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# The NIH MRI study of normal brain development (Objective-2): Newborns, infants, toddlers, and preschoolers

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**The Magn. Reson. Imaging (MRI) study of normal brain development currently conducted by the *Brain Development Cooperative Group* represents the most extensive MRI study of brain and behavioral development from birth through young adulthood ever conducted. This multi-center project, sponsored by four Institutes of the National Institutes of Health, uses a combined longitudinal and cross-sectional design to characterize normal, healthy brain and behavioral development. Children, ages newborn through 18-plus years of age, receive comprehensive behavioral, neurological and multimodal MRI evaluations via Objective-2 (birth through 4-years 5-months of age) and Objective-1 (4-years 6-months through 18 years of age and older). This report presents methods (e.g., neurobehavioral assessment, brain scan) and representative preliminary results (e.g., growth, behavior, brain development) for children from newborn through 4-years 5-months of age. To date, 75 participants from birth through 4-years 5-months have been successfully brain scanned during natural sleep (i.e., without sedation); most with multiple longitudinal scans (i.e., 45 children completing at least three scans, 22 completing four or more scans). Results from this younger age range will increase our knowledge and understanding of healthy brain and neurobehavioral development throughout an important, dynamic, and rapid growth period within the human life span; determine developmental associations among measures of brain, other physical characteristics, and behavior; and facilitate the development of automated, quantitative MR image analyses for neonates, infants and young children. The correlated brain MRI and neurobehavioral database will be released for use by the research and clinical communities at a future date.**
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## Introduction

Knowledge of structural and functional development of the human brain is advancing through evolution of magnetic resonance (MR) technology, and the development and refinement of analytical methods for anatomical MR imaging (aMRI and Diffusion Tensor Imaging [DTI]), MR Spectroscopy (MRS), and functional MR imaging (fMRI) (e.g., Ball, 2000; Barkovich, 2000; Giedd, 2004; Huisman et al., 2002; Huppi, 2001; Martin and Marcar, 2001; Neil et al., 1998; Poldrack et al., 2002; Rivkin, 2000; Sowell et al., 2004). Reports using MR to describe and characterize 'typical' brain development of children from school

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and adolescence ages have been increasing at a relatively rapid rate since the early 1990s (e.g., Blanton et al., 2001; Blatter et al., 1995; Courchesne et al., 2000; Giedd et al., 1996; Gogtay et al., 2004; Jernigan et al., 1991; Kanemura et al., 2003; Lange et al., 1997; Mukherjee et al., 2001; Paus et al., 1999; Pfefferbaum et al., 1994; Schaefer et al., 1990; Schmithorst et al., 2002; Sowell and Jernigan, 1998).

Fewer MR studies of brain development from newborn through preschool ages are available (e.g., Ashikaga et al., 1999; Barkovich, 1998; Barkovich et al., 1988; Holland et al., 1986; Martin et al., 1991; McGraw et al., 2002; Mukherjee et al., 2001; Neil et al., 1998), and many of those studies have used clinical populations of sedated infants and young children. Consequently, the currently available studies do not provide MR data for this young age range that can confidently be accepted as representative of truly healthy brain development. This gap in our knowledge of early, healthy brain development is not limited to MR imaging studies. Indeed, the anatomical brain data derived from autopsy specimens of young children (which are few in number for the < 5 year old age range) are not strictly representative of 'normal or healthy,' since death was generally caused by, or related to, some type of pathology (e.g., Benes et al., 1994; Brody et al., 1987; Dekaban and Sadowsky, 1978; Dobbing and Sands, 1973; Huttenlocher and Dabholkar, 1997; Yakovlev and Lecours, 1967).

MRI has become the premier tool for the quantitative, noninvasive study of childhood brain development. Developmentally accurate MR data are critically needed to determine the actual ranges of variation in brain structure and function that can be expected for healthy infants and young children. Achievement of such an MR data resource would provide standards to permit clinicians and researchers to better identify and define brain pathology that is associated with behavioral, neurological, and/or psychiatric disorders for infants, children, and adolescents.

The NIH has recognized that the scientific and clinical communities have a need for a developmental neuroimaging and behavioral database for normal, healthy children ranging in age from birth through adolescence. In 1999, NIH funded the current, ongoing multi-center project to provide research and clinical communities with a correlated MRI and behavior database that would be demographically diverse and generally representative of gender, race, ethnicity, and income level established by the United States Census Bureau (2000) and United States Department of Housing and Urban Development's Office of Policy Development and Research (2003). Six Pediatric Study Centers (PSCs) serve as principal recruitment and data acquisition sites and are located at Children's Hospital Boston, Children's Hospital Medical Center of Cincinnati, University of Texas Health Science Center at Houston, University of California-Los Angeles, Children's Hospital of Philadelphia, and Washington University Medical Center in Saint Louis. A Data Coordinating Center (DCC; Montreal Neurological Institute, McGill University) coordinates the imaging and database aspects of the project. A Clinical Coordinating Center (CCC; Washington University Medical Center in Saint Louis) coordinates and maintains quality control for the sampling plan, screening and recruitment, and neurobehavioral measures. Centralized data analysis of DTI data is provided by a Diffusion Tensor Imaging Data Processing Center (DTI-DPC; NIH, NICHD, Intramural Program). Spectroscopy data is processed at the University of California-Los Angeles by the Magnetic Resonance Spectroscopy Data Processing Center (MRS-DPC). All of the Centers listed above participate as full scientific partners in this research endeavor.

The overall project comprises two objectives which span the entire period of childhood development from birth through early adulthood. Objective-1 recruits children between the ages of 4-years 6-months through 18 years, while Objective-2 recruits from birth through 4-years 5-months of age. Notably, children advancing to 4-years 6-months of age and older are transferred to Objective-1 for their further participation in the project. Similarly, children who enter Objective-1 in late adolescence (e.g., 18 years of age) continue to participate in the project through their early twenties. As a result, the entire developmental epoch from birth through early adulthood is represented in the combined Objective 1 and 2 samples of participants. This project will yield a correlated brain MRI and neurobehavioral database for healthy brain and behavioral development from birth through young adulthood that will be made accessible to the scientific and clinical communities for use in research studies and clinical practice. It is anticipated that the process of releasing brain and behavioral data to the public will begin in the near future.

The remainder of this report describes the methodology and preliminary results for Objective-2, which is being conducted by the Boston and Saint Louis sites only. An overview report, generally describing the sample and methods of the entire project, is available (see Brain Development Cooperative Group, 2006).

## Methods

Objective-2 combines both *cross-sectional* and *longitudinal* designs to characterize normal, healthy development of the pediatric brain and behavior from the neonatal period through 4 years of age (Fig. 1). The sample comprises 11 cohorts of children that enter the study at predetermined ages, i.e., the cross-sectional component. Each of the 11 cohorts is re-evaluated at least two additional times at specified ages, i.e., a minimum of three scans for the longitudinal component.

### *Participants*

A sample of at least 106 normal, healthy children (newborn through 4-years 5-months of age) is being enrolled in Objective-2 (Fig. 1). The sample is diverse and reflects the demographics of the population of children established by the United States Census Bureau (2000). All participants recruited are born full term (>37 weeks, 3 days), with equal representation of males and females. Race, ethnicity, and income are distributed in a demographically-balanced sample to mirror proportions defined by the United States Census Bureau (2000, see Table 1). Measures of family income level, parental education level, and parental occupations serve as indices of socioeconomic status. Income levels are corrected for geographical region and family size based on United States Department of Housing and Urban Development's Office of Policy Development and Research (2003). At least 60 children are being recruited in Saint Louis, MO, and at least 46 children are being recruited in Boston, MA. Institutional Review Board (IRB) approval of the project has been obtained at both sites, and signed informed consent is obtained from parents at *each* of their child's study time-points (i.e., ages at which brain and neurobehavior evaluations take place).

### *Age calculations and representations*

Ages of all participants are adjusted to a 40-week gestational age baseline (even though all children are born 'full term'). This

Fig. 1. Objective-2 sampling plan showing the number of participants recruited and studied (left-hand side of horizontal rectangles) for the 11 age-cohorts from 0:0 (neonatal) through 4:0 (4 years). Independent groups of participants were recruited, tested and scanned at each of the 11 age cohorts (cross-sectional component), and each participant received at least three testing-scanning sessions (longitudinal component). The second and third numbers within each age-cohort rectangle represent the anticipated participant attrition rate of 20% over the second and third testing ages. The sampling plan provided for a minimum of 106 participants receiving at least 248 brain scan plus behavioral testing sessions across the 11 age-cohorts.

adjusted age is based on the child's "due date" (i.e., Expected Date of Confinement, EDC), and is applied to all age calculations from birth through 4-years 5-months of age for Objective-2. Further, throughout this report, EDC-adjusted age is represented in the "years:months" format, such that "1:3" represents an EDC-adjusted age of "1-year 3-months of age." Using this notation, the age of a newborn infant would be represented as "0:0". However, the actual "target" age used for the newborn scan and neurological exam is 10–14 days post-EDC. This delayed target age for the newborn time-point was selected to allow for physiological adaptation to the extra-uterine environment. To ensure that all newborn infants received an adaptation period, the scan/exam time-point could not occur earlier than 7 days post-delivery.

*Sampling plan*

The overall sampling plan is stratified by four factors: (1) *age* (11 cohorts), (2) *gender* (approximately equal numbers of male and females), (3) *income* (divided into low, medium, or high income level by PSC site and family size based on United States Department of Housing and Urban Development's Office of Policy Development and Research (2003)), and (4) *race/ethnicity* (see Table 1), to provide a diverse and demographically-balanced sample of participants based on the United States Census Bureau (2000, Table 1).

Table 1
Race/ethnicity distribution for US census-2000 and Objective-2 sampling plan

| Race/ethnicity | %Census | %Sampling plan |
|---|---|---|
| White/Caucasian | 69.1 | 69 |
| Black/African American | 12.1 | 12 |
| Asian | 3.6 | 4 |
| American Indian/Alaska native | 0.7 | 1 |
| Native Hawaiian/other Pacific Islander | 0.1 | 1 |
| Hispanic or Latino (of any race) | 12.5 | 13 |
| Some other race + Two or more races | 1.9 | – |
| | 100% | 100% |

In laying out the design for Objective-2, there were no appropriate data available for children in this age range to allow calculation of power or effect sizes. Thus, the design adopted was influenced by practicalities with respect to sampling frequency and participant number given the constraints of conducting non-sedated brain scans with a demographically-balanced sample of normal healthy infants and young children to the age of 4-years 5-months.

Fig. 1 presents the Objective-2 sampling plan and demonstrates the age cohorts and their respective numbers. Both cross-sectional and longitudinal design features are evident. The cross-sectional component consists of 11 age groups ranging from newborn (0:0) through preschool (4:0–4:5) ages (see staggered horizontal rectangles in Fig. 1). The minimum total number of children at each age is presented at the bottom of the figure along the *X*-axis and is the sum of the numbers in rectangles above this number. The longitudinal component is represented by each of the staggered 11 cohorts viewed horizontally, wherein each child receives full evaluation (i.e., MRI, neurological and behavioral testing) a minimum of three times during the project.

The time intervals between individual age-cohorts range from only 3 months for the youngest children (i.e., 0:0 through 1:6 cohorts) to 12 months for the oldest children (i.e., 3:0 to 4:0/4:5 cohorts), with larger sample sizes for children of younger ages whose developmental epochs of brain growth and structural change are expected to be most rapid. The longitudinal component of the design allows for an individual child to be evaluated at multiple time-points (i.e., >3 time-points), thereby increasing the number of

Table 2
Exclusion categories and examples of exclusion factors

*I. Demographic*
Child adopted; Both Parents uncomfortable reading and understanding English documents; Medical history of biological parents unknown

*II. Pregnancy*
Maternal birth age <16 years or >44 years; Intrauterine exposure (Smoking [>10 cigarettes/week], Alcohol [>2 drinks/week], Medications with known/suspected CNS effects [steroids], Drugs of abuse [marijuana]); Medical conditions (Antepartum hemorrhage, Pre-eclampsia, Infections); Pre-delivery hospital admission; General anesthesia

*III. Delivery*
Multiple births; Malpresentation; C-section with fetal or maternal distress; High forceps or Vacuum extraction

*IV. Birth-neonatal*
Born <37 weeks 4 days or >42 weeks 3 days; Growth measures <5%; Apgar score <8 (5 min); Hyperbilirubinemia requiring treatment; Anemia; Respiratory distress; Admitted for specialized care; Chromosomal/ Congenital anomalies; Infections (ToRCHeS); Tumors

*V. Child development*
Child growth measures <5%; Non-fluent in English; Major surgery; Congenital anomalies; Heart problems; Seizures, CNS Infection or radiotherapy, Head injury, Hearing/Visual impairment requiring treatment; Significant language/learning disorder; Lead treatment; Muscle disease; AD/HD; Tic disorder; Mood/conduct disorder; Exclusionary maternal medications during breastfeeding

*VI. Family psychiatric history—1st degree relatives*
Schizophrenia; Bipolar/manic disorder; Major depression; Pervasive developmental disorder; Alcohol dependence; AD/HD; Inherited neurological disorder; non-traumatic mental retardation

*VII. Child behavioral testing/Parental reports*
Poor scores (>2 sd below mean) on developmental, language, intelligence testing; High scores (>70, CBCL) parental report indicating child behavior problems

*VIII. Child neurological exam*
Facial dysmorphisms; Dystonia; Strabismus; Dysmetria; Ocular motility, Strength <4/5; Tics

children that are being evaluated at each of the ages indicated in Fig. 1. Notably, the sampling plan represented in Fig. 1 is open to increasing numbers of children and increasing numbers of time points for data acquisition per child. Indeed, our preliminary results indicate that the sampling plan of 106 children receiving 248 time-point evaluations over the duration of the project will be exceeded (see Results and discussion section). Power and effect sizes will be reported in subsequent reports following completion of data acquisition for Objective-2.

## Procedure

### Recruitment and screening of child and family

Objective-2 children are recruited to fill specific, predetermined cells (based on age, gender, race/ethnicity, and income factors) of the sampling plan in order to achieve a demographically-balanced sample corresponding to the United States Census Bureau (2000). Only one child from a given family is eligible to be recruited into the study. Participant recruitment within the greater Boston and Saint Louis metropolitan regions is conducted using a community-based strategy that includes hospital venues (e.g., maternity wards and nurseries, satellite physician offices, and well-child clinics),

Table 3
Child neurobehavioral testing battery

| Name of Test/Scale | Administration ages [a] | Function/ability assessed |
|---|---|---|
| Neurological Examination (NEURO) | 0:0 to 4:5 | Neurological development |
| Bayley Scales of Infant Development-II (BSID-II) | | |
| Mental Scale | 0:3 to 2:6 | Mental development |
| Motor Scale | 0:3 to 3:0 | Motor development |
| Behavior Rating Scale | 0:3 to 2:6 | Behavioral development [b] |
| Preschool Language Scale-3 (PLS-3) | | |
| Auditory Comprehension Subscale | 0:3 to 4:5 | Receptive language |
| Expressive Communication Subscale | 0:3 to 4:5 | Expressive language |
| Total Language | 0:3 to 4:5 | Total language |
| Differential Abilities Scale (DAS) | | |
| Block Building | 3:0 to 3:5 | Fine motor, visual perceptual |
| Verbal Comprehension | 3:0 to 4:5 | Receptive language |
| Picture Similarities | 3:0 to 4:5 | Reasoning, recognition |
| Naming Vocabulary | 3:0 to 4:5 | Expressive language |
| Pattern Construction | 3:6 to 4:5 | Visual–spatial orientation |
| Early Number Concepts | 3:6 to 4:5 | Math concepts/skills |
| Copying | 3:6 to 4:5 | Fine motor, visual perceptual |
| General Conceptual Ability | 3:0 to 4:5 | Intelligence |
| Handedness-1:0 (HAND-1) | 1:0 to 2:11 | Hand preference |
| Handedness-3:0 (HAND-3) | 3:0 to 4:5 | Hand preference |
| Verbal Fluency (NEPSY) | 3:0 to 4:5 | Semantic fluency |
| Purdue Pegboard (PEG) Half-Board Version | 3:0 to 4:5 | Fine motor coordination |
| Cambridge Neuropsychological Test Automated Battery (CANTAB) | | |
| Motor Screening | 4:0 to 4:5 | Reaction time/ accuracy |
| Spatial Span | 4:0 to 4:5 | Figure/sequence memory |
| Spatial Working Memory | 4:0 to 4:5 | Memory/plan/ monitor |
| Big Circle–Little Circle | 4:0 to 4:5 | Category rule/ reversal |
| Intra/Extra Dimensional Set-Shift | 4:0 to 4:5 | Rule shifting/ interference |

[a] Administration Ages—years:months (e.g., 2:6 = 2-years, 6-months).
[b] Behavioral development includes: attention, engagement, emotional regulation, motor quality, etc.

community organizations (e.g., day-care centers, schools, churches, and other types of community centers), and siblings of children participating in other studies.

Once signed informed consent is obtained, a comprehensive screening process is conducted with the parents to ensure that the candidate child and family do not possess medical and/or family histories that exclude them from participating in the project. Table 2 presents eight major areas of participant screening and exclusion, with examples of specific criteria that exclude a child/family from participation in the project (see www.bic.mni/mcgill.ca/nihpd_info, for a complete inclusion/exclusion listing). In addition, questionable brain scan findings are reviewed by two board certified neuroradiologists, plus the neuroradiologist at the specific site of the brain scan, and agreement that the finding is abnormal or pathological leads to child exclusion. Other questionable conditions/situations that have the potential to be exclusionary are reviewed by a group of physician investigators to determine inclusion or exclusion by simple majority.

Each successfully enrolled child receives 'full evaluation' at a series of pre-selected ages (i.e., time-points) that are defined by the child's age-cohort (see Fig. 1). Full evaluation at each time-point includes: (1) a re-screening evaluation of the child and family (Table 2), (2) behavioral and neurological evaluation of the child (Table 3), (3) evaluation of the child and family via parental questionnaire (Table 4), and (4) MR evaluation of child's brain (Table 5). A child may be excluded from the study if a relevant child or family member exclusion factor is determined to be present for any of the four evaluation areas listed above, at any of a child's full evaluation time-points (ages shown in Fig. 1). The comprehensive and strict nature of the screening-exclusion criteria, and the repeated and ongoing screening-exclusion process at each evaluation time-point, serves to ensure that the final study sample is representative of normal, healthy brain and behavioral development. The exclusion/inclusion factors are very similar between Objectives 1 and 2.

*Age "Windows" for evaluation time-points*

Age windows for full evaluations at selected time-points (Fig. 1) were established to maintain specificity of the age cohorts and age time-points. For the newborn time-point (0:0), an extra 7 days is added to the time-point age-range of 10–14 days post-EDC, yielding a window of 10–21 days post-EDC. A ±2-week window is used for the time-points between 0:3 and 1:3 (e.g., 2 months and 2 weeks through 3 months and 2 weeks defines the boundaries of the 0:3 time-point), and a ±4-week window is used for the 1:6 to 3:0 time-points. The 4:0 time-point boundaries range from 3:11 to 4:5. The window size around a specific cohort age is age dependent, such that narrower windows are designated for the younger infants/children undergoing more rapid brain growth rates (e.g., ±2 weeks for 0:3). These windows are designed and used to ensure adequate separation of the 11 target age-groups (cohorts) in Objective-2, and to allow for statistically valid data analyses of discrete age-groups (see 'Sampling plan' section, and Fig. 1). The "extra" number of days added to time-point windows carries a practical implication by providing some latitude for rescheduling of neurobehavioral testing and/or brain scans due to family or child illness, a failed brain scan, transportation difficulty, and so on.

Superimposed on the "age windows" defined above, a maximum time interval for completion of neurobehavioral plus neuroimaging data acquisition events is defined as the *data acquisition window*. For newborns, the neurological exam and the brain scan must be completed within a 7-day epoch (e.g., if the neurological exam was administered at 10 days post-EDC, the brain scan had to be completed by 17 days post-EDC). The data acquisition window for the 0:3 through 2:6 cohorts is 2 weeks, and within 4 weeks for the 3:0 and 4:0 time-points.

Table 4
Parent questionnaire battery

| Name of questionnaire | Administration ages [a] | Function/ability assessed |
|---|---|---|
| Family Interview for Genetic Studies (FIGS) | 0:0 to 4:5 | Family psychiatric history |
| Family Biographical History (FBH) | 0:0 to 4:5 | Family demographics |
| *Carey Temperament Scales (CAREY)* | | |
| Early Infant Temperament (EITQ) | 0:3 | Child temperament |
| Revised Infant Temperament (RITQ) | 0:6 to 0:9 | Child temperament |
| Toddler Temperament (TTS) | 1:0 to 2:6 | Child temperament |
| Behavioral Style (BSQ) | 3:0 to 4:5 | Child temperament |
| Parenting Stress Index (PSI) | 0:3 to 4:5 | Parent–child relationship |
| Child Behavior Checklist (CBCL) | 1:6 to 4:5 | Child behavior style |

[a] Administration ages—years:months (0:0 is the age code for "newborn" infants, 0:3 is zero years:three months).

*Child testing battery*

Associated with each evaluation time-point (Fig. 1), a child receives a comprehensive, age-appropriate neurobehavioral test battery (including neurological examination). The behavioral testing battery used in Objective-2 is outlined in Table 3, accompanied by the child age-ranges at test administration, and the behavioral domains assessed by each test. All Objective-2 behavioral tests have standardized administration and scoring procedures.

While assembling the behavioral testing battery during protocol development, it was deemed important that the tests selected for Objective-2 provide comprehensive characterization of behavioral development, and that the testing battery could be completed in a timely fashion, i.e., less than 3 h for the oldest children, less than 90 min for children 1:0 and younger, and within approximately 20 min or less for newborn infants. Rest, bathroom, play, snack and drink breaks are provided as appropriate. The tests selected should also have a strong history of widespread use in both clinical assessment and research on child behavioral development, and the instruments need to be readily available for future studies. It was also important that the results of the behavioral testing could be used for determining brain–behavior correlations. Finally, some of the behavioral tests had to serve the purpose of ensuring that the study sample reflects normal, healthy neurobehavioral development (i.e., a screening-exclusion function).

Exclusionary behavioral assessments include the Bayley Scales of Infant Development-II (BSID-II; Bayley, 1993), the Preschool Language Scale-3 (PLS-3; Zimmerman et al., 1992), and the Differential Abilities Scale (DAS; Elliott, 1990) which are all standardized and normed assessments. Scores greater than two standard deviations below the mean of 100 (i.e., scores <70) are exclusionary for those assessments (see www.bic.mni/mcgill.ca/nihpd_info). The four, age-appropriate Neurological Examinations

Table 5
MR protocol for Objecitve-2

| | Sequence | Sequence timing | Resolution and coverage | Purpose |
|---|---|---|---|---|
| 1 | Axial T1W | TR 500 ms<br>TE 10 ms | 1 × 1 × 3 mm; 1NEX; WU:256 × 192 matrix, FOV; CHB:256 × 192 matrix, FOV 250(0.75) to cover the entire brain, skull and overlying skin | Structural Scan for image segmentation |
| 2 | Axial PD/T2W | TR 3500 ms<br>TE1 17 ms<br>TE2 119 ms<br>Echo Train Length 5<br>Bandwidth 130 Hz/pixel | 1 × 1 × 3 mm; 1NEX; WU:256 × 192 matrix, FOV; CHB:256 × 192 matrix, FOV = 250 (0.75), to cover the entire brain, skull and overlying skin | a. Structural Scan for image segmentation<br>b. T2 relaxometry |
| 3 | Axial<br>IR HASTE (WU)<br>SSFSE (CHB) | TR > 10,000 ms<br>TE 47 ms (effective) (WU)<br>TE 35 ms (effective) (CHB)<br>TI 150, 500, 750, 1000, 1500, 2000, 4000 ms | 1NEX WU:2 × 2 × 3 mm, FOV = 256 × 192, matrix 128 × 96 CHB:1.5 × 1.5 × 3 mm, FOV = 240 × 240, matrix 160 × 160 to cover the entire brain, skull and overlying skin | T1 relaxometry |
| 4A | DTI | TR > 6000 ms<br>TE 80 ms (or minimum)<br>6 directions: (*x*,*y*,*z*) {(1,0,1), ( 1,0,1), (0,1,1), (0,1, 1), (1,1,0), ( 1,1,0)} | 3 × 3 × 3 mm<br>WU: either FOV192 × 192, matrix 64 × 64 or FOV 384 × 384, matrix 128 × 128; CHB: FOV 380 × 380, matrix 128 × 128 to cover the entire brain, skull and overlying skin | For computation of the diffusion tensor |
| 4B | MRS (CHB) | TR 1500 ms<br>TE 144 ms<br>64 averages(i.e., 64NEX)<br>6–20 min | Single Voxel PRESS 1.5 × 1.5 × 1.5 mm<br>1. Left frontal white matter<br>2. Left thalamus<br>3. Occipital gray matter which straddles the mid-line (visual cortex)<br>4. Left parietal white matter | For brain metabolic maturation |
| 5 | T2W dual contrast | TR 3500 ms<br>TE1 80 ms<br>TE2 160 ms<br>Echo Train Length 7<br>Bandwidth 83 Hz/pixel | 1 × 1 × 3 mm; NEX = 1; WU:256 × 192 matrix, FOV; CHB:256 × 192 matrix, FOV = 250(0.75), to cover the entire brain, skull and overlying skin | For T2 relaxometry |

WU = Washington University, CHB = Boston Children's Hospital Boston.
Scan priority rank: 1 = highest through 5 = lowest.
Scans 1 and 2 required for minimal data set, i.e., a successful brain scan session.
Scans 4A (DTI) and 4B (MRS) were optional.

are also exclusionary for certain items, in addition to their provision of neurologic data that characterizes the sample (M.J. Rivkin, P. Filipek, and J.J. Neil, unpublished neurological assessments, 2003; Capute et al., 1986). Examples of exclusionary factors include selected facial dysmorphisms, dystonia, strabismus, and hypotonia, as well as height, weight or head circumference <5th percentile (Chen, 2000; National Center for Health Statistics, 2000; Nellhaus, 1968; see www.bic.mni/mcgill.ca/nihpd_info, for a complete description of the examinations and exclusion factors). The choice and effect of exclusionary criteria "cut-offs" is discussed in the Results and discussion section of this report.

Additional tests used for Objective-2 are presented in Table 3, and those tests (plus the DAS) also extend into Objective-1 through at least the age of 5:11. Two versions (based on age) of handedness tests are used to evaluate development of hand use and lateralization (C.R. Almli, unpublished handedness assessments, 1999, see Table 3). The Handedness-1:0 test presents 8 trials of reaching and grasping an object, while the Handedness-3:0 version presents 10 trials of bimanual manipulations. Handedness can be scored in graded fashion for both test versions, e.g., a child using the right hand on all eight trials to reach for and grasp presented objects on the Handedness-1 test would be classified as "strong" right handed, while use of the right hand to reach and grasp objects on five of eight trials would be classified as "weak" right handed (see www.bic.mni/mcgill.ca/nihpd_info, for a complete description of administration and scoring of the hand use assessments). The half-board version of the Purdue Pegboard is used to assess fine motor dexterity, speed and coordination (Gardner and Browman, 1979; Lafayette Instrument Company, 1985; Wilson et al., 1982). The Semantic Verbal Fluency test of the NEPSY: A Developmental Neuropsychological Assessment (Kemp et al., 2001; Korkman et al., 1998) is used for assessing verbal fluency (i.e., child generates of names of "animals" or "things to eat or drink"). The Cambridge Neuropsychological Test Automated Battery (CANTAB; Cambridge Cognition Limited, 2004; CeNeS Limited, 1999) is a computerized assessment that was selected for this project based on research reporting that individual CANTAB tests were sensitive to cortical (e.g., frontal lobe) and subcortical (e.g., basal ganglia) functions or injuries, and can be used with children as young as 4:0 (e.g., Lee et al., 2000; Luciana, 2003; Luciana and Nelson, 1998, 2000, 2002; Owen et al., 1996).

The behavioral tests (Table 3) used for Objective-2 offer the potential to yield data that can be used for calculating developmentally dependent brain structure–behavior correlations using quantitative, whole or regional brain measures. For example, developmental correlations can be computed between: hand

preference scores and hemispheric volumes; CANTAB "working memory" scores and frontal lobe volumes or cortical thickness; and between Purdue Pegboard scores and motor cortex volume or cortical–spinal tract white matter intensities.

*Parental questionnaire battery*

Table 4 presents a listing of the parental questionnaires used for Objective-2, as well as the child's age at administration and the general history and behaviors assessed. Collectively, these questionnaires provide additional background information for characterizing child and family history, and two serve an exclusionary function, and all may prove useful for computing brain–behavior correlations.

The Family Interview for Genetic Studies (FIGS; Initiative NSaBDG, 1992; Maxwell, 1992) and the Child Behavior Checklist for Ages 1½–5 (CBCL; Achenbach and Rescorla, 2000) are used for screening and exclusion for familial or child psychopathology (see Tables 2 and 4; see also www.bic.mni/mcgill.ca/nihpd_info). The Parenting Stress Index (PSI; Abidin, 1995), Carey Temperament Scales (CTS; Medoff-Cooper et al., 1995), as well as the CBCL, are included for Objective-2 because of their widespread use for clinical and research characterization of child behavior, child temperament, and parent–child interactions.

A Family Biographical History questionnaire was developed specifically for Objective-2, and is the mechanism by which family demographic and child historical information is acquired. Information gathered includes family composition and income, parental education and occupation, parent and child race/ethnicity, as well as specific child development information, such as infant breast and bottle feeding experiences, child care and assistance programs used (e.g., day care, baby sitter, Parents-as-Teachers, Head Start, preschool programs; see www.bic.mni/mcgill.ca/nihpd_info, for detailed description).

*Adaptation and habituation to the MR brain scan process*

The neurobehavioral testing, neurological exam, and the MR scan are conducted during one or more visits to the medical center, depending on parental preference. Preparation of the child and parents for the brain scan process is typically accomplished during a visit to the medical center and/or during parent–child interactions within the family home. The fact that no participants could be sedated for brain scans presented challenges that are addressed as follows.

Newborn infants (i.e., 0:0 [10–14 days post-EDC]), and infants at 0:3, are scanned during daytime or nighttime sleep. Generally, these young infants are fed, swaddled, prepared with sound protection (ear plugs) and monitoring equipment (pulse oximetry), and then the supine infant's head is positioned within the magnet coil. The infant is visually monitored by staff throughout the scan process (e.g., McKinstry et al., 2002; Neil et al., 1998).

Older infants (e.g., 0:6) and children through 4:5 are less likely to fall asleep in the strange environment of the scanner room during the day or night, i.e., they are more challenging to scan without sedation than are neonates. As a result, infants and children ranging in age from 0:6 through 4:5 generally receive adaptation protocols that prepare them for the scan, and the scans are conducted at the child's normal night bedtime, i.e., during natural sleep.

Adaptation protocols were designed to maximize the child's (and parent's) comfort with the MRI procedures, so that the child would be able to fall asleep in the scanner room, tolerate application of sound protective materials and head positioning into the quadrature coil, and not awaken in response to the sudden onset and loud volume of scanner noises. Parental education about the scan and testing process is provided via verbal descriptions and a brochure developed for this project that outlined and pictured the scan process (see www.bic.mni/mcgill.ca/nihpd_info). In addition, the parents actually assist with the scan adaptation process for their child while at home. For example, parents are provided with CD or audio tape of the MRI sounds to play for the child at home during the child's sleep and waking (e.g., feeding, playing). This experience serves to habituate the child (and parents) to the scanner sounds. In addition, ear plugs (sponge or wax) are provided so that the parent could place the plugs in the child's external ear canal during play and sleeping for the purpose of habituating the child to the ear plug placement process and the resulting sound changes. Parents are instructed to make "games" of these activities at home and they are encouraged to be a major source of comfort for their child in advance of, during, and following the MR scan.

Two forms of mock scanner environments are made available to the child and family during medical center visits. A 'play-scanner' is made up of a table covered with a sheet to make a bore-like opening, with a "mechanic's creeper" representing a scanner bed. The child can play with this equipment while the scanner sounds are played on CD. The parents and child can also play on a 'mock-scanner' (i.e., the external shell of a real MR scanner, complete with a movable bed platform and a head coil). A Polaroid photograph of the child playing on the mock scanner may be given to the child to take home. As appropriate, the parent and child also visit the actual MR scanner and control room that will be used for the child's scan, as well as see the parking locations that will be available for them during the nighttime scan. The parents are informed about what to bring, or not bring, to the scan appointment. These experiences help the parents feel comfortable prior to and during their child's scan, and help to minimize distress in older infants and children who happen to awaken in the darkened scanner room during scanning.

*Preparation for brain scanning*

Prior to entering the scanner room, an MRI Safety Screening Form (signed by adults) is discussed for each person (children and adults) entering the scanner room. In addition, all parties (including the child) are further screened for materials on their person that are unsafe within the magnetic field of the scanner. A plastic rocking chair and small crib/playpen (both magnet-safe) are available in the darkened scanner room for use by the parent to feed, comfort, and put the child to sleep.

The children are generally brought by the parents to the MRI facility in their pajamas and ready for a nap or bedtime. As quickly as possible, the child and parents are moved into the darkened scanner room to put the child to sleep. One parent generally takes responsibility for getting the child to sleep (e.g., feed and/or rock the child, put the child in crib, etc.). If the child has not fallen asleep and still appears wide-awake after 90–120 min in the darkened scanner room, the scan is typically cancelled. If the parents are agreeable, a "re-scan" is scheduled if there is sufficient time available within the child's time-point window. Otherwise, the child is scheduled for the next time-point age, e.g., no sleep or failed scan at 1:0, child advanced to 1:3 (see Fig. 1).

Once soundly asleep, the child is moved to the scanner bed and may be swaddled or blanketed, as per child preference. Sound

protection is applied (e.g., sponge or wax ear plugs placed in the external ear canals, foam cosmetic pads placed over the ears and held in place by a knit hat). The child is then moved to position the head within the magnetic coil. Additional sound protection is applied (e.g., head phones or ear muffs [Avotec, Inc., Jensen Beach, FL] are placed over the ears and secured with rolled towels and ace bandages around the head, or, are secured by custom designed Styrofoam bead bags that fit snugly around the child's head when air is removed from the bag [separate sizes for newborn, less than 2:0, and less than 4:6; S and S X-ray, Houston, TX]). The rolled towels, ace bandages, and bead bags gently hold the child's head in position, limit head movements, and provide for sound protection. Generally, the child is supine with the head on midline during the scan, however, lateral decubitus (side-lying) or prone positions are also used (depending on child sleeping-position preferences).

Throughout all brain scanning, the child is visually monitored by the staff through the scanner bore for movement, waking, or any signs of distress. Monitoring of younger infants also includes pulse oximetry. If necessary, the child can be removed from the scanner bore within 5 s. At the completion of the brain scan, the child is removed from the scanner and given to a parent. The child and parents are provided with a toy, brain picture, payment for their expenses (e.g., travel, parking, meals, etc.), and they are escorted to their car.

*Magnetic resonance brain scans of normal, healthy infants and children*

Brain maturation over the first few postnatal years appears to be rapid (Mukherjee et al., 2001), thereby presenting challenges in the design of an MR protocol. For example, MR relaxation rates fall dramatically over the first 12 to 18 months, necessitating modifications to an MR protocol that would be suitable for older children and adults (Jones et al., 2004; Nowell et al., 1987). Moreover, white matter microstructure and brain metabolic changes that occur during normal, healthy brain development have never been characterized uniformly, and are likely to be dramatic.

The MRI protocol for Objective-2 (Table 5) is designed to gather structural MRI data for segmentation and parcellation studies of brain throughout early childhood, to characterize brain maturation throughout early childhood using T1 and T2 relaxometry, to characterize the normal, healthy developmental change in white matter microstructure with diffusion tensor imaging (DTI), and to elucidate a profile of brain metabolic maturation using magnetic resonance spectroscopy (MRS). MR scanners from two different vendors are used for Objective-2 (General Electric at Boston, Siemens at Saint Louis), however, both are 1.5 Tesla (T) field strength. The MR pulse sequences, timing parameters and image resolution are selected so that comparable image contrast and quality is achieved across both imaging centers.

Neuroimaging data are acquired at 1.5 T field strength. Sample design, recruitment method, as well as neurobehavioral, neurologic and neuroimaging protocols were planned to provide relevant reference data for both clinical and research domains independent of the field strength employed for imaging. In addition, while imaging at 3 T field strength has entered the clinical domain, the 1.5 T MRI scanner is likely to remain the predominant field strength used for MR imaging for some time. Finally, the imaging protocols employed in the current research were optimized to produce maximum contrast among constituent brain tissues scanned at 1.5 T field strength. Despite differences in T1 and T2 relaxation times, MR spectra and diffusion times found at different magnet field strengths, the methods of optimizing features of pulse sequences in order to maximize contrast between component tissues described in this study remain relevant irrespective of the field strength employed (Huisman et al., 2006).

All Objective-2 MRI scans are acquired without sedation, and since natural sleeping periods may be short due to environmental novelty for the child, or because of scanner noise, the constituent pulse sequences of the protocol are prioritized to ensure that critical portions of the scan protocol are obtained soon after the child has fallen asleep. Thus, neuroanatomical acquisitions (T1-weighted, proton density and T2-weighted scans) are followed by T1 relaxometry, then either DTI or proton MRS, and finally supplemental T2 relaxometry scans.

In order to achieve continuity with Objective 1 of the project, the core of the MR protocol is designed for image segmentation. A 2D T1-weighted multi-slice spin-echo (SE) data set is acquired first (Table 5, Fig. 2). While not a volumetric acquisition, this pulse sequence produces neuroanatomical data in less time than a 3D T1W protocol in subjects that are predisposed to movement during the scan. Images with $1 \times 1 \times 3$ mm spatial resolution are acquired

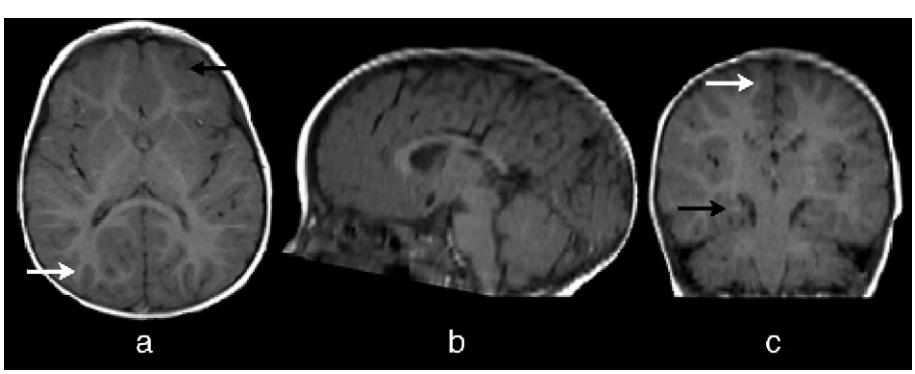


Fig. 2. T1-weighted structural MR images from a toddler (female) at 18 months of age (1:6). (a) Axial image oriented parallel to the anterior/posterior commissure line at the level of the Foramen of Monro and the basal ganglia. (b) Sagittal and (c) coronal images obtained by reformation from the axial images centered on the brainstem. Note that by 18 months of age, the gray/white matter contrast in the parietal lobes has already attained the adult-like pattern (white arrows). An immature pattern of myelination is noted in the subcortical regions of the frontal lobe and in the mesial temporal lobes (black arrows). Note that the same child is presented in this figure and Figs. 2–7, and that radiologic convention is followed for presentation of axial and coronal images in which the left side of the image corresponds to the right side of the participant.

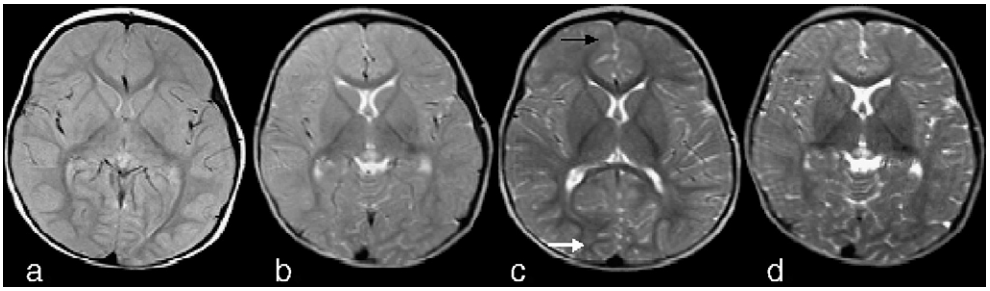


Fig. 3. Proton density and T2-weighted images for segmentation and T2 relaxometry. Images from 18 month old (same child as in Fig. 2) obtained parallel to the anterior/posterior commissure line at the level of the Foramen of Monro and basal ganglia. Two dual (effective) echo turbo spin echo image pairs are shown. The first acquisition (a and c) was performed after the T1-weighted structural images (see Fig. 2). The echo times, TE of 17 and 119 ms, are the same as those used with the older children under study in Objective-1 of the project. This will facilitate image segmentation and provide the necessary images to estimate the slow component of T2 relaxation. The second acquisition (b and d) was performed as the last series. The echo times, TE 83 and 165 ms, improve the estimate of T2 relaxation times in younger children where T2 values are much greater. The black arrow in (c) illustrates an immature pattern of myelination with ill-defined gray/white contrast in the subcortical region of the right frontal lobe. The white arrow in (c) demonstrates a mature, adult-like pattern of myelination in the occipital lobe.

parallel to the AC–PC line to cover the vertex of the skull through the foramen magnum in less than 5 min. The T1-weighted (T1W) scan is given highest priority and should unacceptable subject motion occur, the scan is repeated until acceptable data are obtained (see also, 'quality confirmation process').

The second set of scans for image segmentation includes a dual-echo series consisting of proton density and T2-weighted (PD/T2W) fast/turbo spin echo images that are acquired to match the orientation, spatial resolution, and slice locations established by the T1-weighted acquisition (Table 5, Fig. 3). The PD/T2W images are generated in less than 5 min. Again, the scans are repeated until motion artifact-free before moving to the next phase of the MR protocol. The T1W and PD/T2W images constitute the *minimum dataset* for a successful MR examination.

Following completion of the core structural MR sequences, T1W relaxometry is performed (Table 5, Fig. 4). For T1 relaxometry, an inversion recovery (IR) sequence developed by Haselgrove et al. (2000) was adapted for this study. A few participants recruited early in the study were scanned with the IR echo planar imaging (EPI) sequence. Since then, the image acquisition in the IR sequence has been a single shot fast/turbo spin echo. The images are acquired to match the 3 mm slice orientations and locations established by the T1W and PD/T2W acquisitions. In-plane resolution is reduced to 2 mm as dictated by single shot readout.

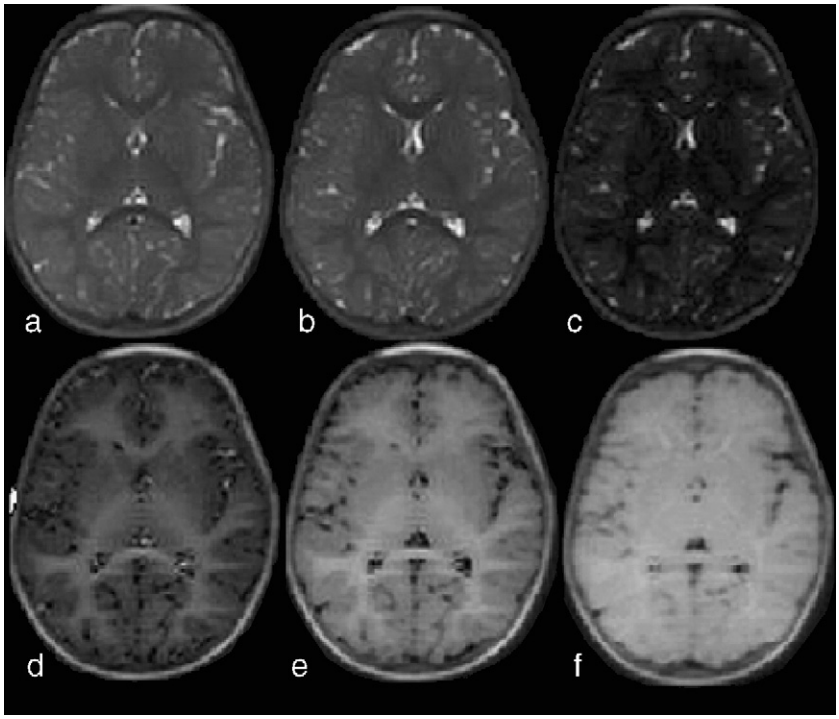


Fig. 4. Inversion recovery images for T1 relaxometry. Image oriented parallel to the anterior/posterior commissure line at the level of the Foramen of Monro and the basal ganglia (same 18 month old toddler as Fig. 2). Varying the inversion time (TI) allows for the computation of the T1 relaxation time in each voxel. (a) No inversion, (b) TI = 50 ms, (c) 400 ms, (d) 800 ms, (e) 1200 ms, (f) 2000 ms.

Good quality, multi-component T2 relaxometry can only be performed one slice at a time using 32 or more echoes and at least 6 min/slice. This is impractical for a sleeping child, and whole brain coverage is prioritized over the established accuracy of the single slice multi echo method. Therefore, the protocol incorporates a dual (effective) echo fast/turbo spin echo acquisition to estimate the T2 relaxation time for a single compartment model throughout the brain. The PD/T2W structural imaging sequence serves double duty in this regard (Table 5, Fig. 3). A second double echo sequence is added with an intermediate and very long echo time which improves the T2 estimate in the youngest children who have very long T2 relaxation times (Table 5, Fig. 3). However, this second acquisition is deemed lower priority to the DTI and MRS protocols. Thus, the second dual echo acquisition is acquired as the last element of the overall imaging protocol. Again, the second dual echo image acquisition is acquired to match the orientation, spatial resolution and slice locations established by the T1-weighted acquisition. Imaging time again is about 5 min duration.

Diffusion tensor images (DTI, Table 5, Fig. 5) are acquired to cover the whole brain with 3 mm isotropic voxels. Single shot, spin echo diffusion-weighted images are acquired using an echo planar sequence. Since diffusion is faster in the brains of the youngest children, a single diffusion-weighting ($b$ value) is not optimal. As such, DTI is obtained in separate acquisitions: $b=0$, 1000 s/mm$^2$, and $b=0$, 500 s/mm$^2$). The gradient orientations are the standard 6-direction set originally established by Pierpaoli et al. (1996) and subsequently implemented by the MR vendors [$x$, $y$, $z$ gradient unit vectors: (1,0,1), ( 1,0,1), (0,1,1), (0,1, 1), (1,1,0), ( 1,1,0)]. In recognition of the likelihood for subject motion, signal averaging is not employed. Rather, separate acquisitions are repeated, i.e., two acquisitions of the $b=0$, 1000 s/mm$^2$, two acquisitions of the $b=0$, 500 s/mm$^2$, and finally two more acquisitions of the $b=0$, 1000 s/mm$^2$. Cardiac-gating, which improves data quality, is deemed impractical for this study as it increases scan duration and complexity.

At the outset of the current study, acquisition of DTI data in children ranging in age from the newborn to 4:5 years was designed to provide quantitative assessment of diffusion tensor attributes such as mean diffusivity and fractional anisotropy during normal brain development in young human subjects. This goal is being met. Importantly, in a supplementary study of the current sample, additional DTI data sets are being acquired using 6 different b-factors and a maximum of 50 unique directions. These data will certainly provide opportunity for quantitative fiber tract analysis and will be the subject of subsequent manuscript(s).

Single voxel magnetic resonance spectroscopy (MRS) is also performed at Children's Hospital Boston (Table 5). Spectroscopy is performed using four single voxel acquisitions as the time required for multi-voxel spectroscopy is too great for use with this non-sedated cohort of sleeping children. Spectra are acquired from left frontal white matter, left thalamus, bi-occipital gray matter and left parietal white matter. A moderate TE (TE 144 MS) PRESS acquisition, with voxels measuring 15 × 15 × 15 mm (3.375 cc), and 64 signal averages produces acceptable SNR spectra in a scan time of less than 3 min per voxel.

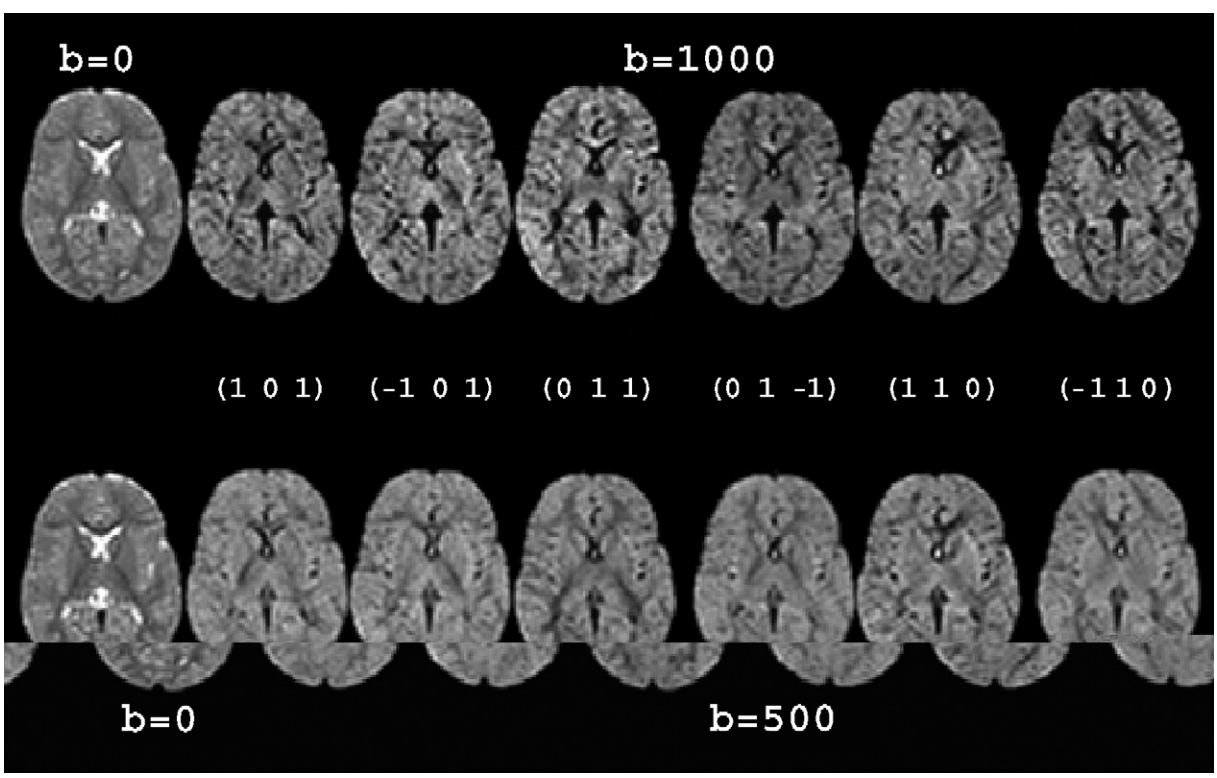


Fig. 5. Diffusion weighted images from an 18 month old (same toddler as Fig. 2). Images were acquired in the axial plane at the level of the Foramen of Monro and basal ganglia. Two separate acquisitions are employed. The top row acquired $b=0$ s/mm$^2$ and 6 directions of $b=1000$ s/mm$^2$. The second acquisition acquired $b=0$ s/mm$^2$ and the same 6 directions of $b=500$ s/mm$^2$. The numbers in parentheses are the diffusion gradient directional unit vector ($x$, $y$, $z$). The first acquisition was repeated twice, then the second acquisition was repeated twice, finally, the first acquisition was repeated twice more. The 6 separate acquisitions were used to compute the diffusion tensor at each voxel location.