Assessment of Brain Function in Clinical Pediatric Research: Behavioral and Biological Strategies

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Abstract

Psychobiological research in child psychiatry requires rigorous assessment of behavior and multiple perspectives on brain function through neurochemical, neuroendocrine, psychophysiological, and other advanced methods. The serious neuropsychiatric disorders of childhood, such as autism, attention deficit disorder, and language disorders, can be studied in complementary clinical protocols aimed at explicating patterns of behavioral and metabolic dysfunction which characterize various clinical syndromes. Clinical research with children raises sensitive ethical issues; the ethical problems can be addressed when children and families are active collaborators with the investigators and a long-term relationship is established. In this setting, participation in research can facilitate better treatment for a child. The use of novel biological strategies, such as pharmacological challenge tests, permits evaluation of the relation of specific neuronal systems to behavioral dimensions in clinical disorders. The development of a new treatment for Tourette's syndrome illustrates the integration of basic and clinical research methods.

The goals of psychiatric research in childhood are the determination of etiologies and treatment methods for specific disorders; specification of brain-behavior relationships; delineation of neuropsychiatric developmental sequences; clarification of the contributions of endowment and experience; determination of biological components of emotional states such as anxiety, arousal, attention, fear, and depression; neurobiological description of the phenomena of brain plasticity and learning; and integration of data from these different domains. Our investigative program moves toward these goals in the context of the pressing needs of the children and families carrying the burden of the most severe neuropsychiatric disorders of childhood. Research approaches, conceived in terms of fundamental theoretical goals, are framed in response to the realistic requirements of families asking for counsel and active support in determining the best care for their children. Clinical research that retains a fidelity to the child and his family is rooted in a knowledge of the family unit which is active and continuous. Our research reflects this integration of clinical and metabolic viewpoints, with the invigoration of one by the other (Cohen 1974; Young and Cohen 1979).

Severe Neuropsychiatric Disorders of Childhood

The severe neuropsychiatric disorders of childhood, phenotypically and genotypically diverse behavioral syndromes apparent during the first years of life, affect up to 3 percent of all children. Organically definable causes include structural malformations of the brain, chromosomal abnormalities, inborn errors of metabolism, anoxia, infection, head trauma, and numerous other types of inborn and acquired pathological influences on central nervous system (CNS).
maturation. When such medically explicable disorders are excluded, the neuropsychiatric disorders consist of a broad range of syndromes of unknown etiology involving the normal unfolding of linguistic, cognitive, and social competence. In their most severe forms, such as primary childhood autism, all spheres of development may be disturbed pervasively; in less severe syndromes, one or another facet of development is more prominently involved (such as expressive and receptive language in the developmental language disorders, or the regulation of attention and motor activity in attention deficit syndromes) (Cohen 1976; Cohen, Caparulo, and Shaywitz 1976; Rutter 1977; Rutter and Schopler 1978).

Various types of neurological and metabolic abnormalities have been postulated as the basis for the disorganization, desynchronization, and retardation in maturation that characterize such severe disorders as autism and schizophrenia. The viewpoint that these disorders rest upon an organic substrate is supported by a range of suggestive evidence. The disorders sometimes are manifest during the first weeks of life, as when the parents of autistic children recognize that the child is not normally attentive to their faces or responsive to their care. The syndromes occur throughout the world, with a fairly predictable incidence (e.g., 1:3,000 live births for autism) and with a characteristic clinical presentation and natural history. Children with neuropsychiatric syndromes often have minor atypicalities on neurological examination or on electroencephalography and have a high incidence of minor physical anomalies. The response of the disorders to medication may sometimes be dramatic (as when children with the attention deficit syndromes respond therapeutically to amphetamine or those with multiple tics respond to haloperidol) and pharmacologically explicable in relation to theories of neurotransmitter function (Cohen, Caparulo, and Shaywitz 1978). The families of some children with neuropsychiatric disorders may have an increased incidence of developmental or psychiatric pathology—for example, language difficulties in families of children with autism or personality disorders in families of hyperactive, attentionally disabled children. Finally, the neuropsychiatric disorders for which no organic basis is now known often bear striking similarities to disorders of known organic causation, such as lead encephalopathy, homocystinuria, and the rubella syndrome.

Although organic factors appear to play the central role in the origin of the severe neuropsychiatric disorders of childhood, experiential factors occasionally precipitate or markedly exacerbate a child's constitutional difficulties. For most children with these disorders, eventual competence represents the complementary relations between endowment and the quality of the child's care, the degree of family stress, the opportunities for adequate stimulation, and luck. Interactions between environmental influences and CNS metabolism, and their mutual impact on natural history, remain to be investigated (Cohen 1974; Young and Cohen 1979). The children afflicted with the severe neuropsychiatric disorders might be characterized as those in greatest need of new research-based knowledge to improve their care and as those in whom a metabolic contribution to their deficit might be most likely to be specified.

Human Investigation: Ethics, Procedures, and the Family

A discussion of the ethics of clinical research might be initiated with the thought that operation of a university-based assessment and treatment program without a research component should be discouraged. The potential pitfalls of neglecting research are exemplified by the questions raised by parents in relation to the widespread use of stimulant drugs for children with attention deficit disorder with hyperactivity (ADD–H). While these drugs are useful in the short term, they are most often administered without other concurrent modes of therapy because clinicians feel such therapeutic approaches to be "outmoded" or too "soft" and time-intensive. Yet, a lack of improvement in the long-term outcome of ADD–H children treated solely with stimulants led some clinicians to feel discouraged with this treatment. A 3-year followup indicates that multimodality treatment of this disorder, in which stimulant medication is combined with one or more psychosocial therapies according to individual needs, leads to less antisocial behavior, better academic performance, and improved adjustment at home and school than in a group receiving less treatment (Satterfield, Satterfield, and Cantwell 1981). In retrospect, it does not seem surprising that
treatment directed toward several areas of a child's function would be more useful than drug treatment alone. Nevertheless, if this research had not been done, unnecessarily restricted treatment methods would have continued for at least an additional decade. These complex disorders of childhood, with interacting contributions from endowment and environment, will only be understood when comprehensive and continuous research is undertaken. Without this research, children and families will be vulnerable to the oscillating interests of clinicians when seeking treatment. This is most clearly the case for children with the severe neuropsychiatric disorders.

In spite of the potential for an unhappy long-term outcome for individuals with such disorders as ADD-H, autism, and pervasive developmental disabilities, there has been a tendency to regard biological methods of assessment as too intrusive when included in a research protocol. We now wonder whether it is sensible to neglect biological research when caring for these children, in view of the severity and intractability of these disorders. Should not the child's disorder and currently favored treatment methods be carefully investigated as part of a comprehensive, humane approach to the problems of the child and family? Intensive psychotherapies represent a major intrusion into the life of a family, yet there has been an unwillingness to assess the utility of these therapies because of the difficulties such research presents. The lack of evidence demonstrating etiologies and optimal treatments for childhood disorders makes it clear that all possibilities must be scrutinized if children are to have the best care available. If urine and blood sampling and other physiological assessment techniques are acceptable procedures in serious nonpsychiatric medical disorders, then the manifest suffering and impairment attributable to the childhood neuropsychiatric disorders justifies the most comprehensive biological and behavioral research.

In our studies of children with developmental disabilities, we have adopted several methods for protecting the rights of children and families, minimizing potential patient risk, and obtaining clearly understood consent. These have included careful selection of patients; preparation of children and families; close collaboration with parents, who can room-in with children who are hospitalized; the clinical presence of the research staff directly responsible for children's care and studies; and special training of nursing and other personnel involved with research patients.

Only families who are completely able to understand the nature and purposes of our research are selected for participation. Families are not invited to join in research studies when they are undergoing unusual family stress or when they are unable to form a stable research collaboration. For those families who cannot be involved in a research collaboration, appropriate medical referrals are arranged. Families involved in research are given copies of publications. The research is described in detail during several outpatient visits, usually extending over the course of months. During the course of evaluation, the researchers review the child's previous development, reports from other professionals and schools, and results of standard laboratory and clinical evaluation procedures. On the basis of these studies, an assessment is made of the child's developmental needs and developmental disturbances. Children who are at unusually sensitive points of development (for example, who seem to be going through periods of accelerated language acquisition) are studied only with the least intrusive cognitive, language, and blood assay methods.

Children who are able to understand the protocol are asked to give written assent on specially designed forms. Parents are given copies of research protocols and encouraged to study them both in the presence of the physician and at home at their leisure. Before parents give written consent, it is determined whether they fully understand the nature and purposes of the research study to which they are consenting. When children are to be hospitalized for special studies, parents and children are encouraged to visit the specially designed Children's Clinical Research Center before hospitalization. Before procedures are performed, the nurse explains each step and ensures that the child and family understand the study.

Of all research procedures, the lumbar puncture with or without probenecid loading presents the most concern to researchers and families. In our research, lumbar puncture and cerebrospinal fluid (CSF) examination are performed only with children who are suffering from severe, lifelong disorders of unknown etiology for which there is no specific, available treat-
ment (for example, childhood autism, aphasia, and Tourette's syndrome). For these children, lumbar puncture and CSF examination are part of an intensive, clinical evaluation of the child's extremely disabling disturbance. It is explained to the families that the results of any particular child's examination are unlikely to reveal information relevant to the child's current treatment. During the past 10 years, lumbar puncture and CSF examination have been performed on over 100 children in our studies. The most common side effects have been (1) nausea from probenecid, the medication given for 12 hours before lumbar puncture for metabolic studies of catecholamine metabolism, and (2) lumbar puncture headaches. The probenecid-induced nausea typically occurs during the last part of the probenecid loading and persists for several hours following lumbar puncture.

Blood and urine studies pose no physical threat to children. To the degree possible, the procedures are carefully described to children and are performed as thoughtfully as possible. Children are especially prepared for electroencephalograms and other procedures by research staff members, nurses, and special educators at the schools that the children attend. In our experience, the most important aspect of the preparation and performance of these studies is the presence of the child's parents and other individuals with whom the child is comfortable. With careful preparation, we have generally been able to complete studies, such as electroencephalograms, for children who have never been able to have these procedures done satisfactorily before. None of the measures of attention, vigilance, or performance are intrusive, and all have been used with young children. For these behavioral studies, there are no associated risks.

Central to our philosophy of human investigation is the idea that collaboration in research studies is not episodic. Children and families who become involved in research are part of an ongoing research collaboration. They can, of course, terminate this collaboration at any time; what is essential to the relationship is the explicit knowledge that all information will be shared by the investigator and that the goal of the research is to understand the disorder experienced by the individual child. The results of all studies are made available to families through discussion and in written form. Detailed reports are prepared for professionals involved with the child and are reviewed with the parents, who are given copies. Recommendations based on findings are shared with the family and placed in the context of the child's general care. Often, the results of the evaluation are used to help the family make decisions about special education or pharmacological intervention. Children and parents are seen following their inpatient studies on an individual basis. Regular school reports are reviewed with the families. Research reports are presented to parent advocate groups and to the staffs of schools involved with children who have been studied. Published articles are circulated to parents. In addition, there is an annual meeting to which all families involved in the research, as well as interested professionals, are invited. At this annual meeting, the progress of research during the past year and plans for the future are described.

**Neuropsychiatric Research in Childhood: Clinical Assessment**

**Diagnosis and Development.** The foundation of the research program of the neuropsychiatric investigatory group at Yale has been the detailed study of specific syndromic groups, using multiple techniques and personnel with varied professional skills. In each case we have attempted to fill in a comprehensive picture of the disorder, while at the same time recognizing individual variation and using inconsistently represented traits for the formation of subgroups. The likelihood of observing a biological homogeneity underlying a specific set of symptoms should be relatively high in a group of children with similar clinical presentations, and may differentiate them from neighboring groups along the clinical spectrum. Our method has been to weave into clinical care quantifying instruments for each of the domains of data, ranging from operational criteria for inclusion or exclusion from a given category, and behavioral assessment with reliable rating scales, through the more naturally numerical physiological and biological measures. This is particularly helpful for children who have characteristics which do not clearly place them in a specific diagnostic group. Differences and similarities to other groups are highlighted, and underlying biological continuities become visible across groups, leading to the in-
vestigation of clinical and biological dimensions cutting across diagnostic boundaries.

Diagnosis has an especially dynamic side in the childhood disorders, as it occurs in a developmental perspective. This gives particular significance to a detailed knowledge of natural history in developmental disturbances, since the recognition of a possible diagnosis at age 12 years may depend on an awareness of the outcome of an early onset disorder (so that it can be considered), and a clear sense of the major clinical components of the illness during the very early years (in order to arrive at the substantiating criteria). Childhood autism, of course, is an excellent example of such a situation, and a disorder that can lead to strikingly different outcomes during adolescence and adulthood.

On the other hand, diagnosis is critically important in childhood because children with early onset disorders are one of the major groups at risk for adult disturbances; often it is possible to trace the continuity between child and adult disorders. Continuity also facilitates the discrimination of treatment effects on the childhood disorders when examined over many years in the perspective of the “natural history” of other children who were not treated.

Finally, respect for heterogeneity and subgrouping within discrete diagnostic groups in childhood may increase the likelihood of recognizing identifying dimensions of behavior in the early history of a patient being examined as an adult; what did not initially look like a classical childhood disorder sometimes falls within a familiar spectrum for the diagnosti- cian who is aware of the significance of these behavioral dimensions.

**Diagnostic Assessment and Instruments.** All aspects of scientific investigation in pediatric neuropsychiatry depend on valid and reliable clinical assessment. A major thrust of the clinical investigation at Yale has been the refinement of the diagnostic process for children with severe neuropsychiatric disorders (Cohen 1976; Cohen, Caparulo, and Shaywitz 1976; Shaywitz, Cohen, and Shaywitz 1978; Shaywitz 1982). These activities have included the study of diagnostic reliability (Cohen et al. 1978a) and of the usefulness of various procedures involved in standard evaluation procedures, such as the electroencephalogram (Waldo et al. 1978), genetic screening (Lowe et al. 1980), and computerized tomography (Caparulo et al. 1981). Yale investigators have devised new instrumentation for assessment of children’s personality (such as the Childhood Personality Scale) which have achieved broad use in studies of normal and developmentally disturbed children (Cohen 1974; Cohen, Dibble, and Grawe 1977a, 1977b) and a series of rating scales which have been used for the assessment of children with developmental disorders and examination of the relation among dimensions of behavioral disturbance and biological variables. For example, in one study, children were rated on the Behavior Rating Instrument for Autistic and Atypical Children (BRIAAC), devised by B. Ruttenberg, and the Behavior Rating Scale, devised by the Yale investigators, and a high degree of intermethod agreement was found (Cohen et al. 1978a). These reliable behavioral measures were then related to CSF monoamine metabolite values in autistic and other neuropsychiatically disturbed children; important patterns were elucidated, relating behavioral competence to serotonin metabolite concentration (Cohen et al. 1980b).

In collaboration with the Clinical Methodology and Data Processing Unit of the Yale Mental Health Clinical Research Center, we created a new instrument for clinical and epidemiological studies of Tourette’s syndrome (Jagger et al. 1982). This instrument has now become a standard part of the clinical assessment of these patients, with good agreement between epidemiological and clinical data.

Sally and Bennett Shaywitz have developed a series of instruments for obtaining consistent information about children with neuropsychiatric disorders. These parent-, physician-, and school-related instruments are based on, and replace, a series of less sophisticated forms and are state-of-the-art instruments for obtaining and recording information about children’s family background, development, and current status.

Each child involved in the research protocols receives a thorough neuropsychiatric assessment and a general data base is created; depending on the specific diagnosis and protocol, additional information is added to the general data base. In the practice of neuropsychiatric diagnosis, no test or procedure can replace the detailed, lengthy, and repeated interview and examination of the child and family. In our experience, the initial evaluation usually involves 3–5 hours of assessment.
by a pediatric clinician, at the minimum. This face-to-face discussion, observation, and interview is supplemented by a variety of other sources of information: for example, parent forms; teacher ratings; psychological tests; neuromaturational examination; attentional measures; and neurological, metabolic, or physiological studies. The following sections describe both the general data base and the additional measures obtained for children involved in research protocols. It should be noted that the diagnostic assessment must be conducted with concern for the needs and strengths of the child and family. In some situations, the process can be completed over the course of several weeks; for other families, the pace must be slower and the procedures more limited.

Clinical interviews. An experienced pediatric clinician meets with the family and child to obtain a history of their difficulties, using an open-ended format with specific probe questions. The clinician assesses the child's gestation, delivery, first months of life, and motor, social, emotional, and cognitive development; areas of symptomatology; natural history of the disorder; responses to previous intervention; current treatment program; and family history and structure. The evaluation includes observation of the child in a free play situation, engagement of the child in structured play activities, and general assessment of cognitive, social, and emotional competence. The clinical interviews (3–5 hours) provide a general portrait of the child's development in the context of genetic, experiential, familial, educational, and social factors. Data from this evaluation include a narrative statement covering the various areas noted, a diagnostic formulation, a specific DSM–III (American Psychiatric Association 1980) diagnosis, and rating scale information.

Clinical rating scales. Accurate and precise assessment of behavioral dimensions occurring across syndromes is the fundamental requirement for a more rational clinical understanding of childhood disorders. The use of clinical rating scales has increased the orderliness of research and the multiaxial system is essential for understanding the complex relations between symptom patterns that may appear in various combinations and with differing degrees of severity. In previous research we developed methods for parental and clinical reports on children's competence (in areas such as sociability, attention, and language), parent-child relations, and family stress (Dibble and Cohen 1974; Cohen, Dibble, and Grawe 1977a, 1977b). These measures have been used with normal and developmentally disturbed children. In clinical research studies, we have developed clinicians' rating scales for the major dimensions of disturbance in childhood psychosis (Children's Behavior Rating Scale), and have performed first-phase reliability studies (Cohen et al. 1978). In current studies, children are involved in various structured and nonstructured tasks and their behavior is assessed using newly devised rating scales (e.g., Achenbach's Child Behavior Checklist) as well as measures which have achieved broad use in child psychiatry (such as the Connors symptom checklist). In addition, the Kiddie-SADS, a structured interview particularly appropriate for higher level children with affective disorders, may become an important aspect of methodology in protocols involving children with attentional, cognitive processing, and affective disturbances.

Parent reports. During the course of the first evaluation interviews, the family is asked to complete detailed forms about the child's history and current status and his specific involvement with academic institutions. These forms include the Yale Neuropsychoeducational Assessment Scales (Shaywitz 1982) and other scales.

Physical and neurological examination. All children receive a thorough general physical evaluation. Specific neurological examination includes assessment of the general neurological status of the child, a neuromaturational examination, and examination for minor physical anomalies. This battery of instruments has been validated with a cohort of normal and developmentally disturbed children (Shaywitz 1982).

Cognitive and language evaluation. The more severely impaired children are often unable to work with clinicians in unraveling the puzzles of their disorders. This led to the development of evaluation strategies which accurately portray the patients' cognitive, linguistic, social, and emotional functioning without requiring self-reporting by the patient. The most productive method of behavioral study has coupled clinical research interviews with home and school observations and individual sessions of psychological testing. The interviews demonstrate the child's spontaneous behavior in a situation which becomes increasingly familiar to him. They are repeated
on several occasions, with the child's family present in order to interpret the child's responses. After the child is maximally engaged, cognitive and language tests are selected according to the child's needs. The measures of general cognitive functioning are standardized and commonly used in clinical assessment, but the method of administration is modified in response to the child's capabilities. The assessment instruments include the Wechsler Preschool and Primary Scales of Intelligence, Wechsler Intelligence Scale for Children-Revised, and Arthur Adaptation of the Leiter International Scales of Performance (traditionally used for children with hearing impairments or severe motor impairments such as in cerebral palsy, and used by us with subjects with language disorders ages 2 years through adulthood).

It is necessary to supplement one or another of the above measures with additional psychometric assessment. The following are used to complement the basic intelligence battery or to provide a cross-validation of particular areas of functioning: McCarthy Scales of Intelligence, Yale Developmental Schedule (combines observation of object manipulation, motor skills, and object relations with testing of problem-solving abilities and language comprehension), and the Peabody Picture Vocabulary Test. Measures of language development include the Sequenced Inventory of Communicative Development, the Receptive-Expressive Emergent Language Scales developed by Bzoch-League (a detailed, 120-item inventory of chronologically sequenced questions filled out by parents for all children with language impairment), and the Illinois Test of Psycholinguistic Abilities.

The tests of cognitive, language, and perceptual-motor competence are supplemented by tests assessing academic achievement. Such tests, which are especially important in the assessment of children with attentional and cognitive processing disturbances, and in the evaluation of the impact of medication, include the Wide Range Achievement Test, Grey Oral Reading Test, and the Woodcock-Johnson Achievement Tests.

This group of cognitive, linguistic, attentional, and achievement measures yields both summative and process measures of children's underlying competence and performance. Measures are operationally defined, normalized, and quantitative. In addition, careful observation of children during the process of formal testing can reveal cognitive strategies, emotional responses, defensive operations, and overall approach to tasks and to the tester that can greatly enrich the observations of the less structured interviews.

**Behavioral measures of attention, perception, and movement.** The assessment of complex states such as attention is based on the characteristics of the group of children being evaluated. Pervasively disturbed children, who are often nonverbal and who have a restricted range of activities within which they can be cooperative, require discrete tasks preselected with their capabilities in mind. Other children have subtle impairments which are more readily elicited in a comprehensive, well-standardized battery of test items. Our range of behavioral assessment items is grouped into those that may be administered to all children, even the most profoundly disturbed, and a standardized test battery that can be used for normal and less severely impaired children.

1. Reaction time and continuous performance. Difficulties in focusing and maintaining attention are found in a variety of psychiatric disturbances in children and adults (Silverman 1964; Mostofsky 1970). Attention, arousal, and unusual sensitivities have been particularly prominent theoretical issues in the study of children with early onset psychoses such as childhood autism (Ornitz and Ritvo 1976). Detailed analyses of attentional processes in autistic and other severely disturbed children have been impeded by difficulties associated with the children's behavioral problems, such as hyperactivity and lack of cooperation. To assess arousal, attention, and perceptual processing, investigators have made use of a variety of nonverbal, physiologic, and behavioral measures.

Two types of psychological tasks which assess processes are reaction time measures and tasks which require continuous engagement. In reaction time studies, a child is presented with a stimulus (e.g., a tone, light, or particular geometric form) on the occurrence of which he must respond (e.g., press a button) as quickly as possible. Dependent measures include: latency to respond (usually in the range of 300 msec for normal children in response to a light); errors of omission (where child did not respond); and errors of commission (responses without the presentation of the stimulus). Various factors influence reaction time per-
formance, including initial motivation, habituation, training, presence of a warning, and nature of the stimulus. In continuous performance tasks (CPT), a child must perform an action (e.g., keep his finger on a moving knob or respond to a particular geometric shape when it is flashed on a screen) over long periods of time. Dependent measures are similar to those in reaction time which can be conceived as a type of CPT.

Care must be exercised when testing for attentional capabilities in children with severe neuropsychiatric disorders. The deficits of cognitive and social function in these children may mask the underlying attentional capabilities and deficits. In many cases it is necessary to train children on tasks used to test attentional competence. In order to make these studies possible, we have established a laboratory at Benhaven, our collaborating school for autistic and communication-impaired children, incorporating our studies into the daily curriculum of the children. In this fashion we are able to study children's reaction times to auditory, visual, and tactile stimulation repeatedly, thus obtaining data that are representative of a child's competence by taking into account day-to-day variation in performance. Also this type of training maximizes performance on the tasks during psychophysiological monitoring and enables a more refined analysis of the relationship between performance and attention.

2. General attentional, cognitive, and perceptual-motor tasks. Fine-grained, operationally defined measures of perceptual and attentional function are essential for clarifying diagnostic subgroups and the relations between domains of disturbances. As part of our multidisciplinary research activities, we develop new measures as well as use standardized tasks involving attention, perception, and motor control.

Tasks are selected to assess fine motor, gross motor, perceptual-motor, motor persistence, and cognitive competencies. They include novel devices we have developed (Continuous Performance Road Tracking task, Automated Sequencing task, Pinball task); subtests from the Wechsler Intelligence Scale for Children–Revised (Block Design, Mazes, Coding, and Similarities); and adaptations of other measures previously used in studies with children (Harcherik, Carbonari, and Cohen 1982). To assess “soft” or “minor” neurological signs, measures of motor impersistence, sequenced movements, laterality, and various gross motor tasks are used. Also included are RT measures, marble sorting tasks, and pegboard tasks; these are sensitive measures of fine motor or perceptual-motor maturity (Shaywitz, Cohen, and Shaywitz 1978; Harcherik et al. 1982).

Medical evaluation. Children with neuropsychiatric disorders may suffer from specific medical disorders that are etiologically related to their neuropsychiatric impairment. In addition, children with developmental disturbances may have other medical difficulties that have not been observed during the course of their standard medical care. Children involved in the Yale neuropsychiatric investigations routinely receive intensive medical evaluation. At times, this has led to interesting research observations. For example, we have found that many children with autism have elevated levels of blood lead, secondary to their mouthing of physical objects. Each child in the research protocols receives extensive laboratory screening of urine and blood samples. All children receive an electroencephalogram (EEG), with sleep deprivation when indicated, and computerized brain tomogram (ACTA scan). Additional biomedical evaluation is obtained, as indicated. For example, chromosomal studies are obtained on children with stigmata, including special banding techniques and culture for Fragile X syndrome which has recently led to the definition of a new biological subgroup of developmentally disabled and autistic children.

Specialists in pediatrics, human genetics, endocrinology, and neuro-ophthalmology provide further consultation.

School reports. Most children involved in the neuropsychiatric evaluations, except for the youngest children, are students in regular classrooms or special programs. Schools can provide rich descriptions of children's cognitive competence, since teachers have the opportunity to observe children for many hours daily and over many months. The Yale researchers are particularly fortunate in the close relationship between Yale and the major special schools and programs for developmentally disturbed children in the New Haven area. Senior Yale investigators are intimately involved as consultants with the major institutions serving the children in most of the protocols. The schools regularly provide investigators with detailed academic reports and progress notes; teachers also com-
plete specialized forms, such as the Parent-Teacher Questionnaire, Childhood Personality Rating Scale, Yale Child Behavior Rating, Yale School Report, and Tourette Syndrome List. Through detailed discussions with teachers and supervisors at the special programs, it is often possible to achieve greater certainty about the nature of a particular child’s difficulties (e.g., in the, at times, difficult discrimination between autism and central language disorders, or in the specification of the nature of a child’s attention difficulties).

Videotape recording. Videotape recording is a standard part of behavioral recording, supplementing and enriching the narrative account of a child’s mental status and current behavior (e.g., in Tourette’s syndrome); to assess inter-observer reliability in scoring behavior; to follow developmental changes and the impact of interventions such as medication; to review a series of patients during the process of data analysis (e.g., in developing rank orderings for a particular dimension of behavior); and to teach other clinicians about features of developmental psychopathology. All children in the research protocols are videotaped as part of their initial evaluation and then as indicated in specific protocols, often in standard situations: a simple interview with a familiar clinician; while engaged in a cognitive task (with an educator) at the child’s usual level of academic achievement; and during a meal or snack. These three situations allow assessment of a child during interpersonal interaction, while confronted with structured cognitive material, and when allowed to function independently.

Inpatient observation. Children admitted to the Children’s Clinical Research Center (CCRC) are intensively observed, providing rich behavioral information that extends data obtained from other sources. CCRC nurses are trained in behavioral observation and description, and are familiar with the range of neuropsychiatric disorders covered by the research protocols. The opportunity for intensive observation during inpatient evaluation may reveal unsuspected competencies (e.g., cognitive strategies in severely autistic children) or areas of difficulty (e.g., excessive anxiety about sleep or provocative behavior in children with attentional or tic syndromes).

Assessment of change.
Behavioral assessment and rating instruments have only recently attained general use in child psychiatry, and are particularly inadequate in areas that clinicians would emphasize as most significant when evaluating a patient, i.e., predominant emotions and their determinants, interpersonal interactions, social involvement, and function at work or school (Rapoport 1978). Productive research will be impeded until scales for these psychosocial parameters are developed. While measurement of change in areas of individual function is a fundamental necessity for clinical research, it presents additional problems, particularly in relation to the difficulty in distinguishing among simultaneously operative sources of change and in adapting measures to the differing questions arising from short- or long-term observation periods.

Changes in the status of a child’s disorder may simply indicate the process of development (e.g., the decrease in activity of attentionally disordered children moving into adolescence), reflect transient alterations in a child’s world (e.g., the exacerbation of chronic, multiple tics with emotional tensions near a holiday), or show the effects of specific treatment interventions (e.g., educational programming, behavior modification, psychopharmacological agents, parent involvement, or psychotherapy). It is often difficult to separate these factors as determinants of change in a group of indices. To assess change, some of the individual scales described above can be administered several more times, generating a repeated measures design which adds to the value of the studies, particularly when they involve a small number of subjects. Yet, the requirements of individual studies frequently do not permit this solution, either because of the nature of the source of the change (as above), or because there are radical differences in the time frame involved. For example, some protocols examine change over long periods of time (such as studies of the natural history of a disorder or metabolic changes with development, requiring 5- to 10-year intervals), while others reflect changes occurring over a few hours (such as the neurochemical and neuroendocrine response to stress). Existing scales can be used to delineate change in clinical parameters (e.g., the Timed Behavioral Rating sheet developed by I. Cohen and M. Campbell for psychotic youngsters), but basic work in the development of new scales is critically necessary.
Neuropsychiatric Research in Childhood: Biological Measures

Because clinical biological studies are conducted without the opportunity to examine brain tissue directly, alternative strategies for coping with this limitation are required, including (1) studies on CSF metabolites of central amines, which provide the closest approximation of a sample of the biochemical function of the human brain now possible in the clinical situation, and (2) assessments of brain activity through measurement of each of the effector arms of the brain (the somatic muscular system, the autonomic nervous system, and the neuroendocrine system). While the somatic arm of the nervous system is invariably studied in psychiatry through behavioral measures and observations, attention to the other effector arms is more recent. The sympathetic nervous system (SNS) is a responsive indicator of "state" in behavioral studies, so that it is an especially informative measure in investigation of the impairments in the regulation of attention, arousal, anxiety, and general emotional state characteristic of many severely disturbed children. It can be monitored through a variety of psychophysiological indices (e.g., cardiovascular correlates) and through biochemical compounds associated with postganglionic neurotransmission. Biochemical investigation of SNS function is centered on the catecholamines, the postganglionic sympathetic neurotransmitter, and on compounds involved in their synthesis (e.g., dopamine-β-hydroxylase; DBH) and modulation (e.g., thyroid hormones).

The concept of the neuroendocrine system as an effector arm of the brain is derived from a convergence of evidence from clinical, morphological, and embryological studies (Pearse 1968; Mason 1975; Pearse 1977). Classical distinctions between the endocrine and nervous systems have been blurred as their common neuroectodermal origin and functional and morphological similarities have been clarified. Since cellular function in both systems typically involves secretion to external fluids and cells, the transport distance of a specific endocrine compound has been a basis for the distinction of the classical endocrine system (in which blood-borne hormones travel relatively long distances) from the nervous system (where the "local circuitry" of electrical and chemical information exchange may take place across the minute distances of opposed dendritic surfaces). From the vantage point of clinical neuroendocrinology, the distinction is not critical and the fullest examination of brain function includes the fluctuating activity of neuroendocrine systems. This triad of the effector arms of the brain (somatic-behavioral, autonomic, and neuroendocrine) is used as an organizing perspective in clinical biological studies.

Cerebrospinal Fluid Metabolites

Biogenic amine metabolism in the human brain can be studied by assay of CSF for the major metabolites of dopamine, serotonin, and norepinephrine (homovanillic acid, HVA; 5-hydroxyindoleacetic acid, 5-HIAA; and 3-methoxy-4-hydroxyphenylethylenglycol, MHPG, respectively) (Bowers 1972; Van Praag et al. 1975). The biogenic amine metabolites are excreted from the brain into the CSF, where their concentration is thought to reflect the state of the parent amines, or their turnover rate, in the brain. Diseases and medications alter CSF metabolite levels.

There are several methodological problems, including the need for lumbar puncture, differences between ventricular and lumbar CSF, possible moment-to-moment variability, and differences in contribution to CSF from various brain areas (Van Praag et al. 1973; Garelis, Young, and Sourkes 1974). Some of these problems have been overcome through the use of the probenecid method. For 10–12 hours before lumbar puncture, patients are given probenecid, a benzoic acid derivative which inhibits membrane transport and blocks the egress of the acid metabolites from the CSF. This produces increasing levels of these metabolites and a more accurate reflection of brain metabolism over a more representative period of time. We have determined the range of CSF metabolites in children with various neurological and neuropsychiatric disorders, such as autism, aphasia, cognitive processing disturbances, epilepsy, and nonautistic psychoses (Shaywitz, Cohen, and Bowers 1975; Cohen et al. 1980b). CSF cannot be obtained for study from normal children. Instead, it has been necessary to contrast one diagnostic group with another and to use children suffering from various other conditions in which a lumbar puncture (LP) and examination of CSF are indicated (e.g., headache and disc disease). In our methodology,
probenecid is administered orally in four divided doses over 10–12 hours, for a total dose of 125–150 mg/kg. A lumbar puncture is performed between 8:00 a.m. and 9:30 a.m., and the second or third 5 cc aliquot is immediately frozen for assay of metabolites and probenecid (Bowers 1972; Korf and Van Praag 1971; Cohen et al. 1974, 1977; Anderson, Young, and Cohen 1979; Anderson et al. 1981b). CSF is also examined for cells, protein, glucose, immunoglobulins, and folate.

Studies with adults indicate that membrane blockade becomes effective when a substantial amount of probenecid has been ingested (usually 100 mg/kg orally over 18 to 20 hours), or when CSF probenecid levels above 20 µg/ml have been achieved (Tamarkin, Goodwin, and Axelrod 1970). However, interpretation of metabolic data without actual measurement of CSF probenecid may be misleading because of the relationship between metabolite concentrations and CSF probenecid levels at lower CSF probenecid concentrations (Sjostrom 1972). In a study of 43 children, the levels of metabolites were highly correlated with the levels of probenecid achieved (Cohen et al. 1977).

Special methods of data transformation have been used to take into account the metabolite-probenecid relation. The amine metabolites may be statistically adjusted by analysis of covariance, or the metabolites may be expressed as a ratio to probenecid (nanograms of metabolite per micrograms of probenecid), which we designate as the HVA/P or 5-HIAA/P ratio (Cohen et al. 1974, 1977). The importance of measuring probenecid and relating the monoamine metabolites to the levels of probenecid achieved in the CSF has recently been reinforced by a report of observations similar to ours in adult patients (Berger et al. 1980).

Without probenecid loading, the concentrations of the two major metabolites of dopamine and serotonin (HVA and 5-HIAA) in autistic children are low, closely clustered, and within the range roughly defined for adults. Determined in six autistic boys (ages 6–15), HVA ranged from 45 to 100 ng/ml (mean ± SE = 65 ng/ml ± 7.7), and 5-HIAA ranged from 36 to 60 ng/ml (mean ± SE = 41.2 ng/ml ± 4.2). Following 10–12 hours of oral probenecid, the accumulations of amine metabolites increased significantly. The acid metabolites were correlated with the levels of probenecid achieved in the CSF. In a study of 34 other neuropsychiatrically impaired children using probenecid loading, the correlation between HVA and 5-HIAA was .57 (p < .001). The relation of each metabolite to CSF probenecid was statistically significant: probenecid and HVA, r = .47 (p < .001) and probenecid and 5-HIAA, r = .50 (p < .001). For this population of autistic and other neuropsychiatrically disabled children, the major difference between diagnostic groups was a reduced level of CSF 5-HIAA accumulation in the autistic children as compared to the age- and sex-comparable, nonautistic, early onset psychotic children (Cohen et al. 1977). This finding may be related to differences in the severity of the disorders, since the autistic children were more pervasively afflicted.

The functional relations between HVA and 5-HIAA may differ among diagnostic groups, reflecting differences in the balance between the parent neurotransmitter systems. To assess this relation, regression curves may be compared or a ratio of 5-HIAA/HVA may be constructed both for individuals (within a group) and for diagnostic groups. This ratio may be especially important in light of the different roles played by serotonergic and dopaminergic mechanisms in the organization of behavior. For example, the serotonergic midbrain raphe system may subserve sensory modulating or gating functions, while the dopaminergic system appears to be involved in motor activation and arousal (Singer et al. 1978). As will be discussed, the ratio of the metabolites of these two systems may be related to aspects of behavioral disorganization.

Most studies of adult schizophrenia and depressed patients using the probenecid method have reported a ratio of 5-HIAA/HVA between .5 and .7. For autistic children, the 5-HIAA/HVA ratio is at the lower end of the adult range. This may reflect, in part, the relatively higher HVA levels observed during childhood and a negative relation between CSF dopamine metabolites and age.

CSF metabolite concentrations span a considerable range within the autistic and nonautistic early childhood psychosis groups, and the detection of between-group differences is difficult. Thus, we have used alternative strategies which (1) delineate subpopulations within diagnostic groups and (2) correlate metabolites with dimensions of behavior. In studies of childhood psychosis, we have scored dimensions such as lan-
guage comprehension and expression, activity, movement abnormalities, and social relatedness, using rating scales completed by clinicians, parents, and teachers (Cohen et al. 1980b). One subgroup within the autistic population was found to have elevated levels of CSF HVA, both absolutely and in comparison with 5-HIAA (as reflected in 5-HIAA/HVA ratios). This subgroup was behaviorally distinguished by the greatest degree of stereotypic, repetitive behavior (flapping, twirling, finger flicking, and the like) and locomotor activity, and was overall the most severely afflicted group.

Correlations between CSF metabolites and ratings of behavioral dimensions, completed by clinicians familiar with all aspects of the child's history and condition and by other clinicians "blind" to everything but current behavior, have suggested hypotheses about serotonin and dopamine metabolites and the organization of behavior. For example, for 10 autistic children, HVA/P was negatively correlated with behavioral ratings of social responsiveness and attention: the ratio of 5-HIAA/HVA was also highly correlated \( r = .97, p < .001 \) with these behavioral ratings. Autistic children with higher competence had higher levels of 5-HIAA and lower levels of HVA in their CSF. For 33 neuropsychiatically handicapped children, social responsiveness and attention were positively related to 5-HIAA \( r = .31, p = .08 \) and 5-HIAA/log P \( r = .39, p = .03 \). Thus increased serotonin turnover, as assessed by measurement of metabolite concentration following probenecid, was associated with a lesser degree of impairment in social and attentional functioning in autistic and neuropsychiatically disturbed children. In recent studies with medical pediatric patients, repeated examinations of CSF without probenecid loading suggest that metabolite concentration may be a relatively stable, individual characteristic (S. MacIntosh et al., unpublished observations). The availability of reliable and sensitive HPLC methods for assaying metabolites and the use of various neuropharmacological approaches, in which CSF metabolites are the dependent variable, may reduce the need for probenecid loading for certain types of clinical studies. The use of probenecid provides the advantages described above: the investigation of CSF metabolites without probenecid will reduce the nausea and methodological difficulties related to probenecid. Continuing research will indicate which approach is best in a particular study.

While CSF MHPG levels cannot be determined in normal children, because of ethical constraints on performing a lumbar puncture, they might be measured in essentially normal contrast groups (children worked up for recurrent headaches, dizziness, etc., with no abnormal neurological or laboratory findings). When CSF MHPG levels of adult neuropsychiatric patients were used for comparison, the CSF MHPG levels of six medication-free autistic boys were normal. Recent data on pediatric medical patients have produced consistent findings, with MHPG ranging from 7 to 12 ng/ml. CSF MHPG measured in six children with Tourette's syndrome of chronic multiple tics (TS), each of whom either received probenecid loading or recently discontinued a medication, were distributed into two groups. Four patients were within the "normal" range, while two TS patients had increased CSF MHPG levels (near 15 ng/ml). The two patients with increased CSF MHPG levels suggest a subgroup of Tourette's syndrome patients with increased noradrenergic activity, perhaps responsive to treatment with agents which reduce central noradrenergic activity, such as clonidine (Young et al. 1981b).

The early-onset, pervasive behavioral disturbance and the high incidence of seizures and other neurological findings in children with autistic-like syndromes suggest that this syndrome might sometimes be caused by a congenital or early viral syndrome. Further support for this hypothesis is the high incidence of autistic-like syndromes in children with congenital rubella (Chess 1971; Fish and Ritvo 1979). CSF protein, immunoglobulins, and colloidal gold were measured in autistic children without neurological findings and were found to be normal (Young et al. 1977). Since this study, similar measures have been obtained in other children with primary and secondary autistic syndromes with negative results. The failure to detect abnormal CSF immunoglobulins does not rule out an etiological role for a slow-acting virus. However, the progressive improvement observed in some children with autism and the absence of other findings of CNS degeneration are unlike other disorders caused by slow virus infections.

Folate and vitamin B₁₂ metabolism were examined in more than 60 children with childhood neuropsychiatric disorders, including 20
children with primary childhood autism. Folate in CSF, serum, and red blood cells, and B12 in serum, were normal in autistic patients, other neuropsychiatric patients, and normal controls. The findings also suggested that there is an active transport system regulating CSF folate independent of blood levels, except in cases of extreme folate deficiency (Lowe et al. 1981).

In summary, CSF metabolites in the serious, early-onset neuropsychiatric disorders of childhood show great variability within each diagnostic group, with no major differences between groups. However, there are relations between CSF metabolites and specific dimensions of behavioral impairment; in general, following probenecid administration, more disorganized children have relatively lower concentrations of CSF 5-HIAA and higher concentrations of CSF HVA.

**Sympathetic Nervous System**

**Psychophysiological Measures.** Difficulties in focusing and maintaining attention are found in a variety of psychiatric disturbances in children and adults (Silverman 1964; Mostofsky 1970). Attention, arousal, and unusual sensitivities have been particularly prominent theoretical issues in the study of children with early onset psychosis, such as childhood autism.

Detailed analyses of attentional processes in autistic and other severely disturbed children have been impeded by difficulties associated with the children's behavioral problems, such as hyperactivity and lack of cooperation. To assess arousal, attention, and perceptual processing, investigators have made use of a variety of nonverbal, physiological measures. These have included EEG, event-related potentials (ERP), and elicitation of vestibular nystagmus. Cardiovascular indices of psychophysiological processes have not been so intensively studied with children suffering from neuropsychiatric disturbances as they have in normal infants and adults.

In adults, heart rate, blood pressure, and respiratory rhythm have been used to assess general arousal and anxiety. Measures of peripheral blood flow are particularly sensitive for psychophysiological correlation with emotional and attentional states (Whitney 1953; Matthews and Lader 1971). Blood flow to muscles can be assessed by measuring the increase in the volume of an extremity when venous flow is occluded and arterial flow remains unimpeded (venous occlusion plethysmography). Using various plethysmographic methods, studies have demonstrated that threats, fear, imagined scenes, and a difficult mental task all may lead to vasodilation and increased muscle blood flow.

Peripheral blood flow changes may be associated with more specific aspects of attention (Williams et al. 1975). During tasks which elicit an external direction of attention, such as reading a blurred word on a screen, individuals show decreased forearm blood flow and increased resistance. With internal direction of attention (e.g., mental arithmetic) and sensory rejection, there is increased forearm blood flow and decreased resistance (Williams et al. 1975). These indices have been correlated with biochemical indices of catecholamine metabolism.

The sensory intake-sensory rejection hypothesis is quite relevant to children with disturbances of attention (such as in childhood autism). We have performed the first systematic studies of peripheral blood flow and resistance in normal and disturbed children during various attentional tasks (Cohen and Johnson 1977). Normal children responded like normal adults to attentional demands: increased flow with inner direction to attention and decreased flow with external direction of attention. Autistic children were markedly abnormal in two respects: their baseline levels of flow were significantly increased, and they failed to show task-related changes in their cardiovascular indices of attention (Kootz and Cohen 1981). These findings were interpreted as consistent with a persistent state of sensory rejection and increased arousal. When young adults were studied during two types of interviews (stressful and nonstressful) and various sensory intake and rejection tasks (Kootz, Gold, and Cohen 1979), the most salient parameter relating to cardiovascular indices was the degree of mental effort that was required, as hypothesized by Pribram and McGuinness (1975).

Autistic children who were higher functioning intellectually acquired a reaction time task, while lower functioning children failed to learn the task even after 25 practice sessions. The higher functioning children displayed the normal pattern of cardiovascular...
correlates of external direction of attention (Kootz, Marinelli, and Cohen 1982); the lower functioning children did not. Autistic children as a group appear to be in a state of increased arousal and relative sensory rejection (see below). When they are able to acquire a task that requires attention to the external world, however, they show the normal accompaniment of cardiovascular correlates.

Several studies have been done to evaluate the cardiovascular correlates of attention in autistic children. In the first of these studies, 14 autistic children were compared with 16 controls during conditions of rest, social interaction, and a reaction time task. The autistic children had a significantly higher resting heart rate; the resting mean blood pressure was also elevated in the autistic group, but not to a significant degree. These findings suggest increased autonomic nervous system activity in autistic children. However, in the interview and reaction time situations, the autistic children did not display the significant changes in heart rate, mean blood pressure, peripheral blood flow, and peripheral vascular resistance that were seen in the normal control group. These findings are consistent with theories of a heightened state of sensory rejection in autism (Kootz and Cohen, in press).

A second series of experiments was designed to assess the hypothesis that autistic children prefer proximal (touch, taste) to distal (sight, hearing) sensory modalities. Autistic children were compared to control children by analyzing response times to auditory, visual, and tactile cues. Response times of the autistic children were significantly slower than those of the control subjects and were significantly related to mental age within the autistic subgroup. However, the autistic children had the same pattern of response as the normal children, responding most rapidly to auditory stimuli, followed by visual and tactile stimuli in that order. This suggests that autistic children’s preference for proximal stimulation reflects a continuation of immature behavior, rather than a basic disturbance in sensory receptor sensitivity (Kootz, Marinelli, and Cohen 1981).

**Blood and Urinary Indices.** Various biochemical measures can be assessed as indices of sympathetic nervous system (SNS) function, including catecholamines, DBH activity, and thyroxine indices. Assessment of SNS activity by multiple techniques is particularly important in the childhood psychoses, since “vasovegetative” disturbances (presumably representing autonomic dysfunction) have been reported in clinical studies for many years.

**Plasma and urinary catecholamines.** Catecholamines are present in body fluids in very small quantities because of the processes of immediate reuptake and metabolism by monoamine oxidase (MAO) and catechol-O-methyltransferase (COMT); catecholamines in the plasma are measured in picogram per ml amounts and subject to rapid fluctuation. Older fluorometric assay methods did not achieve a level of sensitivity and accuracy sufficient for analyzing such clinical specimens as plasma and CSF. Radioenzymatic assays are now available which are less complicated but very sensitive and reliable, enabling the assay of a greater number of samples in a shorter period of time. An alternative method, which does not require the use of radioisotopes, couples ion exchange liquid chromatography (LC) and electrochemical detection (Hallman et al. 1978). We have established this LC method in our laboratory because of its reliability, efficiency, and sensitivity.

The measurement of plasma norepinephrine (NE) has been used as a method for the evaluation of normal sympathetic function; the increment in plasma NE (over basal levels) has been assessed while using such standard stresses of the SNS as postural change, exertion (isometric hand grip), and the cold pressor test. These indices have been applied to the investigation of patients with autonomic dysfunction, contributing to the delineation of subgroups of patients with primary orthostatic hypotension (Lake, Ziegler, and Kopin 1976; Ziegler, Lake, and Kopin 1977), and demonstrating the importance of age correction in comparative studies of plasma NE between normotensive and hypertensive groups (Ziegler, Lake, and Kopin 1976; Lake et al. 1977). The use of plasma NE as an index of sympathetic function rests on the assumption that the plasma levels are derived essentially from the postganglionic sympathetic nerve endings, while it is well known that NE is also secreted from the adrenal medulla (Louis, Doyle, and Anavekar 1973; Lake, Ziegler, and Kopin 1976; Taylor et
al. 1978). It has been suggested that there is a differentiation in the functional source of NE because under normal physiological conditions the SNS is the major source of plasma NE, while, when there is a stress present, the adrenal contribution to plasma NE increases (Popper, Chiuhe, and Kopin 1977). A careful study was performed in which the stress component was minimized while using conscious, unrestrained rats; although plasma NE does have a dual origin, at baseline levels of functioning the primary source of plasma NE was the sympathetic nerve endings (Micalizzi and Pale 1979). When there is an increase in plasma E levels, this seems to be an indication that the adrenal contribution to plasma NE levels has also increased and might no longer be an accurate index of SNS function (Peuler and Johnson 1977; Landsberg and Young 1978; Micalizzi and Pale 1979).

The marked responsiveness of plasma catecholamines to psychological and physical stimuli requires that blood collection procedures be meticulously standardized to minimize the effects of venipuncture, posture, and general environmental stimulation. Basal NE levels are obtained while the subject rests supine in a quiet room, at least 20 minutes after venipuncture. A standard assessment of the integrity and responsiveness of the SNS appears to be most satisfactorily achieved by obtaining the basal plasma NE level, then adding two additional increments of physical stress; sampling after standing for 5 minutes, followed by squeezing a hand dynamometer for 5 minutes at 30 percent of maximal effort.

The tilt test, cold pressor test, and heavy work on a bicycle ergometer were not found to be so useful as the above strategy (Lake, Ziegler, and Kopin 1976; Kopin 1979).

Normetanephrine (NMN) is a major metabolite of NE and appears to reflect the degradation of newly synthesized and released NE; it is produced by O-methylation at a rate proportional to nerve stimulation, and is derived from both adrenal and peripheral sympathetic sites. Plasma NMN levels are 20–25 percent of plasma NE levels in man, and the two vary in parallel in response to suppression or activation of sympathetic nerve activity. The significant correlation of plasma NE and NMN indicates that plasma NMN levels can be used as an index of sympathetic function (Kobayashi et al. 1980).

A simple, sensitive, reliable method (using high performance liquid chromatography with fluorescent detection) is available for the determination of urinary dopamine (DA), norepinephrine (NE), and epinephrine (EPI) (Anderson et al. 1981a). This is of special value in pediatric research because venipuncture and the relatively large amount of blood required for serial catecholamine (CA) samples can be avoided when caring for small children. For example, in a study using urine samples collected over periods of only an hour or more, urinary CA levels were assessed in relation to performance measures in children and adolescents. In general, higher levels of CA excretion were associated with better performance in terms of speed, accuracy, and endurance. Similarly, in relation to enduring psychological characteristics, increased EPI excretion was associated with higher IQ and school achievement and greater "ego strength" and "emotional stability" (Frankenhaeuser 1975). These interesting studies require replication and extension.

Our initial studies of 24-hour urinary free catecholamines in autistic boys indicated significantly reduced levels in the group with severe cognitive and adaptive impairments. In more recent studies of urinary CA levels, we are examining DA, NE, and EPI separately, and using 1- to 8-hour collection periods in order to establish a procedure which will be more responsive to the short-term influences which the CA may be particularly well-suited to detect. Plasma catecholamines are being measured in challenge studies with clonidine and methylphenidate, as described below.

**Plasma and urinary CA metabolites.** Several research groups have studied brain NE metabolism in children by measurement of MHPG, the major metabolite of NE in the brain (Maas and Landis 1968; Schanberg et al. 1968), based on the possibility that levels of MHPG in body fluids would provide an index of noradrenergic activity (Korf, Aghajanian, and Roth 1973; Maas et al. 1977, 1979; Crawley et al. 1978; Crawley, Maas, and Roth 1979; DeMet and Halari 1979).

Autistic boys have reduced 24-hour urinary MHPG levels when compared to same-aged normal boys also studied as inpatients (Young et al. 1979). Similar urinary volumes and creatinine excretion in the two groups indicate that the difficult urine collections from these children were complete. The reduction in MHPG excretion is
not related to activity level, since most autistic patients were very active even while hospitalized.

Simultaneous measurement of free catecholamine excretion in these autistic children showed them also to be reduced in comparison to the control group (Young et al. 1978). The method for the determination of urinary free catecholamines measures NE and EPI, but not DA. Free NE accounts for 80–90 percent of the free catecholamines measured (Crout 1961). While free catecholamines are predominantly derived from peripheral noradrenergic activity, two points suggest that this measure could be useful in studies of infantile autism. First, disturbances in attention and arousal can be monitored through cardiovascular indices of sympathetic function and might be correlated with peripheral concentration of NE, the sympathetic neurotransmitter. Cardiac correlates are altered in autism (Cohen and Johnson 1977). Second, there is reason to expect a correspondence between urinary levels of free NE and MHPG because the locus ceruleus may exert a regulatory influence on peripheral sympathetic function through extensive pathways to the brainstem and spinal cord. In this sense, even peripherally derived MHPG is responsive to prevailing central noradrenergic activity (Crawley, Maas, and Roth 1980; Crawley, Roth, and Maas 1979).

The disordered regulatory mechanisms in autism (including repetitive stereotypic movements, rushes of anxiety, impaired attention and arousal, and hyperactivity in many children) led to the anticipation of increased noradrenergic function in autism. The unexpected reduction might reflect a developmental vulnerability or a response to persistent stress and anxiety, but its actual origin, and whether it is primary or secondary, needs further study (Young et al. 1978).

Although 24-hour urinary MHPG levels have not been measured in mentally retarded children, the levels in seven adults with Down syndrome were not different from levels in controls; 24-hour urinary HVA levels were significantly reduced in Down syndrome patients (Mann et al. 1980). These results suggest that urinary MHPG levels are not simply reduced in all severe neuropsychiatric disorders of childhood, but might be the basis for discrimination among some groups.

Plasma free MHPG levels of normal boys were within the "normal" range for adults. Autistic and TS patients who had not received any medication for a month or longer also had plasma MHPG levels in this range. However, among seven TS patients taking medication, two had increased plasma free MHPG levels; each had discontinued a medication active at the dopamine receptor 3 weeks before venipuncture (pimozide and haloperidol) (Young et al. 1981b). Elevated plasma free MHPG in these children might indicate a subgroup of TS patients with impaired noradrenergic function or might be drug induced. Increased plasma free MHPG levels in these patients could be related to an interaction of the NE and DA neuronal systems, secondary to a supersensitive DA receptor following withdrawal of an agent active at the receptor. In recent studies in our laboratory, baseline levels of plasma and urinary MHPG appear to be normal in TS.

A strong correlation between plasma free and urinary total MHPG levels measured simultaneously in eight normal boys suggests that noradrenergic function may be sufficiently stable to be reflected in both plasma and urinary measures (Young et al. 1981b). This is in contrast to the lack of correlation between plasma free MHPG and urinary total MHPG observed in nine adult psychiatric patients, although an "outlier" may have confounded this potential relation (Sweeney et al. 1980). In studies of normal adults, significant positive correlations between (1) plasma free or conjugated MHPG and CSF total MHPG (Jimerson et al. 1981) and (2) urinary total MHPG and CSF free MHPG (Maas et al. 1982) indicate that there might be a correspondence among MHPG levels in the three body fluids. The small number of subjects in these studies, developmental changes in the pattern of NE secretion and metabolism, sampling inconsistency due to the secretion of free MHPG into the plasma in pulses (rather than continuously), the conversion of MHPG to VMA in the periphery (Blombery et al. 1980), and the underlying disease in the patient group are among the factors that might contribute to discrepancies. The number of patients with simultaneous MHPG determinations in two or three body fluids must be increased, and specific conditions affecting their relations studied, before the relations among these measures can be established.

Serum DBH. Dopamine-β-

1Personal communication.
hydroxylase (DBH) catalyzes the conversion of DA to NE, and its release from sympathetic nerves is proportional to that of NE (Weinshilboum and Axelrod 1971). It has not proved to be the simple indicator of SNS activity first hoped for, and its clinical usefulness is now controversial. Animal studies have indicated that serum DBH is increased by such sympathetic stresses as forced immobilization, acute swim stress, or the cold pressor test (Lamprecht, Williams, and Kopin 1973; Wooten and Cardon 1973). Its sympathetic origin has been demonstrated by the lack of effect of adrenalectomy on serum levels, as contrasted to the reduction of serum DBH following destruction of sympathetic nerves with 6-hydroxydopamine or disease (Weinshilboum and Axelrod 1971; Noth and Mulrow 1976; Cubeddu et al. 1979). However, DBH is not sensitive to other stresses of the sympathetic system, the changes in DBH are quite small relative to the stress when it does elicit an increase, and there is no relationship between prevailing blood pressure and DBH (Goldstein et al. 1974). Although the enzyme is quite stable for an individual with repeated measures over a period of months, there is wide variation (in the range of 100-fold) in serum DBH activity among adults with apparently comparable and normal sympathetic function. Further difficulties in the interpretation of serum DBH activity concern a variety of technical problems in its measurement, such as the presence of endogenous inhibitors and difficulties with each of the several assays in use.

The broad range in serum DBH activity in the normal population reflects the strong genetic component influencing serum DBH activity (Weinshilboum et al. 1973), so that interpretation of measurements between groups must be cautious; repeated longitudinal measures of activity in the same individuals will provide helpful information. Serum DBH has been measured in a variety of medical illnesses, such as hypertension (Reid and Kopin 1974; Laduron 1975; Schanberg and Kirshner 1976), with conflicting results and disputed interpretations as to their meaning; it has also been measured in several psychiatric populations: affective states (Shopsin et al. 1972); psychotic states (Wise and Stein 1973); Down syndrome (Coleman et al. 1974); childhood autism (Goldstein et al. 1976; Lake, Ziegler, and Murphy 1977; Young et al. 1980); and hyperactivity syndrome (Rapoport; Quinn, and Lamprecht 1974).

It has been suggested that most DBH present in the serum is not the product of exocytotic release from sympathetic nerve terminals, but is derived from continuous "shedding" of membrane bound DBH. This hypothesis would fit the lack of correlation between sympathetic neuronal activity and serum DBH turnover, as well as the reduction in serum DBH activity following damage to the SNS (Grzanna and Coyle 1978). Obviously, it also represents a further challenge to our understanding of the significance of serum DBH activity, as it suggests serum DBH activity is not an index of SNS activity.

On the other hand, studies have indicated that CSF DBH more accurately reflects the synaptic release of DBH, and that CSF DBH and serum DBH are derived from different pools. For example, the expected changes in CSF DBH activity following treatment with phenoxybenzamine (an increase) and clonidine (a decrease) are not accompanied by a parallel alteration in plasma DBH activity. This suggests that CSF DBH activity may reflect central noradrenergic activity (Lerner, Dendel, and Major 1980). In a preliminary clinical examination of this possibility, low DBH activity in CSF was related to an elevation of a specific profile on the Minnesota Multiphasic Personality Inventory characterized by "a general distrust of people, a suspicious questioning of their motives, and a fear of emotional involvement with others in spite of an exaggerated need for attention" (Major et al. 1980, p. 309).

A dissociation between plasma NE concentration and plasma DBH activity in clinical studies emphasizes the difficulties encountered when attempting to use plasma DBH activity as an index of SNS function. Assessment of SNS function in Down syndrome subjects replicated the finding of reduced plasma DBH activity, but the plasma NE levels were significantly elevated (Lake et al. 1979).

In general, DBH has given way to the plasma catecholamines as a sensitive and reliable biochemical estimation of sympathetic activity. However, the longer half-life of DBH raises the possibility that it could reflect sympathetic activity in a manner different from the immediately responsive plasma catecholamines; for example, it might be hypothesized that plasma DBH mirrors the cumulative effect of a longer period of sympathetic activity and provides a measure complementary to that of the plasma catecholamines.

Sympathetic effects on the
cardiovascular system are under the influence of central mechanisms, so that to this degree serum DBH activity may reflect CNS function. Treatment directed toward CNS mechanisms, such as psychotherapy, has shown a beneficial effect on mean blood pressure which is accompanied by a reduction in serum DBH (Stone and DeLeo 1976).

Our initial studies of serum DBH activity have replicated the wide distribution of DBH activity in psychiatric patients and normal controls reported by others, determined its relation to age during childhood, indicated the relative levels in several diagnostic categories, demonstrated familial clustering, and indicated that it is not related to thyroid function in euthyroid psychiatric patients and controls (Young et al. 1980). These results are further evidence that findings in clinical studies using serum DBH activity must be interpreted conservatively until this measure is better characterized.

**Thyroxine indices.** The interaction between thyroid hormones and the SNS has long been apparent to clinicians. These observations have been complemented by research specifying points of metabolic relations. For example, we have reported a negative relationship between CSF HVA levels in psychotic children and serum thyroxine measured at the time of the lumbar puncture (Cohen et al. 1974). Thyroid hormone has profound effects on brain development and function, and its possible role in childhood psychoses was indicated by the clinical observation that triiodothyronine (T3) has beneficial effects when administered to psychotic children (Sherwin, Flack, and Stokes 1958; Campbell and Fish 1974). The basis for the possible therapeutic effect of T3 in severely disturbed children is not known, but several lines of evidence suggest that thyroid-catecholamine interactions might play a role. We have conducted investigations of thyroid hormone in autistic children (Cohen et al. 1980), thyroid influences on enzymes related to catecholamine function (Young et al. 1982a, 1982c; Young, Kyprie, and Cohen 1982), and on age-related changes in serum thyroxine indices (Young et al. 1982b).

**Neuroendocrine System**

**Conceptual Issues.** The close relation of neuroendocrine regulatory centers in the hypothalamus to other brain areas makes this system ideally suited as an indicator of fluctuating brain activity. Fundamental physiological relations within and among these systems have now been established, so that pathological deviations in function are more readily identifiable.

While there has been technical and conceptual progress in neuroendocrinology, there has been corresponding amplification of the complexity of functional systems, and the means required for adequate investigation of specific components. For example, basal levels might be normal, while dynamic stimulation and suppression studies reveal impairment; dissociated neuroendocrine responses to apparently unitary phenomena, such as stress, require careful definition of experimental variables; unanticipated changes in hormone levels in a direction opposite to that expected reflect the impact of one hormonal system on another; attempts to clarify normal and abnormal neurotransmitter function through pharmacological intervention with neuroendocrine measures are confounded by multiple neurotransmitters affecting the same hypothalamic cellular system, and the same transmitters acting on multiple neuronal systems, in the hypothalamus and throughout the brain. Only precise, systematic investigations will clarify aspects of neuroendocrine function in normal and impaired children. Our overall strategy for neuroendocrine investigation reflects the awareness that a profile of neuroendocrine response to a specific stimulation gives a more informative picture of possible stress/endocrine associations.

**Neuroendocrine and Neurochemical Challenge Tests.** Clinical assessment of neuroendocrine function in individual patients is often accomplished by measurement of a body fluid hormone at a single time point, recognized as an essentially static evaluation technique. Standard challenge tests for evaluation of human endocrine function are well tested in clinical medicine. This "dynamic" approach uses specific stimulatory or inhibitory compounds to probe for altered responsiveness in a hormonal system. Specific examples are L-dopa, propranolol, and insulin tolerance tests of growth hormone and prolactin, thyrotropin releasing factor stimulation of thyroid-stimulating hormone, and metyrapone inhibition of adrenocortico-steroid compounds.

One weakness of some clinical tests is the nonphysiological level of stimulation or inhibition used. The pharmacological stimulation of a neuroendocrine system through doses hundreds or thou-
sands of time greater than the physiological fluctuation of naturally occurring compounds limits the potential of the test to an absolute “present” or “absent” assessment of the overall competence of the system. Most classical tests give no indication of the system’s responsiveness within the usual range of variation when reacting to typical physiologically activating events. In order to measure modulatory competence and thresholds of neuroendocrine response, a range of stimulus intensities must be incorporated into the dynamic tests. Contemporary studies of neuroendocrine mechanisms require less severe, graded stimuli, capable of defining response thresholds and charting stimulus intensity-response curves for each neuroendocrine axis.

Challenge tests can be conceptually organized in several ways. The usual framework has been categorization of the challenge by the postulated anatomical locus of its principal effect. This is often done by designation of the hypothalamic-pituitary-target organ axis which is affected by the challenge; e.g., challenge of the pituitary by thyrotropin-releasing hormone or challenge of extrahypothalamic brain areas by psychological stress (Brown and Seggie 1980). Organization of challenge tests according to the hypothesized cellular locus of action of a pharmacological probe refers to actions on processes related to neurotransmitter function at the synapse; e.g., neuropharmacologic actions principally affecting synthetic enzyme function, the reuptake process, or the postsynaptic receptor (Brown, Friend, and Chambers 1979).

A range of other strategies incorporating a challenge test exist; while clinical investigators typically use them in an overlapping fashion, it is useful to separate them on a conceptual basis (table 1).

The fundamental strategy is the use of one drug as a (hypothetical) probe of a single hypothalamic neurotransmitter system, monitoring it through a specific peripheral neuroendocrine compound. The use of this strategy in a research protocol is organized according to the stage of clinical investigation of the drug probe (Haskett and Rose 1981). Initially, the challenge represents a simple clinical test of postulated neurochemical pathophysiology in a disorder (e.g., probing noradrenergic effects on the pituitary adrenal axis with amphetamine) (Sachar et al. 1981) or the attempted replication of findings anecdotally reported. If open tests merit continuing interest, the same challenge strategy is embedded in a rigorous research design which will determine the critical features and applicability of the drug probe. This standardization and validation of the drug challenge includes specification of its sensitivity (“true positives”: the percentage of persons with the specific disorder who are correctly identified by a positive challenge test result) and specificity (the percentage of persons not affected with the specific disorder who are correctly identified by a negative challenge test result, the remainder indicating the “false positives”). The “predictive value” or “diagnostic confidence” is the critical variable when a test is used for diagnostic purposes, and indicates the percentage of total abnormal test results that are associated with (true positive for) the index illness (Vecchio 1966; Galen and Gambino 1975; Akiskal et al. 1979; Carroll et al. 1981). The final stage of the

<table>
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<th>Table 1. Clinical strategies for pharmacological challenge of neurochemical and neuroendocrine function</th>
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<td>a. Simple test of hypothesized neurochemical pathophysiology in a disorder</td>
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clinical application of a challenge test is its cautious extension to questions regarding natural history, treatment, and prognosis (Haskell and Rose 1981).

When possible pathophysiological mechanisms are considered, it is obvious that a single neurotransmitter neuronal system can be probed by several drugs (each affecting different features within the sequence of synaptic transmission) yet monitored through the same neuroendocrine index. For example, drugs such as clonidine, desipramine, amphetamine, and insulin have effects on the noradrenergic regulation of growth hormone release. A modification of this strategy is to choose a range of drugs which affect different neurotransmitter systems, but monitor the physiological effects through a single neuroendocrine response index. An example of this approach is challenging the hypothalamic-pituitary-adrenal axis with dexamethasone, amphetamine, and physostigmine, or probing growth hormone release with insulin, arginine, L-dopa, and clonidine. Finally, several drugs with specific effects on a single neurotransmitter system might be chosen, and the response monitored through measurement of several neuroendocrine response indices. For example, the effects of clonidine, desipramine, amphetamine, and methylphenidate on the noradrenergic system can be examined through measures of plasma cortisol, growth hormone, and luteinizing hormone.

Specific pharmacological challenge strategies are typically an intrinsic part of a more general plan for investigating a target disease through a series of drug challenges. A pattern of responses may indicate features of the underlying pathophysiology, or several challenge tests may improve on existing diagnostic procedures. This may be the case, for instance, when the dexamethasone suppression test, plasma cortisol response to d-amphetamine, cortisol secretory patterns, sleep studies, and the thyroid-stimulating hormone response to thyrotropin-releasing hormone administration are all considered in research studies of affective disorders.

Another type of challenge test strategy focuses on the reproduction of the symptoms of a disease and components of its physiological abnormalities. While such a strategy is usually not possible in the sense of preplanned administration of a drug which will elicit or aggravate symptoms of an illness, the clinician can utilize observations on patients who have administered drugs to themselves (e.g., psychotomimetics; Bowers 1977). Similarly, a pharmacological challenge is sometimes given to an unaffected relative of a patient with a known genetic disease in order to estimate the likelihood of the onset of symptoms at a later date for the relative (e.g., L-dopa in Huntington's disease). A more common strategy is to choose a drug which will correct a neurochemical or neuroendocrine abnormality in a syndrome, with the hope that it will also reduce symptoms as well; an example is the administration of clonidine in Tourette's syndrome, first attempted in order to reduce the apparently increased noradrenergic function in a subgroup of these patients.

Finally, a drug challenge test can be administered to multiple members of each of several families in order to determine if genetic patterns of response exist, and in what way they may or may not be related to a clinical disorder.

Clonidine and Methylphenidate Challenge Tests. We have designed challenge tests which are integrally related to central clinical questions. Challenge tests are administered initially in a baseline, pretreatment condition, followed by further challenges at subsequent posttreatment intervals judged to be appropriate on a clinical basis; changes in the metabolic response are examined in the light of clinical change. Placebo-controlled, double-blind designs are used after initial open trials. Metabolic responses are examined in responder and nonresponder groups as a possible key to discriminating them and identifying the physiological basis for their response.

Clonidine is a partial noradrenergic agonist which, when administered in low doses, preferentially acts at the afferent input to the principal nucleus of noradrenergic cell bodies in the brain, the locus ceruleus. Low doses of clonidine reduce the firing rate of neurons in the locus ceruleus and the production of MHPG in brain by selective activation of presynaptic alpha2 receptors (Anden et al. 1970; Svensson, Bunney, and Aghajanian 1975; Cedarbaum and Aghajanian 1977). Elevated CSF free MHPG in a TS patient suggested a subgroup of TS patients with increased noradrenergic activity which might be responsive to an agent which reduces brain noradrenergic activity (Cohen et al. 1979a). An open trial of clonidine indicated that it achieves substantial symptom reduction in approxi-
mately 50–70 percent of TS patients (Cohen et al. 1979b, 1980a)

In our initial open studies, CSF free MHPG was measured in a single patient before and after a challenge dose of clonidine. His baseline level of 14.8 ng/ml was reduced to 10.4 ng/ml free MHPG after 1 month of clonidine treatment, accompanied by a good clinical response (Young et al. 1981a).

Three TS patients, unmedicated for at least 1 month, were given challenge doses of medication, and plasma free MHPG levels were obtained at baseline and 2 and 22 hours following medication administration. A 25–40 percent reduction in plasma free MHPG was observed following a challenge dose of clonidine (two severely disturbed patients) or haloperidol (a single patient with mild symptoms). All three patients had a good clinical response when treated with the challenge medication. Plasma free MHPG has not yet been measured in a medication-free nonresponder (Young et al. 1981a).

Challenge doses of clonidine given while a (responder) patient is on a maintenance dose indicate that a metabolic response can still be achieved. Plasma free MHPG, which had been very high at baseline in one patient, was reduced in the two postclonidine samples. The other responder given a clonidine challenge dose (while on a maintenance dose) had a 40 percent increase in plasma free MHPG at the 2-hour postclonidine point, before it was eventually reduced below baseline at 22 hours. One patient, who did not have a good clonal response while on maintenance clonidine, also did not have a decrease in plasma free MHPG following clonidine challenge (Young et al. 1981a). Further studies of clinical nonresponders will help clarify the relation between plasma free MHPG and clinical response to clonidine.

The use of other medications at the time of a clonidine trial tends to obscure plasma free MHPG changes. For example, while plasma MHPG might decrease during haloperidol treatment, it might increase during acute withdrawal from haloperidol.

A trial of clonidine was also attempted for two adolescent girls and one young adult man with infantile autism and led to a slight aggravation of their symptoms. Clonidine challenge produced a 40 percent increase in plasma free MHPG in the autistic girl in whom it was measured (Young et al. 1981a).

In recent protocols we have used clonidine and methylphenidate as pharmacological challenges to CA function in TS patients and attention deficit disorder while monitoring a greater range of variables. For example, we have designed the clonidine challenge test in TS to include blood pressure and sedation measures on the clinical side, as well as plasma clonidine, MHPG, HVA, and human growth hormone on the metabolic side.

Changes in MHPG levels following clonidine were accompanied by effects on behavior, altered levels of other metabolites, and a selective neuroendocrine response; the various effects are not simultaneous and vary in magnitude. The marked increase of growth hormone following clonidine (Lal et al. 1975; Gil-Ad, Topper, and Laron 1979) is a more robust, acute response than the plasma MHPG change (Leckman et al. 1980; Leckman, Maas, and Heninger 1981; Young et al. 1981a), suggesting that clonidine might have clinical application as a growth hormone stimulation test and that growth hormone response might be a sensitive test of brain function in some neuropsychiatric disorders. In a more basic way, the discrete effects of clonidine on specific clinical parameters (blood pressure, sedation, motor regulation) reflect the distinct action of clonidine on specific noradrenergic neuronal subsystems (e.g., hypothalamic, brainstem), in relation to the more general noradrenergic effect presumably reflected in MHPG levels. Since these effects are neither simultaneous nor of the same magnitude, evaluation of other regulatory influences (such as dopaminergic or serotonergic effects) will help to dissect components of critical control mechanisms acting on individual parameters.

An example of the initial direction of such studies is a comparison of plasma MHPG and HVA levels before and after a 12-week trial of clonidine in six TS patients. There were significant increases after 12 weeks of clonidine in the baseline morning levels of both plasma MHPG (15 percent increase) and plasma HVA (77 percent increase) (Leckman et al. 1982). While the specific physiological mechanism underlying these metabolic changes will require extensive investigation, the data suggest an interaction between the noradrenergic and dopaminergic systems in these patients. This interaction may mediate an increase in central DA turnover from the apparently low baseline turnover found in TS (Cohen et al. 1978b, 1979a; Leckman et al. 1982).
Using a similar strategy in protocols evaluating boys with attention deficit disorder, we have been able to examine the pharmacokinetics of methylphenidate (MPH), demonstrate dose effects on plasma MPH levels and a relation between plasma MPH concentration and clinical response, and indicate the rise in plasma human growth hormone and decline in plasma prolactin following MPH administration (Shaywitz et al., in press a). The large number of children taking stimulant medications, and the questions surrounding their long-term efficacy, make it mandatory that carefully designed, discretely targeted clinical and metabolic studies such as these be continued if we are to use stimulant medications knowledgeably. While there is a growing base of information concerning the attention deficit disorders and their treatment with stimulants (Shaywitz et al., in press b; Shaywitz and Shaywitz 1982), further application of the challenge test strategy promises an improved understanding of biological influences which will lead to optimal clinical care or to troubling side effects (Young 1981; Lowe et al. 1982).

The Development of Monoamine Neuronal Systems During Childhood

Normal Ontogeny. Another perspective in neurochemical research relevant to autism is examination of the development of functional balance of neurotransmitter neuronal systems in childhood and adolescence. Concepts guiding these studies are that (1) while levels of a compound in a fluid may be within the normal range established for adults, they may be unusual for a given period in childhood because of developmental changes; and (2) while levels of a compound may appear to be within a normal range for that age, an abnormal developmental profile of neuronal systems in a specific diagnostic group may be most visible when the relative functional interaction among systems is examined and found to be atypical compared to contrast and normal groups. Biochemical studies of childhood autism have required establishment of normal ontogeny of neurotransmitter neuronal systems through a composite assessment of transmitter, enzymes, and metabolites in body fluids in relation to age, and the delineation of overall functional relations among these systems (Young et al., in press).

Examination of maturational changes in biogenic amines, their metabolites, and related synthetic and degradative enzymes has suggested consistent developmental effects on aminergic neuronal systems. While dopaminergic activity appears to decrease over the period of childhood and adolescence, noradrenergic function appears to increase, and there is stable or decreasing serotonergic activity. These age-related changes may have fundamental behavioral effects. Increased dopaminergic activity leads to increased locomotor activity and stereotypic movements, effects modulated by relative levels of both noradrenergic and serotonergic activity. The apparent developmental reduction in dopaminergic activity parallels the decrease in motor activity as a child matures. Imbalance among monoamine systems, due to differences in developmental rates, might critically affect a child’s behavior (Young et al., in press). Developmental changes in neuromodulators, such as hormones, might also be important determinants of behaviors associated with neuronal maturation (McEwen 1976).

Pathology of Neurotransmitter Ontogeny. As profiles of neurotransmitter maturation in various tissues and body fluids are established with more assurance, it will be possible to investigate the relation of abnormal developmental sequences at the molecular level to familiar clinical pathology. Pilot work along these lines is just beginning.

Sex Differences. As with age effects, there has been little systematic investigation of differences in monoamine function between males and females. Such studies may be of importance in understanding the difference between boys and girls in the incidence of certain types of disorders. For example, attention deficit syndrome, Tourette’s syndrome, and childhood autism are three- or four-fold more common among boys. In neuropsychiatrically impaired children, we found a considerably higher dopamine turnover rate in boys than in girls. This difference did not appear to be related to diagnostic clustering of the boys and girls in different neuropsychiatric subgroups.

We have analyzed CSF sex effects in a larger population of patients, ages 3–20 years. In this child and adolescent population, the mean CSF HVA, following probenecid, for males \( (n = 49) \) was 204 ng/ml; for females \( (n = 22) \), HVA was 164 ng/ml. The HVA/probenecid ratio was strikingly
higher in the boys (15.7) than in the girls (9.5, \( p < .01 \)). Diagnostic differences between the boys and the girls must be considered in evaluating the meaning of the increased HVA and HVA/probenecid measures in males. Also, since the females tended to be older, the age effects noted in the previous section may account, at least in part for observed differences between the sexes (Leckman et al. 1980).

Clearly, considerably more research will be required to assess the relations of age and sex to monoamine neuronal systems and the implication of any trends for psychopathology. Age effects may clarify vulnerable periods for the emergence of psychiatric disorders (e.g., adolescence); also, there may be important interactions among neurochemical development, endocrinological maturational, and the onset or exacerbation of behavioral disturbances at periods of biological upheaval (such as moodiness at the transition into adolescence or the marked increase in aggression among autistic children at this phase of development) (Fish 1977).

**Laboratory and Clinical Research: Treatment of Tourette’s Syndrome With Clonidine**

The syndrome of chronic, multiple tics described by Gilles de la Tourette consists of multiformal motor and phonic tics and compulsive actions originating in childhood and persisting for years (Gilles de la Tourette 1885; Woodrow 1974). The initial hypothesis that this was a biologically mediated syndrome was later displaced by psychoanalytic theories postulating a predominantly psychogenic origin, based upon many observations of the precipitation or aggravation of symptoms by psychological stress. The subsequent use of haloperidol as a specific pharmacological treatment, coupled with the emergence of more sophisticated laboratory techniques, resulted in renewed biological research on Tourette’s syndrome. Catecholamine metabolism was further implicated in the etiology of the illness by exacerbations produced by dopaminergic agonists, such as dextroamphetamine (Feinberg and Carroll 1979). Another line of evidence for a neurological substrate was the high incidence (40 percent in our series) of patients with abnormal or borderline EEGs. Abnormal findings related to maintenance of posture or to performance of sequential movements are found in 50 percent of patients; a similar percentage have cognitive processing disturbances (Shapiro et al. 1973; Harcherik et al. 1982).

Genetic mechanisms were suggested in the original paper by Gilles de la Tourette, who described a marked preponderance of males and possible familial clustering of the disorder. These observations have been replicated repeatedly, with most authors finding 3 to 8 times more male than female Tourette’s syndrome (TS) patients. More sophisticated genetic studies support a strong genetic component, with simple and chronic multiple tics representing, in families with TS probands, an expression of the underlying vulnerability (Kidd, Prusoff, and Cohen 1980; Pauls et al. 1981).

Central nervous system metabolism in children with Tourette’s syndrome has been assessed by measuring the cerebrospinal fluid metabolites of dopamine (HVA) and serotonin (5-HIAA) with and without the administration of probenecid. These studies revealed a reduced accumulation of CSF HVA and 5-HIAA, which may represent a primary decrease in the brain turnover of the parent compounds or a long-term adaptation to overactivity in these systems as a result of changes in receptor sensitivity (Cohen et al. 1978b).

The involvement of noradrenergic mechanisms in TS was suggested by (1) elevated MHPG in the CSF of a severely disabled child (Cohen et al. 1979a), (2) exacerbation of the syndrome by stress and anxiety, and (3) similarities between the clinical phenomena and some aspects of behavior elicited in animals by stimulation of the brain center which regulates noradrenergic activity, the locus ceruleus (Cohen et al. 1979a). A separate line of research in other laboratories at Yale used single cell recording techniques to investigate the neuropharmacology of the locus ceruleus, and the mode of action of clonidine, an imidazoline derivative used in the treatment of hypertension (Svensson, Bunney, and Aghajanian 1979). Clonidine is a partial adrenergic agonist. However, at low doses, it diminishes central noradrenergic activity by stimulating inhibitory presynaptic, \( \alpha \)-adrenergic autoreceptors on neurons which regulate the locus ceruleus. For example, in a clinical application, clonidine has been found to suppress dramatically the noradrenergically mediated symp- toms of methadone withdrawal in adults (Gold, Redmond, and Kleber 1978; Gold et al. 1980) and,
as shown by our recent research, also in infants (Hoder et al. 1981). The elevated spinal fluid MHPG in a child with TS, coupled with these basic and clinical studies of clonidine, suggested assessment of the usefulness of clonidine for TS. We have administered clonidine to 50 patients over the last 3 years. All were individuals who had not responded to haloperidol, had developed tolerance, or had discontinued it because of side effects. In our experience, 50–70 percent of patients have shown improvements in behavioral symptoms and tics, with no serious problems with side effects or tolerance (Cohen et al. 1979b, 1980a; Leckman, Detlor, and Cohen, in press; Leckman et al., in press).

Clonidine's ameliorative action on the symptoms of TS provides a gratifying example of the movement of data and hypotheses from basic science laboratories to the clinic, a course explicitly fostered by the organization of research within a university. Coordination of hypotheses and experimental results across laboratories, and the development of complementary strategies among investigators, play a critical role in generating research with relatively early clinical application. This process was aided in TS by the relative specificity of the symptoms, the biochemical measures, and the psychopharmacological agent. By analogy, it might be predicted that advances in understanding childhood neuropsychiatric disorders will be based on careful diagnostic criteria, specific behavioral dimensions, and precise biological theories and techniques. At all phases of the research, animal studies are needed to clarify points that cannot be investigated in humans. This model for collaborative research offers the maximal opportunity for clarification of fundamental physiology and pathology, and successful application in treatment for children.

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