The MRI Study of Normal Brain Development

Sponsored By

The National Institute of Mental Health
The National Institute of Child Health and Human Development
The National Institute of Neurological Disorders and Stroke
The National Institute on Drug Abuse

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INTRODUCTION

The purpose of the study is to collect a representative sample of normal, healthy infants and children for a magnetic resonance imaging study that will serve two purposes: 1) to provide the largest normative database to date of the developing human brain for comparison with brain scan studies of children with neurological, developmental, and psychiatric disorders; and 2) provide longitudinal data for investigating brain maturation in relationship to behavioral and cognitive development in a normal sample. Such data will allow a greater understanding of deviations in brain structural development associated with pediatric brain disorders.

The study involves six different sites in the United States, as well as a Data Coordinating Center (DCC) in Montreal, Canada, and a Clinical Coordinating Center (CCC) in St. Louis, Missouri. The U.S. sites include three Children's Hospitals (Boston, Cincinnati and Philadelphia), and Washington University in St. Louis, the University of Texas Health Science Center in Houston, and the University of California in Los Angeles. A seventh center, the University of California in Irvine, was initially involved in recruiting subjects who were subsequently followed at UCLA.

These sites will collaborate in the recruitment of a representative sample of approximately 546 children, ages 10 days through 18 years 3 months (at first scan), who will be studied using anatomic magnetic resonance imaging (aMRI), diffusion tensor imaging (DTI), magnetic resonance spectroscopy (MRS) and behavioral testing at three (or more) different time points over a five to six year period. The children will be scanned with techniques that will provide state of the art information concerning the structural and metabolic development of the brain, including the development of specific structures. Developmental changes in the brain and in specific regions of the brain will be related to maturational changes in behavior and cognition. Image analysis tools sensitive to brain developmental changes will be developed as part of this effort. A goal is to develop growth curves for various aspects of brain development.

The information gained will be made widely available to researchers and clinicians. This will permit physicians and researchers to determine possible differences in brain structure and metabolism in children with known or suspected brain-based problems. The information and tools produced may also allow physicians to track brain-related changes associated with disease and to evaluate the effects of various treatments on the brain.

Objectives

The Pediatric MRI study of Normal Brain Development has two main objectives and two ancillary objectives.

Objective 1: Anatomic MRI and cognitive/behavioral cross-sectional and longitudinal studies of children between the ages 4 years, 6 months and 18 years, 3 months (at time of first scan).

Objective 2: Anatomic MRI and cognitive/behavioral cross-sectional and longitudinal studies of children between the age of newborn and 4 years, 5 months (at time of first scan).

Ancillary A: MR Spectroscopy (MRS) of children between the ages of newborn (10-14 days) and 18 years, 3 months (at time of first scan).

Ancillary B: Diffusion Tensor Imaging (DTI) of children between the ages of newborn (10-14 days) and 4 years, 5 months (at time of first scan). However this was broadened to include subjects of all ages. An expanded DTI (eDTI) protocol was developed and acquisitions began in the spring of 2006.

Principle Study Centers (PSCs) and Objectives

Table 1: PSCs, Objectives and Ancillary Studies

PSC	Objective 1	Objective 2	Ancillary A MRS/MRSI	Ancillary B DTI	
				Standard DTI	Expanded DTI
Boston	x	x	x	x	x
Cincinnati	x			x	
Houston	x				
Irvine‡	x‡				
Los Angeles	x		x	x	
Philadelphia	x	x†	x	x	x
St. Louis 1	x			x	x
St. Louis 2		x		x	x

‡ Irvine (since discontinued as a site) initially recruited subjects who were subsequently followed at the UCLA site
† Philadelphia initially recruited some Objective 2 subjects who were discontinued

2. RECRUITMENT

The study will recruit 546 volunteers. The normative database will be used for the purpose of comparison with data from patient populations. Accruals are broken down by objective and site as follows:

Table 2a: Contracted PSC Accruals

	Contracted Accruals		
PSC	Objective1	Objective2	Total
Boston	70	46	116
Cincinnati	100	0	100
Houston	50	0	50
Irvine	50*	0	50*
UCLA	60	0	60
Philadelphia	50	0	50
St. Louis 1	60	0	60
St. Louis 2	0	60	60
TOTAL	**440**	**106**	**546**

*A number of subjects recruited at Irvine were followed at UCLA. The remainder of the accruals, for which Irvine was contracted, was distributed among the other sites.

Recruitment Process

a. Zip code demographic data compiled from PSC region:

Specific detailed zip code based census data for zip codes within a 1-hour traveling distance of each PSC (See St Louis example table 2b) will be obtained. Based on these demographic statistics, summary data of PSC regional demographics will be compiled. Demographic data includes regional median household income as an indicator of socio-economic status and population distribution by race and ethnicity. Regional zip codes will be grouped by: 1) race and ethnicity and 2) by household median income classified as low, medium and high categories.

b. PSC Target accrual tables:

Target accrual tables for each PSC will be created. Tables are constructed to reflect the actual PSC regional population demographics and are planned for recruiting target numbers for each PSC. Adjustment by Nick Lange will be completed to assure that the overall recruited population from the 6 sites reflects the overall US population demographics. After the initial majority of recruitment is completed, target tables may be combined across PSCs to create group targets for all PSCs to jointly attain in order to maximize recruitment efficiency (see section "Sampling Strategy" for additional information/ specifications).

c. Agency list for purchase:

All PSCs will use zip code lists for subject recruitment for Objective 1. These lists will be purchased, in electronic form, from Donnelly Marketing/Info USA. This marketing company has been used successfully in the past by several of the PSC leaders to identify subject samples within their communities. The data will consist of site-specific lists of 10,000 names of local households containing children aged 0 to 17 years, at a cost of $800.00 per PSC. There is an additional charge of $80 per PSC for batching the list of 10,000 into three batches of size 4,000, 3,000, and 3,000 to be received during months 0, 3 and 6 of the accrual period in order to gain more timely

information. Each PSC will receive, in each batch, in six different text files per site. These files will contain the addresses (all households) and telephone numbers (most households) of families with at least one child within the ages of 4-5, 6-11, and 12-17 years, cross-classified by the sex of the child**.**

d. The sources for the InfoUSA lists are:

- Birth Announcements
- Hospital Records
- Lamaze Classes
- People who purchase cribs
- Other Proprietary Sources, Including:
  - Baby Food Manufacturers (Gerber, P&G, Beechnut, etc.)
  - Magazines
  - Daycare
  - Photographers

e. Specification of List Requests by PSC zip codes:

The initial selection of zip codes selected to request lists from the above agency is determined to reflect the regional demographics and PSC targets. Specifications of zip codes and the number of families to request from different zip codes will be created and distributed by the CCC. The households provided will be balanced by Household Income and Race across the zip codes within the PSC region. At least 50% oversampling in lower income zip codes is planned to reflect possible increased exclusion rates and potential recruiting difficulties. Using this approach will enable us to derive a sample that is as representative as possible of the total U.S. population. Using distributed random numbers, 200-300 households will be selected and the introductory letter will be sent to these households. This procedure will be repeated until the desired accruals targets have been reached, subject to the stratification constraints described in item 2c below. After lists of the specified zip codes were created there was a comparison of the demographics of that proposed sub-sample, to the demographics obtained for the entire PSC specific sample (see Table 2e).

f. Mailing Lists & Tracking Log Incorporation:

Mailing lists provided will then be read into a tracking database log created by the CCC and distributed to PSCs. Three batches of zip codes will be received at months 0, 3, and 6 of the accrual period, and will be of sizes 4000, 3000, and 3000, respectively. Random numbers lists obtained from Nick Lange will be used to specify the order of contact to select families for initial mailing. The recruitment process then begins when the PSC mails out the introductory letter and reply card to candidate families, and flags these families as contacted in their local tracking log database.

g. Initial Contact & Screening:

Using distributed random numbers, 200-300 households will be selected and the introductory letter will be sent to these households. This procedure will be repeated until the desired accruals targets have been reached subject to the stratification constraints described in item 2c below.

Table 2b. Compiled Demographic Census Data from Zip Codes within 50 miles of PSC (St. Louis region data)

St. Louis Region Zip code sample is	79.53% white	18.25% Black	.096% Asian Pac	.024% Amer Indian/Eskimo	1.03% Hispanic
		TOTAL	Low income	Med. Income	High Income
Zip population		2,279,435	341,821	1,575,151	362,463
Minority	**20.47%**	466,629	**242,654**	**202,384**	**21,592**
Blacks	18.25%	416,029	235,250	171,144	9,634
Asian Pac. Islander	0.96%	21,829	2,077	11,945	7,807
Amer Indian	0.24%	5,365	1,031	3,802	533
Hispanic	1.03%	23,406	4,296	15,493	3,617
White	**79.53%**	**1,812,806**	**99,167**	**1,372,767**	**340,871**

Table 2c. Demographics of Zip Code Data Grouped by Income Ranges (St. Louis region)

	Low	High	Population				
Low Income Range =	$10,243.00	$24,999.00	341,821	15.00%	of zip pop.	60.62% minorities	39.38% white
Medium Income Range =	$25,000.00	$49,999.00	1,575,151	69.1%	of zip pop.	6.86% minorities	93.14% white
High Income Range =	$50,000.00	$118,174.00	362,463	15.90%	of zip pop.	6.66% minorities	93.34% white

Table 2d: Target Sampling Population – First Third of Sample (3000 families): St. Louis

First Third of sample	15% of 3000=	450	low income	8 zip codes in 1st third
	69% of 3000=	2070	med income	39 zip codes in 1st third
	16% of 3000=	480	high income	5 zip codes in 1st third
		3000	families	

Table 2e. Comparison of Proposed Target Zip Code Sample vs. Total PSC Population Sample

	Median Income	# residents	# minority	% Minority	Entire sample
Low Income	$20,227	82,152	49,803	60.62%	70.99%
Med. Income	$35,403	468,969	32,166	6.86%	12.85%
High Income	$67,244	96,061	6,401	6.66%	5.96%
OVERALL		647,182	88,370	13.65%	20.47%

Mailing List

Each PSC will obtain a list of a large number of households in which there is a child between the ages of 4 and 18 - this list will be obtained from a national marketing agency:

InfoUSA
5711 South 86th Circle, P.O. Box 27347, Omaha, NE 68127
Contact: Mr. Randy Johnson, 888-677-7438, randy.johnson@infousa.com

A list of 10,000 households can be obtained for approximately $800.00. Households for a given metropolitan area with children between 0 and 17 years of age will be requested by zip code. The study introductory letter will be sent to 200-300 households that have been selected at random from this large list. The letter will be followed by a telephone call from the study coordinator at each PSC if a telephone number is available from the list. If a telephone number is not available from the list, then the mailing will ask the household to contact the PSC if interested. After screening and enrollment, if additional subjects are needed, another 200 to 300 households will be selected from the list.

To assist with recruiting lower SES families with combined incomes of <$35,000, 6,666 additional names, addresses and telephone numbers were ordered from an alternative agency, Creative Mailers.

3. STUDY SAMPLING PLAN

Stratified Random Sampling

a. Stratified random samples according to sex, race and socioeconomic status (measured by regionally specific, HUD adjusted income of the parents as a surrogate variable) will be obtained at each PSC in order to match sample proportions of these factors, marginally across all PSCs, to those of the entire U.S. population.

b. In order to address concerns regarding a "super-normal" sample population, we will analyze groups of data stratified according to the IQ of the subject.

Age Targets, Limits and Groups

Target ages will be staggered at entry with 20% compounded attrition for both Objectives; please see Sampling Strategies for more detail.

Age at Study Entry	**Minimum**	**Maximum**
Objective 1	4 years 6 months	18 years 3 months
Objective 2	10 days	4 years 5 months

It is noted that sampling overlap to provide continuity between Objectives 1 and 2 is achieved through lowering the Objective 1 age limit, only, due to rapid cognitive development between ages four and five years and the unsuitability of some behavioral instruments chosen for objective 2 beyond age four years. Children crossing over from Objective 2 to Objective 1 will receive the behavioral battery designed for Objective 1. It is also noted that the maximum age at study entry for Objective 1 is extended upward slightly in order that we will not exclude the possibility of studying developmental changes between 18 and 21 years of age, during which one observes a significant incidence of new onset psychiatric disorders, e.g., first-break young adult psychoses.

Sampling Strategy for Objective 1

Sampling Plan for Attaining Adequate Statistical Power and Sample Representativeness:

As is well known, the only way to measure individual change over time is by a longitudinal study. Our longitudinal anatomical measurements will consist of time series of brain structure volumes for each child. Modern growth curve analyses of longitudinal data include linear, non-linear and flexible individual-specific curves that accommodate temporal, demographic and behavioral factors. The aims of our sampling plan are two-fold: (1) to obtain sufficient data to obtain at least 80% probative power to detect growth and changes in growth in key brain structures over time, and (2) to provide these analyses for a representative sample of growing children across the United States.

Sample Representativeness

We aim to provide representative target accruals derived by scaling down US Census 2000 data on households cross-classified nationwide by Income and Ethnicity to our target sample size of N = 440 children; Tables 3, 4, 5 and 6. Income levels in Tables 3 and 4 refer to originally reported household income. We also use HUD-adjusted incomes as well in all reporting and analyses (Table 5). For Ethnicity/Race, we report Hispanic as a separate category here, yet accommodate for the fact that Ethnicity data are reported in all other categories for non-Hispanic and Hispanic in our reporting and analyses.

Table 3: Distribution of American Households by Income and Ethnicity/Race from the US Census 2000. Key: AI, NA: American Indian or Native Alaskan; A, NH, OPI: Asian, Native Hawaiian; OPI: Other Pacific Islander.

	White	Hispanic	AI, NA	Black	A, NH,	OPI
Total:	83,697,584	9,272,610	770,334	12,023,966	3,129,127	100,151
Less than $10,000	6,583,869	1,150,289	128,129	2,293,890	314,182	9,319
$10,000 to $14,999	4,954,959	722,783	67,676	1,038,360	143,128	5,454
$15,000 to $19,999	4,973,929	739,289	63,055	959,680	142,820	5,600
$20,000 to $24,999	5,302,703	768,327	62,567	934,783	152,512	6,712
$25,000 to $29,999	5,259,418	726,561	56,454	874,249	145,680	6,508
$30,000 to $34,999	5,280,510	681,695	53,618	787,384	157,206	6,480
$35,000 to $39,999	4,953,486	604,712	47,195	697,230	149,470	6,259
$40,000 to $44,999	4,794,031	545,550	42,124	624,259	153,933	5,996
$45,000 to $49,999	4,248,660	465,694	35,452	521,514	136,472	5,171
$50,000 to $59,999	7,833,554	772,953	59,838	878,583	267,395	9,473
$60,000 to $74,999	9,160,744	796,071	61,647	918,284	348,503	11,526
$75,000 to $99,999	9,121,339	682,668	50,831	797,301	396,123	11,220
$100,000 to $124,999	4,695,227	296,226	20,652	342,882	246,993	5,127
$125,000 to $149,999	2,285,321	127,400	8,757	147,771	135,890	2,333
$150,000 to $199,999	2,022,901	96,671	6,271	105,509	127,885	1,742
$200,000 or more	2,226,933	95,721	6,068	102,287	110,935	1,231

Table 4: Percentages by Race and Income for Target Sample, US Census 2000, and Their Difference. Differences in Percentages for Target Sample v. US Census 2000 are due in large part to originally reported, non-HUD adjusted Incomes. *Key*: Ethnicity/Race: AI, NA: American Indian or Native Alaskan; A, NH, OPI: Asian, Native Hawaiian or Other Pacific Islander; Originally Reported Income: Low $: less than $35,000 per year, Med $: $35,000-75,000 per year; High $: greater than $75,000 per year.

	(1) Target Sample (%)			(2) US Census 2000 (%)			Difference (1) – (2)		
	Low $	Med $	High $	Low $	Med $	High $	Low $	Med $	High $
White	22.73	24.55	25.91	29.69	28.43	18.67	-6.96	-3.88	7.24
Hispanic	4.09	2.73	2.73	4.39	2.92	1.19	-0.30	-0.19	1.54
AI, NA	0.68	0.68	0.45	0.40	0.23	0.08	0.28	0.45	0.37
Black	6.82	3.18	2.95	6.32	3.34	1.37	0.50	-0.16	1.58
A, NH, OPI	0.23	0.91	1.36	1.01	1.01	0.95	-0.78	-0.10	0.41

Table 5: Representative Target Accruals (N = 440), a 2-Way Table of Ethnicity/Race x Income. *Key*: Ethnicity/Race: AI, NA: American Indian or Native Alaskan; A, NH, OPI: Asian, Native Hawaiian or Other Pacific Islander; HUD-Adjusted Income: Low $: less than $35,000 per year, Med $: $35,000-75,000 per year; High $: greater than $75,000 per year.

	L	M	H	Total
White	100	108	114	322
Hispanic	18	12	12	42
AI, AN	3	3	2	8
Black	30	14	13	57
A, NH, OPI	1	4	6	11
Total	152	141	147	440

Tables 6 – 8 contain cross-classifications of our target accruals by demographic factors. The age distribution is customized for sampling younger children due to rapid brain growth in the first 18 months or 2 years of life. Target ages will be sampled longitudinally in cohorts according to our accelerated longitudinal design strategy. We sample staggered ages at entry, assume 20% attrition, and accrual of younger children. The sex distribution is split 50-50 between boys and girls.

Table 6 consists of a multi-way cross-classification of children by the following four demographic factors: Age of Child, Sex of Child, Income of Parent(s), Ethnicity/Race of Child, and are posted on the website. Each such four-dimensional (4-D) table is also summarized in a few of the possible marginal tables, such as Age x Sex x Income (3-D Table 7) and Age by Sex (2-D Table 8) with 1-D marginal totals.

Table 6: Representative Target Accruals (N = 440): 4-Way Table, Age x Sex x Income x Ethnicity

	L		M		H		Total
	M	F	M	F	M	F	
4:6-5:11							
White	5	5	7	6	4	5	32
Hispanic	2	1	1		1	1	6
AI, AN							
Black	1	2	1	1	1	1	7
A, NH, OPI	1			1	1		3
6:0-6:11							
White	4	5	5	5	5	5	29
Hispanic	1	2	2	1	1	1	8
AI, AN		1					1
Black	2	2	1	1	1	0	7
A, NH, OPI						2	2
7:0-7:11							
White	6	4	5	7	8	6	36
Hispanic	1		1				2
AI, AN							
Black	1	2		1	1		5
A, NH, OPI							
8:0-8:11							
White	3	4	4	4	7	6	28
Hispanic	1	2		1		1	5
AI, AN	1			1			2
Black	1	1		1		1	4
A, NH, OPI					1		1
9:0-9:11							
White	4	4	3	3	3	6	23
Hispanic	1		1			1	3
AI, AN			1				1
Black	1	1		1	1		4
A, NH, OPI					1		1
10:0-10:11							
White	4	3	4	4	4	5	24
Hispanic		1				1	2
AI, AN							
Black	1	1	1		1	1	5
A, NH, OPI				1			1
11:0-11:11							
White	4	4	3	4	5	4	24
Hispanic		1	1			1	3
AI, AN							
Black	1	1			1		3
A, NH, OPI			1				1

Table 6 (continued)

	L		M		H		Total
	M	F	M	F	M	F	
12:0-12:11							
White	4	3	5	5	4	3	24
Hispanic				1		1	2
AI, AN	1						1
Black	1	1				1	3
A, NH, OPI							
13:0-13:11							
White	3	3	4	3	4	4	21
Hispanic		1	0	1			2
AI, AN					1		1
Black	1	1	1	1		1	5
A, NH, OPI							
14:0-14:11							
White	3	4	3	3	3	3	19
Hispanic	1			1	1		3
AI, AN						1	1
Black	1	1	1		1		4
A, NH, OPI							
15:0-15:11							
White	3	3	4	3	3	3	19
Hispanic	1	1			1		3
AI, AN							
Black	1	1		1	1		4
A, NH, OPI						1	1
16:0-16:11							
White	3	4	4	3	3	4	21
Hispanic				1			1
AI, AN				1			1
Black	1	1	1				3
A, NH, OPI							
17:0-17:11							
White	4	4	4	3	4	3	22
Hispanic	1				1		2
AI, AN	0						
Black	1	1	1				3
A, NH, OPI				1			1
Total	**76**	**76**	**70**	**71**	**74**	**73**	**440**

Table 7: Representative Target Accruals (N = 440), 3-Way Table: Age x Sex x Income

	L	M	H		L	M	H		L	M	H
	4:6-5:11				6:0-6:11				7:0-7:11		
M	9	9	7		7	8	7		8	6	9
F	8	8	8		10	7	8		6	8	6

	8:0-8:11				9:0-9:11				10:0-10:11		
M	6	4	8		6	5	5		5	5	5
F	7	7	8		5	4	7		5	5	7

	11:0-11:11				12:0-12:11				13:0-13:11		
M	5	5	6		6	5	4		4	5	5
F	6	4	5		4	6	5		5	5	5

	14:0-14:11				15:0-15:11				16:0-16:11		
M	5	4	5		5	4	5		4	5	3
F	5	4	4		5	4	4		5	5	4

	17:0-17:11		
M	6	5	5
F	5	4	3

440

Table 8: Representative Target Accruals (N = 440), 2-Way Table: Age x Sex

	4:6-5:11	6:0-6:11	7:0-7:11	8:0-8:11	9:0-9:11	10:0-10:11	11:0-11:11	12:0-12:11	13:0-13:11	14:0-14:11	15:0-15:11	16:0-16:11	17:0-17:11	Total
M	25	22	23	18	16	15	16	15	14	14	14	12	16	220
F	23	25	20	22	16	17	15	15	15	13	13	14	12	220
Total	48	47	43	40	32	32	31	30	29	27	27	26	28	440

Rules for Slight Target Adjustment "Swaps" When Needed

At the study's commencement, in order to achieve representativeness by matching US Census 2000 proportions to sample proportions, some necessary yet arbitrary decisions were made on the allocation of the target number to the 390 demographics cells (13 Age x 2 Sex x 3 Income x 5 Ethnicity). Some amount of random allocation was necessary due to one inherent limitation of the task, namely filling 390 cells with only 440 children. Some examples include target numbers of male and female cells for specific ages, incomes or ethnicities, and various assignments of small target numbers of minorities to age categories.

To obtain our N = 440 goal, some of these targets may need to be altered slightly in order to maintain both representativeness and increasing sample size given available candidate children of particular representativeness factor combinations. Thus, as we proceed to the completion of accruals, some latitude will be exercised regarding the dynamic process of filling cells with strict targeted numbers, However, this latitude will be restricted under the rules outlined in the following paragraphs.

The representativeness of our sample with respect to our US Census 2000 target will not be affected. For both maintaining representativeness and increasing sample size, we prioritize the four representativeness factors in the following order: Age, Sex, Income, and Ethnicity. This ranking of factors is based on their known importance to the overall goals of the study. Thus, when making compromise decisions regarding which available child to scan next, we will first aim to retain the target Age distribution, then the Age by Sex distribution, and then Age by Sex by Income distribution.

Operationally, when a decision on scanning a new candidate child is to be made, accrual tables are reviewed by Drs. Botteron and Lange to make target adjustments across specific cells. This decision is based on rules generated from the preceding representativeness factor priorities. These rules in turn guide the "swaps" needed to match the candidate child, current accruals and target accruals.

The rules are as follows:

1. Generate a distance measure between Original Target (OT) and proposed Adjusted Target (AT). This distance measure will be a sum of χ^2 statistics, $(AT - OT)^2 / OT$, over all cells of the 4-way table (Age, Sex, Ethnicity, Income), with separate sums over lower-dimensional tables if required. Call this statistic X, and it's associated p-value p(X).

2. If the p(X) is greater than 0.20, the swap is allowable. If not, the swap is not allowable.

3. While it is true that any single swap is unlikely to tip X to a value large enough to reject the swap, the cumulative effect of such swaps over time could add up to an Adjusted Target that is not close enough to the Original Target. This system prevents the occurrence of such an undesirable event.

4. A weighted sum of χ^2 statistics across marginal tables will also be employed to weight more heavily the prioritized representativeness factors: Age, Sex, Income, and Ethnicity. This statistic will make it more difficult to accept swaps that affect the prioritized factors.

Objective 2 Sampling Plan

Table 9 provides representative target accruals also derived by scaling US Census 2000 proportions of cross-classified household percentages of Income and Ethnicity to match our target sample size of N = 106 children for Objective 2. Again, the age distribution is customized to meet our need for younger children due to rapid brain growth and the sex distribution is split approximately 50-50 between boys and girls.

Table 9: Objective 2 Sampling Plan (N = 106). Representative Target Accruals, 2-Way Table: Income x Ethnicity

	L	M	H	Total	% Total
White	27	27	24	78	70%
Hispanic	3	3	4	10	9%
AI, AN	1	2		3	3%
Black	6	5	5	16	14%
A, NH, OPI	1	1	2	4	4%
Total	38	38	35		

Note: Table total is five children more than target to allow for five extra degrees of freedom due to random subject arrivals.

Table 10 is of a multi-way cross-classification of children by the following four demographic factors in order of inferential: Age of Child, Sex of Child, Income of Parent(s), Ethnicity of Child. Each such four-dimensional (4-D) table is also summarized in a few of the possible marginal tables: Age x Sex x Income (3-D Table 11), Age x Sex (2-D Table 12), Sex x Income (2-D) Income x Ethnicity (2-D). Marginal 1-D totals are provided for the 2-D tables.

Table 10: Objective 2 Sampling Plan (N = 106). Representative Target Accruals: 4-Way Table, Age x Sex x Income x Ethnicity

	L		M		H		Total
	M	F	M	F	M	F	
0-3 months							
White	5	4	5	3	5	5	27
Hispanic				1		1	2
AI, AN							
Black		2	1			1	4
A, NH, OPI	1			1			2
							35
6-9 months							
White	2	2	4	3	2	2	15
Hispanic			1			1	2
AI, AN		1	1				2
Black	1			1	1		3
A, NH, OPI							
							22
12-15 months							
White	3	2	3	2	3	3	16
Hispanic	1				1		2
AI, AN			1				1
Black		1	1	1	1	1	5
A, NH, OPI					1		1
							25
18-24 months							
White	2	2	1	1	1	1	8
Hispanic		1		1			2
AI, AN							
Black	1			1	1		3
A, NH, OPI					1		1
							14
30-48 months							
White	1	4	3	2	2		12
Hispanic	1					1	2
AI, AN							
Black		1					1
A, NH, OPI							15

Note: Table total is five children more than target to allow for five extra degrees of freedom due to random subject arrivals.

Table 11: Objective 2 Sampling Plan (N = 106). Representative Target Accruals, 3-Way Table: Age x Sex x Income

	L	M	H		L	M	H		L	M	H
	0-3				6-9				12-15		
M	6	6			3	3	1		4	5	3
F	2	3	4		2	2	2		3	2	4

	18-24				30-48		
M	3	1	2		1	3	
F	3	3	1		4		1

Note: Table total is five children more than target to allow for five extra degrees of freedom due to random subject arrivals.

Table 12: Objective 2 Sampling Plan (N = 106). Representative Target Accruals, 2-Way Table: Age x Sex

	0-3	6-9	12-15	18-24	30-48	Total	% Total
M	18	10	10	7	8	53	48%
F	17	12	15	7	7	58	53%
Total	35	22	25	14	15		

Note: Table total is five children more than target to allow for five extra degrees of freedom due to random subject arrivals.

Table 13: Objective 2 Sampling Plan (N = 106). Representative Target Accruals, 2-Way Table: Income x Sex

	M	F	Total	% Total
L	18	20	38	34%
M	21	17	38	34%
H	19	16	35	32%
Total	58	53		

Note: Table total is five children more than target to allow for five extra degrees of freedom due to random subject arrivals.

4. GENERAL OVERVIEW

Exclusion Criteria for Objectives 1 and 2

Below is a general overview of some exclusion criteria. Details can be found in the Objective 1 and 2 Procedure Manuals (appendices A and B respectively).

Exclusion Categories	Examples
I. Demographic	Adopted, English fluency/Language, Parental history unknown
II. Pregnancy	Mother's age at birth of child: <16 years or >44 years Exposure: Smoke & Alcohol (as specified in each manual), Meds & Drugs Medical: Pre-eclampsia, Anesthesia, Gestational diabetes
III. Delivery	Multiple births, Malpresention, Fetal distress, Forcepts/Vacum excraction
IV. Birth-Neonatal	<37 wks 4 dys or >42 wks 3 days, Growth (outside acceptable limits specified in manuals), AS<8@5m Seizures, Hyperbilia, Abnormal face/limbs, PKU, RDS, Infection
V. Child Development	Growth (below acceptable limits specified in manuals), non-English, Breast-drugs, Medical: Major medical illness, Congenital abnormalities, Heart problems, Cancer, Lead poisoning Neuro: Seizures, CNS Infection, Head injury, Significant hearing loss Interven: Language disorder Psychiatric: Mood disorder, Conduct, AD/HD, Tic, Eating disorders,
VI. Psychiatric—1st Degree	Bipolar, Chronic depression, Psychotic, AD/HD, Drug dependence, PDD
VII. Child Testing / Parental	Intelligence, Achievement, BSID & PLS-3 (<70), CBCL>70
VIII. Child Neuro Exam	Hyper/Hypo-tonia, Ocular motility, Face, Motor or reflex abnormalities, Tics

Behavioral Measures

The primary purpose of the behavioral tests included in the battery is: i) to ensure selection of a normal sample and ii) to document the neurobehavioral variability of this research group. Some of the behavioral measures chosen will allow for brain behavior correlations.

Selection of behavioral instruments was guided by the following requirements:

- Description of cognitive and sensory motor abilities of the sample
- Description of academic skills
- Quantification of memory and executive functions

To meet the first requirement, standardized neuropsychological tests available for use by others such as the WASI (or the Bayley for Objective 2), Purdue Pegboard and Nepsy verbal fluency were included in the battery. The second requirement is met by including three sub-tests from the Woodcock-Johnson-III Achievement Battery and the Pre-School Language Scale. Verbal learning and memory is assessed using the California Verbal Learning Test (CVLT). Working memory is assessed with the Spatial Span subtest of the CANTAB. And executive functions are quantified using the Intra/Extra-dimensional set shifting and Spatial Working Memory subtests of CANTAB.

Objective 1

Subjects

Children between the ages of 4 years, 6 months and 18 years, 3 months will be recruited across sites to reflect the current demographic composition of the U.S. population with respect to gender, socioeconomic status, and race/ethnicity. Approximately 440 children will participate in Objective 1 with equal distribution across ages and gender. Subjects will be recruited from the general metropolitan area associated with each study site through lists provided by a national marketing agency.

The agency will provide lists of household addresses within various income levels, known to have children between specified ages. A subset of the subjects will participate in an additional MRS study that may require a second scanning session.

Recruitment and Screening

An introductory letter describing the study, the personnel involved, and a forthcoming phone call from a study recruiter to answer questions about the study will be sent to addresses provided by the marketing agency to each study site.

A brochure describing the study, a stamped, addressed post card, and an incentive (a fridge-magnet with the title of the project) will be sent along with this letter. An initial recruiting telephone call will be made to all families except those who asked not to be contacted in the returned post card. The initial recruiting phone call will screen to determine that the family has children within the age range of the study, that English is the child's primary language, that at least one parent is proficient in reading English, and that the potential child participant does not have a diagnosed chronic medical psychiatric condition, learning, or neurological disorder as assessed in this Brief Telephone Screening Interview. The recruiter will answer general questions about the study and ask whether the family would like to participate in a longer screening interview to further determine eligibility for participation.

If the family is interested in participating, they will be informed that they will receive a letter, a brief description of the study, and a telephone interview screening consent form with an addressed and stamped envelope. This form includes a telephone number that parents may call if they have questions. Included with the consent will be the CBCL, a behavior rating scale to be completed by the parent and returned with the signed consent form. Also included will be an outline of the Full Telephone Screening Interview. The family will be informed that a telephone call will be made within 10 days to ascertain if they received the study documents and at this time a phone appointment will be made to conduct the Full Telephone Screening Interview.

A follow-up phone call will take place (if documents have not been received) to inquire if the family has mailed the required forms (the parent CBCL is used for screening purposes and therefore is needed for the interaction with the family to proceed), and to offer assistance if needed.

At the time of the phone appointment for full telephone screening, any remaining questions the parents have about the interview or the consent form will be answered. If the signed consent form has not been received, a verbal consent will be obtained and documented, and the parents will be asked to sign the consent form and mail it back to the investigators with the completed behavior rating forms.

The Full Telephone Screening Interview will consist of the administration of a medical and developmental history questionnaire and a review of any missing information on the rating scale returned with the screening consent form. If the subject remains eligible, a structured psychiatric interview (the

Diagnostic Interview Schedule for Children [DISC] for subjects over 7 years of age), and the Family History Interview for Genetic Studies (FIGS) will be administered to the parent. Adolescents over age 11 will also complete the DPS interview. Following this phone call, and assuming all inclusion criteria have been reviewed and met, an appointment for an on-site evaluation and MRI will be made with eligible families. A telephone call to confirm the next-day hospital appointment will be made.

On-Site Procedures

The on-site evaluation and MR scans may require up to two days. Consent forms, (Objective 1 {parent, adolescents 18 and older} and Objective 2) describing the study and all evaluations will be signed and witnessed. Children aged 6-17 will give their written assent. Portions of the evaluation that may determine the final screens for eligibility and exclusion will be conducted prior to MR scanning, as will further behavioral testing that will be used for brain-behavior correlation. A physician will conduct a physical/neurological examination (including a Tanner Staging Questionnaire when appropriate).

Two saliva samples will be collected for the measurement of sex hormones as a more sensitive indicator of stage of maturation than chronological age. In addition, a urine sample will be collected for endocrine measurements, which will complement Tanner staging and growth data. Adrenal and gonadal steroids will be measured in saliva and urine.

The physical/neurological examination, will serve as an additional screen for eligibility and exclusionary criteria. In adolescent girls of childbearing potential (who have begun menses) a urine test will be taken to rule out pregnancy (using a stick test). A positive pregnancy test will lead to a laboratory test to rule out a false positive. A refusal for pregnancy testing will result in exclusion from the study.

Following these initial on-site evaluations, a psychologist or trained research assistant will conduct testing with a battery of neuropsychological tests. The results of these tests will be used for purposes of brain-behavior correlations and also to determine continuance in the study; e.g., an IQ rating < 70, or scores on any achievement test greater than 2 SD below the mean would exclude the child. Refer to Table 14 on page 44 for a list of Objective 1 procedures.

The MR scans will be conducted in addition to the clinical/behavioral evaluations described above, but possibly on another day depending upon the preference of the family, the temperament/fatigue of the child, the availability of scanning slots, and best practices of the site in terms of eliciting cooperation and motion-free scans. For example, young children may be scanned late at night to encourage them to sleep in the scanner. Parents will have the option to be with their child in the scanner room, if they desire, and if they have no contraindications for entering the MRI scanner room. The actual scanning time for the anatomic MRI is approximately 30-40 minutes. Those children participating in the additional MRS will have an extra 6-20 minutes of scanning time. The complete scan session will be approximately 35-60 minutes. The entire procedure from arriving to the MR suite to leaving the suite upon completion of the MR studies will take approximately 1 to 1-1/2 hours. For the youngest children and those children who express anxiety about the MR procedure, one or more 15–20 minute sessions with a mock scanner may be scheduled. At one site selected subjects may be asked to return for a more prolonged (70 minute) MRS study scheduled at a different time. Participants will be asked to return two more times at two-year intervals. The full telephone interview, behavior rating scales, on-site evaluations, and MR scans will be repeated each time. Participants will sign, in full, new consents and assents at each follow-up visit.

Subjects will be compensated for their time (in the order of $50-100 per day; final amounts to be decided by each PSC and made known to the other centers to maintain consistency, whilst keeping in mind regional factors dictating differences), and travel, parking, and meal expenses will be covered.

Objective 2

Subjects

Children ranging from newborn (10-14 days old) to 4 years, 5 months of age will be recruited across two sites (Boston, and St. Louis) participating in Objective 2. The sites will attempt to recruit a sample that is representative of the U.S. population with respect to gender, socioeconomic status and race/ethnicity.

A total of 106 children will participate in Objective 2. Newborns will be recruited on-site at hospital maternity wards and from associated well-baby clinics. Neonates recruited for study must be between 37 weeks 4 days and 41 weeks 3 days at birth. Children between 3 months and 4 years, 5 months will be recruited from satellite physician offices affiliated with the institution sites and/or the hospital maternity wards. Subjects may be recruited from community centers as well as the general metropolitan area associated with each study site through lists provided by the marketing agency.

Recruitment and Screening

For the majority of potential participants, except parents of newborns and young infants (2 – 11 months old), recruitment will occur through well-baby and well-child clinics. Staff will introduce the study during medical visits and/or by telephone, the study will be explained to parents, and any questions answered. Additional screening will be conducted in person or by telephone. Informed consent will be obtained prior to the screening. If the parents wish to participate, the appointments will be made for on-site evaluation and MR scanning after passing the screening procedures.

For newborns, parents of potentially eligible infants will be approached as soon after delivery as appropriate, and the study will be explained to them. Children recruited as newborns will receive the first time-point evaluation (neurobehavioral, neurological, and MRI examination) 10 – 14 days after the expected date of confinement (EDC or due date). Consent will be obtained and the evaluation and MR study will be scheduled not sooner than 10 days after birth, a time when initial physiologic post-delivery adjustment has been accomplished for most infants.

On-Site Procedures

All evaluations will be conducted on site. The evaluation and MR study may take more than one appointment day, depending upon the preference of the parents and the temperament and fatigue of the child; however, this should be within the time window as specified on page 27.

The project coordinator will call the family of the recruited child the night before each evaluation to ensure that the child is in good health. The parents will be asked whether the child has acquired an illness such as a cold or gastrointestinal disturbance.

A physician will conduct the physical/neurological examination. A psychologist or trained research assistant will conduct testing with a battery of neuropsychological tests. Results of these tests will be used for purposes of brain-behavior correlations and also to determine continuance in the study. Refer to Table 17 on page 46 for a list of Objective 2 procedures.

MR scanning will be conducted in addition to the clinical/behavioral procedures. The average scanning time for anatomical MRI, DTI, relaxometry and MRS is approximately 40-60 minutes. For children who do not remain comfortable and cooperative the study may be terminated early. The entire

procedure from arriving to the MR suite to leaving the suite upon completion of the MR studies should take approximately 1 to 1-1/2 hours (2-2.5 hours for newborns and young infants).

Children who do not fall asleep or who express anxiety about the MR procedures may be brought back for the scan at a different session. Families will be asked to return for follow-up evaluations and MR scans at intervals that vary depending upon the age at which the child enters the study.

The following procedures will be used to monitor the children while they are in the scanner:

- Pulse oximetry (subjects <3 months)
- Visual inspection by a skilled attendant. This person will be trained and knowledgeable in a) rapidly removing the infant from the bore should that be needed, and b) neonatal/child CPR and resuscitation.

Hazards and Precautions for Both Objectives

Foreign Objects

The Full Telephone Screening Interview requests information regarding the presence of metal objects or electronic devices in or permanently attached to the child's and parent's body. Parents and/or subjects are required to complete and sign an MRI Safety form. Prior to entering the MRI suite, children and parents will again be questioned regarding the presence in/on the child's and parent's body of:

(a) Electronic devices (e.g. pacemaker, defibrillator, neurostimulator etc.)

(b) Metal objects (e.g. skull plate, metal pins, dental brace, bullet or bullet fragments, metal slivers, non-removable piercing etc.).

These objects represent either safety issues (pacemaker, defibrillator, neurostimulators, metal slivers) or image quality concerns (plates, pins, braces). They are grouped into categories (a) and (b) above, rather than on safety and image quality criteria, because the former groupings are easier to understand during a telephone interview. The presence of objects of either category in subjects constitutes exclusion criteria for the study.

MRI Scanning

Although there are no known adverse effects of undergoing an MRI scan, including during pregnancy, absolute safety is difficult to definitively establish and it is plausible that there are long-term effects not yet observed. Current clinical practice therefore follows the prudent practice of avoiding MRI examination of pregnant women unless clinically indicated. Female subjects with onset of menses must undergo a urine pregnancy test prior to the scan. Pregnant females or females at undetermined risk for pregnancy will be asked to stay outside the scanning suite.

Incidental MRI Findings and Adverse Events

A clinical neuroradiologist will assess all MR scans. Any clinically abnormal findings will be communicated to the study investigator who will notify the parents and with permission, forward the results to the child's primary care physician as designated by the parents. Further referrals to appropriate clinicians will be made as appropriate.

Incidental MRI findings and MR-related adverse events are reported to the DCC and on the *Scanning and Evaluation Report.* Adverse events must be reported to the NIH. Incidental MRI findings reported by the site's clinical radiologist will be reviewed by Dr. R. McKinstry and Dr. W. Ball.

Incidental Behavioral Findings

If as a result of the testing or interviews with parents and their child, there is an indication of a clinically significant medical, learning, emotional or behavioral problem, this information will be shared with the parents. With permission, the child's doctor will be notified for follow-up, and referrals to appropriate clinicians will be provided for further evaluation as necessary and desired by the family and child's physician.

Time Windows (Intervals) For Scanning And Behavioral Testing For Both Objectives

Suggested Intervals Between Completion Of Behavioral Testing Versus Completion Of Brain Scanning For A Child

The intent is to complete the behavioral testing and brain scanning of a child within a matter of a few days. However, if circumstances require a longer interval between the scanning and the testing (e.g., a problem with the scan and/or testing) we will use the following time windows:

SCAN-TEST AGE	TIME WINDOW
Objective 1:	
AGE 4:6 and older	± 4-WEEKS
Objective 2:	
AGE 0:0 (10-14 DAYS EDC)	+ 1-WEEK (NO MINUS)
AGE 0:3 THROUGH 2:6	± 2-WEEKS
AGE 3:0 THROUGH 4:5	± 4-WEEKS

Target Ages And Windows For Timing Of Brain Scan & Behavioral Testing For Objective 2

We should try to scan/test the child within the time windows indicated below for children of different ages (e.g., [1] a child representing the 0:3 cohort should receive the scan/test within ±2-weeks of 3-months of age, [2] a child representing the 4:0 cohort should be scanned/tested at between 3:11 and 4:5).

SCAN-TEST AGE	TIME WINDOW
AGE 0:0 (10-14 DAYS EDC)	+ 1-WEEK (NO MINUS)
AGE 0:3, 0:6, 0:9, 0:12 & 0:15	± 2-WEEKS
AGE 1:6, 2:0, 2:6, & 3:0	± 4-WEEKS
AGE 4:0	AT ANY AGE BETWEEN 3:11 - 4:5

NOTE: ALL AGES ADJUSTED FOR 'EXPECTED DATE OF CONFINEMENT (EDC, WITH A BASE/REFERENCE POINT OF 40 WEEKS GESTATIONAL AGE AT BIRTH). 0:0 MONTHS IS ACTUALLY AT 10-14 DAYS POST-EDC.

Inconvenience or Discomfort to Subject

The child or parents may become tired or feel nervous as the testing sessions progress. Participants will not be pressured to respond to questions, and will be allowed to take breaks or seek information at any time. The MR procedures are free of pain. Potential discomforts include the loud sound from the MR scanner (all children will receive comfortable ear protection) and the need to lie still. During the MR scans a child may become anxious. Should this be the case, the procedure can be interrupted and stopped if necessary.

Estimated Duration of Study

The estimated duration of subject participation in the study is between 5 and 6 years.

Description of Behavioral/Clinical Procedures for Both Objectives

A list of procedures corresponding to Objective 1 and 2 is available in sections 6 and 7 respectively.

Bayley Scales of Infant Development II (BSID-II)

Mental Scale (MDI) – includes assessment of a variety of abilities: sensory/perceptual acuities, discriminations, and response; acquisition of object constancy; memory, learning and problem solving; vocalization. Beginning of verbal communication; basis of abstract thinking; habituation; mental mapping; complex language and mathematical concept formation.

Motor Scales (PDI) – includes degree of body control, large muscle coordination, finer manipulatory skills of the hands and fingers, dynamic movement, dynamic praxis, postural imitation, and stereognosis.

Behavior Rating scale – A 30 item questionnaire that seeks information divided into four main factors:

1. Attention/Arousal
2. Orientation/Engagement
3. Emotional Regulation
4. Motor Quality

Standard scores and percentile ranks are reported and the administration times are:

Birth to 15 months	**25-35 minutes**
15 months to 3 years, 6 months	**60-90 minutes**

Standardization included 1,700 children stratified according to the 1980 census. And reliability coefficients are .88 for the mental, and .84 for motor scales. Test-retest stability coefficients are .87 for mental, .78 for motor scales.

Behavior Rating Inventory of Executive Function (BRIEF)

The BRIEF is an 86-item parent-completed behavior rating scale that taps eight sub domains of executive functioning via ratings of everyday behavior. The scales are Initiate, Working Memory, Plan/Organize, Organization of Materials, Monitor, Inhibit, Shift, and Emotional Control. Factor analysis of the parent and teacher normative samples and the BRIEF with other parent/teacher behavior rating scales administered to clinical samples (CBCL/TRF {Teacher Report Form}, ADHD {Attention Deficit Hyperactivity Disorder Rating Scale-IV}) results in a consistent two-factor model – Behavior Regulation (Inhibit, Shift, Emotional Control) and Metacognition (Initiate, Working Memory, Plan/ Organize, Organization of Materials and Monitor). *(BRIEF professional Manual, Gioia et al, PAR Inc, 2000)*

The items that comprise the BRIEF are behavioral descriptors within the eight sub domains (e.g., Initiate items - Is not a self-starter, Has trouble getting started on homework or chores; Shift items – Resists change of routine, foods, places; Tries same approach to problem even when it does not work). The Parent rates whether the behavior has been a problem for the child over the past 6 months by circling Never, Sometimes or Often.

The BRIEF scales demonstrate appropriate reliability, internal consistency is high, and the measure is stable over a 2 to 6 week period. Within the parent normative sample (n = 54), the mean test-retest correlation across the scales was .82, ranging from .76 to .88 over an average two week time span. For the parent clinical sample, the mean correlation was .80, ranging from .72 to .84 over an average three week period. Correlational analyses with other behavior rating scales (CBCL/TRF, BASC

Parent/Teacher, ADHD Rating Scale, and Conner's Parent Scale) provide evidence of convergent and divergent validity for the Parent and Teacher Forms of the BRIEF. Executive functions as measured on the BRIEF correlated in an expected fashion with other measures of general behavioral functioning (e.g., Inhibit more highly with hyperactivity or aggression; working memory with Attention Problems) and typically less strongly or not at all with measures of emotional functioning. *(Gioia et al, PAR Inc, 2000)*

In general, it is hypothesized that the Behavioral Regulation scales would be correlated with orbital-medial cortical regions while the Metacognition scales would be correlated with dorsolateral prefrontal regions. Presumably white matter would be the developmental substrate.

The BRIEF was developed with a variety of childhood disorders in mind including learning disabilities, attention deficit hyperactivity disorder, traumatic brain injury, lead exposure, pervasive developmental disorders, depression and other conditions affecting brain integrity and/or behavioral competency. A variety of clinical populations have been studied to date including reading disabilities (with and without ADHD), ADHD, Tourette Syndrome (with and without ADHD), mixed PDD (Pervasive Developmental Disorder), High Functioning Autism, several years post moderate-severe Traumatic Brain Injury, early treated PKU, documented brain lesions (frontal and extra frontal), low birth weight, and mental retardation. Varying group profiles emerge in these samples.

The 86-item questionnaire takes approximately 10 minutes to administer and can be administered and scored by a research assistant.

Justification for Inclusion: During the age range to be addressed by Objective 1 there are two overwhelming developmental changes that will swamp any effects of more "modular" capacities that we may wish to measure: these are behavioral regulation and processing capacity. While cognitive measures of "executive function" are available, these may be less sensitive to the profound changes that occur during childhood in moment-to-moment behavior. The BRIEF provides an economical port of entry to both behavioral regulation and more cognitive issues (metacognition). The downside for our purposes is that the raters' responses are always age referenced, and so it may not fully capture the developmental changes we wish to chronicle (e.g., a well regulated seven year old may be equivalent behaviorally to a poorly regulated ten year old). What it will do for us is characterize the population well and serve as a reference point for all the developmental disorders with which investigators may want to compare this database. The BRIEF will not be used as an exclusionary measure.

California Verbal Learning Test (CVLT)

This test measures verbal learning and memory and examines the strategies and processes involved in learning and recalling verbal material. The CVLT-C was normed on a standardization sample of 920 children aged 5 to 16 years old, including 459 females and 461 males. The proportions of children in the White, African American, Hispanic, and Other race/ethnic groups were based on March 1988 census data. The standardization sample was also stratified on parent education level and geographic region. The CVLT-C uses a shopping list format to present two lists of to-be-remembered words: List A is presented 5 times, List B one time. Both lists consist of items in semantic categories. It provides scores for immediate-, short-, and long-delay recall, free and cued recall, proactive and retroactive interference, semantic clustering, and recognition. It also provides error measures, including perseveration and intrusion errors. *(CVLT-II Manual, Dellis et al., The Psychological Corporation, 2000)*

The brain structures/systems involved are likely left temporal lobe. The test takes about 30 minutes to administer. The domain of verbal learning and memory is critical for adequate description of a subject's neuropsychological status. It is important for a number of neurological and developmental disorders. It is known to have specific anatomic substrates.

For adolescents older than 16 years old, the CVLT-II will be employed. *(CVLT-II Manual, Dellis et al., The Psychological Corporation, 1994)*

Cambridge Neuropsychological Test Automated Battery (CANTAB)

The CANTAB is an automated computerized test, mostly non-verbal, visually presented using a touch screen.

The following five subtests will be included and administered to children aged 4 and above:

I) Motor Screening Task. This simple reaction time test measures psychomotor speed and accuracy and is designed to screen for psychomotor impairments that would interfere with later task performance.

II) Spatial Span Task. This task, based on the Corsi block task *(Milner, 1971)*, measures memory for a figural sequence and is believed to activate the right ventrolateral prefrontal cortex (Robbins et al., 1996).

III) Spatial Working Memory Task. This self-ordered searching task *(Petrides & Milner, 1982)* measures working memory for spatial stimuli and requires the subject to use mnemonic information to work towards a goal. PET imaging studies indicate that this task activates both the dorsal and ventral prefrontal regions *(Owen, Evans & Petrides, 1996)*.

IV) Big Little Circle. This is a two-stimuli visual discrimination and category achievement test.

V) Intradimensional/Extradimensional Set-Shifting Task. This task measures discrimination and reversal learning under conditions whereby the subject is required to shift attention to changing patterns of visual stimuli. A full description can be found in Downes, Roberts, Sahakian, Evenden, Morris & Robbins(1989). A functional dissociation between prefrontal regions is suggested by a recent lesion study in marmosets *(Dias, Robbins, & Roberts, 1996)* that has implicated the dorsolateral prefrontal cortex in between-category set shifting (e.g., as in the shift in responses from lined figures to colored shapes) and the orbitofrontal cortex in within-category reversal shifts (as in the shift in responses from one lined figure to another previously non-reinforced lined figure) using this task.

The administration time varies from 35 to 45 minutes depending on the stamina and cognitive maturity of the child. Because all tasks are graded in difficulty with easy items presented first and more difficult items presented last, children who are able to solve more difficult task items generally have longer testing sessions. The CANTAB will be run on a portable computer connected to a large desktop touch screen.

CANTAB samples predominately frontal lobe function and given the long time course (up to 25 years) associated with frontal-lobe development the CANTAB is elegantly suitable for the present project. Subtests of the CANTAB sample temporal, parietal and basal ganglia function.

Carey Temperament Scales

Child temperament is believed to be fundamental in determining how a child interacts with his/her environment. Patterns of interaction that develop early on in life can contribute to the development of both child and adult psychopathology. *(Carey, McDevitt: Behavioral-Developmental Initiatives, 1977-95)*

The Early Infant Temperament Questionnaire (EITQ; 1-4 months); Revised Infant Temperament Questionnaire (RITQ; 4-8 months); Toddler Temperament Scale (TTS; 1-2 years); and Behavioral Style Questionnaire (BSQ; 3-7 years) are concise and well-validated temperament questionnaires. They have

the advantage of being a part of a series of temperament scales, allowing for the measurement of this construct from age 1 month to 12 years. The primary caregiver of the child completes the questionnaires.

The questionnaire measures the nine New York Longitudinal Study temperament characteristics:

1. Activity level
2. Rhythmicity
3. Approach-withdrawal
4. Adaptability
5. Intensity
6. Mood
7. Attention span and persistence
8. Distractibility
9. Sensory threshold

The RITQ (EITQ is very similar) was standardized with a sample of 203, primarily Euro-American participants of middle socioeconomic status, living in the eastern United States. The age range of the sample was between 4-8 months. Standardization norms may not apply to infants in specialized settings or subgroups. Users who desire to use this measure with a specialized population should consult the author, and consider performing a re-standardization with item analysis.

The TTS was standardized with a sample of 304, primarily Euro-American participants of middle socioeconomic status, living in the eastern United States. The age range of the sample was between 1-2 years. Separate norms were developed for infants 1 and 2 year-old. Standardization norms may not apply to toddlers in specialized settings or subgroups. Users who desire to use this measure with a specialized population should consult the author, and consider performing a re-standardization with item analysis.

The BSQ was standardized with a sample of 350, primarily Euro-American participants of middle socioeconomic status, living in the eastern United States. The age range of the sample was between 3-7 years. Standardization norms may not apply to children in specialized settings or subgroups. Users who desire to use this measure with a specialized population should consult the author, and consider performing a re-standardization with item analysis.

The Carey Temperament Questionnaires contain approximately 100 items that are rated on a 6-point scale ranging from almost never to almost always. The item scores are tabulated into a category score for each temperament subscale, which can then be compared to the normative scores for that category.

As the measure was designed to assess a wide range of behaviors at a number of developmental levels, it is assumed to be sensitive to the full range of normal performance. This questionnaire is designed to measure the quality and quantity certain of behaviors and affect that are associated with the indicated developmental period. Individual differences in these abilities/characteristics will be reflected by comparing scores with the mean scores of each subscale.

The questionnaire is completed by the primary caregiver of the child and takes about 20-30 minutes to complete.

Childhood Behavior Checklist (CBCL/1.5-5, CBCL/6-18, YASR/18+)

This is an empirically based questionnaire for assessing children's behavioral /emotional problems. It is designed to be completed by parents and others who interact with the child in a variety of every day context. The relevant parent reported CBCL (1.5-5, 6-18) and CBCL/YASR, young adult self-rating, (18 years+) will be used as part of the mailed-out screening to families for subject selection. Children and adolescents would be excluded if they had a T-score > 70 on any CBCL problem scale or subscale. The selection of these cut off scores was based on published research with the relationship between CBCL scores and diagnoses from the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV). The aim of choosing cut off scores was to exclude subjects at an early point in screening if they were highly likely to meet criteria for an Axis I DSM-IV disorder. In addition to their use for subject screening, CBCL subscale scores would be used for brain-behavior correlation.

There are 113 items covering a range of behaviors. The 113 items are scored as follows: 0 if the item is not true of the child, 1 if the item is somewhat or sometimes true and 2 if it is very true or often true. Scales have been derived using factor analytic methods from large populations. The 1991 version generates scores for 7 subscales and 2 overall scales described as "internalizing problems" and "externalizing problems". The seven subscales are: 1) withdrawn, 2) anxious depressed, 3) social problems, 4) thought problems, 5) attention problems, 6) delinquent behavior, 7) aggressive behavior.

The scale has been extensively normed on general population samples in the US and many other countries as well. The most recent norms reported were from 1991 and were based on principal components analyses of parents' ratings of 4,455 clinically referred children, and normed on 2,368 children aged 4 to 18. The normative sample was representative of the 48 contiguous states for SES, ethnicity, region, and urban-suburban-rural residence. Children were excluded from the normative sample if they had been referred for mental health or special education services within the past year *(Achenbach, 1991)*. Norms are currently available for the 1.5-5 and 4-18 age ranges. New norms are under development for the 5-11 & 12-18 age ranges and will reportedly be available within the next 2 years. Scales are separately normed for boys and girls. The CBCL/4-18 Total Problem score has a one-week test-retest reliability of r = .93, while inter-parent agreement is r = .76 *(Achenbach, 1991).*

The CBCL/1.5-5 is 100-question item set is rated on a 3-point scale and it is written at a 5th grade reading level. Six statistically robust syndromes were identified in the analysis of both sexes combined: anxious/depressed, withdrawn, sleep problems, somatic problems, aggressive behavior, and destructive behavior.

The Administration time is about 10 minutes and the standardization sample included 368 children stratified by region and SES. Test-retest reliability is = mean .85 (7.7 days interval) and interparent agreement = .63.

The brain structures/systems involved would be widely varied based on subscales. It is hypothesized that anxious depressed behaviors are mediated through medial and orbital prefrontal/striatal/ thalamic limbic regions *(Price 1999, Mayberg et al, 1999, Drevets et al., 1992)*. Attention is thought to be mediated through prefrontal/cingulate/striatal/parietal cortex regions *(Berger & Posner 2000, Castellanos, 1997)*. Aggression is hypothesized to be related to prefrontal, amygdala, ventral medial prefrontal circuits *(Raine et al., 2000; Adolphs et al., 1998).*

There are clearly gender specific developmental changes that occur in the subscales of the CBCL over preschool, school age, adolescent and young adult norms. To pick a few specific hypotheses, there are decreases in anxious withdrawn scores over early childhood (2-5 years) that could correlate with structural development in medial and orbital prefrontal cortex and amygdala regions. Later in development anxious depressed scores increase in a gender specific manner. Decreases in attention problem scores also occur over the ages of 4 to 18 and may be related to developmental changes in

specific prefrontal regions such as the DLPFC, anterior cingulate and basal ganglia. Aggression scores also decrease over childhood and may relate to developmental changes in the amygdala as well as developmental progress in prefrontal regions important in inhibitory control.

The forms are completed independently by the informant (parent or child). The parent version takes approximately 20 minutes to complete and the adolescent self-version also takes about 20 minutes. Scoring is computerized.

Some of the advantages for the CBCL are its extensive use in many studies involving normal children and children with disorders. It has been extensively normed in large populations. It has been specifically studied in how it relates to DSM-IIIR and IV diagnoses and has many useful properties for the use in screening out children who would meet criteria for Axis I disorders on structured psychiatric interviews *(Biederman et al., 1990, 1993; Bird et al., 1988, 1991; Edelbrock and Costello 1988; Steingard eta al., 1992; Weinstein et al., 1990)*. It is easily suited to use for screening by mail. There is substantial data on developmental changes in subscale scores from both cross-sectional and longitudinal studies. There is data available regarding the contribution of genetic, shared environmental and unique environmental influences on syndrome scores and their developmental changes *(Hudziak et al, 2000; Gjone et al, 1997; Schmitz et al., 1995).*

Diagnostic Inventory Schedule for Children (DISC) & Diagnostic Inventory Schedule for Children Predictive Scales (DPS)

The purpose of the DISC is to screen and identify the presence of psychiatric disorders. It is a structured interview that was designed for epidemiological screening. The DISC is a lay-interviewer administered test and is keyed to DSM-IV.

The DISC has several versions of which the C-DISC-4 and a shortened version, the DISC Predictive Scales (DPS-4) will be used.

The DISC is organized in modules: Introductory; Anxiety; Mood; Disruptive; Substance use; Schizophrenia; and Miscellaneous (eating disorders, tic, elimination disorders, pica, trichotillomania) that are primarily scored "Yes" or "No." The modules are organized by DSM-IV and some ICD-10 disorders. The DISC has 358 stem questions that extent to a total of about 3,000 items. It can be programmed to skip or omit modules (e.g., psychosis) and all the scoring is accomplished via a computer algorithm and the data is saved automatically. The DISC output provides DSM-IV diagnoses.

The DISC has a reliability of 0.43 to 0.85 for symptoms, 0.46 to 0.93 for criterion counts and 0.45 to 0.68 for categories. Attenuation has low reliability.

Test-Retest Reliability:

ADHD	(Attention Deficit Hyperactivity Disorder)	0.79
ODD	(Oppositional Defiant Disorder)	0.54
CD	(Conduct Disorder)	0.43
Specific	Phobia	0.96
SAD	(Separation Anxiety Disorder)	0.58
GAD	(Generalized Anxiety Disorder)	0.65
MDD	(Major Depression/Dysthymic Disorder)	0.66

Validity:

There is good agreement with the clinician interview *(Schwab-Stone et al., 1996).*

ADHD	(Attention Deficit Hyperactivity Disorder)	0.72
ODD	(Oppositional Defiant Disorder)	0.59
CD	(Conduct Disorder)	0.74
Any anxiety		0.63
MDD	(Major Depression/Dythymic Disorder)	0.60

Differential Ability Scales (DAS)

The DAS yields an overall General Conceptual Ability (GCA) score, and cluster scores assessing Verbal Ability and Nonverbal Ability. These are represented in standard score format. Individuals scoring 2 standard deviations below the mean (70) GCA score would be excluded. *(DAS Manual: Psychological Corporation, 1990. C.D. Ellict, 1983)*

There are 6 core subtests. Three subtests comprise the Verbal Ability cluster, and three comprise the Nonverbal Ability cluster.

Verbal Ability:

i. Verbal Comprehension: Assess receptive language. No items require an oral response. Taps ability with syntax, prepositional and relational concepts; ability to formulate and test hypotheses, ability to follow verbal directions; and short term auditory memory. The first items use a picture of a Teddy Bear on which the child points to several features. Next the child is shown an array of toys that samples the child's understanding of names, of commands, and of functions. The next level measures ability to understand prepositions, and finally items assess ability to understand complex instructions.

ii. Picture Similarities: Nonverbal subtest assessing reasoning ability. Task does not require verbal response. Reflects ability to solve nonverbal problems; identify pictures; formulate and test hypotheses; use verbal mediation; and attach meaning to pictures. For each item, the child is shown a row of pictures or designs in a booklet. The child places a card with a single picture or design below the stimulus picture that it best goes with. The child is asked to recognize a relationship based upon a common concept or element. The child must perceive various, possibly relevant features of drawings and engage in hypothesis testing to select the correct elements of commonality.

iii. Naming Vocabulary: Assesses spoken vocabulary. Measures expressive language ability; ability to match; general language development; and word retrieval from long-term memory. Items require the child to recall words from long-term memory rather than recognize or understand the meaning of words. The subtest consists of two objects and a booklet of colored pictures of objects that the child is shown one at a time and asked to name.

Nonverbal Ability:

Pattern Construction: Assesses visual-spatial ability; perception of spatial orientation; analysis of visual data; and nonverbal reasoning. Booklet is 2-dimensional while blocks are 3-D. Task requires child to make a 2-D construction while ignoring the 3rd dimension. Young children create designs using foam squares with sides of black or yellow. Older children use 3-D blocks with sides that are black, yellow, black and yellow divided diagonally and black and yellow divided vertically.

Early Number Concepts: This subtest assesses 10 areas of number concepts and skills. Among the areas are reciting, counting, matching, comparing, recognizing, and solving number concepts.

Copying: Assesses fine motor ability and the ability to perceive similarities between figures. Items start very simple (straight line) and progress to more complex geometric
figures. All items are not timed. Child sees design entire time.

The DAS was normed on 3,475 children; 175 for each 6-month age group from age 2-6 through 4-11, and 200 per year for ages 5-0 through 17-11. Evenly divided by sex. Four race/ethnicity categories: White, Black, Hispanic, other (Asian, Eskimo, etc.). Black and Hispanic children were statistically over sampled, to reduce item bias. Stratified by 4 geographic regions, parent education, and educational preschool enrollment.

The DAS is specifically designed to be culture-fair test. There is no country-specific content; and there are no questions about social values.

Individual subtests are scored as T-scores, while cluster and overall GCA scores are standard scores.

The DAS is child-friendly, is based upon more recent research in cognitive development, has a neuropsychologically based design and each DAS subtest is designed to stand alone, assessing a unique cognitive construct.

Family History Interview for Genetic Studies-modified (FIGS)

The Family Interview for Genetic Studies (FIGS) was developed by principal investigators in the NIMH Schizophrenia and Bipolar Disorder Genetics Initiatives and NIMH extramural program staff as a guide for systematically collecting information about relatives in family/genetic studies of these disorders. The FIGS does not elicit self-report data; rather, subjects are asked to provide information about others. FIGS administration proceeds in three steps: 1) a pedigree is drawn and reviewed with the informant; 2) general screening questions are asked in reference to all known relatives; and 3) based on the informant's responses to the general screening questions, a Face Sheet and possibly one or more of the symptom checklists (depression, mania, alcohol and other drug abuse, psychosis, paranoid/schizoid/schizotypal personality disorder, tic disorders, obsessive compulsive disorder (OCD), antisocial disorder, ADHD) are completed for each first-degree relative, spouse, or other relative well known to the informant (and optionally for second-degree relatives). A particular symptom checklist is completed if, based on the informant's responses to the general screening questions, the interviewer suspects that the psychopathology assessed by the particular symptom checklist is present.

The interview will be used to screen for family history of the following Axis I disorders in any first degree relative: Schizophrenia, bipolar affective disorder, alcohol dependence, OCD, Tourette's disorder, recurrent major depressive disorder (MDD) or chronic MDD episodes, pervasive developmental disorder or attention deficit hyperactivity disorder. The rationale for exclusion of subjects with a close family history of the above Axis I disorders is based on two major concerns: 1. There are research findings demonstrating altered neuromorphometry in relatives of individuals with some of the above Axis I disorders (schizophrenia, and affective disorders); and 2) children are being recruited at young ages, potentially before the age of onset of many of the above disorders, we wanted to decrease the potential for the subjects to develop a major Axis I disorder during the course of study, or following completion of the study when they will not be followed. It is essential for the database to contain information about psychiatric disorders in the relatives of the normal subject database in order for it to be useful as a potential comparison group for studies of early onset psychiatric disorders.

The research assistant would administer the interview over the phone. Completion for first-degree relatives in families without extensive psychiatric disorders would take an average of 15 minutes.

Handedness

The child is asked to demonstrate handedness. Prior to administering the Handedness Test, child's caregiver is asked which hand the child typically uses to reach for and manipulate objects (e.g., a rattle): **RIGHT; LEFT;** or **BIMANUAL**.

There are 3 versions of the Handedness test:

Version 1: Hand for ages 1:0 – 2:11
Version 2: Hand for ages 3:00 – 5:11
Version 3: Hand B Pantomime for ages 6:0+

The Handedness tests are video taped to aid in scoring. Scoring of Handedness uses the Unimanual Laterality Index *(ULI; Michel et al., 1985).*

The score is the total number of activities carried out with each hand where a right-handed response is given a 1 and a left handed response is given a 0. A score < 7 = non right handed. (G.F. *Michel, M.R. Ovrut and D.A., 1985.)*

$$\textbf{ULI} = \frac{\textbf{(\# right-hand grasps) - [(\# left-hand grasps) + (\# bimanual-hand grasps)]}}{\textbf{v (\# right-hand grasps) + (\# left-hand grasps) + (\# bimanual-hand grasps)*}}$$

NOTE: * In the ULI formula, "v" represents the sign for square root.

Based on the Unimanual Laterality Index, Handedness categories are:

Right-Handed: ULI > +1
Left-Handed: ULI < -1
Mixed-Handed ULI is between -1 and +1

Reference: Michel, G.F., Ovrut, M.R., & Harkins, D.A. (1985). Hand-use preference for reaching and object manipulation in 6- through 13-month-old infants. Genetic, Social and General Psychology Monographs, 111(4), 409-427.

Junior Temperament and Character Inventory (JTCI)

There are 108 items on both the observer and self-versions covering a range of personality features. The test was originally developed for adult populations to measure four "temperament" and three "character" traits. The four temperament traits are: 1) novelty seeking, 2) reward dependence, 3) harm avoidance and 4) persistence. The three character traits are 1) self-directedness, 2) cooperativeness and 3) self-transcendence. Items have been modified for use in toddlers, children and adolescents. Scores are derived for the seven scales.

There are small-scale normative studies in children. The scale has been normed more thoroughly in adult populations in the US and abroad. Luby et al (1999) reported on a community population sample of 322 boys and girls 9-13 years of age. They confirmed the four-factor model for temperament features

(novelty seeking, harm avoidance, reward dependence and persistence) and report means and SD along with Cronbach alphas for this population. Some of the "character" factors performed less well. There was more intercorrelation between scales than observed in adults and this was hypothesized to be related to personality development and the observation that some personality features are not as well differentiated in childhood and early adolescent. From this study it was demonstrated that the instrument was well tolerated by parents and children over the age of nine. Further this study concluded that there did appear to be some developmental differences in some of the personality constructs in comparison to the adult studies. A version for toddlers (parent or teacher report) has also been developed and initially tested with teacher reports in a sample of 317 children ages 2-5 years from the general population, and tested with parent reports in 76 thirty-month-old children. Cronbach alphas for items in each of the 7 factors were acceptable (range 0.76 – 0.85). Inter-rater reliability was reported to be good (0.52 – 0.63 range).

The JTCI is a paper and pencil measure that subjects and parents can complete independently with very little instruction. The questionnaire consists of 108 items consisting of short descriptive sentences that the subject or parent circles the most appropriate descriptor (True/ False or based on a 5-point scale: definitely false, mostly or probably false, neither true nor false, mostly or probably true, definitely true). The JTCI will be mailed to subjects ages 10 and above to be completed at home by the subject prior to coming for their testing and MRI appointment. There is a child version that would be completed by subjects ages 10-15 and an adult version that would be completed by subjects over the age of 15. Subjects less than 10 years of age will only have a parent version completed. The parent will complete the JTCI questionnaire rating of their child during the on-site appointment while the child is occupied with other testing. For Objective I, parents will complete the parent JTCI for all subjects ages 4 years six months to 18 years.

There are not well-established scales of personality that have been used extensively in child or adolescent psychiatric disorders. Understanding of the underlying structural correlates of normal functioning in these temperament and character scales could be important in understanding disruptions in brain structure related to childhood psychiatric disorders that are associated with abnormalities in constructs measured in the scales. For example individuals with affective and anxiety disorders demonstrate elevations in harm avoidance. Individuals with attention deficit disorder demonstrate problems with persistence and elevations on novelty seeking scores. Individuals with antisocial behaviors have elevations in novelty seeking and decreases in harm avoidance and reward dependence. The forms are completed independently by the informant (parent and/or child). The parent version takes approximately 20-30 minutes to complete and the child self-version takes about the same amount of time. The child version could be completed at home and parents could complete their scale while their children are involved in other evaluations on the day of their visit. The scale can be administered either in paper pencil version or a computerized version.

NEPSY – Verbal Fluency

The Verbal Fluency subtest from the NEPSY will be used for all children. The NEPSY has been standardized on a representative sample of the US population of children between the ages of 3 through 12, with 100 children in each age group for a total sample size of 1,000 cases. In addition to the NEPSY, older children (age 12 and above) will be administered the standard FAS test that has appropriate age-specific norms. *(NEPSY Manual: Kerman, Kirk, Kemp, Psychological Corporation, 1998)*

The NEPSY Verbal Fluency test requests animals in 1 min, and as many things to eat and drink as possible in 1 min; and the older child names in addition words that start with F and S in one min (again the examiner records the child's spoken responses).

Parenting Stress Index (PSI)

The PSI was developed as a screening and diagnostic tool for use with parents of children 1 month to 10 years of age. It is designed to identify parent-child systems under stress and at risk for less than optimal parenting and the development of emotional problems in children. *(PSI Manual 3rd edition: Abidan, Par Psychological Assessment Resources Inc., 1995)*

The PSI is normed on 2,633 mothers of children ranging from 1 month to 12 years, 200 fathers of children ranging from 6 months to 6 years and 223 Hispanic parents. The PSI yields 17 scores, including 7 Child Domain scores, 8 Parent Domain scores, and a Total Stress score, plus the optional Life Stress score. The child characteristics are measured in 6 subscales: distractibility/hyperactivity, adaptability, reinforces parent, demandingness, mood, and acceptability. The parent personality and situational variables component consists of 7 subscales: competence, isolation, attachment, health, role restriction, depression, and spouse.

The statistical characteristics of the PSI are suitable for correlation studies of which there are many. The normal range for the Total Score is between the 15th and the 80th percentile. One issue is that the PSI is a clinical tool in which elevated score are usually pathologic. However, extremely low scores can be seen in situation in which 'dysfunction in the mother-child system' is present. These respondents are deemed defensive, dishonest, or disengaged. There is a defensive respondent scale. There is some evidence that the stress score of families with younger children is higher than those with older children. Critical cut-off for 'High Stress' level is age normed.

There is an extensive literature (over 300 articles) of the use of the PSI with a wide range of childhood disorders including children with learning disabilities, autism, motor delays, giftedness, emotional problems and attentional deficits.

This self-report inventory is composed of 120 items (of which 19 are optional). The parent responds to each question on a 5-point response scale: Strongly Agree; Agree; Not Sure; Disagree; Strongly Disagree. The PSI takes 20-30 minutes to complete and 5-10 minutes to hand score. The PSI is a self-report inventory designed to be accessible to a parent with a 5th grade reading level. A testing assistant can read the questions to the parent if the reading level is an issue.

The PSI will provide a measure of the parent's knowledge of child development and management skills and comfort in role as parent. It assesses the parent's sense of social isolation, emotional closeness to child, health status and presence of depression. In the child domain it assesses Distractibility/Hyperactivity as reported by the parent. None of the other proposed measures will provide this valuable information.

Preschool Language Scale-3 (PLS-3)

The age range is from birth to 6 years 11 months and the measures include:

Auditory Comprehension – assessment of attention, vocabulary, concepts of quality, quantity, spatial and time/sequence, structure including morphology and syntax, and integrative thinking skills.

Expressive communication – includes vocal development, social communication, vocabulary, concepts of quality, quantity, spatial and time/sequence, structure including morphology and syntax, and integrative thinking skills.

Scores reported:

Total Language score
Auditory comprehension
Expressive communication
Standard scores, percentile ranks and age equivalencies

Standardization is on 1,200 children stratified according to the 1980 census. Sample size varies at different test ages. Internal consistency reliability coefficients range from .47 to .88 for Auditory comprehension, .68 to.91 for expressive comprehension, and .74 to .94 for total language score. Test-retest – stability coefficients range from .82 to .94. *(PLS-3 Manual: Zimmerman et al, Psychological Corporations, 1992)*

Purdue Pegboard

The Purdue Pegboard measures sensorimotor functions; in particular, fine motor coordination that is independent of educational achievement. There are normative data for preschool children from the ages of 2 to 6 years and 5 to 11 years *(Wilson, Iacoviello, Wilson, & Risucci, 1982);* and for school-aged children from the ages of 5-0 through 16-11 years *(Gardner, 1979)*.

Some research (Wilson, Iacoviello, Wilson, & Risucci, 1982) has shown developmental trends in normal children-demonstrating increased efficiency in peg placement for the right hand, left hand and the bimanual conditions. The scores increase steadily with age. This instrument allows for comparisons between lower level functions and higher-level cognitive functions and provides information about lateralized or bilateral deficits. It is likely that myelination of the cortico-spinal tract will affect performance on this task *(Muller & Homberg, 1992; Paus et al., 1999).*

Half-Size Board:

Instructions for the preschool Purdue pegboard (half board) appear in the Tester's Manual.

Wechsler Abbreviated Scale of Intelligence (WASI)

The WASI yields Full-Scale, Verbal and Performance IQ ratings. Two standard deviations below the mean of 100 yields a cut-off score of 70 and subjects scoring below this level would be excluded. The WASI has four subtests, two on the Verbal and two on the Performance scales. *(WASI Manual: Psychological Corporation, 1999)*

1. **Vocabulary** (to a greater or lesser degree this subtest explores expressive vocabulary, verbal knowledge, fund of information, crystallized intelligence and general
intelligence, memory, learning ability and concept and language development).

2. **Similarities** (verbal concept formation, abstract verbal reasoning ability, and general intellectual ability).
3. **Block Design** (taps abilities related to spatial visualization, visual-motor coordination, and abstract conceptualization).

4. **Matrix Reasoning** (measures non-verbal fluid reasoning and general intellectual ability.

The age range for the WASI is from 6–79 years and was used to obtain developmental norms on 1,100 children ranging in age from 6-16; 100 children at each age level, and the sample was stratified

according to parental education level into five categories < 8, 9-11, 12, 13-15 and >or = 16 years. The sample was drawn from the four major geographic regions as defined by the Census Report.

In addition to the WASI we are adding Coding (visual-motor coordination and concentration) and Digit Span (short-term auditory memory and concentration) from the WISC-III; and Digit Span and Coding from the WAIS-R for children 16 and over.

WISC-III/WAIS-R: Digit Span–forwards and backwards
(short-term auditory memory and concentration).

The Digit Span subtest includes a series of number sequences. Each item consists of two trials with each trial having the same number of digits, but different numbers. *(WISC-III Manual: Psychological Corporation, 1991)*

WISC-III/WAIS-R: Coding/Digit Symbol
(visuomotor coordination, planning, and ability to follow directions).

For the WISC-III Coding Subtest, two forms are available. Coding A is administered to children between ages 6 and 7, while Coding B is administered to 8 to 16 year old children. The child copies simple symbols that are paired with simple geometric shapes (Coding A) or with numbers (Coding B).

For the WAIS-R Digit Symbol Subtest, one form is available.

Woodcock-Johnson Psycho-Educational Test Battery III (WJ-III)

Three subtests of this battery would be used: Letter Word Identification, Passage Comprehension, and Math computations. Any Standard Scores <70 would be exclusionary. The WJ-III is normed from age 5 to adult. The tasks require reading of single words from a list; silent reading of sentences and paragraphs with a blank that the child must fill in orally based on their understanding of the text (cloze procedure); paper and pencil arithmetic computations. Word reading typically shows nonlinear growth to a plateau *(Francis et al., J. Ed. Psych., 1996).* Reading comprehension and math show more linear growth. These tests will be used to exclude Learning Disability. *(WJ-III Manual: Riverside Publishing, 2001)*

The neural correlates of word reading involve temporal, angular gyrus, Wernicke's area, and possibly Broca's area; reading comprehension presumably involves the same areas, along with temporal lobe structures involved in semantic comprehension; precise math calculations involve inferior parietal cortex.

Physical / Neurological Examination

A physician skilled in the neurological examination, when possible, should perform it whenever possible. Clinicians conducting the exam will be complete the certification process.

M. Rivkin will provide guidance to examiners as necessary to ensure examination consistency from site-to-site.

A list of the materials needed for the Neurological Exam can be found in Appendix D.

For the physical examination, height, weight and head circumference data provided by the National Center for Health Statistics will be used. Refer to WEB site under Growth Charts for charts taken from NCHS, or go to their site at: http://www.cdc.gov/nchs/about/major/nhanes/growthcharts/charts.htm

Tanner Staging - Questionnaire for Adolescents

The neurologist performing the neurological examination (or other trained health care professional at the site) will give this questionnaire at his/her discretion. The scientific rationale for using a Tanner Staging Questionnaire derives from the necessity to discern the effect(s) of physiologic change in puberty with changes in brain structure and biochemistry. The collection of both physical data (from the self-rating scales) and endocrinologic data (derived from saliva and urine) will constitute a more complete physiologic profile to correlate with neuroimaging data than would be expected using either one alone. Utilization of a reliable self-rating scale for Tanner staging preserves the privacy of the children in the study and saves the time that would be necessary to gather this information through direct examination. (*Carskadon and Acebo, J Adol Health. 1993;14:190-195, Peterson AC et al. A Self-Report Measure of Pubertal Status: Reliability, Validity and Initial Norms. J Youth Adolesc. 1988;17:117-133)*

Saliva

Assay results from saliva sampling will provide a quantitative measure of pubertal development that may be more sensitive than traditional Tanner staging. Salivary gonadal hormone concentrations will correlate with volume measures of sexually dimorphic brain structures.

At each visit during the assessment day, all Objective 1 subjects will be requested to provide two separate 1-3cc samples of saliva at two time points between 12 and 6 pm. They should be collected while the subject is relaxed and not after potentially stressful procedures (e.g. MRI). An appropriate time would be before and after behavioral measures and cognitive testing. The procedure represents minimal risk.

Samples will be collected, stored at –20 to -80°C, and shipped in batches from each site to UCLA. Samples will be assayed by published RIA methods for testosterone (male subjects) and estradiol (females) in Dr. McCracken's laboratory at UCLA. Results will be reported to the DCC database.

Urine

A urine sample will be collected for endocrine measurements, which will complement Tanner staging and growth data. Adrenal and gonadal steroids will be measured in saliva and urine.

At each visit, all objective 1 subjects will be asked to provide a urine sample. A 10cc sample will be transferred to a conical tube, stored at –20 to -80°C. The remainder of the urine sample will be used for a pregnancy test, when appropriate. Samples will be shipped in batches from each site to UCLA.

Samples will be assayed in Dr. McCracken's laboratory at UCLA. Results will be reported to the DCC database.

Pregnancy Test

For adolescent girls of childbearing potential (who have begun menses), a urine test will be taken to rule out pregnancy. This will be done using QuickVue One-Step HcG Urine Test, manufactured by Quidel. The test is available as a kit of 25 and can be purchased from Fisher Scientific (1-800-766-7000), cat. #043-022. Sensitivity/specificity/accuracy is >99%. The test takes 3 minutes to obtain results. A package insert can be downloaded from the manufacturer's website at: www.quidel.com

If the result of the QuickVue test is positive, an additional test should be done at a local laboratory to rule out a false positive. A refusal for pregnancy testing will result in exclusion from the study.

5. OBJECTIVE 1 PROCEDURES

Table 14: Objective 1 Procedures

INSTRUMENT/PROCEDURE			CONTACT	Time (min)
Consent Form			Parent Leisure	10
Consent or Assent Form			Subject Leisure	10
Childhood Behavior Checklist (CBCL)	**CBCL/1.5-5** (parent report for children ages 4:6 to 5:11 yrs) **CBCL/6-18** (parent report for children ages 6 to 18 yrs) **CBCL/YASR** (young adult self report for ages >18 yrs)		Parent Leisure	20
	Young Adult Self-Report (YASR): age 18 years old +		Subject Leisure	20
Brief Telephone Screening Interview			Phone (P)*	20
Full Telephone Screening Interview			Phone (P)*	20
Diagnostic Interview Schedule for Children (C-DISC-4)			Phone/In-Person (P)	50-60
DISC Predictive Scales (DPS-4)			Phone/In-Person (P)	20
Family History Interview for Genetic Studies (FIGS): age 4:6 to 18 years old			Phone (P)	15-20
Behavior Rating Inventory of Executive Function (BRIEF)			Hospital Visit (P)	10
Junior Temperament Character Inventory (JTCI)	**Parent**: age 4:6 to 18 years old		Hospital Visit (P)	20-30
	Child & Adolescent: age 10 to 14:11 years old		Hospital Visit (C)	20-30
	Young Adult: age 15 years old +			
Physical/Neurological Exam			Hospital Visit (C)**	20
Tanner Staging: age puberty +			Hospital Visit (C)	5
Urine Sampling			Hospital Visit (C)	5
Pregnancy Screening Test: all female subjects who have begun their menses			Hospital Visit (C)	5
Urine Test for Pregnancy: Confirm pregnancy if screening test positive			Hospital Visit (C)	5
Saliva Sampling (administered 2 times)			Hospital Visit (C)	10
Handedness A & B			Hospital Visit (C)	10
Differential Abilities Scale (DAS): age 4:6 to 5:11 years old			Hospital Visit (C)	40
Wechsler Abbreviated Scale of Intelligence (WASI): age 6 years old+		Vocabulary		
		Similarities		
		Block Design		
		Matrix Reasoning		
Wechsler Intelligence Scale for Children (WISC-III): age 4:6 to 16:11 years old		Digit Span	Hospital Visit (C)	10
		Coding		
Wechsler Adult Scale of Intelligence (WAIS-R): Age 17 years old +		Digit Span		
		Digit Symbol		
Woodcock Johnson-III (WJ-III)			Hospital Visit (C)	15-20
California Verbal Learning Test (CVLT)	**CVLT-C:** age 4:6 to 15:11 years old		Hospital Visit (C)	30
	CVLT-II: age 16 years old +			
NEPSY	**Verbal Fluency**: age 4:6 to 11:11 years old		Hospital Visit (C)	5
	Verbal Fluency & FAS: age 12 years old +			
Purdue Pegboard	**Half Board:** age 4:6 to 5:11 years old		Hospital Visit (C)	5
	Full Board: age 6 years old +			
Cambridge Neuropsychological Test Automated Battery (CANTAB)		Motor Screening Task Spatial Span Task Spatial Working Memory Task Big Little Circle Intradimensional/Extradimensional Set-Shifting Task	Hospital Visit (C)	35-45
HOSPITAL VISIT (C) TOTAL TIME: age 4:6 to 5:11 years old				**200-230**
HOSPITAL VISIT (C) TOTAL TIME: age 6 years old +				**170-225**

Table 15: Questionnaires and Putatively Important Brain Regions

	Function	Brain Area	Exclusion Criteria
BRIEF	Executive	Frontal lobes	Not applicable
CBCL	Behavior	DLPFC, Anterior Cingulate, Basal Ganglia	T-score > 70 (Any Scale)
DISC	Psychiatric Conditions	General	Most Axis1 disorders*
DPS	Psychiatric Conditions	General	Most Axis1 disorders*
FIGS	Psychiatric Conditions	VMPF, OrbF, DLPFC, Amygdala, Hypothalamus, Hippocampus, Mediodorsal Thalamus	Some Axis1 disorders*
JTCI	Personality	Orbitofrontal, MFC	Not applicable

NOTE: ** Schizophrenia, bipolar affective disorder, alcoholism, obsessive compulsive disorder, Tourette's disorder, recurrent major depressive disorder, pervasive developmental disorder, and attention deficit disorder.*

Table 16: Procedure, Function Tested, Putative Brain Area Subserving this Function, and Exclusion Criterion if Applicable.

TEST	Function	Brain Area	Exclusion Criteria
WASI	Intelligence	General	Full Scale IQ < 70
Woodcock-Johnson-III	Achievement	See below	
Passage Comprehension	Comprehension	Frontotemporal	> 2 SD below mean
Math Computations	Math ability	Inferior Parietal, Linf Frontal, Cingulate, & Medial Temporal	> 2 SD below mean
Letter/Word Identification	Basic Reading Skill	Occiptiotemporal	> 2 SD below mean
CANTAB			Not applicable
Motor Screening	RT, eye/hand coord.	Frontopartietal	
Spatial Span	Visuospatial Working Memory	Right DLPFC	
Spatial Working Memory	Planning/Monitoring	DLPFC +VLPFC	
Big/Little Circle	Visual Discrimination/ category achievement	Visual Association Cortex	
Intra/Extra Set-shifting	Rule Shifting/Resistance to Interference	DLPFC + Orbital FC	
Handedness	Laterality	Motor Cortices	Not applicable
NEPSY – Verbal Fluency	Fluency	Dominant Inferior Frontal Gyrus	Not applicable
Purdue Pegboard	Dexterity	M1, Pre-Motor, CST	Not applicable
CVLT	Verbal Memory	Dominant Temporal	Not applicable

6. OBJECTIVE 2 PROCEDURES

Table 17: Objective 2 Procedures

AGE (Months)	INSTRUMENT/PROCEDURE		CONTACT	Time (min.)
Newborn	Consent Form(s)		Parent Leisure	10
	Full Telephone Screening Interview		Phone/In-Person (P)*	20
	Family History Interview for Genetic Studies (FIGS)		Phone/In-Person (P)	15
	Neurological Exam		Hospital Visit (C)**	20
			HOSPITAL VISIT (C) TOTAL TIME	**65**
2 – 11	Consent Form		Parent Leisure	10
	Full Telephone Screening Interview		Phone/In-Person (P)	20
	FIGS		Phone/In-Person (P)	15
	Carey Temperament Scales	Early Infant Temperament Questionnaire (EITQ): 2 – 4 months old	Parent Leisure	20-30
		Revised Infant Temperament Questionnaire (RITQ): 4 – 11 months old		
	Parent Stress Index (PSI)		Parent Leisure	20-30
	Neurological Exam		Hospital Visit (C)	20
	Bayley Scales of Infant Development-II (BSID)	Mental Scale (MDI)	Hospital Visit (C)	25-35
		Motor Scale (PDI)		
		Behavior Rating Scale (BRS)		
	Preschool Language Scale (PLS-3)		Hospital Visit (C)	15-30
			HOSPITAL VISIT (C) TOTAL TIME	**145-190**
12 – 35	Consent Form		Parent Leisure	10
	Full Telephone Screening Interview		Phone/In-Person (P)	20
	FIGS		Phone/In-Person (P)	15
	Carey Temperament Scales: Toddler Temperament Scale (TTS)		Parent Leisure	20-30
	Childhood Behavior Checklist (CBCL/1.5-5)		Parent Leisure	20
	PSI		Parent Leisure	20-30
	Neurological Exam		Hospital Visit (C)	20
	Handedness 1		Hospital Visit (C)	5
	BSID-III	MDI	Hospital Visit (C)	60-90
		PDI		
		Behavior Rating Scales (BRS)		
	PLS-3		Hospital Visit (C)	15-30
	Handedness 1:0 – 2:11		Hospital Visit (C)	5
			HOSPITAL VISIT (C) TOTAL TIME	**210-275**

Table 17: Objective 2 Procedures – continued

AGE (Months)	INSTRUMENT/PROCEDURE		CONTACT	Time (min.)
36 – 53	Consent Form		Parent Leisure	10
	Full Telephone Screening Interview		Phone/In-Person (P)	20
	FIGS		Phone/In-Person (P)	15
	Carey Temperament Scales: Behavioral Style Questionnaire (BSQ)		Parent Leisure	20-30
	Childhood Behavior Checklist (CBCL/1.5-5)		Parent Leisure	20
	PSI		Parent Leisure	20-30
	Neurological Exam		Hospital Visit (C)	20
	Handedness 3:00 – 4:5		Hospital Visit (C)	5
	BSID-III	PDI: 36 – 42 months old	Hospital Visit (C)	15
	Differential Abilities Scale (DAS)		Hospital Visit (C)	40
	Cambridge Neuropsychological Test Automated Battery (CANTAB): 4:0 – 4:5		Hospital Visit (C)	35-45
	PLS-3		Hospital Visit (C)	15-30
	Purdue Pegboard (half-board)		Hospital Visit (C)	5
	Nepsy		Hospital Visit (C)	5
			HOSPITAL VISIT (C) TOTAL TIME	**245-290**

***NOTE*: *(P) = Parent; **(C) = Child**

Hospital Visit Time:	290 minutes
Time for MRI:	75 minutes
Transition and Meals:	75 minutes
Total Maximum Used Time	**440 minutes (7.3 Hrs) Done over 2 sessions**

Table 18: Measure, Function Tested, Putative Brain Area Sub Serving This Function, and Exclusion Criterion if Applicable

	Function	Brain Area	Exclusion Criteria
Newborn			
FIGS	Psychiatric Conditions	VMPF, OrbF, DLPFC, Amygdala, Hypothalamus, Hippocampus, Mediodorsal Thalamus	Axis 1 Disorders*
2 – 11 Months Old			
BSID-II: PDI, MDI	Behavior	General	> 2 SD below mean
PLS-3	Language	Left hemisphere	> 2 SD below mean
Carey Scales	Temperament	Amygdala/PFC	Not applicable
PSI	Parent and Child information	General	Not applicable
FIGS	Psychiatric Conditions	VMPF, OrbF, DLPFC, Amygdala, Hypothalamus, Hippocampus, Mediodorsal Thalamus	Axis 1 Disorders*
12 – 35 Months Old			
BSID-II: PDI, MDI	Behavior	General	> 2 SD below mean
PLS-3	Language	Left hemisphere	> 2 SD below mean
Purdue Pegboard	Dexterity	M1/Pre-Motor/CST	Not applicable
Carey Scales	Temperament	Amygdala/PFC	Not applicable
CBCL	Personality	General	T-score > 70 (Any Scale)
FIGS	Psychiatric Conditions	VMPF, OrbF, DLPFC, Amygdala, Hypothalamus, Hippocampus, Mediodorsal Thalamus	Axis 1 Disorders*
PSI	Parent and Child information	General	Not applicable
36 – 53 Months Old			
BSID-II: PDI	Motor	Motor Cortices	> 2 SD below mean
DAS	Intelligence	General	> 2 SD below mean
PLS-3	Language	Left hemisphere	> 2 SD below mean
Purdue Pegboard	Dexterity	M1, Pre-Motor, CST	Not applicable
CANTAB	Refer to Table 16	Refer to Table 16	Not applicable
Carey Scales	Temperament	Amygdala/PFC	Not applicable
CBCL	Behavior	Refer to Table 15	T-score > 70 (Any Scale)
FIGS	Psychiatric Conditions	VMPF, OrbF, DLPFC, Amygdala, Hypothalamus, Hippocampus, Mediodorsal Thalamus	Axis 1 Disorders*
PSI	Parent and Child information	General	Not applicable
NEPSY	Fluency	Dominant inferior frontal gyrus	Not applicable

NOTE: *Schizophrenia, bipolar affective disorder, alcoholism, obsessive compulsive disorder, Tourette's disorder, recurrent major depressive disorder, pervasive developmental disorder, and attention deficit disorder.

Cognitive Testing with Transitional Groups

For different age categories, various behavioral tests and questionnaires will be administered. Whenever possible, different versions of the same test will be employed to maintain continuity. Thus, different versions of the Carey Temperament Scale, the Child Behavior Checklist, the Family Interview of Genetic Studies, and the Junior Temperament and Character Inventory will be used. In older children the Differential Ability Scale (DAS) will replace the Bayley Scale of Infant Development. The DAS will be replaced by the WASI beginning with age 6. In younger children the Verbal Fluency subscale of the NEPSY will assess language skills whereas in older children the NEPSY plus the FAS Verbal Fluency test will be given. The CVLT-C will be replaced by the CVLT-II at age 16. The Purdue Pegboard Half-Board will be used up to age 5:11 when it will be substituted by the Full-Board version thereafter. At age 16, the Digit Span and Coding subtests of the WAIS-R will replace the same subtests from the WISC-III.

7. MRI PROTOCOLS

The MRI protocols presented in the following section have been developed as a joint effort between the DCC, the PSCs, and NIH advisors using the original NIH Statement of Work as the guiding document. While Objective 1 and Objective 2 protocols were developed somewhat independently, there was serious consideration given to the goal of consistency and continuity between the two age groups and therefore the protocols were constructed to contain overlapping data.

MRI acquisition details specific for scanner type, parameters, instructions for labeling and transfer instructions are provide in the MRI Procedure Manual (appendix C).

MRI Protocol for Objective 1

Rationale

The overarching goal of the imaging portion of Objective 1 is to use MRI for the in vivo characterization of developing brain structure and function in children aged 4 years 6 months to 18 years 3 months. The MRI data should be collected in a time period feasible for this age range and should be amenable to automated computer analysis to determine global and regional brain volumes, regional morphological measurements, and global and regional measures of tissue composition. In the view of the DCC, PSCs, and NIH advisors this translated into goals of 30-45 minute acquisition duration, 1-2 mm spatial resolution, whole brain coverage, and multiple contrast weightings (T1, T2, and PD).

The most important portion of the Objective 1 MRI Protocol is the T1-weighted whole brain acquisition since it provides the best data for brain tissue segmentation in this age range. For this portion of the protocol a 3D T1-weighted spoiled gradient recalled (3D SPGR) echo sequence has been selected and optimized for this specific study. The only realistic alternative that was considered was a 3D magnetization prepared gradient echo sequence (3D MPRAGE) but after extensive multi-center testing the conventional 3D SPGR was found to provide superior (higher signal-to-noise [SNR] and contrast-to-noise [CNR]) and more consistent results for this specific application. The protocol has also been designed to provide 1 mm isotropic data of the entire head. Since this is clearly the most important MRI data for Objective 1, it is to be acquired immediately following the localizer scan (the specifics imaging parameters of which are not critical) and represents the bare minimum of data for a session to be considered useful. If significant motion artifacts are observed on this scan (as judged by the appropriate PSC representative) it should be repeated prior to proceeding with the PDW/T2W acquisitions. Because the impact of movement and other artifacts (such as B field inhomogeneity) on the subsequent segmentation analysis is quite different from their impact on scans acquired for clinical usage only, the DCC, together with representatives from the PSC will develop a short pamphlet of guidelines and examples describing unacceptable artifacts to avoid during each scan procedure. The duration of this 3D SPGR scan can vary between 10-17 minutes depending upon the number of slices required to cover the entire head (ear to ear). On GE scanners the maximum number of slices may be limited (12) in which case the slice thickness will be increased to give whole head coverage (slice thickness range of 1.0 - 1.54 mm). The acquisition will be sagittal because this is the most efficient way to cover an entire head with a 3D volumetric measurement and high quality reformatting is possible with such isotropic or near-isotropic data.

The second half of the Objective 1 MRI protocol is a dual contrast, proton density and T2 weighted, acquisition that provides additional information for automated multi-spectral tissue classification/segmentation algorithms as well as being standard contrast mechanisms used in diagnostic

studies. Given the increased robustness for classification/segmentation and the potential value of a normative developmental MRI database for future clinical studies, it was felt that PDW and T2W acquisition should be included in this protocol. A 2D multi-slice dual echo fast spin echo (FSE) sequence was selected and optimized for this purpose. The only alternate sequence that was considered was a 3D FSE but this was rejected after first round testing due to numerous technical issues (large inter-site performance variations, intra-volume signal variability, etc.). The slice thickness for the 2D FSE protocol was selected to be 2 mm as it is the minimum achievable on most systems and generally represents a practical minimum for this type of acquisition; however when not technically feasible 3 mm thickness will be used. Unlike the T1W measurements, an axial orientation was selected for the PDW/T2W measurements. While the orientation of these data will not affect image segmentation and analysis, the potential future use of the data in a radiological atlas context provided strong impetus to collect the image in a native axial orientation. Reformatting 1x1x2mm sagittal or coronal images into an axial format would produce suboptimal data for a radiological atlas. Consistency between Objective 1 and Objective 2 acquisitions was also an incentive for an axial PDW/T2W acquisition. This acquisition should occur only after successful completion of the T1W scan and should be repeated only if significantly degraded by motion artifacts (as judged by the appropriate PSC representative) and there is a reasonable expectation that the subject will be able to successfully complete the study. The duration of this portion of the protocol is approximately 7-9 minutes depending upon the exact number of slices acquired.

The total duration of the Objective 1 MRI Protocol should be approximately 40 minutes or less (2-5 minutes of setup, 1-2 minutes of localizer scanning, 2-4 minutes of planning, 10-17 minutes of T1W acquisition, 1-2 minutes of inter-scan delay, 7-9 minutes of PDW/T2W scanning, 1-2 minutes of subject removal).

Objective 1 Aquisition Details

The following collection of MRI acquisition details represents the final agreed upon protocol for Objective 1. The data are to be acquired in the order presented below:

3D T1 weighted:

Sequence:	3D RF-spoiled gradient echo sequence
TR:	22-25 ms
TE:	10-11 ms
Excitation pulse angle:	30^o
Orientation:	Sagittal
FoV:	IS: 256 mm AP: 256 mm LR: 160-180 mm *(to cover head)*
Matrix:	IS: 256 mm AP: 256mm LR: as needed to give ideally a 1 mm isotropic resolution *(On systems with a 124 slice limitation, slice thickness should be adjusted to cover the entire head with 124 slices)*
Signal averages:	1

2D PD weighted / T2 weighted:

Sequence:	Fast / Turbo spin echo. ETL/Turbo factor = 8
TR:	3500
TE1 (effective):	15-17 ms
TE2 (effective):	05-119 ms
Refocusing pulse:	Nominally 180^o *(can be reduced if needed to reduce SAR)*
Slices:	Thickness 2mm, Gap 0
Orientation:	'Oblique Axial' tilted to be parallel to AC-PC
# slices:	Cover apex of head to bottom of the cerebellum
FoV:	AP: 256 mm LR: 224 mm
Matrix:	AP: 256 mm LR: 224 mm

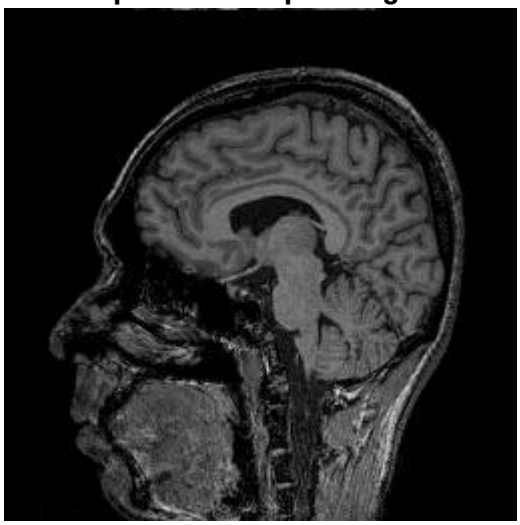
Alternative Sequences for Objective 1:

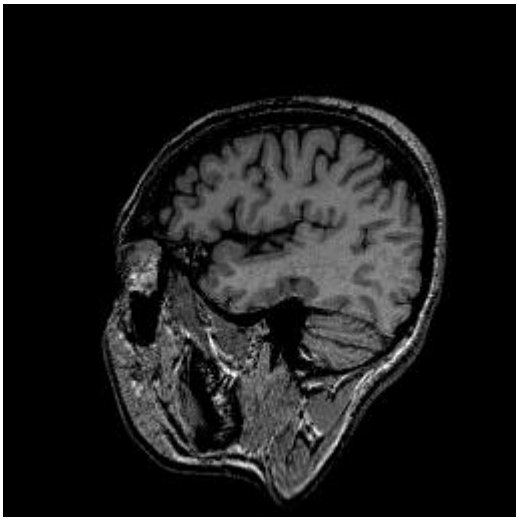
If a subject fails the above T1 and T2 sequences, shorter alternative sequences have been specified. These are the T1W and the double echo PD & T2 weighted used for Objective 2 subjects as detailed in that section.

Objective 1: Sample Data

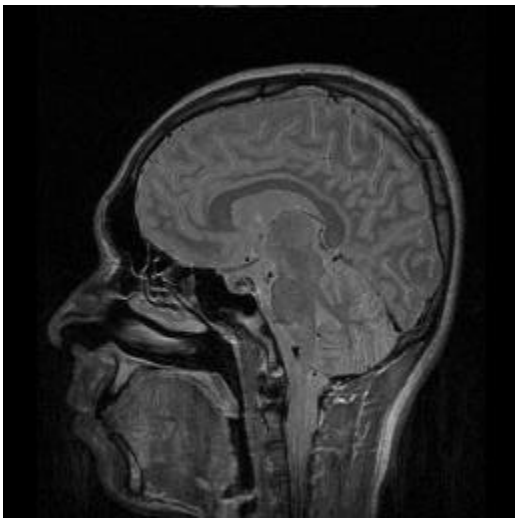
The following sample data were acquired from an adult man with the above protocol and are provided courtesy of Dr. McKinstry, Washington University, St. Louis.

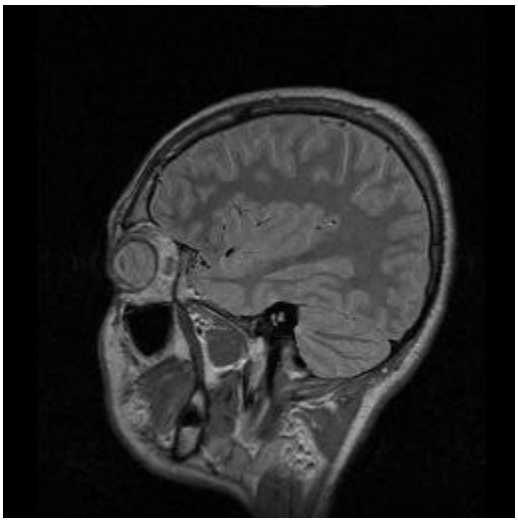
T1W acquisition sample images:



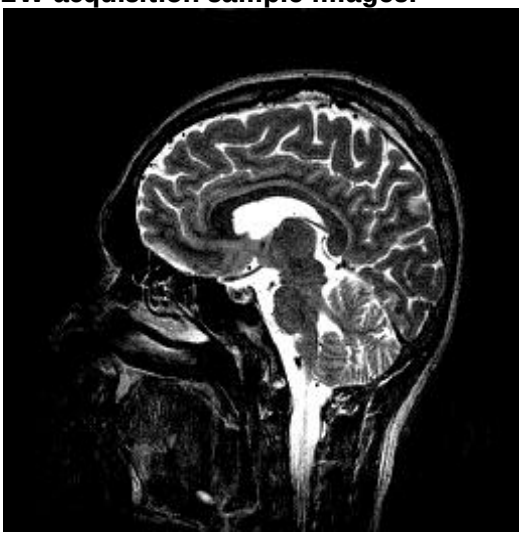


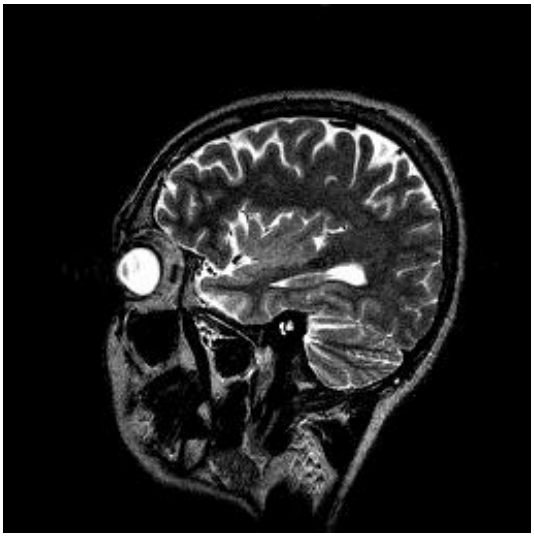
DW acquisition sample images:





T2W acquisition sample images:





MRI Protocol for Objective 2

Rationale

In its simplest conceptual form Objective 2 would merely extend the Objective 1 study into the newborn - 4:5 age range. However, brain development in the newborn – 4:5 age range is rapid and MR relaxation rates vary dramatically over this period. It would therefore be naïve to attempt to apply the Objective 1 protocol directly to this age range. Furthermore, practical scanning considerations require much shorter acquisitions that are more robust in the presence of subject motion, which is more likely in this age range. Thus, the Objective 2 MRI protocol must provide whole brain data similar to Objective 1 but must also provide quantitative relaxation data and do so in a fast, practical, and robust way. Given these requirements, the development of the Objective 2 MRI Protocol clearly involved many more compromises.

The first and most important portion of Objective 2 protocol is the acquisition of data similar to Objective 1 that can be used for classification/segmentation. While a whole brain 3D T1W 1 mm isotropic acquisition represents the current state-of-the-art in neuroanatomical MRI it was considered unrealistic in the 0-4 year age range due to the relatively long acquisition time and the sensitivity to motion. Instead a T1-weighted multi-slice (MS) conventional spin-echo (SE) was considered the best practical compromise. The data will be collected in the axial orientation along contiguous planes parallel to the AC-PC line with a 1x1x3 mm spatial resolution (3 mm slice thickness). As with the Objective 1 protocol, it was felt that the radiological standard was axial and that this should be adhered to in order to maximize the potential future value of these data (e.g. for use in a radiological atlas). Furthermore, it was noted that the high-resolution T1W data of Objective 1 can be easily reformatted to match the orientation and resolution of the Objective 2 data. This MS SE data can be collected in 3-5 minutes and should be repeated if significantly degraded by motion artifacts (as judged by the appropriate PSC representative).

The second component of the Objective 2 protocol is the acquisition of PDW/T2W data in the same axial orientation and with the spatial resolution as the MS SE T1W acquisition. The sequence type (dual contrast FSE) and parameters are otherwise identical to those used in Objective 1 and thus provide continuity between Objectives 1 and 2. As with the T1W acquisition, this measurement will take 3-5 minutes and should be repeated if corrupted by motion. While this acquisition will provide PDW/T2W data that is consistent with Objective 1, it was felt that a stronger T2-weighting would be preferable for very young infants. To this end, a second dual contrast FSE sequence, with stronger T2-weighting, was added as an additional scan in Objective 2. However, that sequence, with echo times of 83 ms and 165 ms, was considered a lower priority and should be performed at the end of the session (second to last scan) if the subject can tolerate the additional 3-5 minutes.

The final component of the Objective 2 protocol is the acquisition of quantitative relaxometry data. Considering T2 relaxometry first, it was recognized by all that good quality multi-component T2 relaxation measurements could only be performed one slice at a time using 32 or more echoes and would require a scan time of at least 6 minutes per slice. While multi-component T2 data has the potential to provide very exciting information regarding myelination, practical technical limits prevent it from being used to acquire data over the entire brain. On the other hand, it was recognized that the dual (effective) echo FSE data could be used to calculate an estimate of T2 for a single compartment model. Thus, the compromise arrived at was the use of the FSE data for whole brain single-component T2 calculation and the collection of a single slice multi-echo data set later in the protocol (after quantitative T1 measurement, DTI, and MRS).

For T1 relaxometry, a sequence developed by Dr. Haselgrove (CHOP) has been adopted *(JMRI, 11:360-367, 2000).* The technique uses inversion recovery (IR) preparation in conjunction with fast EPI (echo planar imaging) readouts to sample the inversion recovery curve at multiple points. The sequence

has already been provided to Dr. McKinstry in St. Louis (also at a Siemens equipped site) and Dr. Mulkern will develop an equivalent sequence for the GE scanner used in Boston. The acquisitions will again be in the axial plan and have a slice thickness of 3 mm. However, the in-plane resolution will be reduced to 2 mm as dictated by a single shot EPI readout.

With regard to Dr. Mulkern's implementation, the final exact details of that sequence remain to be determined and will obviously be constrained by the limitations of the hardware available. While the same basic sequence will be implemented it is very likely not to be identical. This point is an unavoidable reality and also applies to the T2 relaxometry, DTI, and MRS studies discussed below. What is more important than identical raw data is agreement in the derived parameters (T1 in this case). This will be established at the onset of the project and at regular intervals throughout the study via a calibration phantom and the smart phantom.

Objective 2 MRI Protocol – Aquisition Details :

The following collection of MRI acquisition details represents the final agreed upon protocol for Objective 2. The data are to be acquired in the order presented with the exception of the single-slice multi-component T2 measurement, which will follow the MRS and DTI acquisitions (the overall scan priorities and ordering is discussed again in a separate section).

T1_weighted:

Sequence:	Spin echo
TR:	500 ms
TE:	12 ms
Flip angle:	90 degrees
Slices:	Thickness 3 mm, Gap 0
Orientation:	'Oblique Axial' tilted to be parallel to AC-PC
# slices:	Cover apex of head to bottom of the cerebellum
FoV:	AP: 256 mm LR: 192 mm
Matrix:	AP: 256 mm LR: 192 mm
Signal averages:	1

Double echo PD & T2 weighted:

Sequence:	Fast / Turbo spin echo ETL/Turbo factor = 8
TR :	3500
TE	15-17 ; 115 - 119 ms
Slices:	Thickness 3 mm, Gap 0
Orientation:	'Oblique Axial' tilted to be parallel to AC-PC
# slices:	Cover apex of head to bottom of the cerebellum
FoV:	AP: 256 mm LR: 192 mm
Matrix:	AP: 256 mm LR: 192 mm
Signal averages:	1

Quantitative T1:

Sequence:	Any sequence that will generate data from which quantitative T1 values may be calculated.
FoV/Matrix:	Cover brain with in-plane resolution of 2-3 mm
Slices:	Thickness 3 mm, Gap 0
Orientation:	'Oblique Axial' tilted to be parallel to AC-PC
# slices:	Cover apex of head to bottom of the cerebellum.
Signal averages:	1

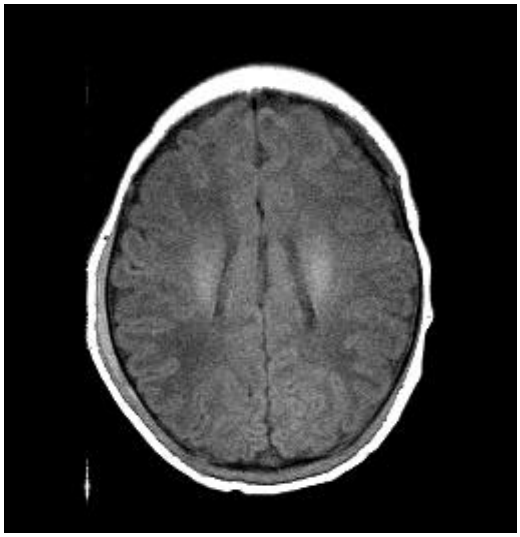
Dual Contrast T2W:

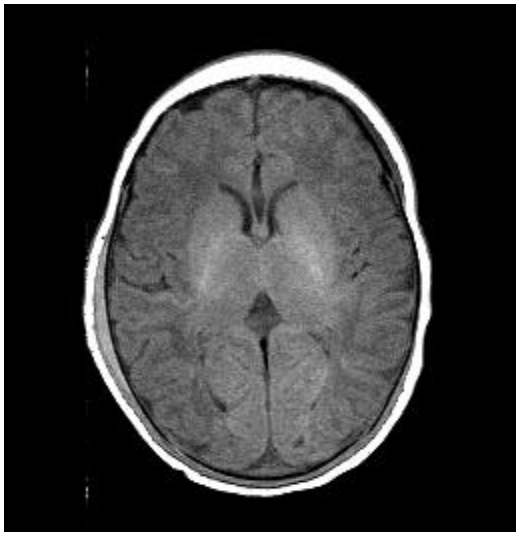
Sequence:	Any sequence that will generate data from which quantitative T1 values may be calculated.
TR:	3300
TE1 (effective):	83
TE2 (effective):	165
Slice thickness:	3 mm
Slices:	30-60 slices to cover apex of head to bottom of the cerebellum
Orientation:	axial (same slice alignment as the T1W acquisition)
FoV:	256 mm AP x 192 mm LR (rectangular FoV)
Matrix:	256 x 192
Signal averages:	1

Objective 2 MRI Protocol: Sample Images

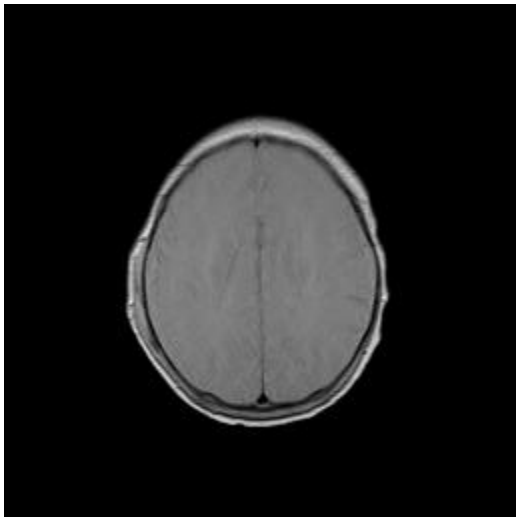
The following sample data were acquired from a normal girl aged 4 days (unless stated otherwise) with the above protocol and are provided courtesy of Dr. McKinstry, Washington University, St. Louis. Note: this hardcopy format dictates 2D sample images only but the complete volumetric data sets are available online on our web page.

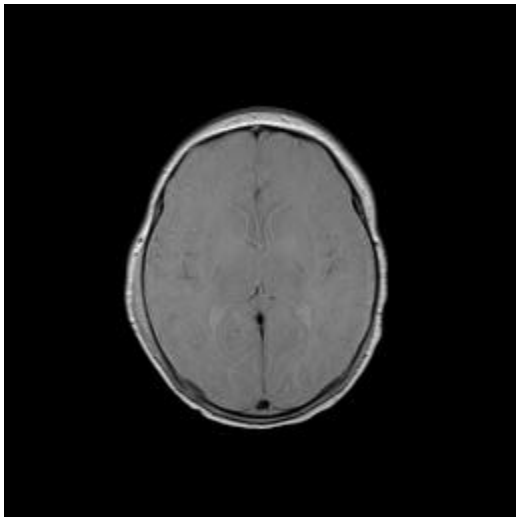
T1W acquisition sample images:



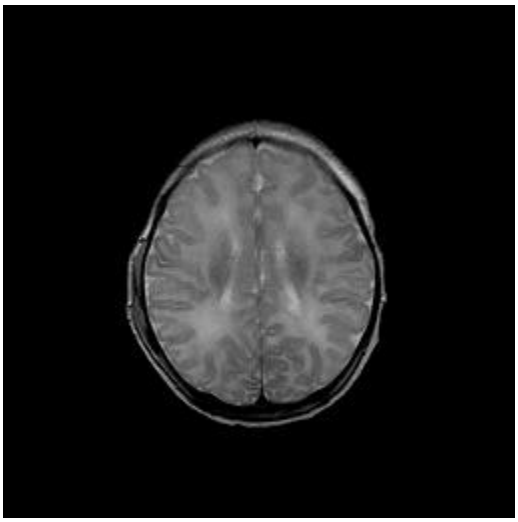


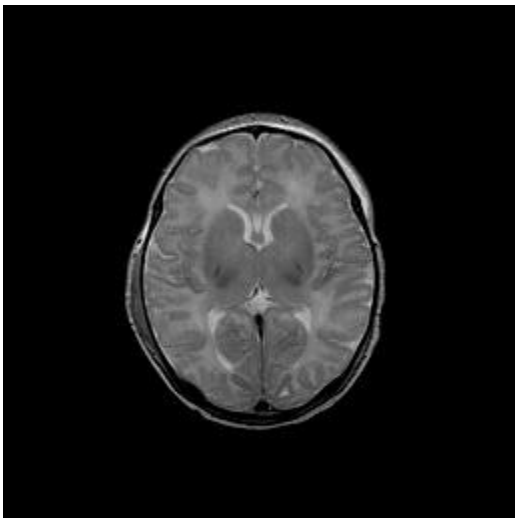
PDW acquisition sample images:





T2W acquisition sample images:





Ancillary A – Magnetic Resonance Spectroscopy (MRS) Protocol

Rationale

The objective of this ancillary study is to obtain normal developmental data on the metabolites detectable via *in vivo* proton MR spectroscopy. There are at least two notable points regarding this ancillary study. Firstly, it will be conducted by PSCs involved in only Objective 1 as well as Objective 2. Second, it will not (in the core acquisition) provide *image* data.

MR spectroscopy detects metabolites with concentrations many orders of magnitude lower than water and is therefore SNR limited. This demands much reduced spatial resolution and increased signal averaging to produce useable data. This, combined with the additional time required for shimming, resulted in the conclusion that only single voxel MRS acquisitions should be performed and that MRSI (MR spectroscopic imaging) was simply too much to include in a single MR session (particularly for the 0-4 age range). With this point agreed upon, discussions focused on the optimal MRS acquisition parameters and the number and location of voxels to acquire. It was agreed that a moderate TE PRESS acquisition would provide the most information with the best SNR and should use voxels measuring 15x15x15 mm (3.375 cc) with 64 signal averages. A maximum of four voxels will be acquired (depending upon subject tolerance) and will be collected in the following order of importance: left frontal white matter, left thalamus, midline occipital gray matter, and left parietal white matter. Including prescan shimming, each voxel will take approximately 3-5 minutes to acquire. Ratio (normalized to creatine) spectral peak areas will be the prime information stored in the database but raw data and processed spectra will also be included.

As discussed above, every effort will be made to keep the acquisitions as similar as possible across platforms but the final critical evaluation of the success of these efforts will be the derived (ratio) data acquired on the calibration and living phantoms.

Two sites, Philadelphia and UCLA, have been funded and are very interested in a more ambitious MRSI study consisting of multiple slice acquisitions. It was felt that this could only be accommodated via a return visit specifically for MRSI and should not preclude acquisition of the single voxel MRS data. Furthermore, it was cautioned that the additional burden upon the subjects and their families should not significantly interfere with their continued participation in the study. With these points agreed upon no further consideration of the MRSI protocol pursued. Details of the Philadelphia and UCLA MRSI protocols are thus not given here and are under the control of Dr. Wang and his colleagues at CHOP and Dr. McCracken and his colleagues at UCLA. Note however, that these data can and will be included in the final database.

MRS Protocol: Acquisition Details

The following acquisition details represent the final agreed upon protocol for single voxel MRS measurements. These are to be performed after the anatomical MRI, T1 relaxometry, and DTI scans.

Single voxel proton MRS:

- Sequence type: single voxel PRESS
- Siemens sequence: custom to site
- GE sequence: custom to site
- Single voxel shimming
- TR: 1500 ms
- TE: 144 ms

- voxel size: 1.5 x 1.5 x 1.5 cm (3.375 cc)
- signal averages: 64
- 2-4 voxel locations (in order of priority)
  - left frontal white matter
  - left thalamus
  - midline occipital gray matter (visual cortex)
  - left parietal white matter
  - scan time: 6-20 min

Proton MRSI:

To be specified by Dr. DJ Wang (Philadelphia) and Dr. James McCracken (UCLA).

Processing of Spectroscopy Data

Site-to-site differences in post-processing contribute to variability in MRS and MRSI endpoints. This variability can be appreciable and can negatively influence the efficiency and success of multicenter studies. As a major normative study, the NIHPD should strive to minimize this variability. Adoption of common automated MRS post-processing software, such as LCModel (Provencher 1993,2001) and agreement on data-processing protocols across sites go far towards this goal. Yet, subtle between-site differences in post-processing arise, including differences in MRS voxel tissue content determination and MR spectral quality control. Establishment of a central Spectroscopy Processing Center (SPC) at one NIHPD study site, in this case at UCLA, would assure uniformity of post-processing and thus minimize variability, as is already being done for DTI and structural MRI data.

The SPC would be responsible for timely post-processing and quality control of MRS data from all PSCs, thus relieving PSC workload and expenses, and maintain a central archive of raw data and processed output. The SPC would also perform cross-site checks of data comparability and study-wide statistical analyses on an on-going basis, including analysis of MRS and, if desired, MRSI data collected on the NIHPD "living phantom". Details are provided in Appendix I.

Dr. McCracken as UCLA PI will have ultimate responsibility for the SPC, sharing the supervisory responsibility with Dr. Alger and Levitt; and Dr. O'Neill will have day-to-day responsibility for administering the SPC. All are full-time faculty at UCLA; Drs. Alger and O'Neill are specialized in MRS.

Ancillary B – Diffusion Tensor (DTI) Protocol

Rationale

This ancillary study aims to collect normative diffusion tensor data (orientationally averaged diffusivity, diffusion anisotropy, and principle directions of diffusion) in newborn – 18 years, 3 months age range. This information is potentially very valuable, as it should provide more specific information on white matter maturation and fiber orientation in the normal developing brain. This ancillary study is being conducted at Boston, Cincinnati, Philadelphia, St. Louis and UCLA and while a basic approach has been agreed upon the precise acquisition sequence implementation will vary between sites. Sequence details are provided on the succeeding page.

The consequences of the site-to-site implementation variations will again be assessed via calibration and the living phantoms.

Ancillary B: Multi-slice DTI Protocol

The following acquisition details represent the final agreed upon protocol for DTI scanning. These data will be collected anatomical MRI and T1 relaxometry measurements.

Objective 1

- Sequence type: diffusion encoded spin echo EPI
- GE sequence: custom to site. Siemens sequence: EP2D_diff
- minimum TR: 3s. (TR=9s for 60 slices)
- TE: minimum full (minimum achievable TE with full echo acquisition)
- excitation pulse angle: 90 degrees
- orientation: Axial (i.e. perpendicular to the z axis of the magnet, not oblique)
- FoV, matrix, and slice thickness adjusted to give 3 x 3 x 3 mm voxels. If brain fits within a 19 cm Fov, use FOV 192, matrix 64x64, otherwise use FOV 384, matrix 128x128
- slices: 48-60 contiguous slices (as needed to cover from bottom of the cerebellum to apex of head)
- b-values: 0, 1000
- 6 diffusion sensitization directions: **{ (**1,0,1), (-1,0,1), (0,1,1), (0,1,-1), (1,1,0), (-1,1,0) **}**
- 4 series acquired with NEX=1, for a total of 28 images/slice (4 *(1*b=0 + 6*b=1000)).
- Images to be reconstructed at their native resolution, without zero filling or interpolation.

Objective 2

- Sequence type: diffusion encoded spin echo EPI
- GE sequence: custom to site. Siemens sequence: EP2D_diff
- minimum TR: 3s. (TR=9s for 60 slices)
- TE: minimum full (minimum achievable TE with full echo acquisition)
- excitation pulse angle: 90 degrees
- orientation: Axial (i.e. perpendicular to the z axis of the magnet, not oblique)
- FoV, matrix, and slice thickness adjusted to give 3 x 3 x 3 mm voxels. If brain fits within a 19 cm Fov, use FOV 192, matrix 64x64, otherwise use FOV 384, matrix 128x128
- slices: 48-60 contiguous slices (as needed to cover from bottom of the cerebellum to apex of head)
- b-values: 0, 1000; 0, 500
- 6 diffusion sensitization directions: **{ (**1,0,1), (-1,0,1), (0,1,1), (0,1,-1), (1,1,0), (-1,1,0) **}**
- 6 series acquired with NEX=1, for a total of 42 images/slice (4 *(1*b=0 + 6*b=1000) + 2*(1*b=0 + 6*b=500)).
- Recommended acquisition order: [0, 1000], [0, 1000], [0, 500], [0, 500], [0, 1000], [0, 1000]

Expanded DTI Protocol

Rationale

In 2005, the NIH Neuroscience Blueprint Panel approved an expansion of the DTI component of the project. A new expanded DTI (eDTI) protocol was developed. Acquisition commenced in early 2006. The main enhancements of the expanded protocol over the conventional DTI are i) improved resolution (2.5 x 2.5 x 2.5mm instead of 3 x 3 x 3mm) and ii) increased number of diffusion directions and a broader range of b-values. The aim is to provide an improved quality of eDTI datasets available for more sophisticated data analysis. Potentially, this eDTI data could be analyzed with more sophisticated diffusion models.

For Siemens Scanners

- 60 slices
- 2.5 mm slice thickness
- FOV 24 cm, 96 x 96 matrix
- Orientation: IMPORTANT that image plane be straight axial not oblique
- Image data reconstructed without zerofilling or interpolation
- Images are acquired in 10 series sequentially using all schemes from 17 to 7 directions
- total acquisition time is about 20 minutes
- With the maximum b-value set to 1100 s/mm2 the sequence will acquire the following diffusion weighted images at each slice location:

  b=0 10 images
  b=100 10 images
  b=300 10 images
  b=500 10 images
  b=800 30 images
  b=1100 50 images

Completion of Acquisition:
On Siemens scanners, 80% or the first 7 series are required for the acquisition to be considered complete. Sites should not transfer data that are less than 80% complete.

For GE Scanners:

- 60 slices
- 2.5 mm slice thickness
- FOV 24 cm, 96 x 96 matrix
- Orientation: IMPORTANT that image plane be straight axial not oblique
- Image data reconstructed without zerofilling or interpolation
- Images are acquired in 7 series of 17 images each using entries prescribed in the cdiflist04 file provided separately
- total acquisition time is about 30 minutes with current hardware
- With the maximum b-value set to 1100 s/mm2 the sequence will acquire the following diffusion weighted images at each slice location:

  b=0 9 images
  b=100 10 images
  b=300 10 images
  b=500 10 images
  b=800 30 images
  b=1100 50 images

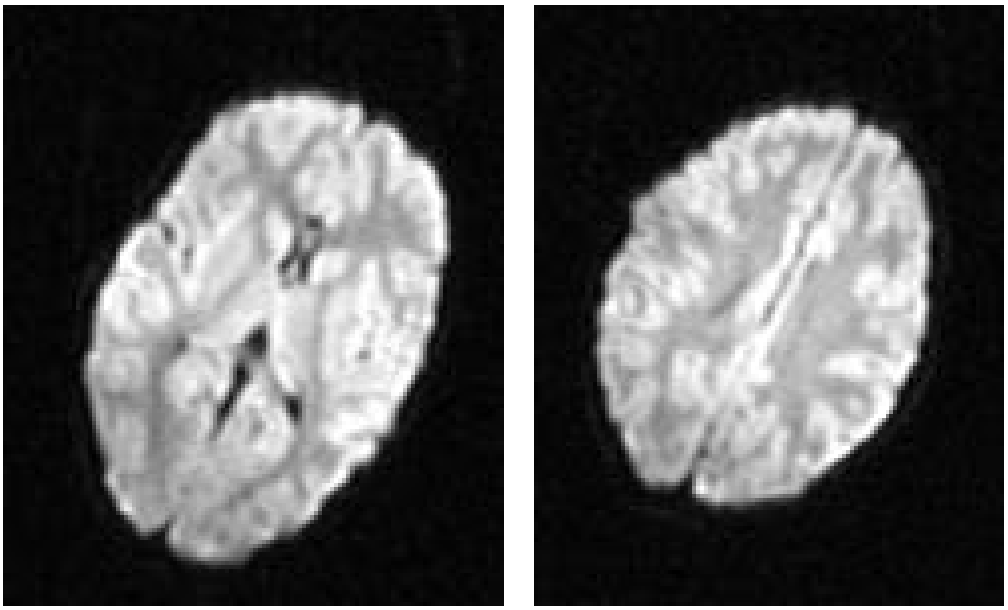
Completion of Acquisition:
On GE scanners, a minimum of 80% of the data (i.e. the first 6 series) are required for the acquisition to be considered complete. Sites should not transfer data that are less than 80% complete.

Processing of DTI Data

A central DTI Processing Center (DPC) was established under the responsibility of Dr. Carlo Pierpaoli at
The National Institute of Child Health and Human Development. The creation of a DPC for DTI data was recommended because DTI data processing requires procedures and computer algorithms that differ from those used for other MRI modalities. Investigators in the NIH intramural program have expertise in DTI data acquisition, processing and interpretation and have already developed several computer algorithms for DTI data processing. Details regarding can be found in Appendix

Ancillary B - DTI Protocol: Sample Data

Dr. McKinstry at St Louis collected the following sample diffusion data in an unsedated normal 4-year-old girl. Shown are two trace images.



Overall Acquisition Priorities and Ordering

While it is hoped that every study will run perfectly smooth and all data will be collected in every subject without motion artifacts, this is simply unrealistic and thus some prioritizing and scan ordering is necessary.

For children in the 4 years 6 months to 18 year 3 months age range, the most important and first acquisition is the T1W 3D volume scan. This should be repeated if necessary and must be successfully completed before proceeding to the PDW /T2W FSE acquisition. After successful completion of these scans, the Ancillary A (MRS) data will be collected with the voxel locations acquired in the order listed above.

For children in the newborn – 4:5 age range the T1W and PDW/T2W acquisitions are both critical (PDW/T2W may be more valuable for the under 1 year old group and the T1W may be valuable for the 1-4 year old group) and both should be repeated until they are satisfactorily acquired or the session aborted. They should be followed by the T1 relaxometry, DTI, MRS and the second dual contrast FSE in that order with each being successfully completed before proceeding to the next. The success rate for these scans will only be determined with experience and some of these priorities may have to be revisited.

Concluding Remarks

The Objective 1 study is clearly the most straight forward and is therefore the most advanced in terms of protocol testing and optimization. The MRI protocol for Objective 1 is not expected to change at all after initial calibration and living phantom scanning. The Objective 2 and Ancillary A and B studies are more complicated due to the nature of the data to be collected and the difficulties associated with scanning unsedated children in the 0-4 age range. While the protocols presented here are considered optimal given the constraints of the study, some minor refinements may be required in the initial phases of phantom testing as well as possibly in the initial phases of subject testing. While it would be preferable to absolutely lock all protocol details in advance of all subject scanning, experience acquired during the initial phase of scanning unsedated children in the 0-4 age range should be considered and the possibility of protocol refinement maintained. Such refinements should, of course, involve the input of all participants and advisors and be carefully considered in light of the project goals.

Objective 1: The following example test data for OBJECTIVE 1 was processed using a basic image analysis pipeline developed at the McConnell Brain Imaging Centre. Fully automated processing included correction for image intensity non-uniformity [Sled98]; linear stereotaxic registration of the T1-weighted data [Collins94]; mutual-information-based multimodal registration of the T2 and PD data to the T1 volume; resampling onto a 1mm isotropic grid; tissue classification to identify grey matter, white matter and cerebrospinal fluid with a self-training artificial neural net classifier (INSECT) [Zijdenbos96]; non-linear registration to a standardized brain model and segmentation (ANIMAL) [Collins96,97].
www.bic.mni.mcgill.ca/nihpd/protocols/mri/obj1_processed/

Objective 2: The following example test data for OBJECTIVE 2 was processed using a simplified image analysis pipeline that included image intensity non-uniformity correction [Sled98]; semi-automated linear stereotaxic registration of the T1-weighted data [Collins94]; mutual-information-based multimodal registration of the T2 and PD data to the T1 volume; and resampling onto a 1mm isotropic grid.
www.bic.mni.mcgill.ca/nihpd/protocols/mri/obj2_processed/

8. Follow-Up Visits

Follow-Up Visits: Subjects to be invited back for Visits 2 and 3:

1. All subjects who successfully completed the first visit
2. Subjects who completed the first visit but failed QC
3. Subjects who partially attempted but did not complete the first visit
4. Subjects with **MINOR** abnormal MRI findings following review from McKinstry & Ball
5. Subjects who have developed disorders or meet other exclusionary criteria **AFTER** the first visit

Follow-Up Visit Exclusions:

1. Subjects who had **SIGNIFICANT** abnormal MRI findings following review from McKinstry & Ball
2. Subjects with direct physical brain damage (i.e. accident with penetrating cranial trauma)
3. Subjects who had developed disorders or meet other exclusionary criteria prior to the first visit

The Medical Exclusion Committee via the CCC will review these on a case-by-case basis as they present themselves.

Procedures Administered at Visits 2 and 3

Subjects will undergo all the age-appropriate procedures at subsequent visits with the exception of the Brief Screener which is only administered at Visit 1.

Time Intervals Between Visits

For Objective 1: The target date for Visit 2 is 24 months after Visit 1
The target date for Visit 3 is 48 months after Visit 1

For Objective 2: The time interval between visits for Objective 2 subjects varies since it depends on the post-EDC age o the cohort at the time of visit. This is specified on page 27.

Time Window for Visits 2 and 3

Objective 1 subjects are allowed a 9-month (-6/+3months) window for Visits 2 and 3. A subject's visit can occur up to 6 months earlier to 3 months later from the target date. *Sites must contact the coordinating centers if a subject cannot be seen within the specified window.*

Objective 2 time window varies since it depends on the post-EDC age of the cohort at the time of visit. This is specified on page 27.

9. NIHPD DATABASE

The marketing agency will provide lists of household addresses known to have children at the specified ages. Since these lists contain names of subjects, they will be stored separately from the database to preserve subject confidentiality. The registration system ensures that private, confidential information (e.g. subject name, address, etc...) will not be present in the database in any phase of the study.

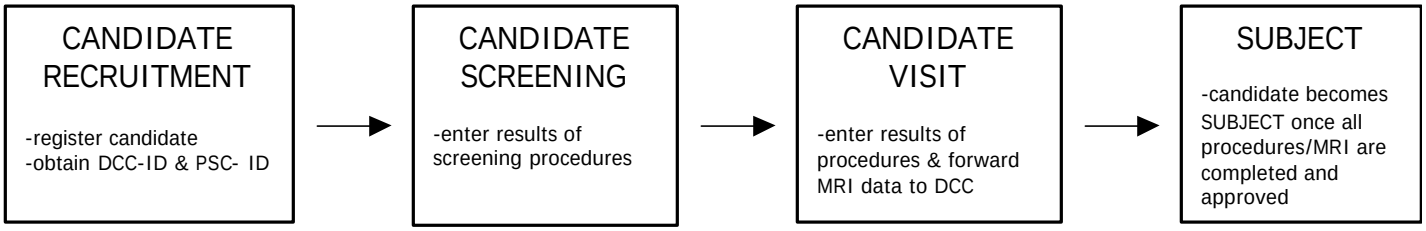
Data Entry

There are three stages in the data entry process prior to a candidate becoming a subject:

1. Candidates are first registered with the database at the **CANDIDATE RECRUITMENT** stage which occurs once the PSC receives a positive response to the recruitment mailing. The candidate's date of birth, sex, ethnicity, highest level of parental education, and the study objective are entered. The database will then provide two identifiers, DCC-ID and PSC-ID, which need to be recorded for future reference in the candidate's file at the PSC.

2. Once the ID's have been assigned following the recruitment, the entry proceeds to the **CANDIDATE SCREENING** stage. The results of screening procedures such as the Brief Telephone Screening Interview, CBCL, Full Telephone Screening Interview, DISC, DPS, and FIGS are entered into the database at this stage.

3. The last stage, the **CANDIDATE VISIT** stage, involves procedures that are administered during the visit(s) to hospital in addition to completing any outstanding screening procedures.



A candidate becomes a **SUBJECT** only once all screening and visit procedures have been completed, forwarded to the DCC, undergone quality control and approved. At this point, the database will assign and store a Subject ID. This ID will be used for future analysis and publications and will not be used during the course of the study by the PSCs.

Assignment of Identifiers

Once a candidate is registered, the database will provide two identifiers, the DCC-ID and the PSC-ID, which will be used throughout the study. The **DCC-ID** is a six-digit number randomly assigned (e.g. 123456). The **PSC-ID** consists of the Site ID and a four-digit number randomly assigned (e.g. cin1234).

Site ID

Boston	bos
Cincinnati	cin
Houston	hou
Irvine (UCI)	irv
Los Angeles (UCLA)	ucl
Philadelphia	phi
St. Louis – Objective 1	st1
St. Louis – Objective 2	st2

Data Integrity

At the PSC, all subject data will be archived onto recordable CD. In addition, it is expected that the MRI data will be archived at the scanner using the standard PSC MRI data archival procedures.

At the DCC, the entire database will physically reside on an on-line mass storage facility (RAID), hosted by a multi-processor SGI file server. Data security will be ensured both by the redundancy provided by the RAID storage facility, and by an off-line tape backup system. Full tape backups of the entire database will be made on a quarterly basis, and incremental tape backups will be made automatically on a daily basis. Backup tapes will be stored off-site. This setup allows fast and complete recovery of all data in case of hardware failures or other calamities. Any data lost between two backup time points can simply be re-sent by the PSC that acquired the data.

In addition to the standard data backup procedures, explicit archive copies of the subject's session data will be made at the DCC on recordable CD media. This will be done only after the data has passed all quality control procedures, and is thus considered valid Study data. Two copies of these archival CDs will be made, one of which will be kept at the DCC for immediate access, while the second copy will be stored off-site.

10. QUALITY CONTROL

Quality Control (QC) Procedures for Screening and Behavioral Data

The issue of quality control and standardization of procedures across sites is an important element in a multi-site study. Therefore specific procedures were developed and implemented in this study to address general QC issues. The goals of the QC plan were the following: 1) to establish uniform application of recruitment procedures and assessment protocols, 2) to establish initial certification procedures prior to the testing of actual protocol subjects and 3) To monitor and evaluate the appropriateness and reliability of the administration of assessment measures.

The QC procedures were developed by process of a subcommittee and are briefly summarized here. These procedures address the maintenance of project-wide quality control and certification for all behavioral, psychological, neurological, psychiatric, biological specimen data collection and recording. This includes periodic assessment of statistical inter and intra-rater reliabilities for selected clinical/behavioral measures.

Certification for in house neurobehavioral testing was based on expert review of the rater's videotaped testing sessions and a review of the associated test scoring. Certification, prior to testing actual subjects, was based on videotaped sessions for several age groups for each rater. If areas of concern were noted and the tape was reviewed as "not passing", then the rater will complete another videotape with a mock/practice subject for the specified test or test battery depending upon the situation. Ongoing QC monitoring consists of random videotaping on their next subject at the request of the coordinating center. An average of every 6th subject is completely reviewed. If the testing procedure is rated as non-standard the specific rater/tester can be asked to repeat the certification process with mock subjects. If recertification was requested, the rater/tester will need to stop testing actual enrolled subjects. Quality control for screening interviews, including structured and semi-structured psychiatric interviews, is based on the review of audio taped interviews. The initial review consists of 5 audio taped interviews will be reviewed/edited per interviewer at each site. Specified criteria were established for definition of passing and non- passing interviews. If the interview were judged as non-passing then the rater would provide audiotapes for review of the next 5 interviews. During the ongoing study phase, 5 additional interviews would be reviewed/edited per interviewer at approximately 4-month intervals. Structured interviews such as the DISC, DPS and FIGS Interviews will be reviewed and recoded to establish inter-rater reliability. QC review of videotaped neurological exams is also completed at the beginning of each sampling period based on review of one passing videotaped exam for each examiner.

Quality Control (QC) for Data Entry and MRI

A comprehensive, study-wide system of quality control has been implemented. Database referential integrity, logic-checks and quality control of MRI data and behavioral instruments are part of this system. There are three levels of Quality Control of the behavioral test data and MRI:

The first level of Quality Control is conducted at each, during and immediately after the data entry. The behavioral test data are checked automatically (validity, range, etc.) as the data are entered in the on-screen test forms, while the MRI scans are visually checked at the MR console. After the image files are transferred to the Study Work Station (SWS) and registered with the database, they are to be verified at the SWS using visualization software that allows multiple simultaneous cross-sectional views. When this is done, the PSC user is expected to check the database record as completed. This is the most efficient level of QC because it can be conducted while the candidate is still in the hospital, probably undergoing other tests in the battery.

The second level of Quality Control occurs at the DCC, and checks the state of the received data and MRI scans, i.e. if the received files were correctly transmitted.

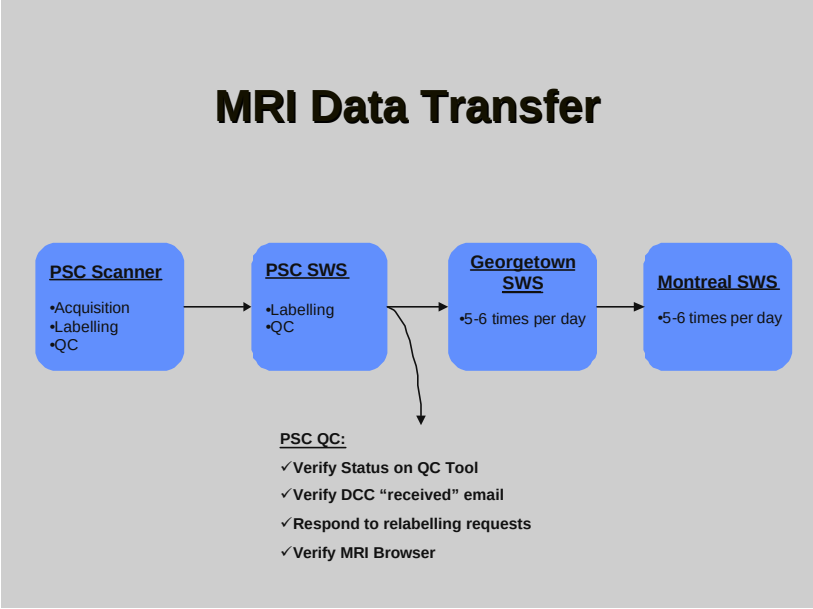
The third level of Quality Control is the most comprehensive, in-depth control for all received information for a Candidate. It is conducted at the DCC, and the validation at this QC level initiates the candidate's "promotion" into the status of an approved subject (when the Subject ID is assigned). At this time, the data is stored in the central database and archived.

Samples of hard copy data from the PSCs for one third of the subject visits will be sent to the DCC for QC by the behavioral liaison team. This QC will focus on the accuracy of data entry.

All quality control results will be recorded in the database with a complete audit trail; i.e., any comments entered by the QC officer will be recorded together with a time stamp, and no comments will ever be erased. This way the compete QC history of a particular subject or test will be retrievable at all times.

MRI Data Transfer and Quality Control (QC)

Flowchart of MRI data Pre-Transfer: Acquisition, Labeling, QC and Transfer



The magnetic resonance imaging (MRI) quality control (QC) procedure was developed to evaluate the quality of the MRI data coming in from the different pediatric study centers (PSCs) and to ensure its applicability for automated processing. The procedure involves verification of parameters for protocol adherence, automated QC estimation of movement, noise and contrast and visual inspection of the MR image data.

Different levels of QC of MRI data occurs at different locations and at different times while the data is transferred from scanner, to PSC study workstation (SWS), to Georgetown, and then finally to the Data Coordinating Center (DCC) in Montreal.

The first level of MRI QC is a visual inspection by the PSC while the subject is scanned. After each volume is acquired, the image data is examined at the scanner console to determine if a rescan or a fallback scan is necessary. Once scanning is completed, all image volumes are transferred to the PSC SWS where they are reviewed by the PSC principal investigator (PI) or his/her designate. Labeling of MRI data with the study identifiers can be done at the MRI console or the SWS. Scans are transferred to Georgetown. If the site is uncertain of the quality of the scans, the PI can request an expedited review.

At Georgetown, a basic DICOM file format level QC is completed to make sure that the scan is complete and that the checksum for each file corresponding to each image slice is correct. If the data is good, it is forwarded to the DCC in Montreal. If the parts of the data are incorrect, the Georgetown SWS will request a resend from the PSC SWS. The request is machine-to-machine and does not involve human interaction.

When the data arrives at the DCC in Montreal it is in DICOM format. This data is archived. The data is then converted to MINC, the medical image file format developed at the Brain Imaging Centre

which is used here and in many other laboratories. The DCC and PSC identification parameters are checked to make sure they match across all volumes sent for the particular subject. The acquisition parameters for each volume for the subject are checked against the scanning protocol automatically. If there is a problem with the identification labels, the PSC is contacted and asked to relabel and resend the data. Once the labels pass verification, the data is inserted into the database with a status flag set to PENDING for the subject and for each image volume. The MRI data are ready for QC of the image quality.

Once each night, a cron job (a program that starts automatically at a given time) checks for all new PENDING volumes. For each volume, this program runs a number of scripts to accomplish the following tasks:

- A verify image is created of the native data containing a mid-transverse, sagittal and coronal image through the volume. These are the images used to organize the visual inspection described below.
- A histogram of the volume is computed.
- Movement artifacts are estimated.
- Noise and contrast are estimated.
- Image preprocessing (cropping, image intensity non-uniformity correction, intensity normalization) is completed.

Using the acquisition parameters, the most likely volumes for T1, T2 and PD-weighted anatomical are pre-selected. Once completed, the data is ready for visual QC.

Visual inspection of all data sets is performed at the DCC using a tool similar to the MRI Browser now available on the NIHPD web site. The goal of the visual inspection was to rate:

- the amount of movement artifacts – either within the slices or volume, or between packets for the multi-packet acquisitions
- the level of intensity homogeneity within slices, between slices and throughout the volume
  the amount of noise in the scan
- the level of contrast between grey matter, white matter and CSF
- the adherence to scanning protocol in terms of coverage of the head/brain from left to right, top to bottom and front to back
- the amount of geometric distortion due to susceptibility artifacts
- the appearance of any other artifacts in the images.

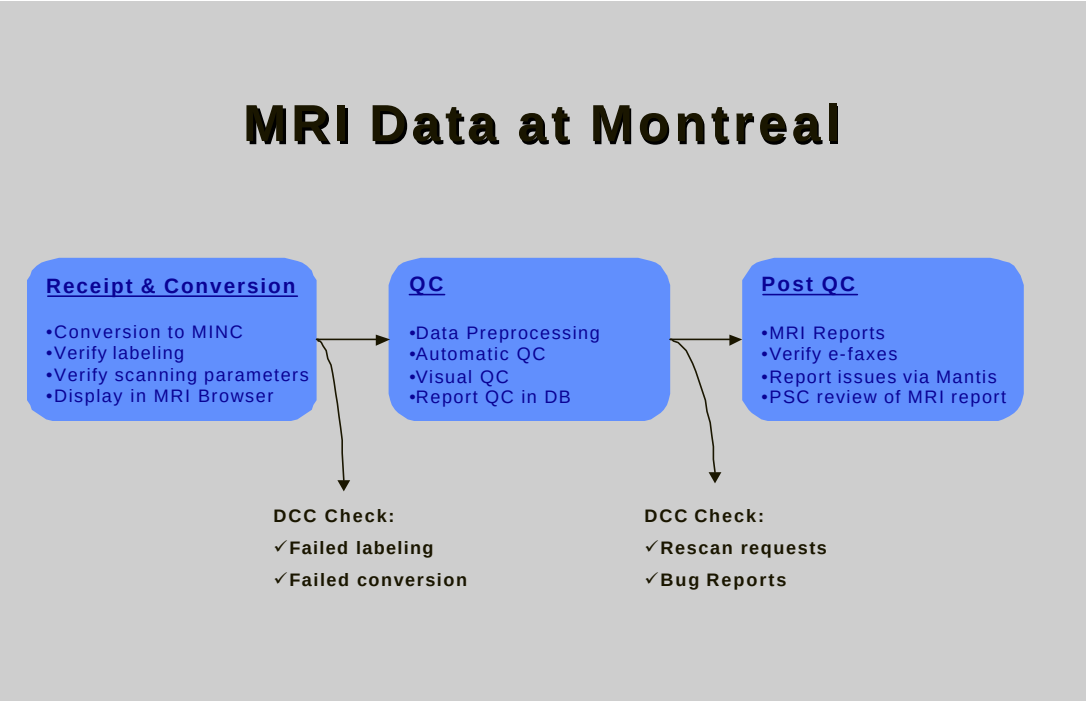
Individual volumes are given a ”PASS” or “FAIL” QC status received based on the categories above.

Passing MRI Datasets: The complete dataset for a subject visit is then given a “PASS” or “FAIL” status determined by the successful acquisition of the structural MRIs (T1W, PD/T2W either initial or fallback). A dataset must have a successful T1W and a PD/T2W to receive a final “PASS” status. If a scan is failed, a rescan is requested (assuming the repeat is acquired within the allowable time window).

Expedited review: When the PSC has a question about the quality of a data set, they can request an expedited review. This often occurs when the data is at the limit of quality in terms of subject movement and the site has acquired a number of primary and fallback scans. The expedited review is completed with 48 hours of the receiving the data at the DCC.

Reporting back to site: Reporting back to the site has evolved over the course of the project. At the beginning, PSCs received a summary report on new scans and QC status once a week. Since October 2003, QC results are available to the PSCs, NIH and coordinating centers via the MRI Browser. Summary reports are available on the database.

Flowchart of MRI data Post-Transfer: QC, Conversion, and Reporting



Inter-Site MRI Quality Control

ACR and Living Phantom Rationale:

Quality control if a major issue in a large multi-center MRI study such as this. A key component of the DCC's MRI QC program is the use of both the ACR and living phantoms. The ACR (American College of Radiology) Phantom is a standardized MRI test object that is to be scanned on a regular basis at all the PSCs. The purpose of this procedure is to monitor the overall performance and stability of the scanners used to collect data at every site. The ACR phantom can be used to assess: geometric accuracy, high contrast spatial resolution, image intensity uniformity, signal ghosting, and slice thickness. Much of this will be automated at the DCC and should allow easy detection of scanner problems and/or changes at the PSCs. It will also allow correction of geometric distortions due to any magnetic field gradient non-linearities. While the ACR phantom can provide considerable information it still has a number of shortcomings (e.g. does not contain the geometric complexity of a human brain) that make the living phantom desirable. The living phantom is a single individual who is scanned at every site at regular intervals throughout the study. This data can then be analyzed using the full pipeline to assess reliability and reproducibility of derived morphometrics (e.g. white matter tissue volume) which are the primary imaging outcomes in this study.

The quality control procedure will consist of acquiring the protocol on an ACR phantom and on a living phantom, Nick Lange, at various time points. The data will be sent to the DCC for analysis. If there are any unusual events at a PSC (quench, power failure, hardware/software upgrade, etc) a new phantom scan should be performed. It is the responsibility of the site to closely monitor QC at their site. All details regarding the ACR and living phantom acquisitions are specified in the MRI Procedure Manual provided by the DCC (appendix C).

Sites recruiting Objective 1 subjects are asked to acquire the FULL Objective 1 protocol for which the site is contracted on the ACR phantom at the beginning of each scanning phase (years 1, 3, and 5). In addition, the site should run the anatomical acquisitions (3D T1W, PD/T2W) monthly on the ACR phantom during each scanning phase.

The FULL protocol should be acquired on the "living phantom" at the beginning of each scanning phase (years 1, 3, and 5). This should be done on the same day as the ACR phantom.

Sites recruiting Objective 2 subjects are asked to acquire the FULL Objective 2 protocol for which the site is contracted should be acquired on both the ACR phantom at the beginning of each scanning phase and repeated every six months.

The FULL protocol should be acquired on the "living" phantom yearly at the beginning of each scanning phase. This should be done on the same day as the ACR. The site should run the anatomical acquisitions monthly on the ACR phantom during each scanning phase. Sites recruiting both Objective 1 and 2 subjects should acquire both sets of anatomical scans.

Note: Due to the size of the ACR phantom, some of the parameters in the anatomical acquisitions required slight revisions in order to obtain complete coverage. ***These revisions apply to the ACR phantom ONLY!*** *Subjects and the "living" phantom should be scanned as per the protocol.*

11. APPENDICES

Appendix A: Objective 1 Procedure Manual

Appendix B: Objective 2 Procedure Manual

Appendix C: MRI Procedure Manual

Appendix D. Materials for the Neurological Exam

Objective 1

a. Paper measuring tapes for head circumference.

b. Nellhaus Charts for Head Circumference Measurement.

c. CDC 2000 Charts for Weight and Stature.

d. Snellen E and Standard Snellen Visual Acuity Charts.

e. Hand-held Ball Painted Bright Red.

f. Stop Watch.

Objective 2

a. Shiny red-painted hand-held ball for use in elicitation of extra ocular movements and for ball-catching milestone assessment.

b. Color patch cards for use in assessment of child's ability to name colors (one card for each of the following colors: blue red, green, yellow).

c. Paper tapes for measurement of head circumference. A tape should used for measurement of a single child's head and then discarded.

d. Pencils for use by children in all drawing items.

e. Thin paged book for page turning.

f. A box of small cubes of different colors for use in grasping and transfer tasks.

Appendix E. CANTAB: Hardware and Software Costs

1. Computer/**LAPTOP**
   Estimated cost: 2960 USD
   Specifications:
   Dell Latitude CPx(h) Pentium II 500,
   256 MB SDRAM
   12 GB HD
   Xircom CBE2 10/100 Cardbus/LAN Card (Network Interface Card)
   24x max CD ROM drive
   Videocard, keyboard, mouse (standard)
   3 year next business day on-site parts and labor warranty deal,
   Windows 98, Second Edition (if preinstalled, **see** item **5.** for details)
   Justification: This computer will be used for the administration of CANTAB, and questionnaires (TCI), and of all demographic, screening, and behavioral measures.

2. EXP Computer, Inc 20x/4x RW Dual Port **CD-RW**; Dell part # 100826
   Estimated cost: 265 USD

3. Computer **MONITOR** with integrated touchscreen
   Estimated cost: 3629.00 USD (monitor with the integrated touch-screen)
   Specifications: Mitsubishi PrecisePoint 820PR 18" (built in touch) from the Monitor Outlet (1-888-476-6161)
   Justification: The 18" monitor is necessary for large-field presentations of visual stimuli. The capacitive touchscreen is required for the administration of CANTAB. This particular model was recommended by Dr. Luciana and by Microtouch technical support.

4. **CANTAB**
   Estimated Cost: 3,500.00 USD per license
   Specifications: CANTAB for Windows 98
   Justification: This is a computerized battery for testing executive functions.

5. **Windows 98 – SECOND EDITION**
   Estimated Cost: 200.00 USD
   Justification: Operating system.

6. 6' **printer cable** (part no. 1335582)
   Estimated Cost: 7 USD

7. Additional equipment:
   - 2m or about 7 foot long **serial cable**
   - 5m or about 20' of 10 BaseT **network cable**

ESTIMATED TOTAL COST: 10,753.00 USD

Appendix F: Study Workstation Budget and Justification

System Description:

The study workstation (SWS) is a PC-based system, capable of running LINUX and MEDx 3.3 software.

OS: RedHat LINUX 6.2

Software: MEDx version 3.3

Hardware:

19" color monitor
768 MB RAM ECC
Graphics
100Base-T
CD RW
80 GB hard disk
3.5" floppy
800Mhz PIII
chassis 300W PS
Intel Seattle MB
Hardware encryption interface

Total workstation cost (includes shipping to PSC)	**$5,787**
Annual Maintenance per workstation (15% of initial cost) (component swap)	**$868**

On site installation costs are estimated at $2700, assuming that 3 days will be sufficient to solve any local problems.

Airfare	$800	1	$800
hotel (per day)	$150	3	$450
Perdiem	$50	3	$150
Taxi	$100	1	$100
labor per day ($50/hr*8hr)	$400	3	$1,200
Total installation cost per workstation			**$2,700**

Total Cost	**$ 9,355.00**

NIH will provide costs for this equipment and materials will be organized and distributed by the DCC.

Appendix G: Tests & Questionnaires, Source, & Cost

TEST	Company	Price
OBJECTIVE 1		
BRIEF	PAR	115.00
CANTAB + Computers + Touch Screen	CeNeS	10,753.00
CBCL/6-18; YASR (Forms & Manuals)	Achenbach UV	180.00
CBCL/6-18; YASR (Software Modules)	Achenbach UV	756.00
CVLT–C (Manual & 25 Forms)	Psychological Corporation	129.00
CVLT–C (Software)	Psychological Corporation	288.00
CVLT–II (Manual, 25 Forms & Software)	Psychological Corporation	450.00
C-DISC-4	Columbia University	100.00
C-DISC-4: DCC Paid Fee	Columbia University	2,000.00
Differential Abilities Scales (DAS)	Psychological Corporation	725.00
DAS software	Psychological Corporation	140.00
DPS-4	Columbia University	250.00
FIGS	Public Domain	0.00
Handedness	N/A	
JTCI - Forms	Dr. Cloninger	200.00
JTCI - Manual	Dr. Cloninger	85.00
NEPSY (Verbal Fluency): (Manual & 25 Forms)	Harcourt Brace	126.00
Parenting Stress Index	PAR	118.00
Physical/Neurological Exam	N/A	
Preschool Language Scale-3 (PLS-3)	Psychological Corporation	145.00
Purdue Pegboard (Full-Board)	PAR	129.00
Purdue Pegboard (Half-Board)	PAR	129.00
Saliva	J. McCracken	
Tanner Staging	N/A	
Urine	J. McCracken	
WASI	Psychological Corporation	198.00
WISC-III (Manual & 25 Forms)	Psychological Corporation	155.00
WAIS-R	Psychological Corporation	174.00
Woodcock-Johnson-III (Achievement - Form A)	Riverside	480.00
OBJECTIVE 2		
Bayley Scales of Infant Development–II	Psychological Corporation	941.00
Carey Temperament Scales (PWRITQ: 4 questionnaires)	Behavioral-Developmental Initiatives	700.00
CANTAB + Computers + Touch Screen	CeNeS	10,753.00
Differential Abilities Scales (DAS)	Psychological Corporation	725.00
DAS software	Psychological Corporation	140.00
FIGS	Public Domain	0.00
Handedness	N/A	
Preschool Language Scale-3 (PLS-3)	Psychological Corporation	145.00
Parenting Stress Index (PSI; Complete kit with Software)	Psychological Corporation	560.00
Purdue Pegboard (Half-Board)	PAR	129.00
Physical/Neurological Exam	N/A	

Appendix H: Proposal for DTI Processing Center

Appendix I: Proposal for Spectroscopy Processing Center