

Brief communication

Volumetric Neuroimaging and Low-Dose Early-Life Exposures: Loose Coupling of Pathogenesis-Brain-Behavior Links

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Abstract

The interface of developmental neuroimaging with developmental neurotoxicology can, broadly speaking, address two complementary concerns. The first is to study the impact of specific exposures on brain development. The second is to study known neurobehavioral disorders with an eye to discerning toxicological contributions to pathogenesis. Pathogenesis targets brain based upon physical properties (receptors, growth factors, etc.) while behavior is modulated by regional and neural systems alterations. The distribution of pathogenesis-brain relationships overlaps only partially with that of brain-behavior relationships. The goal of this paper is to highlight methodological issues involved in designing and interpreting volumetric neuroimaging studies in the light of this loose coupling.

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NEUROIMAGING: LESION STUDIES AND VOLUMETRICS

Perhaps the bulk of the neuroimaging literature related to toxicological exposures has reported on acute effects and on lesions attributable to cytotoxic impact of neurotoxins. However, it is becoming apparent that exposures at doses lower than those necessary to cause cell death or visible focal lesions may still have significant effects on central nervous system development (Markowski et al., 2001). When such exposures are experienced early in life, the consequences may be alterations in brain development (Table 1). The imaging analysis methods required to discern altered brain development include some that

are qualitatively different from those typically necessary to identify more gross neuropathology in brain scans. These different methods derive from those used for the quantitative volumetric assessment of brain differences in neurodevelopmental and neuropsychiatric disorders (Caviness et al., 1996; Kennedy et al., 2002).

Volumetric neuroimaging is a means of identifying differences between brains that may not be visible to the unaided eye. For most individuals with neurodevelopmental and neuropsychiatric disorders, there are no focal lesions, and yet brain function is clearly abnormal. The presumption has been that microscopic alterations of tissue properties in these brains account for the functional differences. Volumetrics has been used as a tool for locating the sites at which these microscopic alterations have macroscopically detectable impacts.

Volumetrics has been described as a developmental brain science in its own right because it addresses the

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Table 1
Neuroimaging findings in high- and low-dose developmental neurotoxicity

Higher-dose neurotoxicity	Low-dose neurotoxicity
Cytotoxic injury	Dysregulation of development
Grossly visible abnormalities	Detection requires quantitative measures
Volume loss, often focal	Scaling changes

regularities of volumetric relationships at the macroscopic level (Caviness et al., 1999). From this vantage point, volume is an evolutionarily and developmentally regulated fundamental property of tissue. Macroscopic volume measures are conditioned by properties at the cellular level that include cell size, shape, number, density of cells within the tissue, and density of cellular components such as processes. Alterations at these microscopic levels may lead to volumetric changes that are striking or subtle.

Volumetric characteristics of the brain presumably reflect a complex and interacting set of constraints (Changizi, 2001) that have evolved over time in relation to systems optimization (Chklovskii et al., 2002). These constraints are quite tight, so that subtle volumetric alterations may reflect changes with significant functional consequences.

It is just such subtle volume changes that may eventuate from subtle and low-dose neurotoxin exposures. At these low levels, the consequences of exposure may not be cell death but rather alterations in gene expression and concentration or function of neurotrophic chemicals including brain morphogenic factors, such as growth factors, neurotransmitters, and receptors. The distribution of volume changes may indicate selective vulnerability traceable to the timing and mechanisms of histogenetic events or injury (Clancy et al., 2000, 2001).

PATHOGENESIS VERSUS BEHAVIOR CORRELATES

On the other hand, when volumetric methods have been applied in such neurobehavioral disorders, the

questions posed have for the most part not been oriented toward illuminating mechanisms of pathogenesis, whether related to neurotoxicology or not. Instead, the focus has been on finding the neuroanatomical correlates of neurobehavioral abnormalities. This endeavor has been informed to a major extent by a model of localization or modularity, leading to the expectation that specific behavioral abnormalities will correlate with equally specific neuroanatomical disturbances. The resultant research program has attempted to establish brain-behavior correlations (Table 2).

The lack of focus on pathogenic mechanisms has largely been a function of the assumption that the etiology of neurobehavioral disorders is to a substantial or overwhelming degree genetic. This assumption leads implicitly to the expectation that pathogenic mechanisms will be discovered through identification of genes strongly associated with a particular disorder. The possibility that there might be some contingent component to pathogenic mechanisms, something other than the unfolding of a genetic program, has slowly been emerging in studies of neurobehavioral disorders, implying that these disorders emerge not simply from genetic factors but from a complex interplay between genetic vulnerabilities and risk factors (Hyman, 2000). An analogous argument has been made regarding neuroanatomy: the disordered brain development should not be expected to produce anatomical abnormalities that are modular, since the factors perturbing development are likely to have more widely distributed impacts (Johnson et al., 2002). But to date this understanding has not systematically penetrated the methods that dominate neurobehavioral or neuropsychiatric volumetric neuroimaging analysis.

INCONSISTENT RESULTS: METHODOLOGICAL OR MATERIAL BASIS?

Unfortunately, the research agenda of brain-behavior correlation has produced strikingly inconsistent results. A clear identification of modules responsible for aberrant neurobehavioral functions has not emerged (Rumsey and Ernst, 2000). The inconsisten-

Table 2
Questions differ depending on the purpose of the investigation

To interrogate volumetric findings for clues to mechanism, ask about:	To interrogate volumetric findings for clues to behavioral underpinnings, ask about:
Regional differences in tissue vulnerability	Specific regional abnormalities
Timing of injury or perturbation	Behaviorally-related neural systems
Inter-regional mutual developmental influences	

cies in findings are frequently attributed to methodological differences between studies. From this perspective, such methodological differences appear to be the only obstacle to finding the true neuroanatomical configurations underlying these disorders. Were methods of imaging analysis, subject characterization, age selection and so forth to be made consistent, the brain correlates of atypical behaviors would be clearly discernable.

There are several alternative possibilities. One is that there is no single brain volumetric profile that correlates with any of the common neurobehavioral disorders. From this vantage point, the observable abnormal behaviors are a final common pathway eventuating from multiple different underlying brain abnormalities. If this is the case, then the task becomes not to find which specific brain abnormalities account for which particular behaviors, but rather to consider how cognitive neuroscience can illuminate the neural system vulnerabilities allowing multiple different neuroanatomic perturbations to lead to the same cluster of atypical behaviors (Herbert, 2004).

This “final common pathway” formulation may be applicable to genetically based disorders. For instance, there are multiple diseases associated with an unusually high incidence of autistic behaviors, such as tuberous sclerosis, in utero rubella infection and fragile X, and there is no evidence at this time for a common underlying biological defect in these diverse disorders. A “final common pathway” formulation may be particularly apt for disorders where neurotoxicity plays a role in pathogenesis during development. The profile of brain alteration associated with developmental neurotoxin exposure may potentially vary as a function of timing of exposure, dose, particular genetic vulnerabilities, health and nutritional status of the child or fetus and mother, other concomitant exposures, and other contextual features. This variability may contribute to a heterogeneity that could skew group means sufficiently to lead to the inconsistencies that have plagued modularly oriented neurobehavioral imaging volu-

metrics. Insofar as this is the case, such variability has a material basis, and is not merely a function of methodology (Table 3).

NON-MODULAR BRAIN ABNORMALITIES

Another possibility, which is by no means mutually exclusive with the first alternative and may be complementary, is that the brain abnormalities underlying the atypical behaviors in these disorders may not be modular (Johnson et al., 2002). Indeed, if the idea that localized regions or neural systems must underlie specific abnormal behaviors is regarded as a hypothesis rather than simply assumed, it is fair to say that in brain-behavior correlation studies in neurobehavioral disorders, this hypothesis has been poorly validated. In fact in autism, the most strongly replicated volumetric finding—larger than normal brains—is not modular but widespread. This widely distributed abnormality raises the question of whether its significance can be reduced to its impact on specific neural systems with which it overlaps, or whether it has a functional impact not via module or specific circuit disruption but via quite different mechanisms—such as network or connectivity disturbances (Just et al., 2004).

Gene expression patterns in development are not strictly modular but involve multiple overlapping morphological gradients. While in certain instances there may be strict boundaries between regions with their associated genes, there may also or instead be an interweaving of multiple influences, with mapping being combinatorial rather than discrete (Kingsbury and Finlay, 2001). Moreover, there is plasticity in later areal specification (Grove and Fukuchi-Shimogori, 2003). Because of this, altered gene expression is more likely to have a broadly regional than narrowly modular distribution. This applies whether the alterations are a consequence of endogenous or exogenous influences, the latter including neurotoxins.

Table 3

Differing expectations, assumptions, and inferences regarding volume change depending on models motivating research

Modular, later-acquired toxicity or genetic determinist model	Developmental low-dose neurotoxicity and/or epigenetic model
Pathogenesis maps directly to behavior	Intervening levels between pathogenesis and behavior
Pathogenic factors directly impact region of interest	Epigenesis and feedforward mechanisms modulate anatomy
Region of interest studies suffice	Volumetric profiles are necessary to detect scaling changes
Focal abnormalities	Widespread abnormalities
Modular differences explains the behavior	Network perturbations contribute to the behavior

PATHOGENESIS AND FUNCTION: NON-IDENTICAL DISTRIBUTION AND LOOSE COUPLING

One particularly important implication of these biological considerations is that as a rule we should not presume a one-to-one mapping of factors influencing regional morphogenesis onto specific changes in neural modules or circuits. Because of this, there is no reason to assume that the identification of abnormalities in specific regions or neural systems can be applied directly to inferences about pathogenesis. Moreover, if pathogenic mechanisms have their impact in a distribution different than that underlying neural systems functions, then there is no reason to assume that the identification of abnormalities in specific functional systems comprehensively delineates the brain abnormalities in a neurodevelopmental disorder.

In essence, then, what we have is a loose coupling between mechanisms of pathogenesis on the one hand and neural systems-behavior relationships on the other (Fig. 1). The coupling can only be loose due to the multiple levels of integration that intervene between the level of mechanisms at which low-dose neurotoxins operate in development and the level of mechanisms of neural systems underlying behavior. Low-dose neurotoxins in fetal and infant life modulate factors regulating cell structure, chemistry, and organization. These microscopic and local physical changes alter features of neural systems organization in ways that are not entirely predictable from characterizing changes at the microscopic and local levels. This is because the impact of these changes reverberates through interconnected parts of neural systems in a fashion that generates feedback loops with inputs that are more than local. Behaviors are in turn influenced by neural systems that bear the influence of these higher-order, non-local feedback loops. There is thus no clear, straight and unambiguous pathway between altered brain development and behavior.

RE-THINKING HYPOTHESIS FORMULATION: INTERROGATING VOLUMETRIC DATA REGARDING PATHOGENESIS

This loose coupling between brain development and behavior, once recognized, ought to inform the way hypotheses are formulated in neuroimaging studies that pursue neurotoxicological questions. Neurotoxicological inquiries are forced to problematize pathogenic mechanisms in a way that cognitive neuroscience inquiries into the brain basis of behavior are not, particularly if the latter tacitly sidestep issues of pathogenesis by assuming that these will eventually be exhaustively explicated by genetic mechanisms. It therefore becomes pertinent to consider how to interrogate volumetric data to yield insights into pathogenesis.

A central set of considerations relates to the probable non-modular distribution of effects of low-dose neurotoxin exposure. This non-modular distribution is also likely to be non-uniform, affecting one region differently from another. In order to detect such phenomena it is thus useful to have measures of the volumes of multiple regions as well as measures of total brain volume. With this type of volumetric profile, it becomes possible to assess not only the volumes of individual regions of interest, but also ratios of volumes to one another as well as regional proportions within total brain volume. These relational assessments permit the detection of non-uniform but widely distributed patterns of volume change that may distinguishes the study group from the control group brains (Table 3).

CASE STUDY: VOLUMETRICS OF AUTISM AND DEVELOPMENTAL LANGUAGE DISORDER

We applied this type of inquiry to a study of segmented gray and white matter regional volumes

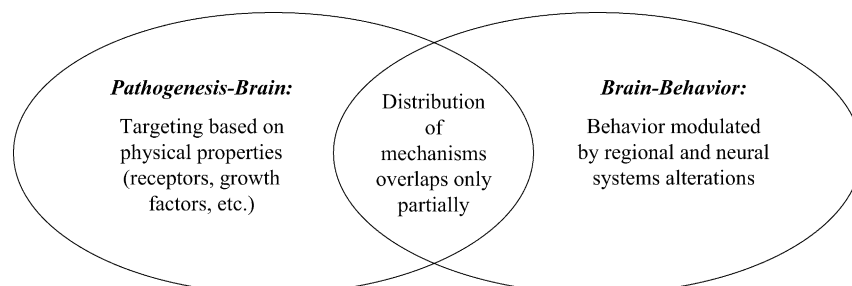


Fig. 1. Loose coupling of pathogenesis-brain with brain-behavior correlations.

in a group of high-functioning autistic children (Herbert et al., 2003a) and a group of children with developmental language disorder (DLD) (Herbert et al., 2003b) as compared with controls. These two disorders are of potential relevance regarding neurotoxicity, since there are reports of increased learning disability incidence, as well as reports of autism prevalence as much as 10 times higher than 15 or more years ago. While some attribute this increased incidence to increased awareness (Fombonne, 2003), it is (at least) equally likely that increased awareness is fueled by increased incidence (Blaxill, 2004). If any of the increase at all is real, then the question of environmental influence needs to be addressed if we are to identify the causes of harm and prevent it from continuing.

For autism, in addition to the above-mentioned frequently replicated finding of increased total brain volume, there are several reports suggesting that brain volume increase occurs after birth, during the first 2 years of life, which suggests, though does not in itself prove, a possible role for environmental factors (Lainhart et al., 1997; Courchesne et al., 2003). Recently, some neuropathology researchers have reported that their new findings do not consistently replicate those they had reported earlier (Kemper and Bauman, 1998) regarding cerebellar Purkinje cell loss (Kemper et al., 2004) or brainstem abnormalities (Thevarkunnel et al., 2004); whereas the earlier findings had been interpreted to suggest that abnormalities occurred in mid-gestation, current findings seem to suggest late gestational or early postnatal processes. Findings of neuroinflammation and microglial activation in the autistic brain (Vargas et al., 2004) also suggest postnatal and even ongoing pathology. It has also been pointed out that certain brain regions and cell types may have particular vulnerability to postnatal environmental insults (Kern, 2003).

Our own results replicated the finding of larger total brain volume in autism and also identified an overall volume increase in DLD, albeit to a lesser degree. In the left three bars in Fig. 2, the mean proportional volumes of the three groups are depicted. One can see a slight increase in the proportion of white matter in autism and DLD as compared with the controls. In the right three bars, on the other hand, one sees a depiction of the regional contributions to brain volume increase in autism and DLD as compared with controls, as well as in autism as compared with DLD. Here we see that white matter accounts for 66% of brain volume increase in autism as compared with controls, and 88% of the volume increase in DLD as compared with

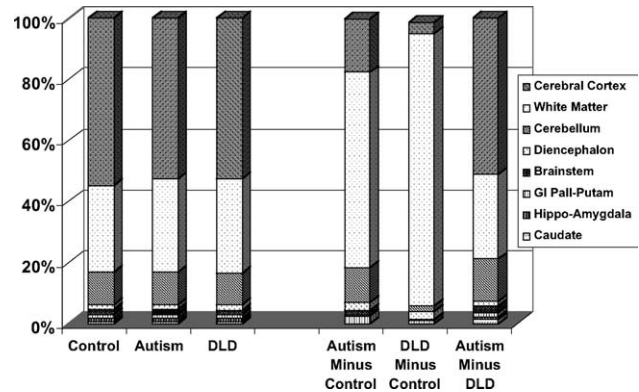


Fig. 2. Proportions of total volume and of volume differences. The three bars on the left depict the contribution of each major brain area to total brain volume. The three bars on the right depict the contribution of each major region to the extra volume in the larger as compared to the smaller group. White matter contributes disproportionately to volume increase of autism and DLD brains over controls, while it contributes the same amount to the volume increase of autism over controls as it does to total brain volume. This indicates a uniform volume enlargement of autism in relation to DLD, but a non-uniform enlargement of both autism and DLD in relation to controls.

controls; in both of these groups there is therefore a non-uniformity of regional volume changes from the typical pattern. On the other hand, white matter contributes only 28% of the volume increase in autism as compared with DLD, a proportion that is nearly identical to the proportion that white matter contributes to total brain volume. The regional volume increases in autism compared to DLD are thus uniform.

SUBTLE AND WIDELY DISTRIBUTED VOLUMETRIC CHANGES

There are several observations and comments to be made about these results. The first is that the total brain volume and regional brain volume changes are not large. Subtle differences of this kind are not detectable to the unaided eye: quantitative measurements are necessary to detect them. Indeed, one of the requirements for inclusion in this study was that the brains needed to be judged by a clinical neuroradiologist as normal. Within the range of “normal”, the volumetric constraints are quite tight. In addition, insofar as the total brain and white matter volume increase may occur postnatally (as mentioned above), constraints are even greater, since the basic anatomical architecture would already have been established before the onset of these developmental scaling alterations (Fig. 3).

A further observation is that total white matter volume does not clearly pertain to any specific neural system. In fact, it is often not measured, both because not all laboratories have the technical capability to



Fig. 3. Division of white matter into outer (subcortical) zone, radiate white matter (R), and deep zone (D), consisting of sagittal and bridging zones. White matter enlargement in autism and developmental language disorder was confined to the radiate zone, which myelinates later.

make the measurement and because white matter is often implicitly considered as little more than a “space between” the “important” gray matter structures, the nodes of initiation of signaling within neural systems. Yet to find that it contributes disproportionately to volume increases raises questions about what kinds of pathogenic processes might cause such an increase. To further clarify the nature of this enlargement, we performed a topographical parcellation of the white matter (Meyer et al., 1999; Makris et al., 1999) and found that the volume increase was localized to the radiate white matter, the subcortical zone of white matter consisting predominantly of corona radiata and short cortico-cortical connections (Fig. 2) (Herbert et al., 2004). Two observations can be made about this second finding. The first is that once again this regional bias of white matter enlargement (which affected all four lobes in the autism sample (though with strongest increases in frontal and especially prefrontal white matter) and all lobes but the parietal (again with greatest increase in prefrontal, though not frontal, white matter) in the DLD sample) does not correspond to any specific neural system, and so would have been missed in any inquiry where measurements were entirely limited to tests of neural systems-based hypotheses. The second is that the radiate and prefrontal white matter myelinate last, so that this finding gives a strong hint that the pathogenic mechanism underlying this volume change is occurring in the late

stages of myelination and is affecting these late-myelinating regions in some fashion.

Yet another observation in these same brains is that there are widely distributed differences in regional cortical asymmetries compared to controls in both groups (Herbert et al., 2005). The asymmetries are, for the most part, the same in both autism and DLD, even though they differ strongly from controls. Most investigations of asymmetry, particularly in DLD, have focused exclusively on language areas due to the language-related phenotype, and would therefore miss such widespread changes that, since they are so similar in both groups, are likely to be the result of systematic rather than random perturbations.

VOLUMETRICS AND MULTIDISCIPLINARY INQUIRY

At this point volumetrics needs to join forces with other levels of inquiry to make further sense of these findings. Other imaging methodologies, as well as neuropathology studies, can be recruited to characterize the tissue basis of the changes underlying these volume increases. In addition, candidate environmental agents, as well as candidate genetic vulnerabilities, can be explored in animal models for their potential to yield a similar pattern of volume alteration. Regarding mechanism, it is conceivable that these agents and vulnerabilities may have their primary impact on another site than white matter, with the volume changes we describe here being downstream of these other changes. Whatever the primary site (or sites) of primary impact, all affected sites may potentially have functional impact. Overall, these potentially pathologically relevant but non-modular, widespread findings were only made possible by a suspension of testing a priori hypotheses about behavior-neural systems correlation, and a careful examination of brain scaling and proportion with an eye toward developmentally relevant volumetric profile alterations. Even so, as it turns out, these findings may indeed have functional relevance. It should be noted that at the levels both of total white matter volume increase and regional bias of this volume increase, as well as of cortical asymmetries, the autism and DLD samples are quite similar. This suggests that these disorders may be related, an inference that can be made on other grounds as well, including genetic findings (Kjelgaard and Tager-Flusberg, 2001). There are similarities between these two disorders from a cognitive neuroscience vantage point as well. Beneath the behavioral profiles

of autism (which involves impaired language, socialization, and behavior impairments) and DLD (which involves speech and language impairments in the absence of physical or social reasons), it is argued that there are core-processing endophenotypes. These underlying processing abnormalities have been variously characterized as weak central coherence (Shah and Frith, 1993), impaired complex processing (Minshew et al., 1997), or underconnectivity (Just et al., 2004) in autism; and impaired rapid processing (Benasich and Tallal, 2002; Kail, 1994) in DLD. Such abnormal processing is described as widespread and as giving rise to deficits in domains most dependent on the types of processing that are impaired, linking them to the specific behaviors discerned at the behavioral level of integration. It should be noted that such widespread processing abnormalities are potentially quite consistent with widespread white matter volume increases. Moreover, and also consistent with this, the cortical asymmetry differences from controls in these brains are most pronounced in higher-order association cortex (Herbert et al., 2005).

SUMMARY AND CONCLUSION

In summary, while it has been possible to identify abnormalities in brain regions associated with functions noted by neuropsychologists to be perturbed in these disorders, these findings are inconsistent; and perhaps more important, the anatomical differences from controls are by no means discretely restricted to these regions. Correlation between anatomy and underlying endophenotype may possibly be better, but such correlation still does not address the question of what accounts for pathogenesis. Regarding neural systems variously identified as having volume or even functional differences from controls, the question remains open whether these regions are primary targets of pathology or whether they are just caught in the crossfire. If the latter, their abnormalities may not be unique or even central in causing the behavioral abnormalities. The more broadly distributed abnormalities may act by disruption of networks or other non-modular mechanisms. A shift of focus to anatomical findings that may suggest pathogenic mechanisms—whether genetic, environmental or both—has the advantage of allowing a fresh look at the changes underlying neurodevelopmental disorders and may yield many more insights as to what goes wrong in development to lead to these disorders. With these insights in hand, it may well also be the case, as our

own findings suggest, that fresh light can be shed on the underpinnings of the functional abnormalities as well. But this cascade of insights will only become accessible once the attachment to a priori brain-behavior correlation hypotheses is relaxed and a neurodevelopmental approach is adopted in its stead.

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