A Test of Dietary Monosodium Glutamate Developmental Neurotoxicity in Rats: A Reappraisal

Charles V. Vorhees

Department of Pediatrics, University of Cincinnati College of Medicine and Division of Neurology, Cincinnati Children's Hospital Medical Center, Cincinnati, OH, USA

Keywords

Monosodium glutamate · Developmental neurotoxicity · Behavioral assessment

Abstract

In 1979, we tested dietary monosodium glutamate (MSG) for developmental neurotoxicity in rats. The study was recently cited for establishing a No Observable Adverse Effect Level (NOAEL) for MSG as a food additive resulting in a change in the acceptable daily intake (ADI). Therefore, I re-evaluated the study [Vorhees et al.: *Toxicol Appl Pharmacol* 1979;50: 267–282]. Sprague-Dawley rats were fed diets containing 0, 1.7, 3.4, or 5.1% MSG prior to conception, throughout gestation and lactation, and the same diets were fed to the offspring until 90 days of age. About 18–20 L were tested per dose with litter and sex factors in data analyses. There were 21 functional tests with 36 dependent variables and 10 body weight and histological outcomes. Of the functional tests, 4 were significant involving 6 effects. Two effects were on swimming ontogeny: one was an improvement and the other an atypical minor delay of no significance. Two effects were on active avoidance: one was a low-dose female-only extinction effect and the other a high-dose male-only acquisition effect, neither providing evidence of consistency. One was on passive avoidance, but was an improvement not a deficit. The last was

on open-field rearing in the absence of its normal association with locomotion changes. Thus, it can be concluded, as was done in 1979 and by the U.S. Food and Drug Administration who sponsored the study, that there is no evidence in these data that dietary MSG is developmentally neurotoxic, hence, the study provides no basis for the establishment of a NOAEL and changing the ADI for dietary MSG.

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In 1979, we published a paper investigating the developmental neurotoxicity (DN) of dietary exposure to monosodium glutamate (MSG) on somatic growth, landmark development, reflex ontogeny, neurobehavioral function, brain cell counts, and Golgi neuronal spine counts [1]. The study was cited in a reevaluation of the safety of MSG and related glutamate-based food additives by the European Food Safety Authority [2].

In this paper, I will review the study noting its strengths and weaknesses, summarize the statistically significant effects, explain their possible meaning, and place them in perspective with progress in doing such evaluations in the 40 years since the experiment was published. In addition, crucial aspects of how the data were analyzed and how they would be analyzed today are highlighted.

The Experiment

Below I will summarize the following: What was the study? What was its purpose? What was the experimental design? What were the methods? How were the data analyzed? What was found? How should the data be interpreted?

Study's Objectives

The study had 2 objectives: (1) create a DN test battery and (2) use the battery to evaluate a series of food additives of interest to the U.S. Food and Drug Administration (FDA) at that time. This was the first study of its kind, and the test additives were MSG and calcium carrageenan (CC) in a unified study design in which both food additives along with control groups would be evaluated simultaneously. The control groups served as controls for both additives but the data for each additive and the 2 control groups were analyzed separately. For the control groups; one was a negative control (C-) and the other was a positive control (C+). The intended C+ group was treated prenatally by i.p. injection with 550 mg/kg of hydroxyurea (HU) on embryonic day 12 (E12; conception was inferred as E0). HU is an established teratogen, but at doses near the teratogenic threshold HU produces neurobehavioral abnormalities in the offspring [3, 4].

Purpose

DN was an emerging field in the 1970s [5]. Several labs began to develop test batteries to screen compounds for DN. This paper was our lab's first contribution in this area as noted by the title: A developmental test battery for neurobehavioral toxicity in rats: A preliminary analysis using MSG, CC, and HU [1]. Therefore, in addition to MSG and CC being tested, the battery was also tested simultaneously. This duality creates an inferential issue: One cannot simultaneously validate a test while using it to test an unknown. The C+ group could have provided the initial validation, unfortunately, the effects of HU were marginal and therefore did not provide the needed validation. The results, therefore, could be due to MSG or issues with the test methods. It is worth noting that this test battery never became a standard in the field; it was superseded by a test battery developed by the U.S. Environmental Protection Agency (EPA) that is referred to as the DN Test (DNT) guideline [6-8].

Animals and Diet

Sprague-Dawley rats were used. Test materials were added to the standard ground Purina rat chow. The experimental diets were provided to the rats prior to breeding, throughout gestation and lactation, and to the offspring throughout the development to the end of the experiment at postnatal day (P)90. Litters were housed in shoebox cages; at all other ages, rats were in wire-bottom hanging cages. Offspring were weaned on P21.

Statistics

Depending on the outcome, 1-way, 2-way, or 3-way analyses of variance (ANOVA) were used to analyze the data. For pre-weaning tests where the dependent measure was days to criterion, 1-way ANOVAs were used. In later tests, sex was an additional factor in 2-way factorial ANOVAs. If there was a repeated measure, then the data were analyzed using 3-way ANOVAs with repeated measures (RM-ANOVA). There were 31 primary outcomes measured: 21 were functional tests and 10 were of body or brain weight or histology. Of the 21 functional tests, several had multiple dependent measures resulting in 36 functional dependent variables that were analyzed. Of the latter 36 ANOVAs, 22 were 1-way, 8 were 2-way, and 6 were 3-way ANOVAs; of the 2-way ANOVAs one had a repeated measure factor; and of the 3-way ANOVAs all had one repeated measure factor. These 36 ANOVAs generated 68 F-tests. Where a treatment effect was significant at $p < 0.05$, follow-up tests were performed. If one considers 68 F-tests one would expect at least 5% to be significant by chance, that is, 3.4. Four were significant.

Where F-tests were significant for a main effect or a group $\times$ sex or group $\times$ day interaction, post-hoc tests were conducted first using simple-effect ANOVAs, followed by Newman-Keuls pairwise group comparisons. Neither of these methods are endorsed by statisticians these days because they tend to inflate alpha. The design of the test battery is shown in Table 1 of the original paper. Body weight and mortality were used as indices of general toxicity. There was no effect of MSG on mortality, although no supporting data were shown in the paper. Body weight showed no effects prior to or after weaning, hence, no general developmental toxicity occurred.

We did control for litter effects. While better methods became available later we handled the problem by treating sex as a within or matching factor to avoid doubling the effective N as if they were orthogonal. Some labs ana-

lyzed males and females separately, but this requires twice as many ANOVAs, reduces sample size, and prevents testing for treatment $\times$ sex interactions. On pre-weaning tests, we used a single measure for the entire litter. On post-weaning tests, we tested 2 males and 2 females per litter but used their averaged performance to represent the litter. In all analyses of post-weaning behavior, group and sex were factors in the ANOVAs.

Exposure

For MSG, the dietary doses were 1.7, 3.4, and 5.1% by weight mixed in ground Purina rat chow. For CC, they were 0.45, 0.9, and 1.8% by weight mixed in ground Purina rat chow. Both were available 24/7 ad libitum.

Methods

Birth was designated P0. On P1, litters were adjusted to 8–12 pups using a random number table. Litters of more than 12 were culled balancing for sex; those with less than 8 pups were not used. Pups were weaned on P21, remained with their littermates until P25, and were housed separately thereafter. About 18–20 L/group were tested. Pregnant females were enrolled on a rotational basis to keep groups balanced over time. Final numbers varied slightly due to an occasional death or because of human error during testing. The number of pups at weaning in the MSG groups for males were: 18, 18, 20 for the 1.7, 3.4, and 5.1% doses; and 20 for the C– group and 20 for the C+ group, respectively; for females they were: 18, 20, and 17 for the 1.7, 3.4, and 5.1% doses, and 20 for the C– group and 20 for the C+ group.

For pre-weaning tests, days to criterion for one male and one female per litter was used as a litter average for pinna detachment, incisor eruption, eye opening, pivoting emergence, forward locomotion (3 stages), surface righting, cliff avoidance, pivoting by time and number of 90 degree turns, auditory startle emergence, visual placing (P18 and P21) were each analyzed by 1-way ANOVA. Swimming ontogeny, active avoidance (3 phases), and appetitive discrimination were analyzed by 2-way ANOVA, with the one for swimming ontogeny a RM-ANOVA by day.

The remaining functional tests were 3-week running wheel (3-way RM-ANOVA), open-field for 3 days, 3-min/day (3-way RM-ANOVA), 2-day rotorod (3-way RM-ANOVA), appetitive position discrimination and rever-

sal (3-way RM-ANOVA), and 3-day passive avoidance (3-way RM-ANOVA). Brain weight, H&E (hematoxylin and eosin) cell counts, and Golgi dendritic spine counts were analyzed by 1-way ANOVA.

Findings

First, it should be noted that there were no significant effects on maternal or offspring body weight at any point during the experiment as reported by Vorhees et al. [1]. One thing missing from the published paper, but which was found through another source and verified, was that there were no significant effects on offspring mortality.

Out of 21 functional tests with 36 dependent measures, 4 were statistically significant. These were for swimming ontogeny, open-field rearing, and active and passive avoidance.

First is the effect on swimming ontogeny. When a treatment affects this test, a plot of the data shows a steady increase in scores across days. A treatment that delays swimming development shows a deflection downward from the normal curve. For a deflection to be meaningful, it must span several days. We published a paper in the same year that shows such delays and should be consulted for comparison with what was seen with MSG [9]. In the case of MSG, the effects occurred on only one day and were small; there were no multiday effects as occurs from neurotoxins. Two swimming effects were seen. The first effect occurred on P6 only in the high-dose MSG group. P6 is the least reliable day of this test because rats have never been placed in water before and their initial response is unpredictable. After P6, the high-dose MSG group performed normally and matched the C– group closely. No effects were found on swimming angle or limb usage for the MSG exposed groups, whereas neurotoxins are known to affect all 3 measures and not just one. Furthermore, the MSG effect was not dose-dependent. The second effect occurred on swimming angle. It occurred in the low-dose MSG group on P12 and was an increase, not a decrease, that is, the rats performed better than those in the C– group. Interestingly, if one examines Figure 1 in the paper, an identical effect occurred in the low-dose CC group on exactly the same day, in exactly the same direction.

Two tests of locomotor activity were conducted; 24/7 running wheel activity for 3 weeks and open-field exploration for 3 days, 3 min/day. There were no effects of MSG on running wheel. There was also no effect of MSG on open-field ambulation, the principal measure on this

test. The one effect was on rearing in the high-dose MSG group. The effect was a decrease of approximately 1.5 rears versus control of about 17 rears; a change so small it is probably not biologically meaningful or replicable.

There were no effects of MSG on rotorod. On active avoidance, rats were required to turn a wheel during a 9 s auditory warning to avoid foot-shock. The criterion was 18 out of 20 trials, avoiding the shock within a single test session of 20 trials. Once the rats reached the acquisition criterion, the contingencies were changed such that they had to learn that the signal no longer warned of impending shock until they reached an extinction criterion of 90% non-responding to the tone. Once the rats reached the extinction criterion, they were not tested for several days and then retested to respond to the tone again, to the same 90% avoidance criterion as during acquisition. There was an increase in days to criterion for males on acquisition in the high-dose MSG group and on extinction in the female low-dose group; there were no effects in other groups or other phases. The male high-dose acquisition lag might be real, but would have to be replicated in a new experiment. The low-dose female extinction, effect does not fit with the rest of the data and it looks like an outlier.

In the appetitive position discrimination and reversal test, there were no effects of MSG.

The last test was passive avoidance. Day-1 was training: rats were placed on the lighted side of a 2-chamber apparatus with the opposite compartment dark. Rats spontaneously cross into the dark side when the door is opened. The divider door is then closed and rats are given a 1 s (0.75 mA) foot-shock. Tests of memory are given 24 and 48 h later. For this, the rat is placed back in the lighted side, the door opened, and it is free to crossover for up to 3 min. Latency to cross is recorded. Rats are then removed and tested a second time the next day. The first session assesses memory consolidation; the second, memory with partial extinction. There were no MSG effects on day-2 or 3, however, the high-dose MSG group remained on the lighted side proportionately longer on day-3 than on day-2. This indicates that they performed better at 48 h than at 24 h, that is, they remembered better, not worse. This is not a deficit, but shows enhanced memory.

Cell counts in 7 μ m paraffin-embedded H&E stained sections through cerebellum, olfactory bulbs, and hippocampus showed a small reduction in cell number in the low-dose MSG group with no change at higher doses or in other regions. There were no effects of MSG on regional brain weights, or on Golgi dendritic spine counts.

Interpretation

The 1979 data provide no persuasive evidence of MSG-induced DN. Given that there are only 4 effects obtained out of 21 functional tests involving 31 dependent variables raises concerns about the meaning of these observations. For instance, the effects in the high-dose MSG group for swimming direction was an isolated 1-day minor delay; it does not fit with what other studies show for this test when a compound is neurotoxic [9], and this reduces the confidence in this observation. The open-field effect on rearing in the high-dose MSG group occurred in the absence of an effect on locomotion which is at variance with the fact that these measures are normally positively correlated [10–12]. There was an effect on active avoidance acquisition in the high-dose male MSG group and in the low-dose female group on extinction. The problem is that the effects are inconsistent across phases, inconsistent across sexes, not dose-dependent, and fail to be biologically plausible. The odds that this pattern would be seen in a replication experiment are slim. The passive avoidance 24–48 h retention difference is an improvement, not a deficit, so this is not evidence of DN. Hence, viewed collectively, none of the findings in the 1979 study suggest that MSG causes DN.

This conclusion is reinforced by related studies, although they have limitations as well. When MSG is injected subcutaneously or intraperitoneally, mice are more sensitive to arcuate lesions than rats, suggesting that mice are a more sensitive species. Based on this species difference, Anantharaman [13] conducted a 3-generation reproductive study in CD-1 mice providing 1 or 4% MSG in the diet with exposure starting during pregnancy of the F0 generation. F1.1 and F3.1 male and female offsprings from each group were taken at P3, P14, and P21 for neurohistological examination. He found no effects on growth, mortality, or reproduction in any generation, and no effects on neuronal density in the arcuate nucleus or other hypothalamic nuclei, or in basal ganglia, hippocampus, thalamus or cortex in the MSG groups compared with controls. However, this study was not peer reviewed and aspects of methods and results are incompletely presented.

Takasaki et al. [14] used ICR mice and compared the effects of dietary to intraperitoneal, gavage, subcutaneous, and dietary MSG given at P10, P23 or as adults and examined brains for effects on plasma testosterone, luteinizing hormone, follicle-stimulating hormone, organ weight, reproductive function, day of vaginal opening, activity in an Animex system, or 3-min open-field test. Ef-

fects were found only if MSG was injected. They also tested Wistar rats injected with 0.5 g/kg MSG from P10–19 then fed high doses in the diet from P20–29: no arcuate lesions were found; however, if injected with 4 g/kg MSG from P2–11 most neurons in the arcuate nucleus were not detected. But if MSG was injected at the same dose, 4 g/kg, from P10–19, no arcuate lesions were seen. Hence, in rats and mice, only early neonatal s.c. or i.p. injections of high doses of MSG induce arcuate lesions, no dietary dose, no matter how high or how early, induced lesions. Neonatal mouse and rat brain, especially during the first 10 days postnatal is, in terms of brain development, comparable to late third trimester intrauterine brain development in humans [15–17] (see also translatingtime.net). This study was also published in a non-peer reviewed source and details of methods and results are incomplete.

Two studies were done in Wistar rats, exposing them to MSG in their drinking water during the last 2 weeks of gestation [18, 19]. In the first study [18], MSG was provided as 5 g/100 mL of water resulting in an estimated dose of 10 g/kg. The offsprings were tested at P20 in a small open-field and again at P35 in a larger open-field, each time for 3-min. Starting at P60 rats were tested for discrimination learning, maze learning, and 2-way active avoidance.

The authors report reduced birth weight but increased body weight at P28 in the MSG group. There were no open-field differences at P20 but at P35 MSG rats were less active and showed less rearing than controls. There were no differences on active avoidance or a 2-choice discrimination task, but the MSG group made more errors in a maze on the last 4 trials compared with controls. However, this study has serious deficiencies. They used only 3 L in the MSG group and 2 in the control group and from these few litters retained 30 MSG and 20 controls for testing without regard to litter membership and no statistical adjustment for litter effects. How the retained pups were chosen is not stated. Because of the flawed design of this experiment, it is not possible to determine if these effects are reliable.

In a second study from this group [19], they gave MSG in drinking water at the same concentration as in their previous study to gravid Wistar rats on the same gestational days (the last 14 days of pregnancy up to E20) as before. The outcomes assessed in this experiment were ³H-choline, norepinephrine (NE), and GABA (gamma-amino butyric acid) uptake into selected brain regions in synaptosomal fractions, and ¹⁴C-acetyl-CoA uptake, as a marker of choline acetyltransferase activity, in crude fractions. They took offspring from 10 MSG-exposed and 10

control litters and analyzed ~6 pups per group at different ages. Choline and acetyl-CoA uptake were done at P15, P21, P28, P60, and P75, whereas NE and GABA were done only at P60. At P15 and P60, they report decreased and increased choline uptake in PFC of MSG rats compared with controls, whereas choline uptake in the hippocampus was increased only at P75. NE uptake in PFC was decreased at P60 and acetyl-CoA was increased at P15 and reduced at P60 in the MSG group, with no changes at other ages and no changes in GABA. Sample sizes were 6 per group except at P60 where males were 6–12, with 6 per group in females. Some effects at P60 were sex-dependent, such as the acetyl-CoA uptake increase in the hippocampus that was seen in males and not females. This study too has problematic aspects to it. There is no indication that they controlled for litter, no information on how pups were chosen for testing from the different litters, no indication of how data were analyzed, no pattern to the small up and down changes in those markers that were changed, nor did they include any behavioral tests as in their first study to corroborate the relationship between these neurochemical changes and the behavioral changes they previously reported. Lastly, the relevance of MSG dissolved in drinking water is unclear as MSG as a food additive is primarily added to food not beverages. As with the first MSG study from this group, these later data are difficult to interpret. This is because the experimental design raises concerns about the small sample sizes and the absence of control for litter effects. Therefore, these data do not contribute to the determination of whether MSG is developmentally neurotoxic.

Taken collectively, the above studies are consistent with our data that dietary MSG does not show evidence of DN in rodents at high doses in mice or rats.

Interpretations Then and Now

Our 1979 data do not support defining a No Observable Adverse Effect Level and therefore, do not support a change in the acceptable daily intake (ADI) for dietary MSG. If our data raise concerns, they should be addressed in a new study with current protocols and contemporary methods; no change in the ADI should be made before a new study is done because if a new study fails to find DN, changing the standard prematurely would cause serious unnecessary difficulties that would then have to be undone.

My lab has investigated developmental neurotoxins for 42 years. Our 1979 data do not resemble any develop-

mental neurotoxin my lab has tested or of any other that I am aware of from the literature.

My coauthors and I interpreted the 1979 study as providing no persuasive evidence of DN. Looking back at the study 40 years later, my view is even stronger that the data provide no evidence of DN. Furthermore, the FDA came to Cincinnati and spent days auditing every page of data from this study and found the data to be in proper order and as we reported it. Subsequently, the FDA reviewed the results and found no evidence of concern for the safety of dietary MSG as a Generally Recognized as Safe (GRAS) food additive and made no change in its status. To the best of my knowledge, no other rodent data have been published since showing any evidence of DN from dietary MSG.

Context

In 1979, there were no guidelines on how DN studies should be done. Later, the EPA's DNT guidelines were developed as were those of the Organization for Economic Cooperation and Development for pesticides and priority commercial chemicals. The EPA's DNT guideline has been the standard regulatory practice for 30 years. The FDA has similar but less specific guidelines as does the ICH (International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human Use). What is important is that almost no test used in our 1979 study is still used. This is because those methods did not turn out to be sufficiently sensitive to detect neurohazards nor predictive of long-term neurotoxicity. This raises the question of how much weight should be put on the 1979 study. No one does swimming ontogeny anymore, therefore, it should not be relied upon. Open-field today is automated and tested for 60 min not 3 min because 3-min data are unreliable. Active avoidance is still

used but not the procedure we used. Passive avoidance is still used, but recent data cast serious doubt on its reliability and sensitivity for detecting DN [20]. In the latter study, we showed that methamphetamine given neonatally results in lasting learning and memory deficits on tests of egocentric, allocentric, and working memory, but had no effect on passive avoidance. We have unpublished data showing this is also the case for trial-to-criterion passive avoidance that some argue is more sensitive than the one-trial method, but our data suggest otherwise.

Current EPA and Organization for Economic Cooperation and Development DNT guidelines leave much to be desired, therefore, do not take my concerns about our 1979 data as an endorsement of current DN protocols. Current DNT protocols have serious limitations, they have not kept up with current science, and are in much need of modernization as described recently [21]. Nevertheless, current DNT guideline studies are still better than what we did in 1979. Therefore, our 1979 data should be interpreted with caution and taken as no more than suggestive. I know our 1979 study better than anyone, I know the methods because I supervised the work, I know the data analyses because I ran them, I know the study's limitations because I wrote the FDA report and the published paper from the report. In my estimation, the 1979 data should not be used to change the ADI for dietary MSG.

Disclosure Statement

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References

- 1 Vorhees CV, Butcher RE, Brunner RL, Sobotka TJ: A developmental test battery for neurobehavioral toxicity in rats: a preliminary analysis using monosodium glutamate calcium carrageenan, and hydroxyurea. *Toxicol Appl Pharmacol* 1979;50:267–282.
- 2 EFSA Panel on Food Additives and Nutrient Sources added to Food (ANS), Mortensen A, Aguilar F, Crebelli R, Di Domenico A, Dusemund B, Jose Frutos M, Galtier P, Gott D, Gundert-Remy U, Leblanc JC, Lindtner OI, Moldeus P, Mosesso P, Parent-Massin D, Oskarsson A, Stankovic I, Waalkens-Berendsen I, Antonius Woutersen R, Wright M, Younes M, Boon P, Chrysafidis D, Gurtler R, Ooback P, Altieri A, Rincon AM, Lambre C: Re-evaluation of glutamic acid (E 620), sodium glutamate (E 621), potassium glutamate (E 622), calcium glutamate (E 623), ammonium glutamate (E 624) and magnesium glutamate (E 625) as food additives. *EFSA J* 2017;15:90.
- 3 Brunner RL, McLean M, Vorhees CV, Butcher RE: A comparison of behavioral and anatomical measures of hydroxyurea induced abnormalities. *Teratology* 1978;18:379–384.
- 4 Butcher RE, Scott WJ, Kazmaier K, Ritter EJ: Postnatal effects in rats of prenatal treatment with hydroxyurea. *Teratology* 1973;7:161–165.
- 5 Vorhees CV: Origins of behavioral teratology; in Riley EPAV, CV (ed): *Handbook of Behavioral Teratology*. New York, Plenum Press, 1986, pp 3–22.
- 6 Agency USEP: USEPA OPPTS 870.6300, Developmental Neurotoxicity Study, Health Effects Test Guidelines, EPA 712-C-98-239. Washington, U.S. EPA Office of Chemical Safety and Pollution Prevention, 1998.

- 7 OECD: OECD Test No. 426. Developmental Neurotoxicity Study, OECD Guidelines for Testing of Chemicals, Section 4: Health Effects. Paris, Organization for Economic Co-operation and Development, 2007.
- 8 U.S.EPA: USEPA OPPTS 870.6300, Developmental Neurotoxicity Study, Health Effects Test Guidelines, EPA 712-C-98-239. Washington, U.S. EPA Office of Chemical Safety and Pollution Prevention, 1998.
- 9 Vorhees CV, Brunner RL, Butcher RE: Psychotropic drugs as behavioral teratogens. *Science* 1979;205:1220–1225.
- 10 Walsh RN, Cummins RA: The open-field test: a critical review. *Psychol Bull* 1976;83:482–504.
- 11 Qu W, Liu NK, Xie XM, Li R, Xu XM: Automated monitoring of early neurobehavioral changes in mice following traumatic brain injury. *Neural Regen Res* 2016;11:248–256.
- 12 Boulle F, Massart R, Stragier E, Paizanis E, Zaidan L, Marday S, Gabriel C, Mocaer E, Mongeau R, Lanfumey L: Hippocampal and behavioral dysfunctions in a mouse model of environmental stress: normalization by agomelatine. *Transl Psychiatry* 2014;4:e485.
- 13 Anantharaman K: In utero and dietary administration of monosodium L-glutamate to mice: Reproductive performance and development in a multigeneration study; in Filer LJ Jr, Garattini S, Kare MR, Reynolds WA (eds): *Glutamic Acid: Advances in Biochemistry and Physiology*. New York, Raven Press, 1979, pp 231–253.
- 14 Takasaki Y, Matsuzawa Y, Iwata S, O'Hara Y, Yonetani S, Ichimura M: Toxicological studies of monosodium L-glutamate in rodents: Relationship between routes of administration and neurotoxicity; in Filer LJ Jr, Garattini S, Kare MR, Reynolds WA (eds): *Glutamic Acid: Advances in biochemistry and physiology*. New York, Raven Press, 1979, pp 255–275.
- 15 Clancy B, Darlington RB, Finlay BL: Translating developmental time across mammalian species. *Neuroscience* 2001;105:7–17.
- 16 Clancy B, Finlay BL, Darlington RB, Anand KJ: Extrapolating brain development from experimental species to humans. *Neurotoxicology* 2007;28:931–937.
- 17 Clancy B, Kersh B, Hyde J, Darlington RB, Anand KJ, Finlay BL: Web-based method for translating neurodevelopment from laboratory species to humans. *Neuroinformatics* 2007;5:79–94.
- 18 Frieder B, Grimm VE: Prenatal monosodium glutamate (MSG) treatment given through the mother's diet causes behavioral deficits in rat offspring. *Int J Neurosci* 1984;23:117–126.
- 19 Frieder B, Grimm VE: Prenatal monosodium glutamate causes long-lasting cholinergic and adrenergic changes in various brain regions. *J Neurochem* 1987;48:1359–1365.
- 20 Jablonski SA, Williams MT, Vorhees CV: Learning and memory effects of neonatal methamphetamine exposure in rats: Role of reactive oxygen species and age at assessment. *Synapse* 2017;71.
- 21 Vorhees CV, Sprowles JN, Regan SL, Williams MT: A better approach to in vivo developmental neurotoxicity assessment: alignment of rodent testing with effects seen in children after neurotoxic exposures. *Toxicol Appl Pharmacol* 2018;354:176–190.