Diminished Experience-Dependent Neuroanatomical Plasticity: Evidence for an Improved Biomarker of Subtle Neurotoxic Damage to the Developing Rat Brain

Christopher S. Wallace,[1,2] Jonathon Reitzenstein,[1] and Ginger S. Withers[1,2]

[1]Center for Research on Occupational and Environmental Toxicology, Oregon Health and Sciences University, Portland, Oregon, USA; [2]Biology Department, Whitman College, Walla Walla, Washington, USA

Millions of children are exposed to low levels of environmental neurotoxicants as their brains are developing. Conventional laboratory methods of neurotoxicology can detect maldevelopment of brain structure but are not designed to detect maldevelopment of the brain's capacity for plasticity that could impair learning throughout life. The environmental complexity (EC) paradigm has become classic for demonstrating the modifications in brain structure that occur in response to experience and thus provides a set of indices for plasticity in the healthy brain. In this study, we have tested the hypothesis that if degradation of experience-dependent cortical plasticity is used as a biomarker, then developmental neurotoxic effects will be detected at doses below those that alter cortical morphogenesis overtly. Pregnant Long-Evans hooded rats received a single injection of either saline vehicle or 1, 5, 10, or 25 mg/kg of the well-characterized developmental neurotoxicant methylazoxymethanol acetate (MAM) on the 16th or 17th day of gestation. On postnatal days 35–39, male offspring were assigned to either a complex environment (EC) or an individual cage (IC) for 28 days to stimulate neuroanatomical plasticity. This response was measured as the difference between the thickness of visual cortex of IC and EC littermates at a given dose. The threshold dose for significant reduction of cortical thickness was 25 mg/kg, but the threshold dose for failure of plasticity was much lower and could be detected at 1 mg/kg, the lowest dose used. No other method of assessment has detected lasting effects of prenatal exposure to MAM at such a low dose. These data suggest that this simple test of plasticity could be an efficient way to detect subtle neurotoxic damage to the developing brain. *Key words:* biomarker, complex environment, cortical plasticity, developmental neurotoxicology, methylazoxymethanol. *Environ Health Perspect* 111:1294–1298 (2003). doi:10.1289/ehp.6088 available via *http://dx.doi.org/* [Online 1 April 2003]

The development of the human nervous system is an extended process. It begins during early embryogenesis with the specification of neural epithelium; it requires that complex synaptic circuits be established [reviewed by Pomeroy and Kim (2000); Volpe (2000)], and it may never truly end in that synaptic connections continue to be modified throughout life, presumably as a mechanism of brain information storage (Black et al. 1998). Therefore, intrusion of a neurotoxicant could produce different defects depending on the timing and level of exposure (Spencer 2000) because it might disrupt populations of neurons engaged in cellular proliferation, migration, differentiation, or maturation (Jensen and Catalano 1998; Rodier 1995). This timing issue alone makes assessing the risk of even a single compound at a single dose a complex task, even discounting individual differences in response. Ideally, a biomarker would assess an important brain function with broad sensitivity. In this study, we questioned whether measuring experience-dependent brain plasticity late in development can reveal subtle lasting deficits caused by exposure to a neurotoxicant early in development.

By late embryogenesis, cortical neurons have been intrinsically programmed to form dendrites of the appropriate architecture (Banker and Waxman 1988). However, dendrites continue to grow into the postnatal period (Becker et al. 1984; Juraska 1982) and remain "plastic," such that extrinsic factors, notably, the unique behavioral experience of the individual, can induce readily quantifiable modifications in dendritic structure throughout life [reviewed by Kolb and Whishaw (1998)]. The therapeutic potential of behavioral stimulation is well established (e.g., Walsh and Greenough 1976), and recent animal studies demonstrate positive plastic changes in brain structure or physiology following housing in a broadly stimulating environment (e.g., Rema and Ebner 1999) or regimens of training designed to address specific functional deficits (Klintsova et al. 1999). The fact that plasticity is often reduced (e.g., Rema and Ebner 1999), or even eliminated after these manipulations (e.g., Berman et al. 1996), however, suggests that measurements of plasticity *per se* could provide an improved biomarker for detecting latent neurotoxic damage relevant to lasting learning and memory deficits.

The environmental complexity (EC) paradigm, originally devised by Hebb (1947), has been a key tool in documenting that behavioral experience produces enduring modifications of brain circuitry and behavior throughout life. It has also been used to identify cellular and molecular changes associated with the long-term storage of information in the mammalian brain [reviewed by Greenough (1976); Rosenzweig et al. (1972); van Praag et al. (2000)]. In this model, litters of rats are divided into conditions of differential environmental stimulation. Rats housed individually in standard cages (IC) are compared with their siblings that have been housed as a group in a complex environment that provides continuing opportunity for exploration, play, and spatial learning. Experience in the EC induces a robust and coordinated growth response in neurons (Kempermann et al. 1997; Turner and Greenough 1985; Volkmar and Greenough 1972), as well as in supporting cells (Black et al. 1987; Jones et al. 1996; Sirevaag and Greenough 1991). This array of cellular changes accumulates in some regions of neocortex to the extent that the thickness of cortex increases significantly (Diamond et al. 1964).

In the present study, we have selected this classic behavioral paradigm to test the hypothesis that cortical plasticity will begin to fail at a lower neurotoxicant dose than will the gross maturation of cortex. Our goal was to establish a laboratory assay for neuroanatomical plasticity using a summary measure such as cortical thickness that would be efficient yet sensitive. Such an assay could then be used to screen a variety of substances, including mixtures such as those found in groundwater surrounding Superfund sites.

As an initial test of this model, we selected a compound with well-characterized effects on brain development in rats, methylazoxymethanol acetate (MAM), a derivative of the cycad plant (Spencer et al. 2000). MAM causes DNA damage, and through this mechanism it is thought to kill actively dividing neurons (Esclaire et al. 1999). When administered

Address correspondence to C.S. Wallace, Dept. of Biology, Whitman College, 345 Boyer Ave., Walla Walla, WA 99362 USA. Telephone: (509) 522-4421. Fax: (509) 527-5904. E-mail: wallaccs@whitman.edu

We thank A. Roussin, A. Riddle, and K. Woods, for technical assistance; M. Lasarev for assistance with statistical analysis; and P.S. Spencer, G. Kisby, G. Higgins, K. Anger, and anonymous reviewers for comments on the manuscript.

This publication was made possible by grant 5P42 ES10338 from the National Institute of Environmental Health Sciences (NIEHS), with funds from the U.S. Environmental Protection Agency (EPA). Contents are solely the responsibility of the authors and do not necessarily represent the official views of the NIEHS or the U.S. EPA.

The authors declare they have no conflict of interest.

Received 4 November 2002; accepted 1 April 2003.

during rat cortical neurogenesis [gestational days (GDs) 14–18], MAM produces a dose-dependent forebrain microcephaly that is most pronounced at GDs 14–15 (Fischer et al. 1972; Haddad et al. 1972; Tamaru et al. 1988). MAM-induced microcephaly has become a common experimental model for studying cortical dysgenesis (Berger-Sweeney and Hohmann 1997; Colacitti et al. 1999). In addition to killing some neurons as they are being generated, prenatal MAM treatment causes detectable elevations in DNA adducts that remain detectable at postnatal day (PD) 30 (Kisby et al. 1999) and generates aberrant patterns of connectivity in the remaining cells (Colacitti et al. 1999). We administered MAM on GD 16 or 17 because this exposure *a*) coincides with the generation of association neurons in the superficial layers of cortex (Bayer and Altman 1995) that express robust plasticity (Juraska et al. 1980; Wallace et al. 1992) and *b*) produces milder morphogenic effects than do exposures at GDs 14–15 (e.g., Balduini et al. 1991). We chose a range of doses based upon data from GD-15 exposures in which the reported threshold dose for obvious reduction of cortical volume by MAM is > 10 mg/kg (Fischer et al. 1972; Haddad et al. 1972; Tamaru et al. 1988) and the reported threshold dose for behavioral deficits (e.g., open field, maze training and avoidance conditioning) is > 15 mg/kg (e.g., Balduini et al. 1991; Ohta et al. 2000). A high dose (25 mg/kg) was included as a positive control for morphogenic effects, but we focused on relatively low doses expected to produce a grossly normal brain (i.e., 1, 5, and 10 mg/kg).

Materials and Methods

Embryonic exposure to MAM. Litters of Long-Evans hooded rats (Simonsen Laboratories, Gilroy, CA) were exposed *in utero* to the neurotoxin MAM (Midwest Research Institute, Kansas City, MO) on GD 16 or 17 via a single intraperitoneal injection to the pregnant dam. The day of sperm plug detection was recorded as GD 1.

Timed-pregnant dams were selected randomly to receive 0 (saline vehicle), 1, 5, 10, or 25 mg/kg MAM in saline (12 dams per GD). For all doses, 2 dams were injected, except 3 dams each received saline or 5 mg/kg MAM injections on GD 16 and 3 dams each were injected with 5 mg/kg or 10 mg/kg treatments on GD 17. Offspring were housed with the dam until weaning on PD 28 and then housed in groups of two or three until the start of differential housing. Because handling per se can apparently induce neuroanatomical changes (Horner et al. 1991), we left rats undisturbed between weaning and assignment to differential housing to maximize EC–IC differences. All animals were handled according to the guidelines of the National Institutes of Health Office of Laboratory Animal Welfare (2002).

Differential housing. At PDs 35–39, male littermates were selected randomly for assignment to differential housing conditions, and these conditions were then balanced for body weight. Two littermate pairs from each dose were assigned to differential housing, with the exception of two litters that produced fewer than four males (one litter treated with saline, one litter treated with 25 mg/kg MAM). Rats were housed either individually in standard laboratory cages (IC) or as a cohort of 12 with the opportunity to play and explore in a large cage filled with toys that were changed daily (EC). As an additional opportunity for exploration, EC animals were placed in an open-field play cage with a novel arrangement of toys for 1 hr daily. All doses of MAM-treated and saline-treated rats were represented in a single EC cage, such that two parallel EC environments were required to accommodate the number of animals involved. Differential housing was continued in this manner for 28 days. Food and water in both conditions were administered *ad libitum*. EC and IC conditions were maintained in the same room under a 12-hr light/12-hr dark cycle to control for ambient conditions.

Cortical thickness measures and quantitative analyses. Cortical thickness was used as a simple, quantitative measure to determine both the effects of MAM treatment and the effects of differential housing. At the end of environmental exposure, rats were euthanized [sodium pentobarbital (Nembutal), 150 mg/kg, intraperitoneally], and their brains were removed, fresh-frozen using isopentane chilled over dry ice, sectioned into 20 µm coronal sections with a cryostat, and stained with a Nissl stain. A standard sample section of occipital cortex (OC) was approximately –4.3 mm from bregma and identified by the presence of both dorsal and ventral hippocampal formation with a narrowing in the middle [comparable with Figure 21 from Zilles (1985)]. The section was scanned to produce a digital image [Polaroid SprintScan 35+ with scan path enabler (Polaroid Corporation, Waltham, MA) using Photoshop 5.5 software (Adobe Systems Inc., San Jose, CA)]. To correct for overall differences in brain size that might arise from toxicant treatment, standard atlas coordinates for cortical subdivisions within the sample section (Zilles 1985) and the spacing of triplicate measures were normalized to the cross-sectional area of each brain. Cortical thickness was measured using NIH Image 1.62 software (National Institutes of Health, Bethesda, MD) as the distance between the lower boundary of layer I and the beginning of the white matter beneath layer VI. All histological processing and data collection were carried out blind to the experimental history of the cases.

The average of the three measurements was analyzed using a general linear model (GLM), allowing for unequal numbers of subjects, with dose, housing condition, and GD of dose (GD 16 vs. GD 17) as main effects. Design and number of animals per condition are reported in Table 1. Unequal numbers arose because of the *a priori* decision to use additional animals in some conditions (particularly intermediate doses), low numbers of males in some litters, and tissue loss during processing. The influences of dose and housing condition on cortical thickness were assessed using *t*-statistics for individual effects estimated from the GLM (Netter et al. 1989).

Results

Experimental design and main effects. We exposed the developing rats to a range of MAM doses via injections to timed-pregnant

Table 1. Experimental design including number of subjects in each treatment condition.

GD	Dose	Housing condition	No.	GD	Dose	Housing condition	No.
16	0	IC	3	17	0	IC	4
16	0	EC	3	17	0	EC	4
16	1	IC	3	17	1	IC	4
16	1	EC	3	17	1	EC	3
16	5	IC	4	17	5	IC	6
16	5	EC	4	17	5	EC	7
16	10	IC	2	17	10	IC	6
16	10	EC	2	17	10	EC	6
16	25	IC	3	17	25	IC	3
16	25	EC	2	17	25	EC	3

For each dose, subjects were assigned as littermate pairs, and housing conditions were balanced for body weight.

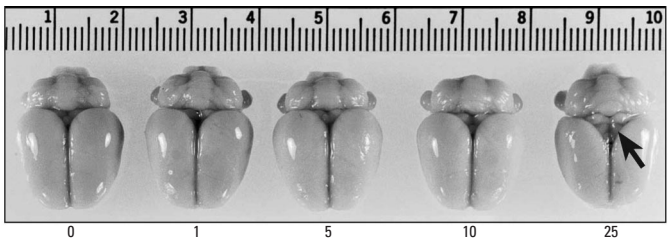

Figure 1. Dose–response analysis of MAM on brain size. The relative effects of prenatal exposure to 0, 1, 5, 10, and 25 mg/kg of MAM are shown in a comparison of brains from rats exposed at GD 16 and photographed at weaning. An enduring reduction in cortical volume was obvious at the highest dose, 25 mg/kg, but was not apparent at lower doses. These defects are most apparent in the extensive exposure of the subcortical colliculi in the posterior cortex (arrow), suggesting that the overlying cortex is diminished in size.

dams on GD 16 or 17, a time when the superficial cortex is undergoing significant development. We then screened for enduring defects when these animals were in late adolescence (PDs 60–64) using two types of biomarkers. The first biomarker was evidence of persistent cortical maldevelopment (i.e., an index of deficient morphogenesis). The second biomarker was the capacity of MAM-exposed cortex to grow thicker in response to behavioral stimulation (i.e., an index of deficient plasticity). Because MAM was delivered *in utero*, it was possible to compare the relative sensitivities of these two biomarkers in littermates exposed to MAM under the same conditions and assigned randomly to the EC or IC condition. Statistical analysis revealed a significant main effect of MAM upon cortical thickness ($p < 0.0001$), a significant main effect of housing condition upon cortical thickness ($p < 0.0001$), and a significant main effect of GD of exposure ($p < 0.0001$). Significant interactions were detected between MAM dose and housing condition ($p < 0.05$) and MAM dose and GD of dose ($p < 0.01$) but not between housing condition and GD of dose ($p = 0.51$).

Disruption of cortical morphogenesis as a biomarker of prenatal MAM exposure. Upon weaning, representative brains from litters exposed at GD 16 were compared (Figure 1). Visual inspection of these brains confirmed that the volume of cerebral cortex was reduced dramatically by a dose of 25 mg/kg, as indicated by the exposed dorsal surface of both inferior and superior colliculi (Figure 1). Using these criteria, however, the brains of rats receiving MAM doses of 1, 5, and 10 mg/kg were not obviously different from those exposed to saline vehicle only (0 mg/kg). Brains of rats exposed to 25 mg/kg MAM on GD 17 also showed obvious reductions of cortical mass (data not shown). The observation of microcephaly at the 25 mg/kg dose served as a positive control for the action of MAM at lower doses where exposed brains showed no overt signs of cortical maldevelopment.

For a more fine-grained assessment of morphogenic defects, we measured the overall thickness of cerebral cortex (Figure 2A). We sampled thickness in the OC because this region is appropriate for assessing both defective morphogenesis (Figure 1) and defective experience-dependent plasticity. Rats (PDs 60–64) housed in the IC were used to assess toxicant effects on thickness of cortex (Figure 2B). A decrease in cortex was observed in animals exposed to 25 mg/kg MAM on GD 16 but was not detected in animals exposed during GD 17. Taken together with our observations of overall brain size (Figure 1), these data suggest that animals exposed to MAM on GD 17 may have less cortical volume, but the thickness of the cortex that survives appears to be relatively normal. Nonetheless, the sensitivity of cortical mass as a biomarker appears limited to relatively high dose effects, with a detection threshold in the present experiment similar to that of previous reports (Fischer et al. 1972; Haddad et al. 1972; Tamaru et al. 1988).

The capacity for robust plasticity emerges from healthy development. The usefulness of experience-dependent plasticity as a biomarker hinges on its reliable and robust induction. Figure 3 demonstrates the stimulatory effects of the EC condition (Figure 3A) compared with housing in the IC condition (Figure 3B) and shows that the thickness of the OC is significantly increased after 1 month of exposure to the EC condition. The mean thickness of cortex was increased by 18% ($p < 0.001$; Figure 3C).

Disruption of cortical plasticity as a biomarker of prenatal MAM exposure. To determine if exposure to MAM interfered with this growth response, we compared differences in mean cortical thickness between EC and IC at each dose of MAM (0, 1, 5, 10, and 25 mg/kg). Although significant differences were detected for both main effects of dose and housing condition with the GLM analysis of variance, the significant interaction between environment and MAM dose revealed that MAM during cortical development altered the

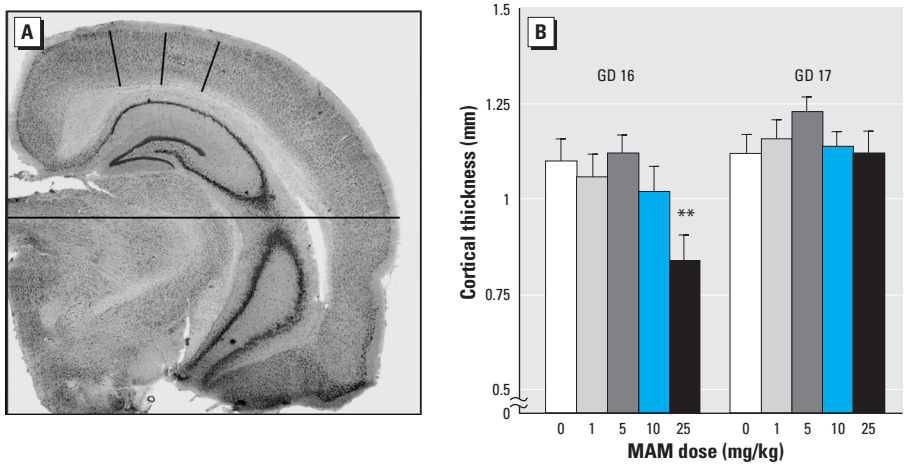

Figure 2. High-dose MAM reduces the baseline thickness of cortex. (*A*) Cortical thickness measured in a representative section of OC from a male rat (PD 60–64); the black lines superimposed on the cortex illustrate the triplicate thickness measures performed on the section. (*B*) Quantitative analysis of cortical measurements for male rats (PD 60–64) after exposure to a single dose of saline (0 mg/kg) or MAM (1, 5, 10, or 25 mg/kg) during GD 16 or GD 17 (see "Materials and Methods" for details). Data are reported as mean thickness for each dose ± SE.
**$p < 0.01$.

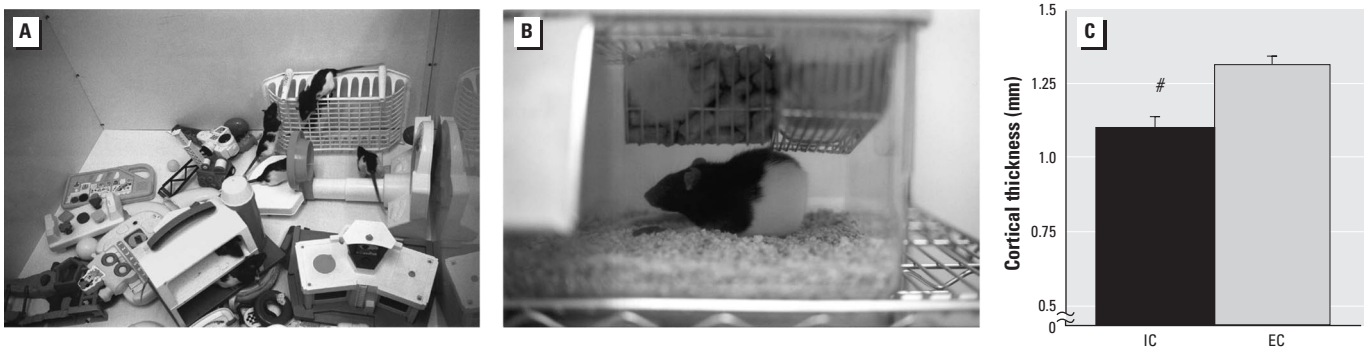

Figure 3. OC grows thicker in response to EC. (*A*) Rats, shown in the play cage, are housed as a group in the EC condition. (*B*) Their littermates reside individually in the IC condition. (*C*) After 28 days of housing in the EC, thickness measurements from the OC of control rats (saline injections) show an increased thickness compared with their IC siblings; data are reported as mean thickness ± SE.
#$p < 0.001$.

capacity for plasticity later in life (Figure 4A, GD 16; Figure 4B, GD 17). Rats exposed to the highest dose of MAM (25 mg/kg) failed to exhibit significant increases in cortical thickness in response to the EC condition. Rats exposed at GD 16, however, showed both a reduction in baseline thickness of cortex (indicating a morphogenic defect; Figure 2B) and a reduction in plasticity, whereas rats exposed at GD 17 showed only reduced plasticity.

A critical test of experience-dependent plasticity as a biomarker of subtle neurotoxic insult is the comparison between the effects of EC and IC conditions at low doses of MAM. At the two lowest doses (1 and 5 mg/kg of MAM), no basal morphogenic defect was detected (Figure 2B), but the effect of the EC condition was blunted or lost (Figure 4). At 1 mg/kg, the response to the EC condition differed with GD of exposure; at GD 16 the magnitude of response was reduced but still significant ($p < 0.05$; Figure 4A), whereas at GD 17 there was no significant effect of EC ($p = 0.23$; Figure 4B). At 5 mg/kg, neither the GD 16 nor the GD 17 rats exhibited a significant EC effect (GD 16, $p = 0.45$; GD 17, $p = 0.69$). Rats exposed to 10 mg/kg MAM also failed to show a significant effect of EC (GD 16, $p = 0.15$; GD 17, $p = 0.13$). The trend for EC rats to have numerically thicker cortices than their IC siblings across the 1–10 mg/kg MAM range suggests that, although reduced significantly, some capacity for growth may have been spared.

To determine if exposure to MAM reduced plasticity in a dose-dependent manner, we tested the hypothesis that the EC cortices would decrease in thickness with increased dose using Jonckheere's test (Jonckheere 1954). With this test, the null hypothesis was rejected both in rats exposed at GD 16 ($p < 0.001$) and at GD 17 ($p < 0.05$). Because these analyses examined only the differences between EC groups across doses, we also determined the correlation of the difference in EC/IC cortical thickness and dose (Figure 5). For rats treated with MAM on GD 16, this analysis produced a correlation coefficient of –0.87 (Figure 5A); for those exposed on GD 17, the correlation was –0.63 (Figure 5B). Together, these results indicate that as the dose of MAM increased, the magnitude of the EC response was diminished.

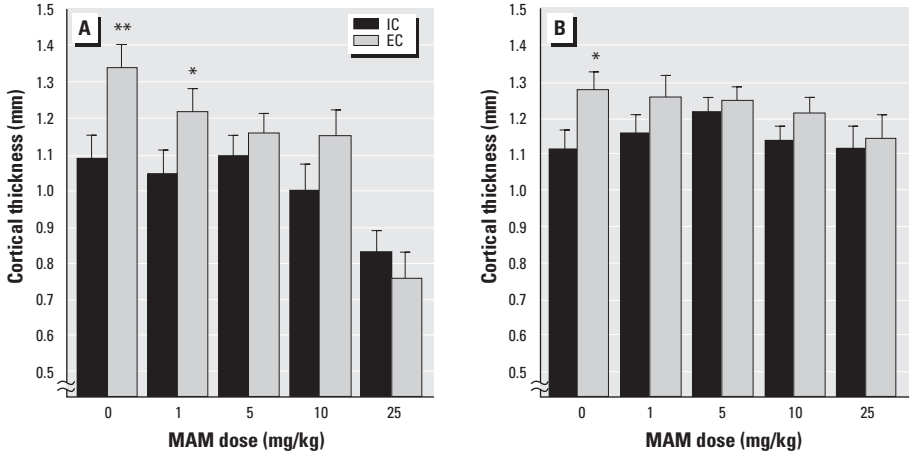

Figure 4. Prenatal MAM exposure impairs postweaning cortical plasticity. OC thickness was measured in rats exposed to either saline vehicle (0 mg/kg) or MAM (1, 5, 10, or 25 mg/kg) at GD 16 (*A*) or GD 17 (*B*) and housed postweaning in either the EC or the IC condition for 30 days. Plasticity failed at MAM doses below those required to reduce baseline cortical thickness (compare with Figure 2). Data are reported as mean thickness ± SE.
*$p < 0.05$. **$p < 0.01$.

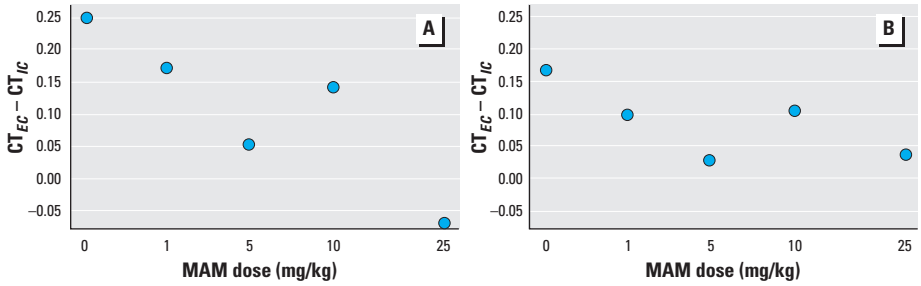

Figure 5. The capacity for plasticity is inversely correlated with dose of MAM; the difference between effects of the EC and IC conditions is plotted by dose for rats exposed to MAM on GD 16 ($r = –0.87$) (*A*) and on GD 17 ($r = –0.63$) (*B*). CT, cortical thickness. These plots show a consistent, systematic decline in plasticity, most notably across the low doses (0–5 mg/kg). They also show an unexpected, but consistent, break in trend at 10 mg/kg. Any exposure to MAM resulted in a diminished plasticity response compared with that of saline-treated control rats.

Discussion

The answer to the question of whether the brain develops normally in the presence of a toxicant may depend upon on how development is measured. One way is use a variety of sensitive neuropathological techniques to assess the integrity of neural circuits and associated metabolic support at the close of a key stage (Garman et al. 2001). Here, as an alternative way to define healthy development, we evaluate experience-dependent plasticity, a late component of brain development that emerges after basic circuitry has formed and remains active into maturity (Black and Greenough 1986). We hypothesized that plasticity would be a more sensitive biomarker because it appears to be an emergent integrative property of a properly assembled brain. The induction of plasticity depends on a rat's active exploration of its environment (Ferchmin et al. 1975), and its expression involves changes in the structure of neurons (Volkmar and Greenough 1972), glia (Jones et al. 1996; Sirevaag and Greenough 1991), and blood vessels (Black et al. 1987). The major finding of this study was that young adult rats could appear to have normal cortical thickness after prenatal exposure to low doses of MAM, yet exhibit significantly diminished experience-dependent neuroanatomical plasticity.

The threshold dose of MAM required to significantly reduce cortical thickness was clearly > 10 mg/kg, but doses as low as 1 mg/kg impaired plasticity. This initial assessment of plasticity as a biomarker demonstrates deficits at lower prenatal exposures of MAM than have been documented previously (Ohta et al. 2000). The potential of this model is in its ability to test the integrity of cellular plasticity directly under conditions relevant to learning and memory. Its strength lies in the ability to detect the expression of experience-dependent plasticity, as well as the degradation of plasticity by neurotoxic insult, through the use of within-litter comparisons. In the present study we did not sample a sufficient number of subjects per dose to fully document the absolute threshold for disruption of plasticity, nor was that its intent. Instead, the study was designed to test the relative sensitivity of this measure using a well-characterized neurotoxicant. Although the present data suggest that prenatal MAM dose and postweaning capacity for experience-dependent plasticity may be inversely related (Figure 5), confirmation of this trend will also require a larger data sample.

Based on this initial study with MAM, we would not predict that exposure to neurtoxicants at the low doses of greatest interest would necessarily eliminate plasticity. In fact, residual plasticity in the face of toxic insult

could play an important role in compensating for brain damage during development and therefore holds therapeutic promise (Jones et al. 1998). Remarkable levels of cognitive function have been reported in individuals with profound defects in brain morphogenesis (Lewin 1980), indicating that the compensatory capacity of human developmental plasticity can be profound under certain conditions. Accordingly, these data suggest that subtle deficits are likely to show up as quantitative differences in the magnitude of plasticity or in the rate at which it is expressed.

The generality of this model awaits assessment in future studies. The EC paradigm has proven quite reliable in inducing robust neuroanatomical plasticity in a variety of species (Rosenzweig and Bennett 1996) and appears to represent a fundamental mechanism of brain information storage relevant to humans (Bertenthal et al. 1984; Karni et al. 1995; Maguire et al. 2000). MAM is a compound that affects the development of cerebral cortex directly (Spencer et al. 2000). We would predict, however, that even if cortical cells are not damaged directly, cortical plasticity is likely to be vulnerable to disruptions in the integrity of the extrinsic afferent systems that trigger it or modulate its expression.

We observed relatively large increases in cortical thickness of untreated rats after 28 days of the EC condition [e.g., Diamond (1967)]. However, this analysis may not be sufficiently sensitive in all species or strains (e.g., Wainwright et al. 1993), and more refined assessments of plasticity based specifically on the growth response of cells may be required. In species in which both neuropathology can be modeled appropriately and experience-dependent plasticity induced robustly, however, the efficiency of a simple measure of plasticity such as cortical thickness has tremendous advantages in screening a large set of toxicants. A testable prediction is that this paradigm can be applied to a variety of neurotoxicants to reveal deficits at lower doses than can routine morphological examination. If so, this novel biomarker based on experience-dependent neuroanatomical plasticity could become an important tool in establishing limits for acceptable levels of toxicants present in the environment.

References

Balduini W, Elsner J, Lombardelli G, Peruzzi G, Cattabeni F. 1991. Treatment with methylazoxymethanol at different gestational days: two-way shuttle box avoidance and residential maze activity in rat offspring. Neurotoxicology 12:677–686.

Banker GA, Waxman AB. 1988. Hippocampal neurons generate natural shapes in cell culture. In: Intrinsic Determinants of Neuronal Form and Function (Lasek RJ, Black MM, eds). New York:Alan R. Liss, 61–82.

Bayer SA, Altman J. 1995. Neurogenesis and neuronal migration. In: The Rat Nervous System (Paxinos G, ed). San Diego, CA:Academic Press, 1041–1078.

Becker LE, Armstrong DL, Chang F, Wood MM. 1984. Dendritic development in human occipital cortical neurons. Dev Brain Res 13:117–124.

Berger-Sweeney J, Hohmann CF. 1997. Behavioral consequences of abnormal cortical development: insights into developmental disabilities. Behav Brain Res 86:121–142.

Berman RF, Hannigan JH, Sperry MA, Zajac CS. 1996. Prenatal alcohol exposure and the effects of environmental enrichment on hippocampal dendritic spine density. Alcohol 13:209–216.

Bertenthal B, Campos J, Barret K. 1984. Self-produced locomotion: an organizer of emotional, cognitive, and social development in infancy. In: Continuities and Discontinuities in Development (Embde R, Harmon R, eds). New York:Plenum, 175–210.

Black JE, Greenough WT. 1986. Induction of pattern in neural structure by experience: implications for cognitive development. In: Advances in Developmental Psychology, Vol 4 (Lamb ME, Brown AL, Rogoff B, eds). Hillsdale, NJ:Lawrence Erlbaum Associates, 1–50.

Black JE, Jones TA, Nelson CA, Greenough WT. 1998. Neuronal plasticity and the developing brain. In: Handbook of Child and Adolescent Psychiatry (Alessi N, Coyle JT, Harrison SI, Eth S, eds). New York:John Wiley & Sons, 31–53.

Black JE, Sirevaag AM, Greenough WT. 1987. Complex experience promotes capillary formation in young visual cortex. Neurosci Letters 83:351–355.

Colacitti C, Sancini G, DeBiasi S, Franceschetti S, Caputi A, Frassoni C, et al. 1999. Prenatal methylazoxymethanol treatment in rats produces brain abnormalities with morphological similarities to human developmental brain dysgeneses. J Neuropathol Exp Neurol 58:92–106.

Diamond MC. 1967. Extensive cortical depth measurements and neuron size increases in the cortex of environmentally enriched rats. J Comp Neurol 131:357–364.

Diamond MC, Krech D, Rosenzweig MR. 1964. The effects of an enriched environment on the histology of the rat cerebral cortex. J Comp Neurol 123:111–120.

Esclaire F, Kisby G, Spencer P, Milne J, Lesort M, Hugon J. 1999. The Guam cycad toxin methylazoxymethanol damages neuronal DNA and modulates tau mRNA expression and excitotoxicity. Exp Neurol 155:11–21.

Ferchmin PA, Bennett EL, Rosenzweig MR. 1975. Direct contact with enriched environments is required to alter cerebral weights in rats. J Comp Physiol Psychol 88:360–367.

Fischer MH, Welker C, Waisman HA. 1972. Generalized growth retardation in rats induced by prenatal exposure to methylazoxymethyl acetate. Teratology 5:223–232.

Garman RH, Fix AS, Jortner BS, Jensen KF, Hardisty JF, Claudio L, et al. 2001. Methods to identify and characterize developmental neurotoxicology for human health risk assessment. II: Neuropathology. Environ Health Perspect 109(suppl 1):93–100.

Greenough WT. 1976. Enduring brain effects of differential experience and training. In: Neural Mechanisms of Learning and Memory (Rosenzweig MR, Bennett EL, eds). Cambridge, MA:MIT Press, 255–278.

Haddad RK, Rabe A, Dumas R. 1972. Comparison of effects of methylazoxymethanol acetate on brain development in different species. Fed Proc 31:1520–1523.

Hebb DO. 1947. The effects of early experience on problem-solving at maturity. Am Psychol 2:306–307.

Horner CH, O'Regan M, Arbuthnott E. 1991. Neural plasticity of the hippocampal (CA1) pyramidal cell–quantitative changes in spine density following handling and injection for drug testing. J Anat 174:229–238.

Jensen KF, Catalano SM. 1998. Brain morphogenesis and developmental neurotoxicology. In: Handbook of Developmental Neurotoxicology (Slikker WD, Chang LW, eds). San Diego, CA:Academic Press, 3–41.

Jonckheere A. 1954. A distribution-free k-sample test against ordered alternatives. Biometrika 41:34–56.

Jones TA, Hawrylak N, Greenough WT. 1996. Rapid laminar-dependent changes in GFAP immunoreactive astrocytes in the visual cortex of rats reared in a complex environment. Psychoneuroendocrinology 21:189–201.

Jones TA, Hawrylak N, Klintsova AY, Greenough WT. 1998. Brain damage, behavior, rehabilitation, recovery and brain plasticity. Ment Retard Dev Disabil Res Rev 4:231–237.

Juraska JM. 1982. The development of pyramidal neurons after eye opening in the visual cortex of hooded rats: a quantitative study. J Comp Neurol 212:208–213.

Juraska JM, Greenough WT, Elliott C, Mack KJ, Berkowitz R. 1980. Plasticity in the adult rat visual cortex: an examination of several cell populations after differential rearing. Behav Neural Biol 29:157–167.

Karni A, Meyer G, Jezzard P, Adams MM, Turner R, Ungerleider LG. 1995. Functional MRI evidence for adult motor cortex plasticity during motor skill learning. Nature 377:155–158.

Kempermann G, Kuhn H G, Gage F H. 1997. More hippocampal neurons in adult mice living in an enriched environment. Nature 386:493–495.

Kisby GE, Kabel H, Hugon J, Spencer PS. 1999. Damage and repair of nerve cell DNA in toxic stress. Drug Metabol Rev 31:589–618.

Klintsova AY, Goodlett CR, Greenough WT. 1999. Therapeutic motor training ameliorates cerebellar effects of postnatal binge alcohol. Neurotoxicol Teratol 22:125–132.

Kolb B, Whishaw IQ. 1998. Brain plasticity and behavior. Annu Rev Psychol 49:43–64.

Lewin R. 1980. Is your brain really necessary? Science 210:1232–1234.

Maguire EA, Gadian DG, Johnsrude IS, Good CD, Ashburner J, Frackowiak FSJ, et al. 2000. Navigation-related structural change in the hippocampi of taxi drivers. Proc Natl Acad Sci USA 97:4398–4403.

Netter J, Wasserman W, Kutner MH. 1989. Applied Linear Regression Models: Regression, Analysis of Variance, and Experimental Designs. Holmwood, IL:Irwin.

Office of Laboratory Animal Welfare. 2002. Public Health Service Policy on Humane Care and Use of Laboratory Animals. Available: http://grants1.nih.gov/grants/olaw/references/phspol.htm [accessed 18 June 2003].

Ohta R, Matsumoto A, Sato M, Shirota M, Nagao T, Tohei A, et al. 2000. Postnatal behavior in Hatano high- and low-avoidance rats following prenatal exposure to low-dose methylazoxymethanol. Neurotoxicol Teratol 22:405–413.

Pomeroy SL, Kim JYH. 2000. Biology and pathobiology of neuronal development. Ment Retard Dev Disabil Res Rev 6:41–46.

Rema V, Ebner FF. 1999. Effect of enriched environment rearing on impairments in cortical excitability and plasticity after prenatal alcohol exposure. J Neurosci 19:10993–11006.

Rodier PM. 1995. Developing brain as a target of toxicity. Environ Health Perspect 103(suppl 6):73–76.

Rosenzweig MR, Bennett EL. 1996. Psychobiology of plasticity: effects of training and experience on brain and behavior. Behav Brain Res 78:57–65.

Rosenzweig MR, Bennett EL, Diamond MC. 1972. Chemical and anatomical plasticity of brain: replications and extensions. In: Macromolecules and Behavior (Gaito J, ed). 2nd ed. New York:Appleton-Century-Crofts, 205–277.

Sirevaag AM, Greenough WT. 1991. Plasticity of GFAP-immunoreactive astrocyte size and number in visual cortex of rats reared in complex environments. Brain Res 540:273–278.

Spencer P, Kisby G, Palmer V, Obendorf P. 2000. Cycasin, methylazoxymethanol and related compounds. In: Experimental and Clinical Neurotoxicology (Spencer P, Schaumburg H, Ludolph A, eds). New York:Oxford University Press, 436–447.

Spencer PS. 2000. Biological principles of chemical neurotoxicity. In: Experimental and Clinical Neurotoxicology (Spencer P, Schaumburg H, Ludolph A, eds). New York:Oxford University Press, 3–54.

Tamaru M, Hirata Y, Nagayoshi M, Matsutani T. 1988. Brain changes in rats induced by prenatal injection of methylazoxymethanol. Teratology 37:149–157.

Turner AM, Greenough WT. 1985. Differential rearing effects on rat visual cortex synapses. I. Synaptic and neuronal density and synapses per neuron. Brain Res 329:195–203.

van Praag H, Kempermann G, Gage FH. 2000. Neural consequences of environmental enrichment. Nature Rev Neurosci 1:191–198.

Volkmar F, Greenough WT. 1972. Rearing complexity affects branching of dendrites in the visual cortex of the rat. Science 176:1445–1447.

Volpe JJ. 2000. Overview: normal and abnormal human brain development. Ment Retard Dev Disabil Res Rev 6:1–5.

Wainwright P, Levesque L, Kermpulec L, Bulman-Fleming B, McCutcheon D. 1993. Effects of environmental enrichment on cortical depth and Morris-maze performance in B6D2F_2 mice exposed prenatally to ethanol. Neurotoxicol Teratol 15:11–20.

Wallace CS, Kilman VL, Withers GS, Greenough WT. 1992. Increases in dendritic length following a brief period of differential housing in weaning rats. Behav Neural Biol 58:64–68.

Walsh RN, Greenough WT, eds. 1976. Environments as Therapy for Brain Dysfunction. New York:Plenum.

Zilles K. 1985. The Cortex of the Rat. Berlin:Springer-Verlag.