

Imaging normal and abnormal brain development: new perspectives for child psychiatry

Judith L. Rapoport, F. Xavier Castellanos, Nitin Gogate, Kristin Janson, Shawn Kohler, Phillip Nelson

Objective: The availability of non-invasive brain imaging permits the study of normal and abnormal brain development in childhood and adolescence. This paper summarizes current knowledge of brain abnormalities of two conditions, attention deficit hyperactivity disorder (ADHD) and childhood onset schizophrenia (COS), and illustrates how such findings are bringing clinical and preclinical perspectives closer together.

Method: A selected review is presented of the pattern and temporal characteristics of anatomic brain magnetic resonance imaging (MRI) studies in ADHD and COS. These results are discussed in terms of candidate mechanisms suggested by studies in developmental neuroscience.

Results: There are consistent, diagnostically specific patterns of brain abnormality for ADHD and COS. Attention deficit hyperactivity disorder is characterized by a slightly smaller (4%) total brain volume (both white and grey matter), less-consistent abnormalities of the basal ganglia and a striking (15%) decrease in posterior inferior cerebellar vermal volume. These changes do not progress with age. In contrast, patients with COS have smaller brain volume due to a 10% decrease in cortical grey volume. Moreover, in COS there is a progressive loss of regional grey volume particularly in frontal and temporal regions during adolescence.

Conclusions: In ADHD, the developmental pattern suggests an early non-progressive 'lesion' involving neurotrophic factors controlling overall brain growth and selected dopamine circuits. In contrast, in COS, which shows progressive grey matter loss, various candidate processes influencing later synaptic and dendritic pruning are suggested by human post-mortem and developmental animal studies.

Key words: ADHD, brain development, childhood schizophrenia.

Australian and New Zealand Journal of Psychiatry 2001; 35:272–281

It has long been an assumption that serious and chronic childhood psychiatric disorders reflect, at least in part, relatively subtle abnormalities of brain development.

Judith L. Rapoport, Chief (Correspondence); F. Xavier Castellanos, Medical Officer; Nitin Gogate, Clinical Fellow; Kristin Janson, IRTA Fellow

Child Psychiatry Branch, National Institute of Mental Health, Building 10, Room 3N202, 10 Center Drive MSC 1600, Bethesda, Maryland 20892-1600, USA. Email: rapoport@helix.nih.gov

Shawn Kohler, Post-Baccalaureate IRTA Fellow; Phillip Nelson, Head

Section on Neurobiology, Laboratory of Developmental Neurobiology Branch, National Institute of Child Health and Human Development, Bethesda, Maryland

Received 17 January 2001; accepted 1 February 2001.

Strong indirect support for this has been provided by the association of childhood psychiatric disorders with numerous neurological and neurodevelopmental disorders [1] and from clinical neuropsychological studies over many decades, indicating abnormal brain function in child psychiatric populations [2]. Because of the limitations of clinical investigation to validate subtle brain abnormalities, child psychiatric research has explored new methods for studying the brain. Brain imaging is an innovative technology that to date has best furthered the goal of understanding normal and psychiatrically abnormal brain structure and function. With the advent of non-invasive brain magnetic resonance imaging (MRI)

methodology, imaging data can now be acquired for paediatric populations. Moreover, these data are converging with new information on the organization and function of circuits in the developing brain, and on the molecular mediators of these changes. It is hoped that imaging studies in child psychiatric populations, will not only define the brain systems underlying illness, but also suggest candidate molecules for genetic studies. Based on the nature, location and temporal pattern of these abnormalities, and preclinical findings, we will be able to make more specific and testable hypotheses about the aetiology of these disorders. The quantitative study of brain development during childhood and adolescence with MRI began in the late 1980s (e.g. [3]). Subsequent cross-sectional [4–7] and mixed longitudinal/cross-sectional studies (e.g. [8]) have confirmed that although total brain volume changes between ages 5 and 18 are negligible, there are robust and complex changes in white and grey matter. White matter volume increases linearly during this age range, reflecting increasing myelination [4,9], while grey matter volume increases until early to mid-adolescence before decreasing during late adolescence [8], presumably from synaptic pruning and reduction of neuropil. A special feature of these normative data is that they were acquired in parallel with prospective clinical studies, so that brain development for psychiatrically abnormal populations can be compared. Elegant studies of known chromosomal abnormalities, such as Down syndrome and Rett's syndrome, all testify to abnormal development in these known retardation syndromes [10,11]. These produce gross disturbances of central nervous system (CNS) development for which the cause is known and, in principle, relatively simple screening could be used to detect and prevent such disorders. More subtle non-dementing disorders, however, have proven more difficult. There remains considerable controversy, for example, about even the validity of the diagnosis of attention deficit hyperactivity disorder (ADHD). Brain morphometric studies may help validate this diagnosis, and are summarized in detail

here. Since prospective longitudinal rescan data is now available, we can address not only how the two patient groups, ADHD and childhood onset schizophrenia (COS), differed from controls at their initial evaluation but also examine their differing developmental course.

Anatomic brain magnetic resonance imaging studies of attention deficit hyperactivity disorder and childhood onset schizophrenia

Attention deficit hyperactivity disorder

Table 1 summarizes some representative anatomic brain studies that have been carried out to date in ADHD. As seen in Table 1, several independent studies have found a smaller total brain volume. This represents a global reduction of grey and white equally (not shown here) [12–16]. There are also subtle and not entirely consistent abnormalities of various basal ganglia structures (Table 2) [12,13,15,17] and, most striking, a consistent and significant reduction of the volume of the posterior inferior cerebellar vermis (Table 2) [16,18,19]. These findings support other biological models of ADHD implicating frontal–basal ganglia and dopaminergic circuits [20].

These abnormalities appear to be a fixed, rather than an ongoing, process. Longitudinal changes during childhood and adolescence did not differ between our 73 ADHD subjects, and 75 healthy matched controls studied prospectively with 2 and 4 year follow-up rescan [21]. These anatomic abnormalities are not due to stimulant drug effects since the 17 medication-naïve patients showed the same brain pattern. Thus, in contrast to COS (described below), the smaller total brain and cerebellar vermis in ADHD, seems due to an earlier process (at least before age 4, the earliest age at which these scans were obtained). Moreover, since the trajectories of the total and regional brain development does not differ between ADHD patients and controls, severe inattention or impulsivity per se is not likely to cause the late progressive abnormalities seen for the schizophrenic group.

Table 1. Anatomic brain magnetic resonance imaging studies in ADHD

Study	Measure	Normal controls (n)	ADHD (n)	% Smaller	Effect size
Aylward <i>et al.</i> 1996 [12]	Representative brain volume	11	10	3.2%	0.60
Filipek <i>et al.</i> 1997 [13]	Right hemisphere volume	15	15	4.8%	0.67
Bullmore <i>et al.</i> 1999 [14]	Grey and white matter voxels	16	18	3.0%	0.29
Castellanos <i>et al.</i> 1996, 2000 [15,21]	Grey and white matter voxels	119	132	4.2%	0.40
	Total/average	161	175	3.8%	0.49

ADHD, attention deficit hyperactivity disorder.

Table 2. Anatomic brain magnetic resonance imaging studies in ADHD: basal ganglia and cerebellum findings

Location	ADHD probands	Contrast subjects	Findings	Comments
Basal ganglia Aylward <i>et al.</i> 1996 [12]	10 boys	11 normal controls; 16 boys with ADHD + TS	Globus pallidus volume smaller in ADHD (significant on left)	Caudate and putamen also smaller in ADHD, although not significantly
Castellanos <i>et al.</i> 1996 [15]	55 health controls	55 healthy controls	Normal symmetry in prefrontal brain, caudate, and globus pallidus significantly decreased in ADHD; cerebellum volume also smaller	Data support hypothesis that prefrontal-striatal-cortical circuitry mediates ADHD, particularly on right side
Filipek <i>et al.</i> 1997 [13]	15 males (same subjects as Semrud-Clikeman <i>et al.</i> 1994) [77]	15 normal controls	Caudates smaller (only significant left) in ADHD; right anterior superior white matter also significantly diminished; posterior white matter volumes decreased only in stimulant non-responder	First study to quantify grey and white matter separately; authors suggest using medication response to subtype patient groups
Mataro <i>et al.</i> 1997 [78]	11 adolescents with ADHD	19 healthy control subjects (three girls in each group)	Right caudate larger in ADHD; larger caudate nucleus areas associated with poorer performance on tests of attention and higher ratings on Conner Teachers Rating Scale	Single slice axial MRI-not volumetric measure; only study to find larger size in ADHD
Cerebellum Berquin <i>et al.</i> 1998 [18]	46 right-handed boys (subset of Castellanos 1996) [15]	47 right-handed boys	Posterior inferior cerebellar vermis volume and area significantly smaller	Contrast survived covariance for total cerebral volume differences
Mostofsky <i>et al.</i> 1998 [19]	12 males	23 males	Posterior inferior cerebellar vermis area significantly smaller	Contrast survived covariance for total cerebral volume differences
Castellanos <i>et al.</i> 2001 [16]	50 girls	50 girls	Posterior inferior cerebellar vermis volume significantly smaller (-12%, same as boys)	Contrast survived covariance for total cerebral volume; most robust and replicated finding in ADHD

ADHD, attention deficit hyperactivity disorder; MRI, magnetic resonance imaging; TS, Tourette's Syndrome.

Based on these findings, candidate processes for the abnormalities in ADHD focus on prenatal or early post-natal events.

Childhood onset schizophrenia

Schizophrenia is a heterogeneous illness both with respect to clinical phenomenology and aetiology. Age of onset of illness has provided an avenue to understanding of disease across all of medicine, with earlier onset cases often having more striking or homogeneous risk factors and/or differing pathophysiologicals. For this reason, a study of COS, defined as onset of psychotic symptom by

the 13th birthday, has been ongoing since 1990 at the National Institute of Mental Health. These severely ill patients are less contaminated with substance abuse and other factors seen in later-onset populations. Moreover, the childhood onset illness appears continuous with respect to clinical and neurobiological measures [22].

A comparison of 46 COS and 84 healthy volunteers extends our previous studies [23,24] showing that COS had decreased total brain volume and increased lateral ventricular volume as seen in adult onset schizophrenia. Unlike the subtle global decrease in grey and white matter seen in ADHD, the decreased brain volume here is due exclusively to the robust 10% decrease in cortical

grey matter, as the total white matter volume does not differ significantly between the COS and healthy groups. These findings are summarized in Table 3.

In addition, Table 4 and Figure 1 show prospective longitudinal brain MRI rescan measures for these COS cases. There is increasing ventricular volume, and decreasing cortical grey and medial temporal lobe structures across 2, 4 and (for a smaller number of cases), 6 years after their initial scan. Here, too, regional grey–white segmentation showed the progressive loss to be for grey matter only [25–28]. Figure 1 shows this progressive loss during adolescence for regional cortical grey matter.

These observations have led us to view adolescence as a time-limited window in which progressive brain changes in schizophrenia may be observed.

Clinically these changes parallel a decline in full-scale IQ [29] and lack of normal maturation of neurological status [30] for these patients. These progressive changes are not likely to be due to medication. Data from a contrast group of 17 children with transient psychotic symptoms treated with the same typical and atypical antipsychotic drugs, but without clinical progression to schizophrenia (MDI or ‘multidimensionally impaired’ group) [31] show no progressive ventricular volume

Table 3. Brain MRI volumes (mL) for childhood onset schizophrenics ($n = 46$) and healthy controls ($n = 82$)

Region of the brain	COS patients ($n = 46$) Mean (\pm SD)	Healthy controls ($n = 82$) Mean (\pm SD)	F*	df	p
Total cerebral volume	1073.0 (123.2)	1102.2 (113.0)	1.92	1,134	1
Total grey	683.7 (81.4)	718.0 (76.3)	6.31*	1,134*	0.05*
Total white	389.3 (51.8)	383.8 (50.5)	13.27	1,133	0.001
			0.35*	1,134*	NS*
			13.27	1,133	0.001
Regional grey volumes					
Frontal					
Left	104.3 (12.3)	111.2 (10.5)	26.39	1,133	0.001
Right	106.2 (13.8)	111.9 (11.5)	7.99	1,133	0.005
Total	210.5 (23.9)	223.1 (21.7)	16.93	1,133	0.001
Parietal					
Left	56.5 (7.4)	60.4 (6.5)	12.13	1,133	0.001
Right	55.4 (7.5)	59.8 (6.3)	19.11	1,133	0.001
Total	111.9 (14.7)	120.3 (12.6)	16.92	1,133	0.001
Temporal					
Left	84.6 (10.5)	87.5 (8.8)	1.02	1,133	0.314
Right	92.5 (11.8)	95.3 (9.4)	0.29	1,133	0.592
Total	177.2 (22.0)	182.8 (18.11)	0.63	1,133	0.429
Occipital					
Left	31.1 (4.7)	32.8 (5.6)	0.93	1,133	0.337
Right	30.4 (4.9)	31.6 (5.5)	0.07	1,133	0.793
Total	61.6 (9.5)	64.4 (10.8)	0.41	1,133	0.521
Lateral ventricles					
Left	8.3 (4.4)	5.7 (3.3)	20.05	1,133	0.001
Right	7.4 (3.7)	5.4 (3.1)	15.51	1,133	0.001
Total	15.7 (7.8)	11.1 (6.2)	18.97	1,133	0.001
Hippocampus					
Left	4.4 (0.6)	4.6 (0.5)	2.37	1,121	0.126
Right	4.4 (0.7)	4.5 (0.4)	0.01	1,121	0.927
Total	8.8 (1.2)	9.1 (0.9)	0.86	1,121	0.356
Amygdala					
Left	2.7 (0.8)	2.5 (0.5)	2.91	1,121	0.0914
Right	2.2 (0.6)	2.2 (0.6)	0.28	1,121	0.596
Total	4.9 (1.3)	4.8 (1.0)	1.82	1,121	0.180
Superior temporal gyrus					
Left	26.3 (4.4)	25.9 (3.1)	2.79	1,121	0.097
Right	24.4 (4.0)	24.2 (3.2)	1.18	1,121	0.280
Total	50.8 (7.7)	50.1 (5.5)	2.58	1,121	0.111

*All probabilities are ANCOVAs except for ANOVAs as indicated by *. COS, childhood onset schizophrenia; MRI, magnetic resonance imaging.

Table 4. Progressive brain changes during adolescence in COS

Findings for COS vs controls	Comments/references
Progressive reduction in superior temporal gyrus, lateral hippocampus at 2-year follow up (n = 10)	Only two time points [27]
Increased lateral ventricular volume at 2-year follow up (COS n = 16; normal controls n = 24)	Clear progression across ages 14–16 [28]
Progressive decrease in TCV and hippocampus; increase in ventricular volume (COS n = 42; normal controls n = 74)	No progression after age 19; longitudinal and cross-sectional (208 scans) [29]
Frontal and temporal grey volume loss for COS ages 13–18	Differences most significant for frontal and temporal grey
Loss = 5% more than for healthy controls (COS n = 15; normal controls n = 43)	Longitudinal (Dx X time X region, $p = 0.004$) [30]
COS, childhood onset schizophrenia; TCV, total cerebral volume.	

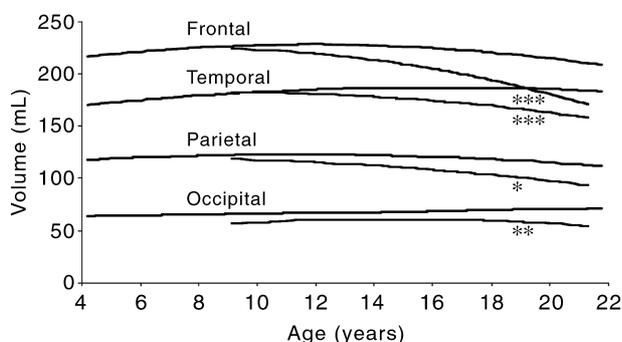


Figure 1. Regional cortical grey matter loss during adolescence for childhood onset schizophrenia.

* $p = 0.6$, ** $p = 0.2$, *** $p = 0.001$. Based on 172 scans from 98 healthy controls and 98 scans from 48 patients with childhood onset schizophrenia. (Based on Rapoport et al. [44] and Giedd et al. [45])

increase or grey matter volume loss relative to healthy controls [32].

These findings are consistent with the neuropathology of schizophrenia [33,34]. The loss of cortical volume is consistent with models of progressive widespread, subtle disruption and decreasing connectivity of multiple cortical regions hypothesized for schizophrenia [35,36].

Regulation of brain development: clinical implications

Brain plasticity

A combination of genetic and environmental factors controls the development of the CNS. The process is highly plastic, involving a series of sequential and parallel events, and a disruption of one step can greatly influence later processes [37].

Because of high malleability in the human brain, it would seem possible that the stress and abnormal thought and behaviour experienced by psychotic patients might be responsible for the progressive loss of grey matter and cytoarchitectonic deficiencies found in these individuals. For example, could schizophrenic behaviour be a *cause* rather than an *effect* of the decreased and decreasing frontal grey brain volume? Could severe inattention and impulsivity produce small grey matter volume? Although the influence of plasticity is most pronounced during the critical periods of postnatal development and declines during adolescence, a significant level of adaptability continues to exist even into adulthood. The fact that developed brains can be induced into schizophrenic states [38] is at least consistent with the idea that changes in synaptic efficacy could play a critical role in the onset of schizophrenia [39,40].

Important preclinical demonstrations of brain plasticity have suggested two distinct forms. A classic study by Hubel, Wiesel and LeVay exemplifies the first [41]. Specifically, monkeys with one eye completely covered from birth were found to develop a greater number of afferents from the lateral geniculate nucleus to the visual cortex of the open eye than to the sutured eye. In other words, synaptic pathways failing to attain the expected level of stimulation by the environment lost efficiency, while those that were properly activated increased in connectivity and gained efficiency [42]. This process has been called experience-expectant maturation. A second form of plasticity can be demonstrated by studies with rats which have suggested that different levels of general sensory stimulation have distinct influences on brain development. Animals raised in 'enriched' environments have been found to have significantly greater cerebral cortical weight and thickness, dendritic length and spine density, total dendritic material per neuron, acetylcholinesterase activity, RNA/DNA ratio, RNA diversity

and brain-specific protein concentrations than animals raised in 'impooverished' environments [43]. These results suggest that environmental stimuli that are not predetermined and vary between individuals in a species contribute to brain structure: a second form of plasticity described as experience-dependent maturation. Applied to the schizophrenic patient, then, could experience-dependent plasticity cause the cytoarchitectonic decline and grey matter deficiencies?

Probably not. In general, experiments involving plasticity apply drastic conditions. In the aforementioned examples, animals were exposed to highly unusual if not devastating visual and sensory environments. Other studies involve equally extreme measures. For instance, plasticity is often studied through the induction of lesions. Neuronal input to a target zone is severed, and anatomical, biochemical and electrophysiological data are collected to determine whether re-innervation has occurred with other healthy afferent areas [44]. 'Postlesional plasticity' has been found in many regions including the cerebral cortex [45] and cerebellum [46].

In considering the relationship between plasticity and the onset of schizophrenia, therefore, it is important to recognize that even the abnormality in environmental input to the brain of a schizophrenic patient is considerably less severe than the conditions involved with most of the experiments exploring plasticity. The possibility that abnormal behaviour per se could induce abnormal connectivity exists, and experimental studies of plasticity using functional imaging may demonstrate such effects. For structural findings reviewed here, however, it seems more probable that schizophrenic behaviours result from these abnormalities rather than the reverse.

Processes that may underlie the brain abnormalities in attention deficit hyperactivity disorder

While total brain volume is slightly but significantly decreased in ADHD, the total and regional growth curves for this group run parallel to normal brain curves [21]. Thus, the events leading to the anatomic differences in ADHD probably occur early in neurodevelopment. The period of critical neurodevelopmental organizational events [47] is influenced by a complex interplay of various genetic and epigenetic factors such as neurotransmitters, neurotrophins and growth factors, and cytokines, along with hormonal influences [48,49]. Even a subtle injury during this vulnerable process of neurodevelopment and organization *in utero* (second or third trimester) can affect the brain development and size globally, thus explaining the changes seen in ADHD [50]. Various phenomenological observations in ADHD may help us understand the mechanism of this global size reduction.

Incidence of pregnancy-related complications, and prematurity are slightly higher in ADHD [51]. However, the strongly genetic nature of ADHD, and lack of specificity of findings for what mediates the effects, for example prematurity, leaves us without a specific hypothesis of what might mediate this subtle global reduction in brain volume. The striking volume reduction of the posterior inferior lobule of cerebellar vermis in ADHD are also important in this regard (see Table 1). This region of cerebellar vermis is highly dopaminergic [52] and appears, like most brain volumetric measures to be highly heritable, [Giedd J, Castellanos X: unpublished data]. The posterior inferior vermis, thus, may be an important part of cerebello-striato-frontal circuitry and hence in the aetiopathogenesis of ADHD. Recently, it was observed that brain-derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) mRNAs (important brain growth modulators) are colocalized to specific ventral mesencephalic dopaminergic neurons [53]. Thus any defect in dopaminergic circuitry could alter these growth modulators and ultimately the brain size and development. Allelic variations of several dopaminergic genes (DA transporter, DA receptor 4) have been associated with ADHD [54], however, these alleles have been examined in ADHD populations and show no significant association with any of these brain volumetric measures [55]. Conversely, subtle abnormalities in BDNF, NT-3 and others may cause localized abnormality of dopamine circuitry. This awaits exploration. Finally, differences in the volume of caudate nucleus could also point us toward aetiopathogenic mechanisms.

The caudate nucleus volume is decreased in boys with ADHD [56,57]. This is interesting as healthy girls have a larger caudate nucleus, probably owing to higher concentration of oestrogen receptors in the region. In the CNS, oestrogen plays a role in regulation of gene transcription, can act as an antioxidant for toxic substances, and can also act as a neuroprotectant [58]. In theory, this might explain the smaller caudate volume in ADHD boys and lower incidence of ADHD in girls. However, this would not account for the fact that brain MRI findings for ADHD boys and girls are very similar (see Table 1).

Candidate processes for childhood onset schizophrenia

The smaller brain sizes in COS and ADHD indicate that some compartment or compartments in the brain are being reduced. Two major theories have been put forth that name different compartments as the source of the loss [59]. The first proposes that the cause is either a developmental failure to produce the proper number of neurons or it is neurodegenerative disease that results in

an overall neuronal loss in the brain. The second theory proposes that the differences in brain volume are due to reduction of or reduced formation of the neuropil, which consists of the axonal and dendritic arbours that make up a large fraction of the grey matter in the cortex [34,60]. This reduction of the neuropil could result from reduction in the numbers of synaptic connections made between neurons. In humans, detailed post-mortem electron microscopy work has shown that there is normally a postnatal increase in synaptic connections followed by a decrease in synaptic connections that extends as far as mid-adolescence in some parts of the brain [61].

As noted above, the decreased volume in ADHD may be related to the first factor (a reduced number of neurons generated during early neurodevelopment) while COS is due to the second factor (an excessive degree of synapse reduction of an initially fairly normal number of synapses and neurons).

Post-mortem studies to determine if there is a reduced cell number in the brains of people with schizophrenia have not produced consistent results in many parts of the brain [34,60]. Conversely, a number of studies using stereological methods have found increased densities of neurons in the prefrontal cortex and temporal lobe [62–64]. In addition, these neurons have smaller cell bodies, and it has been shown that the cell bodies are proportional to the level of dendritic and axonal arborization [60]. Other evidence shows decreased loss of expression of synaptic markers in schizophrenia [65] and most recently, decreased expression of several functional genes important in presynaptic function and development [66]. Thus, while there is not direct evidence for synaptic loss in COS there is a good deal of evidence that supports the reduced neuropil hypothesis. Detailed, quantitative electron microscopy studies are needed to directly address this crucial question as to the cellular locus of the brain abnormality in COS. The two theories are not mutually exclusive, but the evidence is strong in favour of a reduced neuropil playing a prominent role in schizophrenia (see [34,60] for details).

In normal development it is known that excess numbers of synaptic connections are formed initially and as development progresses the extra connections are eliminated or pruned. Considerable information is available concerning some of the mechanisms regulating the generation of neurons, development of the synapses between the neurons and the process by which some synapses are lost during development. We will briefly review some of this information, focusing primarily on the process of synapse elimination, since the available evidence suggest that this may play a key role in COS.

Synapse elimination is particularly well characterized in two systems, namely in the climbing fibre (CF)

system which innervates Purkinje cells (PC) in the cerebellum, and in motor neurons which innervate skeletal muscle fibres, although synapse elimination has been demonstrated in many systems [67]. The possible mechanisms behind pathologic elimination of the synapses are too numerous and complex to thoroughly review here. However, a general idea of the possibilities can be gleaned from the well-studied model of elimination at the neuromuscular junction (NMJ). In this system, at birth in the rodent, all muscle fibres are innervated by at least two axons, and by 3 weeks postnatally all but one axon has been eliminated from all of the muscle cells [68]. This process requires activation of the system because it fails to take place in paralysed preparations. Some of the steps in this coupling between activation and synapse elimination are becoming clarified, and seem to involve a number of protein kinases, enzymes which regulate protein function by altering their state of phosphorylation. In particular, neurotransmitter receptors are known to be targets for kinase action, and their physiology and stability in the membrane of nerve and muscle are greatly affected by the addition of phosphate groups. One major link in the chain leading to synapse elimination, therefore, may be activation of appropriate kinases, phosphorylation of neurotransmitter receptors and subsequent, selective destabilization of the synapses involving those receptors [69]. Loss of synaptic acetylcholine receptors has been shown to be an early step in the loss of synapses at the NMJ [70]. Selective loss of synapses may be due to differential activation of localized kinases, which have different effects on receptor stability.

Evidence for the involvement of a kinase (protein kinase C or PKC) in synapse elimination in the central nervous system has been obtained in experiments on the CF/PC system mentioned above. On average PCs in the cerebellum are innervated by 3.5 CFs at birth but as normal development proceeds, the number of CFs forming a synaptic connection with each PC is reduced to one [67].

Genetic manipulations have given some clues as to the mechanisms involved in synapse elimination. Mutation of one isoform of PKC which inactivates the kinase has been shown to block synapse elimination in the mouse cerebellum [71]. In the mouse cerebellum the expression of the PKC-gamma isoform goes up in PCs during the period during which multiple synapses from CFs are reduced to single innervations. In mutant mice in which PKC-gamma is inactivated there is marked reduction in the elimination of the initial multiple innervations of PCs by CFs. Thus the activity of this particular molecule, PKC, is essential for normal elimination of redundant synapses. It might be that excessive activity of the enzyme would produce the abnormally high degree of synapse elimination postulated to be related to COS.

Trophic factors have been shown to have powerful effects on neuronal survival and synaptic structure and function during development [50,72–74]. Competition for a limited supply of trophic material has been postulated to account for at least a portion of the synapse loss that occurs during development. Blockade of the trophic BDNF has been shown to prevent the normally occurring pruning that is essential for development of the normal architecture of the visual cortex [75] and an inadequate supply could result in inadequate development or survival of cortical synapses.

Cortical neurons are generated during a sharply delimited time window relatively early in development and the total number of neurons can be drastically affected by various manipulations during this period. For instance, it has been shown that a neuropeptide, vasoactive intestinal peptide (VIP) controls the duration of the mitotic cycle in the neuroblasts in the ventricular germinal zone and that this affects the total number of neurons that get born during development. Antagonists of VIP given during the critical neuron-generating period (and only during this period) result in markedly microcephalic animals [74]. Some such interference with the process of neuron generation could be involved in the early deficit in brain size seen in ADHD.

Following on the initial speculation of Feinberg [76], McGlashan and Hoffman [35] and others have used computer modelling of neural networks to test the plausibility of the ‘over-pruning’ hypothesis of schizophrenia. Their experiments characterized the behaviour of over-pruned neural networks and found strong parallels between schizophrenia and the networks’ behaviour.

In summary, as we hope this report illustrates, clinical brain imaging studies are bringing child psychiatry and the developmental neurosciences ever closer. We present here some possible candidates, but expect that much greater specificity and converging information will emerge from genetic linkage and association studies that will be carried out over the next decade. Some of these studies are ongoing with these populations at the NIMH.

References

- Rutter M, Graham P, Yule W. A neuropsychiatric study in Childhood. *Clinics in Developmental Medicine* Nos 35, 36. London: S.I.M.P./Heinemann, 1970.
- Goodman R. Brain disorders. In: Rutter M, Taylor E, Hersov L, eds. *Child and adolescent psychiatry: modern approaches*. 3rd edn. London: Blackwell, 1994:172–190.
- Jernigan TL, Tallal P. Late childhood changes in brain morphology observable with MRI. *Developmental Medicine and Child Neurology* 1990; 32:379–385.
- Reiss AL, Abrams MT, Singer HS, Ross JL, Denckla MB. Brain development, gender and IQ in children. A volumetric imaging study. *Brain* 1996; 119:1763–1774.
- Giedd J, Snell J, Lange N *et al*. Quantitative magnetic resonance imaging of human brain development ages 4–18. *Cerebral Cortex* 1996; 6:551–560.
- Sowell ER, Thompson PM, Holmes CJ, Jernigan T, Toga AW. *In vivo* evidence for post-adolescent brain maturation in frontal and striatal regions. *Nature Neuroscience* 1999; 2:859–861.
- Sowell ER, Thompson PM, Holmes CJ, Batth R, Jernigan TL, Toga AW. Localizing age-related changes in brain structure between childhood and adolescence using statistical parametric mapping. *Neuroimage* 1999; 9:587–597.
- Giedd JN, Blumenthal J, Jeffries NO *et al*. Cerebral cortical gray matter changes during childhood and adolescence: a longitudinal MRI study. *Nature Neuroscience* 1999; 2:861–863.
- Paus T, Zijdenbos A, Worsley K *et al*. Structural maturation of neural pathways in children and adolescents: in vivo study. *Science* 1999; 283:1908–1911.
- Schapiro M, Luxenberg J, Kay J, Haxby J, Friedland R, Rapoport SI. Serial quantitative estimates of brain morphometrics in Down syndrome at different ages. *Neurology* 1989; 39:1349–1353.
- Reiss A, Eliez S, Schmitt JE, Patwardhan A, Haberecht M. Brain imaging in neurogenic conditions: realizing the potential of behavioural neurogenetics research. *Mental Retardation and Developmental Disabilities Research Reviews* 2000; 6:186–197.
- Aylward EH, Reiss AL, Reader MJ, Singer HS, Brown JE, Denckla MB. Basal ganglia volumes in children with attention-deficit hyperactivity disorder. *Journal of Child Neurology* 1996; 11:112–115.
- Filipek PA, Semrud-Clikeman M, Steingard RJ, Renshaw PF, Kennedy DN, Biederman J. Volumetric MRI analysis comparing attention-deficit hyperactivity disorder with normal controls. *Neurology* 1997; 48:589–601.
- Bullmore ET, Suckling J, Overmeyer S, Rabe-Hesketh S, Taylor E, Brammer MJ. Global, voxel, and cluster tests, by theory and permutation, for a difference between two groups of structural MR images of the brain. *IEEE Transactions of Medical Imaging* 1999; 18:32–42.
- Castellanos FX, Giedd JN, Marsh WL *et al*. Quantitative brain magnetic resonance imaging in attention-deficit hyperactivity disorder. *Archives of General Psychiatry* 1996; 53:607–616.
- Castellanos FX, Giedd JN, Berquin PC *et al*. Quantitative brain magnetic resonance imaging in girls with attention-deficit/hyperactivity disorder. *Archives of General Psychiatry*, 2001; 58:289–295.
- Mataró M, García-Sánchez C, Junqué C, Estévez-González A, Pujol J. Magnetic resonance imaging measurement of the caudate nucleus in adolescents with attention-deficit hyperactivity disorder and its relationship with neuropsychological and behavioral measures. *Archives of Neurology* 1997; 54:963–968.
- Berquin PC, Giedd JN, Jacobsen LK *et al*. Cerebellum in attention-deficit hyperactivity disorder: a morphometric MRI study. *Neurology* 1998; 50:1087–1093.
- Mostofsky SH, Reiss AL, Lockhart P, Denckla MB. Evaluation of cerebellar size in attention-deficit hyperactivity disorder. *Journal of Child Neurology* 1998; 13:434–439.
- Castellanos FX, Swanson J. Biological underpinnings of ADHD. In: Sandberg S, ed. *Hyperactivity and attention disorders of children*. Cambridge University Press, Cambridge UK (in press).
- Castellanos FX, Giedd J, Walter J *et al*. Longitudinal MRI in attention-deficit/hyperactivity disorder (ADHD): effects of prior stimulant treatment in cerebellum and total brain [Abstract]. *Society for Neuroscience Abstracts* 2000; 26:1327.
- Nicolson R, Rapoport J. Childhood onset schizophrenia: rare but worth studying. *Biological Psychiatry* 1999; 46:1418–1428.
- Frazier JA, Giedd JN, Hamburger SD *et al*. Brain anatomic magnetic resonance imaging in childhood-onset schizophrenia. *Archives of General Psychiatry* 1996; 53:617–624.

24. Kumra S, Giedd JN, Vaituzis AC *et al.* Childhood-onset psychotic disorders: quantitative magnetic resonance imaging of structural brain abnormalities. *American Journal of Psychiatry* 2000; 157:1464–1474.
25. Jacobsen LK, Giedd JN, Castellanos FX *et al.* Progressive reduction of temporal lobe structures in childhood onset schizophrenia. *American Journal of Psychiatry* 1998; 155:678–685.
26. Rapoport JL, Giedd J, Kumra S *et al.* Childhood-onset schizophrenia: progressive ventricular change during adolescence. *Archives of General Psychiatry* 1997; 54:897–903.
27. Giedd JN, Jeffries NO, Blumenthal J *et al.* Childhood onset schizophrenia. Progressive brain changes during adolescence. *Journal of Biological Psychiatry* 1999; 46:892–898.
28. Rapoport JL, Giedd JN, Blumenthal J *et al.* Progressive cortical change during adolescence in childhood-onset schizophrenia: a longitudinal magnetic resonance imaging study. *Archives of General Psychiatry* 1999; 56:649–654.
29. Bedwell J, Keller B, Smith A, Hamburger S, Kumra S, Rapoport JL. Childhood onset schizophrenia: post-psychotic decline in IQ. *American Journal of Psychiatry* 1999; 156:1996–1997.
30. Karp BI, Garvey M, Jacobsen LK *et al.* Abnormal neurological maturation in early-onset schizophrenia. *American Journal of Psychiatry* 2001; 158:118–122.
31. Nicolson R, Lenane M, Brookner F *et al.* Children and adolescents with psychotic disorder not otherwise specified: a two to eight year follow-up. *Comprehensive Psychiatry* (in press).
32. Nicolson R, Giedd JN, Blumenthal J *et al.* Lack of progressive cortical gray matter with non-schizophrenic psychotic disorders. *Biological Psychiatry* (in press).
33. Glantz L, Lewis D. Decreased dendritic spine density on prefrontal cortical pyramidal neurons in schizophrenia. *Archives of General Psychiatry* 2000; 57:65–73.
34. Selemon L, Goldman-Rakic P. The reduced neuropil hypothesis: a circuit based model of schizophrenia. *Biological Psychiatry* 1999; 45:17–25.
35. McGlashan TH, Hoffman RE. Schizophrenia as a disorder of developmentally reduced synaptic connectivity. *Archives of General Psychiatry* 2000; 57:637–648.
36. Woods BT. Is schizophrenia a progressive neurodevelopmental disorder? Toward a unitary pathogenetic mechanism. *American Journal of Psychiatry* 1998; 155:161–170.
37. Keshavan MS, Hogarty GE. Brain maturational processes and delayed onset in schizophrenia. *Development and Psychopathology* 1999; 11:525–543.
38. Allen RW, Young SL. Phencyclidine-induced psychosis. *American Journal of Psychiatry* 1978; 135:1081–1084.
39. Friston KJ. Schizophrenia and the disconnection hypothesis. *Acta Psychiatrica Scandinavica* 1999; 99:68–79.
40. Benes FM, Davidson J, Bird ED. Quantitative cytoarchitectural studies of the cerebral cortex of schizophrenics. *Archives of General Psychiatry* 1986; 43:31–35.
41. Hubel DH, Wiesel TN, LeVay S. Plasticity of ocular dominance columns in monkey striate cortex. *Philosophical Transactions of the Royal Society of London, Series B. Biological Sciences* 1977; 278:377–409.
42. Singer W. Neuronal mechanisms in experience dependent modification of visual cortex function. *Progress in Brain Research* 1979; 51:457–477.
43. Greenough W, Black J. Induction of brain structure by experience: substrate for cognitive development. In: Gunnar MR, Nelson CA, eds. *Minnesota symposia on child psychology 24: developmental behavioral neuroscience*. Hillandale, NJ: Lawrence Erlbaum, 1991:155–200.
44. Haracz JL. Neural plasticity in schizophrenia. *Schizophrenia Bulletin* 1985; 11:191–240.
45. Rutledge LT. Effects of cortical denervation and stimulation on axon, dendrites, and synapses. In: Cotman CW, ed. *Neural plasticity*. New York: Raven, 1978:273–289.
46. Chen S, Hillman DE. Plasticity of the parallel fiber-Purkinje cell synapse by spine takeover and new synapse formation in the adult rat. *Brain Research* 1982; 240:205–220.
47. Volpe JJ. Overview: normal and abnormal human brain development. *Mental Retardation and Developmental Disabilities Research Reviews* 2000; 6:1–5.
48. Evrard P, Marret S, Gressens P. Environmental and genetic determinants of neural migration and postmigratory survival. *Acta Paediatrica* 1997; 422 (Suppl.):20–26.
49. Riddle DR, McAllister AK, Lo DC, Katz LC. Neurotrophins in cortical development. *Cold Spring Harbor Symposium on Quantitative Biology* 1996; 61:85–93.
50. Johnston MV. Neurotransmitters and vulnerability of the developing brain. *Brain and Development* 1995; 17:301–306.
51. Milberger S, Biederman J, Faraone SV, Guite J, Tsuang MT. Pregnancy, delivery and infancy complications and attention deficit hyperactivity disorder: issues of gene–environment interaction. *Biological Psychiatry* 1997; 41:65–75.
52. Melchitzky DS, Lewis DA. Tyrosine hydroxylase- and dopamine transporter-immunoreactive axons in the primate cerebellum. Evidence for a lobular- and laminar-specific dopamine innervation. *Neuropsychopharmacology* 2000; 22:466–472.
53. Serogy KB, Lundgren KH, Tran TM, Guthrie KM, Isackson PJ, Gall CM. Dopaminergic neurons in rat ventral midbrain express brain-derived neurotrophic factor and neurotrophin-3 mRNAs. *Journal of Comparative Neurology* 1994; 342:321–334.
54. Castellanos FX. Toward a pathophysiology of attention-deficit/hyperactivity disorder. *Clinical Pediatrics* 1997; 36:381–393.
55. Castellanos FX, Lau E, Tayebi N *et al.* Lack of an association between dopamine-4 receptor polymorphism and attention-deficit/hyperactivity disorder: genetic and brain morphometric analyses. *Molecular Biology* 1998; 3:431–434.
56. Castellanos FX, Giedd JN, Eckburg P *et al.* Quantitative morphology of the caudate nucleus in attention deficit hyperactivity disorder. *American Journal of Psychiatry* 1994; 151:1791–1796.
57. Hynd GW, Hern KL, Novey ES *et al.* Attention deficit-hyperactivity disorder and asymmetry of the caudate nucleus. *Journal of Child Neurology* 1993; 8:339–347.
58. Sawada H, Shimohama S. Neuroprotective effects of estradiol in mesencephalic dopaminergic neurons. *Neuroscience and Biobehavioral Reviews* 2000; 24:143–147.
59. Arnold S. Neurodevelopmental abnormalities in schizophrenia: insights from neuropathology. *Development and Psychopathology* 1999; 11:439–456.
60. Harrison P. The neuropathology of schizophrenia: a critical review of the data and their interpretation. *Brain* 1999; 122:593–624.
61. Huttenlocher PR, Dabholkar AS. Regional differences in synaptogenesis in human cerebral cortex. *Journal of Comparative Neurology* 1997; 387:167–178.
62. Selemon LD, Rajkowska G, Goldman-Rakic PS. Abnormally high neuronal density in the schizophrenic cortex: a morphometric analysis of the prefrontal area 9 and occipital area 17. *Archives of General Psychiatry* 1995; 52:805–818.
63. Selemon LD, Rajkowska G, Goldman-Rakic PS. Elevated neuronal density in prefrontal area 46 in brains from schizophrenic patients: application of a three-dimensional stereological method. *Journal of Comparative Neurology* 1998; 392:402–412.
64. Pakkenberg R. Total nerve cell number in neocortex in chronic schizophrenics and controls estimated using optical disectors. *Biological Psychiatry* 1993; 34:768–772.

65. Glantz LA, Lewis DA. Reduction of synaptophysin immunoreactivity in the prefrontal cortex of schizophrenic subjects: regional and diagnostic specificity. *Archives of General Psychiatry* 1997; 54:660–669.
66. Mirmics K, Middleton F, Murquez A, Lewis DA, Levitt P. Molecular characterization of schizophrenia viewed by microarray analysis of gene expression in prefrontal cortex. *Neuron* 2000; 28:53–67.
67. Lohof AM, Delhaye-Bouchaud N, Mariani J. Synapse elimination in the central nervous system: functional significance and cellular mechanisms. *Reviews in the Neurosciences* 1996; 7:85–101.
68. Thompson WJ. Activity and synapse elimination at the neuromuscular junction. *Cellular and Molecular Neurobiology* 1985; 5:167–182.
69. Lanuza MA, Li M, Jia M *et al.* Protein kinase C-mediated changes in synaptic efficacy at the neuromuscular junction in vitro: the role of postsynaptic acetylcholinereceptors. *Journal of Neuroscience Research* 2000; 61:616–625.
70. Balice-Gordon RJ, Lichtman JW. Long-term synapse loss induced by focal blockade of postsynaptic receptors. *Nature* 1994; 372:519–524.
71. Kano M, Hashimoto K, Chen C *et al.* Impaired synapse elimination during cerebellar development in PKC γ mutant mice. *Cell* 1995; 83:1223–1231.
72. Hughes RA, Sendtner M, Thoenen H. Members of several gene families influence survival of rat motor neurons in vitro and in vivo. *Journal of Neuroscience Research* 1993; 36:663–671.
73. Thoenen H. Neurotrophins and neuronal plasticity. *Science* 1995; 270:593–598.
74. McAllister AK, Katz L, Lo D. Neurotrophins and synaptic plasticity. *Annual Review of Neuroscience* 1999; 22:295–318.
75. Cabelli RJ, Shelton DL, Segal DL, Shatz C. Blockage of endogenous ligand of trl: B inhibits formation of endogenous ocular dominance columns. *Neuron* 1997; 19:63–76.
76. Feinberg I. Schizophrenia: caused by a fault in programmed synaptic elimination during adolescence? *Journal of Psychiatric Research* 1982/1983; 17:319–334.
77. Semrud-Clikeman M, Steingard RJ, Filipek P *et al.* Using MRI to examine brain-behaviour relationships in males with attention deficit hyperactivity. *Journal of the American Academy of Child and Adolescent Psychiatry* 2000; 39:477–484.
78. Mataro M, Garcia-Sanchez C, Junque C, Estevez-Gonzalez A, Pujol J. Magnetic resonance imaging measurement of the caudate nucleus in adolescents with attention-deficit hyperactivity disorder and its relationship with neuropsychological and behavioural measures. *Archives of Neurobiology* 1997; 54:963–968.