsivity, inhibition, and stereotypic behaviors. Animals were rated on a scale of 0 to 3 for each category. The scale represented the frequency and/or intensity of the behavior category. Rating was not conducted during training sessions in the WGTA.

2.13. Reward delay (12.5 months of age)

The ability to inhibit responding in order to receive a food reward was measured. The test was performed in the familiar WGTA apparatus. Animals were first adapted to an opaque sliding screen, which was mounted vertically in a groove in front of the test board and was used to reveal the test board and allow the infant to retrieve the food item by displacing a plastic box (previously used as the container for toys in the DNMS task). During adaptation, the screen was removed immediately after presentation of the test board so that the infant could displace the box and retrieve the food item.

For the single test session, the door of the WGTA was opened and the infant was presented with a sliding screen concealing the food well covered by the box. The screen was moved 1 in. at a time over seven 2-s intervals to slowly reveal the box. An additional 16 s (eight 2-s intervals) was

allowed for retrieval of the reward after the box was fully revealed. The interval (0–7) was recorded at which the animal reached out of the WGTA and attempted to displace the screen, reach behind it, or displace the box after it was visible. If the monkey did not wait until completion of the 7th interval, the WGTA door was closed, and no reward was received. If the animal inhibited responding until the screen was completely removed, the box could be displaced and the food reward retrieved. The test session consisted of forty 30-s trials with 10-s intertrial intervals.

2.14. Automated cognitive testing (CANTAB) (13–18 months of age)

Fixed interval (FI) and continuous performance test (CPT) assessments were conducted using the Cambridge Neuropsychological Test Automated Batteries software (CANTAB, Cambridge Cognition, Cambridge, UK). This test battery was designed for and has been used to assess cognitive function in both monkeys and humans [37], including children [29]. The tests were presented on video monitors with touchscreens that displayed visual stimuli and recorded the infants' responses. Monkeys were tested in their home cages between 1300 and 1500 h. A cart with the computer, monitor, pellet dispenser, and food cup was wheeled in front of the monkey's home cage and secured with bungee cords at a distance which allowed ready access to the touchscreen and food cup [17,18]. Program parameters were entered into the computer by investigators, who then left the room during the test period.

Correct responses were reinforced with a small (45 mg) sugar pellet (Noyes Dustfree Sucrose Reward Tablets, Research Diets, Inc. New Brunswick, NJ). The monkeys were not food deprived. Animals in the colony are fed at 0700 and 1500 h each day, and receive enrichment no later than 0900 h. The afternoon ration of food was withheld until the completion of testing.

Computer adaptation and touchscreen training began at 13 months of age. The training criterion was completion of 10 consecutive correct touches (to a small colored square on the screen) within 10 min for two consecutive sessions or five nonconsecutive sessions.

When the training criterion was met, the *fixed interval (FI) assessment with drug challenge* began. Under a FI reinforcement schedule, the reinforcement is contingent on the first response that occurs after a fixed interval of time, in this case 120 s. A stable pattern of responding develops which can be used as a baseline for evaluating drug effects. Daily sessions increased in length over the course of FI training from 11 to 21 min while FIs increased from 30 to 120 s. FI 120 s training continued for 13 weeks or to a weekly criterion of less than 20 (± 2)% variation of total session responses from the mean for the week, whichever came first.

After reaching a stability criterion or completing 13 weeks training, drug challenge sessions were initiated in

all animals that met a participation criterion of 3/5 weekly sessions with >5 reinforcements obtained per session. The drug challenge protocol was adapted from a study in squirrel monkeys [2]. Animals were injected everyday (5 days/week) with either drug or vehicle, and injections were followed 30 min later with a 20-min FI 120 s session. Drugs were given on Mondays and Thursdays for 3 weeks, with vehicle injections on Tuesdays, Wednesdays, and Fridays. This resulted in a minimum of 72 h between drug dosing and allowed for baseline testing between drug exposures.

Drug and vehicle injections were given intramuscularly (i.m.) in the thigh using tuberculin syringes and a 27-gauge needle 30 min before the animal's regular FI testing time. Each drug/dose, based on the literature [2], was administered once in the 3-week experiment. Drugs, dilutions, and doses were as follows: apomorphine HCl hemihydrate, 10 mg/ml, (Sigma-Aldrich, Inc, St. Louis, MO) (0.1, 0.2, 0.3 mg/kg); D-amphetamine HCl, 1 mg/ml, (Alltech Associates, Inc., Deerfield, IL) (0.1 mg/kg); haloperidol, 5 mg/mL (Sicor Pharmaceuticals, Inc., Irvine, CA) (0.02 mg/kg). Doses were based on the body weight of the previous Friday and the weight of the formulated compound. The vehicle for apomorphine and amphetamine was methanol and for haloperidol was sesame oil. The three doses of apomorphine were administered first in random order to determine a dose that increased response rates in the Controls. Next was a session of co-administration of the selected dose of apomorphine (0.2 mg/kg) plus 0.02 mg/kg haloperidol [2]. This was followed by a session of haloperidol alone. One session of amphetamine administration (0.1 mg/kg) was randomly scheduled either before or after the haloperidol session.

Vehicle injections were given at the same volume and vehicle as the previous day's drug injection (0.01–0.32 mL). Following the session of co-administration of apomorphine and haloperidol animals received a vehicle injection of sesame oil. On Wednesdays, animals received vehicle injections of sterile saline.

The second automated CANTAB test, the *continuous performance test (CPT)*, began 1 week after completion of the drug challenges. A requirement of five responses within 30 s to a white rectangle presented on the touchscreen resulted in presentation of the CPT task. Stimuli of three different colors (red, green, white rectangles, 7.8 × 10.5 cm) were then presented on the touchscreen one at a time in a modified random order (balanced for total occurrences in the 10-min session). Each stimulus presentation was 3 s maximum and terminated when the animal responded by touching the stimulus. The interval between stimulus presentations was 2 s. Responses to the white stimulus resulted in delivery of reinforcement, while responses to green or red stimuli resulted in a longer interval (3 s rather than 2 s) between stimulus presentations. CPT testing continued for 30 sessions or to a criterion of 75% correct hits on 5 successive sessions. Attention was evaluated for

the first five sessions completed by each infant, for all sessions completed, and for the five sessions for which the highest number of correct hits were registered.

2.15. Stereotypy (18 months of age)

Each cage of four monkeys was observed one time for 30 min at 0930 h in the home cage room. This period, 2.5 h after the morning feeding and 1 h after distribution of daily enrichment foods, was determined to be the time during which stereotypy was most often seen. A familiar observer sat 2 m in front of the cage and recorded each episode of stereotypy. A behavior was considered stereotypic if the animal performed it two or more times in rapid succession. If the behavior stopped and restarted in less than 3 s, it was considered to be part of the same episode. Behaviors coded as stereotypies were: jump, circle, flip, pace, startle, self-clasp, rock, fur pluck, scratch, self-bite, spin, and bounce. Episodes of body part sucking were also recorded. One person performed all observations.

2.16. Statistical analysis

Endpoints of continuous data were analyzed by ANOVA using Fisher post hoc tests for comparison of Soy and Soy+Mn groups to Controls. To evaluate Mn-related effects, a dose metric was constructed as the total Mn intake of the infants during the first 2 weeks of life (when formula intake was measured daily.) This measure was used in regression analysis of parameters that demonstrated formula group differences to evaluate Mn dose response. In addition, parameters demonstrating formula group differ-

ences were entered into regressions with CSF neurotransmitter metabolite concentrations to explore possible association between behavior and altered dopamine or serotonin.

3. Results

3.1. Growth and health

No formula group differences were seen in statistical analysis of body weights and lengths during the experimental formula feeding from birth to 4 months of age (data not shown). However, between 6 and 9 months of age, the Soy and Soy+Mn groups began to lag behind slightly in weight and height. There was a formula group effect on body weight at 9 months of age ($F(2,21)=3.39$, $p=0.04$, Control vs. Soy, $p=0.01$) and a formula group effect on crown rump length at 8 months of age ($F(2,19)=5.49$, $p=0.01$, Control vs. Soy $p=0.004$, Control vs. Soy+Mn $p=0.04$) and 9 months of age ($F(2,19)=3.59$, $p=0.048$, Control vs. Soy $p=0.03$). Weights were not obtained by animal age after 9 months of age (monkeys were weighed as a group at 2-month intervals). Medical records did not indicate differences in incidence of health problems between the formula groups.

3.2. CSF catecholamine metabolites

Values showed high inter-individual variability, as is usually observed for this assay (Fig. 1). No statistically significant formula group differences were observed by

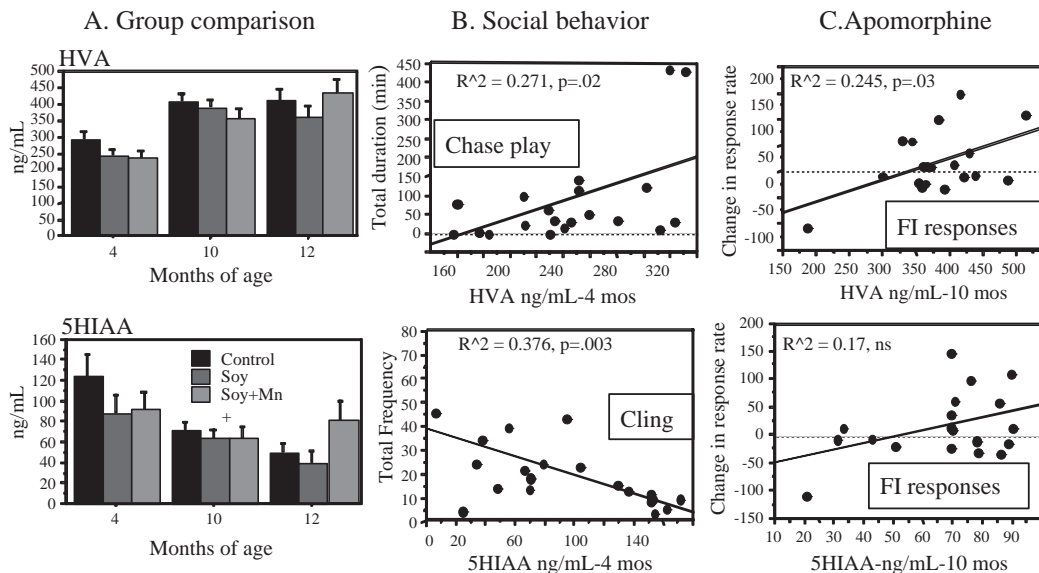


Fig. 1. Cerebrospinal fluid (CSF) concentrations of dopamine metabolite homovanillic acid (HVA) and serotonin metabolite 5-hydroxyindolacetic acid (5HIAA). (A) Concentrations in the three formula groups at three ages. Bars show group means \pm S.E.M. There were no statistically significant group differences. (B) Associations between CSF monoamine metabolite concentrations and social interaction endpoints influenced by formula group. Frequencies and durations were totaled across 16 sessions of dyadic interaction. The regression coefficient is also shown. (C) Associations between CSF monoamine metabolite concentrations and changes in FI response rate (responses/20-min session) after apomorphine injection (0.2 mg/kg).

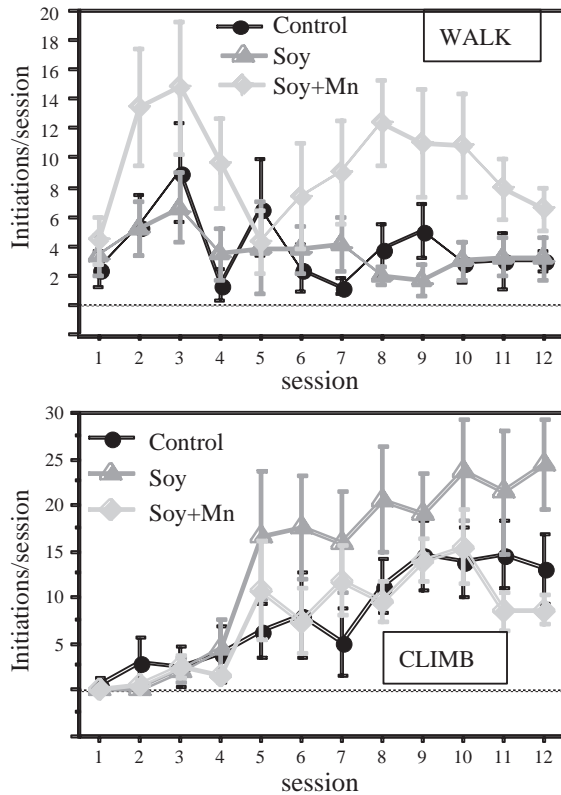


Fig. 2. Maturation of gross motor behaviors. Behavior was observed in weekly 10-min sessions. Top graph: walking (four-foot). Bottom graph: mature climbing. $N=8/\text{group}$.

ANOVA. Age related trends were seen; the 5HT metabolite 5HIAA decreased with age (RMANOVA, $F(2,36)=10.03$, $p=0.003$) and the dopamine metabolite HVA increased (RMANOVA, $F(2,36)=29.31$, $p<0.0001$). Across all individuals, 5HIAA and HVA concentrations were strongly correlated (4 months, $r=0.573$, $p=0.01$; 10 months, $r=0.578$, $p=0.01$; 12 months, $r=0.621$, $p=0.004$). Although there were no group differences at any age, there was a trend for the Soy+Mn group to increase, rather than decrease, metabolite concentrations between the 10- and 12-month timepoints.

3.3. Gross motor maturation

Infants varied in the amount of spontaneous activity they displayed during the twelve 7-min weekly observation sessions. Soy+Mn infants were more consistently active. Seven of eight Soy+Mn infants demonstrated motor behaviors during all observation sessions compared to three of eight Control and Soy animals (Fisher's exact test, $p=0.03$). One Soy infant displayed no motor behaviors in 75% (9/12) of the observation sessions.

Observations for each session were summarized as percent active behaviors, percent mature behaviors, and number of behavior changes per 30-s interval. Diet groups did not differ on percent active or percent mature behaviors for any session or overall. Over all sessions, more total behavior initiations were recorded for the Soy group than for controls. The Soy group also demonstrated a higher number of 30-s intervals with greater than the median number (3) of behavior changes. However, these group differences were not statistically significant. Plots of the data showed that climbing behavior initiations were elevated in the Soy group and walking initiations were elevated in the Soy+Mn group at the ages when these behaviors began to emerge during development (Fig. 2).

3.4. Spontaneous activity; rest/activity cycles

Data are shown in Fig. 3. The mean number of activity counts summarized in 2-min intervals during the sleep and wake periods was generally lower for the Soy+Mn group than Controls, but this difference was significant only for sleep periods at 4 months of age ($F(2,20)=4.91$, $p=0.02$, Control vs. Soy+Mn $p=0.005$). The duration of wake periods was shorter in the Soy and Soy+Mn groups at 8 months of age ($F(2,20)=5.23$, $p=0.02$, Control vs. Soy $p=0.01$, Control vs. Soy+Mn $p=0.02$). (Data for one Soy+Mn infant were missing at 8 months due to equipment failure.) There was a corresponding increase in the duration of the sleep periods. The longest period of inactivity during the wake phase (successive 2-min intervals with 0 activity

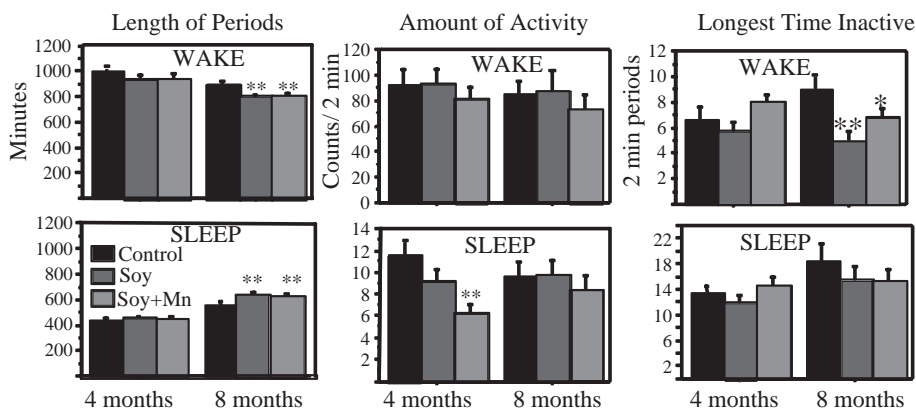


Fig. 3. Activity patterns. Activity was monitored over a 48-h period. Counts were summed over 2-min periods. $*p\leq 0.05$, $**p\leq 0.01$, post hoc comparison to controls. $N=7-8/\text{group}$.

counts) was shorter for the Soy and Soy+Mn groups than for Controls at 8 months of age ($F(2,20)=7.92$, $p=0.003$, Control vs. Soy $p=0.001$, Control vs. Soy+Mn $p=0.048$). The average maximum duration of inactivity at 8 months of age was 18, 10, and 14 min respectively in the Control, Soy, and Soy+Mn groups.

3.5. Dyadic social interaction

Analysis was conducted on the first 16 sessions in which an infant was paired with a similar number of strange partners from the three diet groups (4–6 sessions per diet group). The data are summarized in Fig. 4. Diet effects were demonstrated by ANOVA for the number of bouts of chase play ($F(2,21)=9.38$, $p=0.001$), rough-and-tumble play ($F(2,21)=7.39$, $p=0.004$), and cling ($F(2,21)=5.04$, $p=0.02$). Play was generally lower in pairings including a Soy or Soy+Mn infant (see Fig. 4). Conversely, the frequency of clinging was greater in the pairings that included Soy and Soy+Mn infants. Rough play was particularly affected by the Soy+Mn treatment as reflected in the ratio of

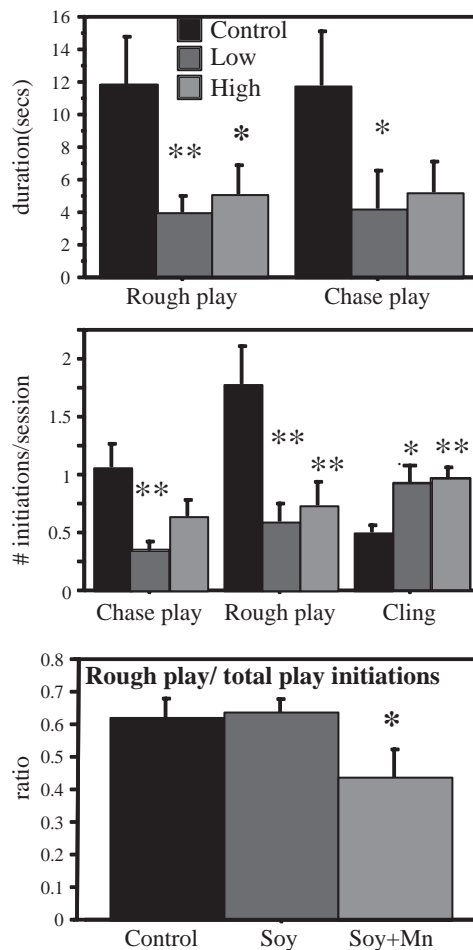


Fig. 4. Dyadic social interaction. Social behaviors (play, cling) were recorded during 16 sessions in which each monkey was paired with one other monkey in the cohort, including all formula groups. $N=8/\text{group}$. * $p \leq 0.05$, ** $p \leq 0.01$, post hoc comparison to controls.

rough to all play bouts (Fig. 4). The total duration of play behaviors over the 16 sessions was also lower in the Soy and Soy+Mn groups for both chase ($F(2,21)=7.93$, $p=0.003$) and rough-and-tumble ($F(2,21)=4.24$, $p=0.03$). The average duration (total duration/frequency) of play bouts was similar for all groups (data not shown).

3.6. Cognitive testing in the WGTA

The WGTA tasks represented the first introduction of the infants to standardized testing, and the performance levels of some infants were not adequate to allow evaluation of the targeted cognitive functions. The numbers of infants in each group failing to reach the performance criterion for each of the cognitive tasks (WGTA and automated testing) are shown in Table 4. These performance criterion were based on experiments with adult and juvenile rhesus, as performance criterion for infants less than 1 year of age were not available. Formula group differences were not found; the Soy group contained the most infants with low participation.

For the *object discrimination* task, one Soy infant failed to meet the training criterion. Two additional infants each in the Control group and the Soy group, and one in the Soy+Mn group failed to meet the object discrimination criterion. Of the infants that reached criterion for the object discrimination (Control $N=5$, Soy $N=6$, Soy+Mn $N=7$) there were no group differences in sessions required to reach criterion or the number of reversals completed after criterion was reached.

For the *DNMS* task, one infant in the Soy group did not meet criterion for participation and another Soy group infant stopped participating at session 6 (of 10). The formula groups did not differ in the number of trials completed per session, the number of balks (trials not initiated), or the ratio of correct choices to total choices summarized across all sessions (data not shown). Infants generally performed this task at chance level, indicating that they did not learn the task in the time allotted.

For *position reversal learning*, one infant (Soy group) did not displace objects. All other infants learned the position discrimination to criterion in 10 sessions or less. The number of reversals completed ranged from 0 to 11. Formula groups did not differ in the number of sessions to attain the performance criterion or the number of reversals completed after learning (data not shown). Response latencies also showed no formula effect, although the number of balks tended to be lower in the Soy+Mn group during the reversal sessions (data not shown).

3.7. Temperament ratings after WGTA sessions

Average temperament scores were calculated for each group using the scaled 4-point rating system for each of the eight categories. No diet group effects were detected in temperament ratings (data not shown). Group average ratings were highly consistent across the three WGTA

Table 4

Number of infants ($N=8$ /group) that failed to reach training criteria on structured cognitive tests

	OD	DNMS	PR	FI	CPT
Control	2	0	0	1	0
Soy	3	2	1	1	3
Soy+Mn	1	0	0	2	1

The training criteria were based on adult monkeys performing the same tasks in the WGTA.

Abbreviations: OD=object discrimination; DNMS=delayed nonmatch to sample; PR=position reversal; FI=fixed interval; CPT=continuous performance test.

tasks. The only category that demonstrated a group trend was that of stereotypy. Average stereotypy rating scores were consistently higher in the Control than in the Soy or Soy+Mn groups but these differences were not significantly different ($0.05 < p < 0.09$).

3.8. Reward delay

There was no performance criterion for this task. Monkeys in the Soy group tended to respond earlier in the reward delay test, particularly when the problem was first shown (no displacement of the screen, interval 0). At interval 0, the problem was first revealed by opening of a door that separated the test cage from the test board. To determine the time to response, the 30-s trial was divided into 2-s intervals corresponding to the screen displacements (0–7). Failure to respond on a trial was scored as the maximum number of intervals (15 intervals or 30 s). The average time to respond was lower for the Soy group than for Controls ($F(2,21)=3.28$, $p=0.057$, Soy vs. Control $p=0.02$) (Fig. 5). Also, the Soy group tended to respond more often in the first 2-s interval, although the ANOVA was not statistically significant. Although the Soy+Mn group had fewer early responses than the Soy group, they were distinguished by lack of improvement in the average response interval over the test session. Improved performance was seen across the four 10-trial blocks in the 40 trial session in both Control (RMANOVA, $F(3,21)=4.58$,

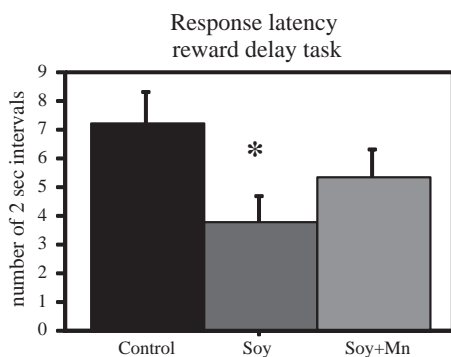


Fig. 5. Response inhibition in the delayed reward task. The figure shows the average number of 2-s intervals before the monkey infant reached for the food during the 30-s trial. $N=8$ /group. $*p \leq 0.05$, post hoc comparison to controls.

$p=0.01$) and Soy groups ($F(3,21)=5.69$, $p=0.01$). The Soy+Mn group did not improve its average performance ($F=2.85$, ns) (data not shown).

3.9. Dopamine drug challenge

Sixteen of twenty-four monkeys passed FI performance criterion before the end of the 13-week training period (average 6 weeks), and an additional four monkeys proceeded to the drug challenge phase at the end of the 13-week training period because they met the participation criterion. Four monkeys (one Control, one Soy, and two Soy+Mn) failed to pass the participation requirement at the end of the 13-week training period and were not included in the drug challenge experiments.

Fig. 6 shows that the Soy+Mn group differed from Controls in the response rate increase caused by 0.2 mg/kg apomorphine, and in the amelioration of haloperidol-induced response rate decrease by apomorphine. Each infant's response rate during sessions with drug administration was compared to its baseline by subtracting the response rate of the preceding vehicle session. The Soy+Mn group showed no increase in response rate with apomorphine, whether administered alone or with haloperidol. The Soy group showed a similar trend, but no comparisons to Controls were significant. The amphetamine dose was not effective in changing response rate in any group (data not shown).

3.10. Continuous performance test

Three monkeys in the Soy group failed to meet a criterion of $>75\%$ correct hits in any of the 30 session test series. One additional monkey in the Soy+Mn group had only four sessions that met this criterion. The average group performance of the CPT task did not differ in terms of the numbers of hits, misses, correct rejections and false alarms,

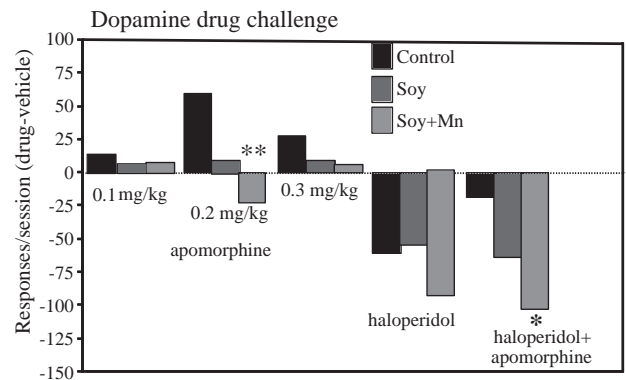


Fig. 6. Response rates under a fixed interval (FI) schedule of food reinforcement. The change in response rate was calculated as the number of responses made per 20-min session after drug injection minus the number made after vehicle injection. Drugs were administered 30 min prior to the session. Control $N=7$, Soy $N=7$, Soy+Mn $N=6$. $*p \leq 0.05$, $**p \leq 0.01$, post hoc comparison to controls.

or the ratios of hits to total responses, or correct rejections to total nonresponses, for all sessions or for the five sessions for each animal with the best performance (data not shown). The Soy+Mn group tended to respond more during the early sessions of training (first five sessions, hits+false alarms, $F(2,21)=3.7$, $p=0.04$, Control vs. Soy+Mn, $p=0.04$), but the proportion of hits to total responses was not different by group.

3.11. Stereotypy

There were no formula group differences in stereotypy. Similar numbers of monkeys in each group engaged in at least one instance of motor stereotypy (jumping, circling, flipping, bouncing) during the observation period (Control 4/8, Soy 4/8, Soy+Mn 3/8). Two monkeys from the Control group and one from the Soy group demonstrated >5 instances of motor stereotypy. None of the monkeys demonstrated rocking or self-clasping (self-directed stereotypies). One Control, three Soy, and two Soy+Mn monkeys demonstrated self-biting/chewing. Most of the infants (Control 5/8, Soy 7/8, Soy+Mn 7/8) sucked a body part, usually fingers, during the observation period.

3.12. Mn dose regressions

Regression analysis was conducted using neonatal Mn intake (first 2 weeks of life) as a predictor for behavioral endpoints that showed the strongest formula-related effects. (Individual formula intake could not be measured once socialization began as multiple infants in the same cage would have access to the same bottle of formula.) There was a significant association between neonatal Mn intake and variables from both the dyadic social interaction and FI drug challenge (regressions not shown). For dyadic interaction, the Mn intake was associated positively with the frequency of cling behavior ($r=0.430$, $p=0.04$) and negatively with the frequency of rough play ($r=-0.517$, $p=0.01$). In addition, the ratio of rough to total play was negatively associated with early Mn intake ($r=-0.492$, $p=0.02$). For FI drug challenges, the behavioral response to 0.2 mg/kg apomorphine alone was negatively correlated with early Mn intake ($r=-0.551$, $p=0.01$). Early Mn intake was not related to behavior changes in gross motor observation, to impulsivity indices in the delayed reward task, or to sessions-to-criterion for position discrimination learning.

3.13. CSF metabolite/behavior regression analysis

Regression analysis was conducted using 5HIAA and HVA as predictors for behavioral endpoints that showed the strongest formula-related effects. For social dyadic interaction, 5HIAA in 4-month CSF samples was a strong predictor of cling behavior ($r=-0.613$, $p=0.003$, $n=21$), while HVA predicted the total duration of chase play

($r=0.520$, $p=0.02$, $n=21$). Lower 5HIAA concentrations in CSF were associated with more cling behavior, while higher HVA concentrations were associated with more chase behavior (see Fig. 1).

For reward delay, HVA from 12-month CSF samples predicted the number of early responses to screen displacement ($r=0.44$, $p=0.03$, $n=24$). The 10-month HVA CSF concentration also predicted this impulsivity measure ($r=0.44$, $p=0.04$, $n=23$) (not shown).

For object discrimination learning, 5HIAA at 4 months was highly correlated with the sessions to criterion measure ($r=-0.674$, $p=0.0008$, $n=20$, $n=21$). 5HIAA at 10 months was also correlated with sessions to criterion ($r=0.467$, $p=0.03$, $n=22$). (The test was conducted at 11.5 months of age (not shown)).

For FI drug challenge endpoints, catecholamine metabolites in CSF samples obtained at 10 months were the best predictors. HVA was a significant predictor of response rate increase induced by 0.2 mg/kg apomorphine ($r=0.50$, $p=0.03$, $n=23$) (see Fig. 1).

4. Discussion

In this study the responses of infants fed cow's milk formula or soy formula with two different concentrations of manganese were compared on a battery of behavioral tests extending to 18 months of age (approximately equivalent to 6 years of age in children). The study design included three formula groups that differed on two dimensions, (1) soy vs. cow's milk base and (2) Mn content. The cow's milk group was designated as the Control group, although clearly infant monkeys do not ordinarily receive their nutrition from commercial cow's milk formula. However, if a control group nursed by their mothers had been used, a standardized rearing situation could not have been provided across groups.

An important finding of our study was the lack of formula group effects on growth, health, developmental milestones, temperament ratings, or stereotypy. Thus the monkeys fed soy formula were not generally debilitated and did not demonstrate grossly aberrant behavior or delayed maturation relative to the monkeys fed cow's milk formula.

In addition, formula group differences did not emerge in the more highly structured testing situations (delayed nonmatch to sample, object discrimination, position discrimination and reversal, continuous performance test) used to assess specific cognitive functions including learning, memory, and attention. Poor engagement of the infants with the formal tests of cognitive function was seen at these ages; additional testing at more mature ages would be appropriate. Three performance characteristics related to formula group did emerge from the structured testing: (1) poorer participation by the Soy group infants across tasks; (2) greater responsiveness in early trials/sessions of the CPT and the position reversals by the Soy+Mn group; and (3) slower attainment of the position discrimination task criteria and

lack of improvement in the reward delay task by the Soy+Mn group.

Behavioral assessments of spontaneous behavior were more sensitive to formula group. A potential effect of infant nutrition with soy vs. cow's milk formulas can be inferred by examination of differences between the Control and Soy groups. Endpoints that were statistically different in the Control and Soy groups were the duration of the daily sleep period, the frequency of cling (passive affiliation) and play behaviors during dyadic interaction, and the premature responding during the reward delay test. On many of these measures, the Soy+Mn group did not differ significantly from controls, but showed a mean difference in the same direction as the Soy group. The endpoints showing similar statistically significant effects in the Soy and Soy+Mn groups compared to Controls were longer sleep periods at night, shorter periods of inactivity during the day, and more cling and less play behavior in social dyads. If soy vs. cow's milk base was the determinant of formula group differences, the Soy+Mn group would be expected to show similar differences from Control as did the Soy group. The lack of effect in the Soy+Mn group on variables such as earlier responses in the reward delay test could be attributable to several factors such as an interaction of Mn effects with soy effects in the Soy+Mn group. It should also be kept in mind that the 3-fold difference in Mn exposure between the Soy and Soy+Mn groups was less than the 6-fold difference between the Control and Soy groups.

Endpoints that demonstrated differences between the Control and Soy+Mn group are potentially reflective of Mn developmental neurotoxicity since these two groups had the greatest differential Mn exposure. These endpoints were: more consistent presence of activity across sessions of motor observation, quality of play (rough vs. chase) in social dyadic interaction, overall spontaneous activity as detected by actimeters, poorer learning rate in the position discrimination task, fewer balks during discrimination reversals, failure to improve performance with experience in the reward delay session, and blunted behavioral response to the dopamine agonist apomorphine. Endpoints that demonstrated relationship with dose, as indexed by neonatal Mn intake, in regression analysis were dyadic interaction and response to apomorphine. This suggests sensitivity of social behavior and dopamine systems to early Mn exposure. The position discrimination learning task (trials to criterion measure) also showed a Mn dose-related trend and was highly correlated with CSF 5HIAA concentrations.

Integration of the behavioral findings is difficult. In the Soy group, the increased behavior changes, altered diurnal rhythms, and reduced play behavior seem to indicate altered regulatory control. In the Soy+Mn group, fewer balks during position reversals, earlier responding during the reward delay test, and greater response rates during early CPT testing seem to indicate a lack of normal wariness, or an increase in impulsivity. Infants did not show perseveration in the position reversal task or impaired inhibitory

control in the continuous performance test, thus ruling out a general problem with response inhibition.

Formula groups did not differ statistically in CSF monoamine metabolite concentrations. These measures were obtained to explore the hypothesis that Mn effects on dopamine and serotonin systems might mediate behavioral changes. A connection between Mn exposure and dopamine/serotonin system damage has been made in the literature through cellular and molecular actions of Mn, and also through the appearance of Parkinsonian symptoms in acute adult Mn neurotoxicity. However, no previous studies have looked specifically at Mn exposure and CSF metabolites. Regression analysis suggested that the performance of several tasks influenced by formula groups was also associated with the infants' CSF 5HIAA levels (social cling, discrimination learning) and HVA concentrations (chase play, early response on the reward delay, increase in response rate by apomorphine). Since CSF measures were obtained both during Mn exposure and after discontinuation of exposure at weaning, concurrent and delayed effects on catecholamines both need to be considered. Hypotheses concerning dopamine/serotonin mediation may be more appropriately explored by using drug challenge or CSF measures taken after the brain has matured. Although Mn exposure has been associated with lower brain dopamine in several situations, increased brain dopamine was found when assessed in immature rats exposed developmentally [9].

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References

- [1] American Academy of Pediatrics, Soy protein-based formulas: recommendations for use in infant feeding, *Am. J. Pediatr.* 101 (1998) 148–153.
- [2] J. Bergman, S. Rosenzweig-Lipson, R. Spealman, Differential effects of dopamine D1 and D2 receptor agonists on schedule-controlled behavior of squirrel monkeys, *J. Pharmacol. Exp. Ther.* 273 (1995) 40–48.
- [3] T. Burbacher, D. Shen, K. Grant, L. Sheppard, D. Damian, S. Ellis, N. Liberato, Reproductive and offspring developmental effects following maternal inhalation exposure to methanol in nonhuman primates, *Res. Rep. Health Eff. Inst.* (1999) 1–117.
- [4] M. Champoux, J.R. Hibbeln, C. Shannon, S. Majchrzak, S.J. Suomi, N. Salem Jr., J.D. Higley, Fatty acid formula supplementation and neuromotor development in rhesus monkey neonates, *Pediatr. Res.* 51 (2002) 273–281.
- [5] A.C. Chao, B.I. Ziadeh, G.Y. Diau, V. Wijendran, E. Sarkadi-Nagy, A.T. Hsieh, P.W. Nathanielsz, J.T. Brenna, Influence of dietary long-

- chain PUFA on premature baboon lung FA and dipalmitoyl PC composition, *Lipids* 38 (2003) 425–429.
- [6] A. Chen, W. Rogan, Isoflavones in soy infant formula: a review of evidence for endocrine and other activity in infants, *Annu. Rev. Nutr.* 24 (2004) 33–54.
- [7] L.A. Davidson, R.E. Litov, B. Lonnerdal, Iron retention from lactoferrin-supplemented formulas in infant rhesus monkeys, *Pediatr. Res.* 27 (1990) 176–180.
- [8] J. Donaldson, A. Barbeaux, Metal ions in neurology and psychiatry, in: B.T. Ho (Ed.), *Neurology and Neurobiology*, Alan R. Liss, Inc., New York, 1985, pp. 259–285.
- [9] D.C. Dorman, M.F. Struve, D. Vitarella, F.L. Byerly, J. Goetz, R. Miller, Neurotoxicity of manganese chloride in neonatal and adult CD rats following subchronic (21-day) high-dose oral exposure, *J. Appl. Toxicol.* 20 (2000) 179–187.
- [10] H. Eriksson, C. Morath, E. Heilbronn, Effects of manganese on the nervous system, *Acta Neurol. Scand., Suppl.* 100 (1984) 89–93.
- [11] H. Eriksson, K. Magiste, L.O. Plantin, F. Fonnum, K.G. Hedstrom, E. Theodorsson-Norheim, K. Kristensson, E. Stalberg, E. Heilbronn, Effects of manganese oxide on monkeys as revealed by a combined neurochemical, histological and neurophysiological evaluation, *Arch. Toxicol.* 61 (1987) 46–52.
- [12] H. Eriksson, P.G. Gillberg, S.M. Aquilonius, K.G. Hedstrom, E. Heilbronn, Receptor alterations in manganese intoxicated monkeys, *Arch. Toxicol.* 66 (1992) 359–364.
- [13] H. Eriksson, J. Tedroff, K.A. Thuomas, S.M. Aquilonius, P. Hartvig, K.J. Fasth, P. Bjurling, B. Langstrom, K.G. Hedstrom, E. Heilbronn, Manganese induced brain lesions in *Macaca fascicularis* as revealed by positron emission tomography and magnetic resonance imaging, *Arch. Toxicol.* 66 (1992) 403–407.
- [14] M.S. Golub, Use of monkey neonatal neurobehavioral test batteries in safety testing protocols, *Neurotoxicol. Teratol.* 12 (1990) 537–541.
- [15] M.S. Golub, M.E. Gershwin, L.S. Hurley, A.G. Hendrickx, W.Y. Saito, Studies of marginal zinc deprivation in rhesus monkeys: infant behavior, *Am. J. Clin. Nutr.* 42 (1985) 1229–1239.
- [16] M.S. Golub, P.T. Takeuchi, C.L. Keen, A.G. Hendrickx, M.E. Gershwin, Activity and attention in zinc-deprived adolescent monkeys, *Am. J. Clin. Nutr.* 64 (1996) 908–915.
- [17] M.S. Golub, C.L. Keen, M.E. Gershwin, Moderate zinc–iron deprivation influences behavior but not growth in adolescent rhesus monkeys, *J. Nutr.* 130 (2000) 354S–357S.
- [18] M.S. Golub, S.L. Germann, C.E. Hogrefe, Endocrine disruption and cognitive function in adolescent female rhesus monkeys, *Neurotoxicol. Teratol.* 26 (2004) 799–809.
- [19] V.M. Gunderson, K.S. Grant-Webster, T.M. Burbacher, N.K. Mottet, Visual recognition memory deficits in methylmercury-exposed *Macaca fascicularis* infants, *Neurotoxicol. Teratol.* 10 (1988) 373–379.
- [20] N. He, J. Bai, M. Champoux, S.J. Suomi, M.S. Lidow, Neurobehavioral deficits in neonatal rhesus monkeys exposed to cocaine in utero, *Neurotoxicol. Teratol.* 26 (2004) 13–21.
- [21] S.L. Kelleher, I. Casas, N. Carbajal, B. Lonnerdal, Supplementation of infant formula with the probiotic lactobacillus reuteri and zinc: impact on enteric infection and nutrition in infant rhesus monkeys, *J. Pediatr. Gastroenterol. Nutr.* 35 (2002) 162–168.
- [22] W.W. Koo, L.A. Kaplan, S.K. Krug-Wispe, Aluminum contamination of infant formulas, *JPEN. J. Parenter. Enteral Nutr.* 12 (1988) 170–173.
- [23] N.K. Laughlin, R.E. Lasky, N.L. Giles, M.L. Luck, Lead effects on neurobehavioral development in the neonatal rhesus monkey (*Macaca mulatta*), *Neurotoxicol. Teratol.* 21 (1999) 627–638.
- [24] B.S. Levy, W.J. Nassetta, Neurologic effects of manganese in humans: a review, *Int. J. Occup. Environ. Health* 9 (2003) 153–163.
- [25] B. Lonnerdal, Manganese nutrition of infants, in: D. Klimis-Ravantzis (Ed.), *Manganese in Health and Disease*, CRC Press, Boca Raton, FL, 1994, pp. 175–191.
- [26] B. Lonnerdal, Nutritional aspects of soy formula, *Acta Paediatr., Suppl.* 402 (1994) 105–108.
- [27] B. Lonnerdal, J.G. Bell, A.G. Hendrickx, R.A. Burns, C.L. Keen, Effect of phytate removal on zinc absorption from soy formula, *Am. J. Clin. Nutr.* 48 (1988) 1301–1306.
- [28] B. Lonnerdal, L. Jayawickrama, E.L. Lien, Effect of reducing the phytate content and of partially hydrolyzing the protein in soy formula on zinc and copper absorption and status in infant rhesus monkeys and rat pups, *Am. J. Clin. Nutr.* 69 (1999) 490–496.
- [29] M. Luciana, C. Nelson, Neurodevelopmental assessment of cognitive function using the Cambridge Neuropsychological Testing Automated Battery (CANTAB): validation and future goals, in: J. Rumsey (Ed.), *Functional Neuroimaging in Child Psychiatry*, Cambridge Press, UK, 2000, pp. 379–397.
- [30] S. Luciana, R.M., B. Martin, K. Morris, I. Greig, C. McKinnell, A.S. McNeilly, M. Walker, Infant feeding with soy formula milk: effects on the testis and on blood testosterone levels in marmoset monkeys during the period of neonatal testicular activity, *Hum. Reprod.* 17 (2002) 1692–1703.
- [31] M.H. Malloy, H. Berendes, Does breast-feeding influence intelligence quotients at 9 and 10 years of age?, *Early Hum. Dev.* 50 (1998) 209–217.
- [32] National Research Council, *Nutrient Requirements of Nonhuman Primates*, National Academy Press, Washington, DC, 1978.
- [33] National Toxicology Program, Evaluation of genistein and soy formula, *Fed. Regist.* 69 (2004) 19444.
- [34] M. Neuringer, J. Sturman, Visual acuity loss in rhesus monkey infants fed a taurine-free human infant formula, *J. Neurosci. Res.* 18 (1987) 597–601.
- [35] P.K. Pal, A. Samii, D.B. Calne, Manganese neurotoxicity: a review of clinical features, imaging and pathology, *Neurotoxicology* 20 (1999) 227–238.
- [36] A.J. Riopelle, P.A. Hale, C.W. Hill, Protein deprivation in primates: VIII. Early behavior of progeny, *Dev. Psychobiol.* 9 (1976) 465–475.
- [37] A.C. Roberts, B.J. Sahakian, Comparable tests of cognitive function in monkey and man, in: A. Sahgal (Ed.), *Behavioral Neuroscience: A Practical Approach*, Oxford University Press, 1993, pp. 165–184.
- [38] S. Rudloff, B. Lonnerdal, Calcium and zinc retention from protein hydrolysate formulas in suckling rhesus monkeys, *Am. J. Dis. Child.* 146 (1992) 588–591.
- [39] H.H. Sandstead, D.A. Strobel, G.M. Logan Jr., E.O. Marks, R.A. Jacob, Zinc deficiency in pregnant rhesus monkeys: effects on behavior of infants, *Am. J. Clin. Nutr.* 31 (1978) 844–849.
- [40] M.L. Schneider, C.F. Moore, E.F. Becker, Timing of moderate alcohol exposure during pregnancy and neonatal outcome in rhesus monkeys (*Macaca mulatta*), *Alcohol Clin. Exp. Res.* 25 (2001) 1238–1245.
- [41] B.L. Strom, R. Schinnar, E.E. Ziegler, K.T. Barnhart, M.D. Sammel, G.A. Macones, V.A. Stallings, J.M. Drulis, S.E. Nelson, S.A. Hanson, Exposure to soy-based formula in infancy and endocrinological and reproductive outcomes in young adulthood, *JAMA* 286 (2001) 807–814.
- [42] M.N. Subhash, T.S. Padmashree, Regional distribution of dopamine beta-hydroxylase and monoamine oxidase in the brains of rats exposed to manganese, *Food Chem. Toxicol.* 28 (1990) 567–570.
- [43] M.N. Subhash, T.S. Padmashree, Effect of manganese on biogenic amine metabolism in regions of the rat brain, *Food Chem. Toxicol.* 29 (1991) 579–582.
- [44] J.M. Swanson, N. Volkow, J. Newcorn, B. Casey, R. Moyzis, D. Grandy, M. Posner, Dopamine and glutamate in attention deficit hyperactivity disorder, in: W.J. Smith, M.E.A. Reith (Eds.), *Dopamine and Glutamate in Psychiatric Disorders*, Humana Press, Totowa, NJ, 2005, pp. 229–316.
- [45] T. Tran, W. Chowanadisai, F. Crinella, A. Chicz-Demet, B. Lonnerdal, Effect of high dietary manganese intake of neonatal rats on tissue mineral accumulation, striatal dopamine levels and neurodevelopmental status, *Neurotoxicology* 23 (2002) 8.
- [46] T. Tran, W. Chowanadisai, B. Lonnerdal, L. Le, M. Parker, A. Chicz-Demet, F. Crinella, Effects of neonatal dietary manganese on brain dopamine levels and neurocognitive functions, *Neurotoxicology* 23 (2002) 645–651.