Part B: Experimental Design and Protocol – ALL APPLICANTS MUST COMPLETE THIS FORM

Please refer to the INSTRUCTIONS MANUAL for assistance in completing the protocol application. The instructions manual is available on the CCI website, under '*Forms-New Protocol/3 Year Rewrite*'.

You may use this web-based form to develop your protocol, or you may insert the protocol and experimental design from another source. Either way, please ensure the final protocol covers the elements listed below as they apply to the research before submission to the CCI.

Part B should be written to reflect the research as it will be conducted at or through Children's Hospital.

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Please provide a brief summary or abstract of this research protocol.

1. Specific Aims /Objectives

Early, severe social deprivation is believed to be associated with increased risk for a variety of behavioral, social, and cognitive abnormalities in middle childhood, presumably associated with underlying differences in brain development. Research on brain development strongly implies that earlier remediation is generally more beneficial than later remediation, but it is quite unlikely that "the earlier the better" rule applies equally across all different developmental domains. It is important to delineate the degree to which specific developmental functions (and the underlying brain circuitry) are more or less vulnerable to adverse experiences (and more or less amenable to remediation) at particular times in development. Sensitive periods have been well described in animal studies, but even there, the focus has been more on perceptual systems rather than on cognitive or social/emotional development.

A major purpose of this application is to conduct a study that examines different domains of development with regard to their amenability to remediation. Another purpose is to extend the focus of previous research on early experiences to complex cognitive operations and to social and emotional domains. The proposed prospective follow-up of young children raised in institutions in Bucharest, Romania will examine the cognitive, behavioral, social, psychiatric, and brain characteristics of the children when they are 8 years old. These children have all experienced varying degrees of serious social and material deprivation from institutional rearing. As such, we will also investigate the role that gene-environment interactions may play in the differential recovery of these children after institutionalization by examining certain common genetic polymorphisms. This sample is uniquely suited to the questions being addressed because they have been followed longitudinally from the mean age of 22 months (range=6-32 months) to 54 months with systematic, repeated assessment of their cognitive, social and brain development.

The domains of inquiry in the current proposal include: IQ, attention and inhibitory control, social and behavioral development, psychiatric status, cognitive development, and brain activity. As a result of prior longitudinal data, the proposed study will be well-positioned to answer fundamental questions about the timing of early intervention in remediating the effects of early severe deprivation. Because of the range of ages of subjects when first removed from the institution, the proposed study will be able to address questions regarding the possibility of sensitive periods in the development of particular cognitive and social skills. And finally, because of the range of domains assessed in the BEIP study, the proposed analyses will be able to examine the relative impact of timing and length of intervention (placement in foster care) as it affects different domains of behavior. These issues will be addressed within the following specific aims:

1) To examine the effects of timing of intervention (placement in foster care), length of intervention, and age at which intervention no longer ameliorates deficits in multiple domains of functioning: IQ, attention and inhibitory control, social behavior, psychiatric disorders, cognitive development, and brain activity, among a group of children who experienced varying degrees of severe deprivation during the early months of their lives. In order to accomplish this aim, we will examine children's performance in these six broad domains of functioning at age 8 as a product of the length of time they were institutionalized, the age at which they leave the institution, and the length of time in intervention

2) To determine if length of institutionalization, number of disruptions in placement, and quality of *early* caregiving environment contribute uniquely, additively or interactively to outcomes in children at age 8 years in:

a) psychiatric disorders/symptomatology/impairment
b) IQ
c) memory and its neural correlates as inferred by event-related potentials
d) executive functioning (planning, inhibitory control, and their neural correlates as inferred by event-related potentials)
e) attentional processes (orienting, detecting deviance, monitoring performance, and their neural correlates as inferred by event-related potentials)
f) social cognition (recognition of facial emotion expression as inferred by event-related potentials, and social information processing).
g) social competence and behavior problems
h) EEG power and asymmetry
i) height, weight, and head circumference
j) speech, language, and reading skills

3) To determine if 8-year-old children who experienced severe early deprivation and who are compared to age-matched children who did not experience severe early deprivation will demonstrate:

a) an increase in psychiatric disorders/symptomatology/impairment
b) lower IQs
c) impairments in recognition memory
d) disturbances in executive functioning
e) disturbances in attentional processes
f) disturbances in social cognition
g) less social competence and more behavioral problems
g) reduced EEG power and more right frontal asymmetry
h) physical growth compromises in height, weight and head circumference
i) reduced vocabulary and deficits in reading and decoding skills

4) To determine the role that gene-environment interactions may play in the differential recovery of these children after institutionalization by examining functional polymorphisms in genes involved in the regulation of norepinephrine (NE), dopamine (DA) and serotonin (5HT) neurotransmission systems. These include:

a) the serotonin transporter gene length polymorphism (5HTTLPR)
b) the catechol o-methlytransferase (COMT) val 158 met allele
c) the val 66 met allele of the brain derived neurotrophic factor (BDNF) gene
d) the VNTR alleles of the dopamine receptor 4 (DRD4) gene
e) polymorphisms in DRD2 (dopamine receptor 2)
f) polymorphisms in DAT (dopamine transporter)

2. Background and Significance

The first three years of life is unprecedented in the human life cycle for the rapidity, complexity, and profundity of developmental changes. In only three years, the human infant progresses from complete dependence upon its caregiver, to a mobile, verbal, and cognitively sophisticated child capable of understanding and participating actively in complex social situations and groups. Further, individual differences in the quality of the caregiving environment have been shown to be powerfully associated with developmental outcomes in young children.

Investigators are only now attempting to understand the developmental brain changes that underlie these remarkable developmental advances. From the standpoint of intervening with children with disadvantageous beginnings, a number of questions are important. How much recovery is possible for children who experience early social deprivation? Are there critical periods that limit recovery from early deprivation? What are the crucial ingredients in facilitating recovery? These questions have been addressed to some degree by investigators studying children adopted out of institutions.

Children in Institutionalization: Previous Research

For most of the 20th century, clinicians and researchers have noted the deleterious effects of institutional rearing on the development of young children. Initially, many of these studies were uncontrolled or poorly controlled, but more recent investigations have been more rigorous and have confirmed earlier findings from descriptive studies suggesting that institutional care was associated with a variety of deleterious outcomes.

Contemporary research has documented many problems in young children adopted out of institutions in Eastern Europe and Russia. Abnormalities include a variety of serious medical problems (Johnson, 1997; Johnson et al., 1992), physical and brain growth deficiencies (Aronson & Johnson, 1999; Benoit et al., 1996), cognitive problems (O'Connor, Rutter, Beckett, Keaveney & Kreppner, in press; Morison, Ames & Chisholm, 1995; Rutter, et al., 1998), speech and language delays (Dubrovina, 1991; Groze & Ileana, 1996; Albers, Johnson, Hostetter, Iverson & Miller, 1997), sensory integration difficulties and stereotypies (Cermak & Daunhauer, 1997; Chisholm & Savoie, 1992), as well as social and behavioral abnormalities (Fisher, Ames, Chisholm & Savoie, 1997; O'Connor, Bredenkamp & Rutter, 1999). The latter include difficulties with inattention/hyperactivity (Rutter, 1999), disturbances of attachment (Chisholm et al., 1995; Chisholm, 1998; O'Connor et al., 1999; in press) and a syndrome that mimics autism (Federici, 1999; Rutter, Anderson-Wood, Beckett, Bredenkamp, Castle, Groothues, Kreppner, Keaveney, O'Connor and the English and Romanian Adoptees [ERA] Study Team, 1999). Most of the data available concern children adopted from Romania, which has been the leading source of international adoptions for families in the United States and many other western countries in the decade of the 1990's.

Some of these abnormalities are associated with risk factors that precede placement in the institutions, but quality of care often is appalling in these institutions, and many problems seem related to the ecology of institutional life (Ames, 1997; Johnson, in press; Muhamedrahimov, in press). One of the distinguishing features of the quasi-autistic syndrome reported in these children, for example, is that the symptoms improve dramatically following adoption (Rutter, 1999). Therefore, a major purpose of the BEIP is to determine which effects are remediable and which are not.

A number of longitudinal studies have been conducted as "natural experiments" to examine the effects of institutionalization on children's development. The first study was initiated in the later 1960's and early 1970's in residential nurseries in London. Barbara Tizard and her colleagues studied young children who were reared in institutions for their first two to four years of life. She studied four groups of children: a group that was adopted between age two and four years, a group of children who was returned to their biological families between two and four years, a group who remained institutionalized, and a group of never-institutionalized children. Nevertheless, group assignment was not random, and selection factors may have been substantially related to outcomes demonstrated (the adopted group looked better on virtually all measures). Further, children who were adopted and those who were returned to biological parents may have differed in important ways from those who remained institutionalized. The other limitation of the Tizard data is that the measures were used nearly 30 years ago, rendering them quite dated by contemporary standards.

Two longitudinal studies have been conducted recently using children adopted from Romanian institutions. Ames, Chisholm and colleagues conducted a longitudinal study of babies adopted from Romanian institutions into Canada. Their investigation included three groups of children: children adopted into Canada after having spent at least eight months in a Romanian institution, children adopted into Canada from Romania at less than four months of age, and a Canadian born (but not adopted) comparison group matched on age and sex to the first group. They found behavior problems, disturbances of attachment, and lower IQs in the group of children who had spent eight months or more in Romanian institutions.

O'Connor, Rutter and colleagues studied 165 children adopted from Romania and compared them at the ages of 4 and 6 years to 52 children adopted within the U.K. They interviewed parents of these children using semi-structured interviews regarding attachment disorder signs and behavior problems at age 4 years and administered a home-based version of the Strange Situation Procedure. They repeated these interviews and administered the McCarthy Scales to children at age 6 years. They found that both at age 4 and age 6, duration of deprivation was linearly related to number of signs of attachment disorders. Children exhibiting indiscriminate sociability at age 6 years had experienced deprivation for twice as long as the cluster of children exhibiting no attachment disorder signs (M = 22 months vs. M = 11 months). Although cognitive recovery was inversely related to age of adoption, social and emotional problems were less clearly related to timing.

O'Connor and Rutter also examined developmental level and attachment disorder behaviors. They reported a modest negative correlation in 6-year-old children adopted out of Romanian institutions between global cognitive index and attachment disorder behaviors. Nevertheless, when duration of deprivation was taken into account, the association between cognitive delays and attachment disorder symptomatology disappeared. These findings suggest that attachment disorder symptomatology and global cognitive impairments were largely independent. Aggressive behavior appears to be largely independent of signs of attachment disorder in institutionalized children, while associations of signs of attachment disorder with language delays and stereotypies are sufficiently low to suggest that another factor (or factors) may be influencing all three of these developmental problems.

Taken together, these findings suggest that although social deprivation may be associated with impairment across a range of developmental domains, the degree of impairment and trajectories of recovery may vary for these different domains. These tentative conclusions must be tempered by the realization that these studies are flawed by lack of randomization and selection bias in who is adopted, lack of data about individual differences in institutional experiences, and lack of adequate comparison groups (i.e., native children who have never been institutionalized).

Neurobiological abnormalities

Given all of the dramatic behavioral abnormalities observed in institutionalized and formerly institutionalized children, it seems reasonable to explore neural systems that might be associated with those behavioral abnormalities. Previous research on institutionalized children has not included measures of brain functioning, although some assessments have been conducted with children adopted out of institutions. For example, Chugani and colleagues conducted a 2-deoxy-2-[^{18}F]fluoro-D-glucose PET study in 10 children (average age = 8 years) who had been adopted after living in a Romanian institution. Nearly all children had been placed in the institution before age 1½ months, and had lived in the institution an average of 38 months before being adopted. Compared to a control group of healthy adults, the adoptees showed significantly reduced brain metabolism in the orbital frontal gyrus, the infralimbic prefrontal cortex, medial temporal structures (including the amygdala and head of the hippocampus), the lateral temporal cortex, and the brain stem. Compared to a sample of 10-year-old children with medically refractory epilepsy, the adoptees showed significant decreases in glucose metabolism in the left orbital frontal cortex, left medial temporal structures, and the left lateral temporal cortex. Behaviorally, the adopted children were described as suffering from mild neurocognitive impairments, impulsivity, attention and social deficits.

Collectively, results from this study, the first of its kind, point to the serious neurobiological sequelae of early and prolonged institutionalization. In particular, these children suffered from metabolic deficits in the areas of the brain believed to be involved in higher cognition, emotion and emotion regulation. Unfortunately, this study suffers from the same shortcomings as other post-adoption studies noted earlier, making it unclear to whom the results generalize.

The proposed investigation will improve upon previous research in this area in a number of ways:

1) The study will include baseline assessments and randomization to assure comparability in the intervention (foster care) and the institutionalized control groups. No previous investigations have used random assignment and suffer from potential biases about which children get adopted from institutions.

2) The proposed study will include a never-institutionalized Romanian group matched on child age and gender. Because the measures we are using have not been standardized nor widely used in Romania, the addition of this group will allow an examination of how much recovery is possible following early institutionalization. No previous study has used a never-institutionalized Romanian control group.

3) In addition to state of the art measures of social interaction, social communication, attachment, behavior problems, and global cognitive development, this will be the first study of institutionalized children to use measures of brain function, including a visual discrimination paradigm, designed to measure specific brain functions and studied extensively in humans and primates. Results from this measure will provide a preliminary way of assessing brain function in young children who have experienced profound early deprivation.

4) This project also will be the first to assess brain electrical activity in institutionalized and non-institutionalized children by conducting EEGs and examining for frontal asymmetry. These results will tie into other work demonstrating that infants of depressed mothers and young children at risk for anxiety disorders have right frontal activation.

5) Finally, this will be the largest longitudinal investigation of institutionalized children less than two years old at the time that the intervention begins. This will allow a more fine-grained look at issues of timing of intervention and recovery than previous studies that have included children with histories of deprivation longer than two years.

3. Preliminary Studies/Progress Report

Romanian Context

Romania is a country of 22,000,000 people, more than 80% of whom are ethnic Romanians, located in Southeastern Europe between Central Europe and the Black Sea. (National Institute for Statistics, 2002). Situated at the gateway of Europe, near both Russia and the Middle East, Romania has throughout history been the battleground of rival Empires. Following Roman occupation and the invasions of migratory tribes, Romania was fragmented, divided between the Ottoman, Austro-Hungarian, and Russian Empires (Iorga, 1970). As such, Romania has existed as a unified and independent nation for less than 200 years.

From 1945 to 1989, Romania was an Eastern European satellite under the sphere of influence of the USSR. In 1965, Nicolae Ceaucescu, a former shoe cobbler, became Secretary General of the Communist Party. He instituted a number of draconian economic and social policies designed to enhance Romania's productivity, but which instead, had devastating effects, especially on young women and families. Among these measures, he oversaw passage of a law that required all women under the age of 40 to produce five children in order to increase the number of workers by creating the "Romanian Workers Army." This law was associated with various incentives for compliance and harsh penalties for failure to comply. These included higher income taxation for "incomplete families" and visits from a gynecological corps dubbed "the Menstrual Police" which had free reign to interrogate and even examine young women who did not seem to be in compliance. Abortion and all forms of contraception were illegal, and carried severe punishments, so many families who were unable to support their children handed them over to be raised by the state (Moskoff, 1980).

Romania began the transition from communism a mere 15 years ago when Nicolae Ceausescu was overthrown in a revolution in 1989. At present, Romania is a democracy, committed to a free market economy, and civil rights and freedom for all citizens. However, the effects of Soviet communism combined with Ceausescu's totalitarian rule placed Romania far below the level of development of its neighbors, and thus it continues to struggle today with economic reform, ranking among the lowest income per capita in the region. Low wages, poor living conditions, civil inequities, and judicial corruption also have slowed the process of Romania's desired admission into the European Union (EU Country report: Romania, 2004).

Orphanages in Romania

Institutions for young children in Romania date back at least to the 19th century. Nevertheless, in the Communist era, widespread poverty and coerced childbirth led to many more unwanted children. In addition, the Communist ideology de-stigmatized institutional care because the state, according to this rationale, could raise good and loyal workers. These factors converged and are believed to have led to significant increases in the orphanage population in the latter half of the 20th century. Thus, at a time when orphanages for young children were disappearing in the West and foster care was becoming the preferred form of care for orphaned and abandoned children (Hacshi, 1997), in Romania and other Soviet satellites, institutions for young children flourished. Although exact numbers are difficult to ascertain, estimates are that as many as 200,000 children were institutionalized in 1989 when Ceaucescu was overthrown (Rosapepe, 2001).

Despite putative ideological support, most communist orphanages in Romania were poorly financed, and children often were raised in appalling conditions of social and material deprivation. This was documented dramatically by Western media reports about Romania's institutions soon after Ceaucescu's overthrow, as well as by Human Rights Watch (www.hrw.org/children/abandoned.htm).

Reform efforts began in earnest with legislation in 1997 designed to encourage prevention of child abandonment by provision of social supports to parents considered at high risk, and through efforts to support local governmental efforts to develop alternatives to institutional care. This was a major transformation, as institutional care had been the only form of child protection in Romania for decades and was the official State policy even after Ceaucescu was overthrown. These legislative initiatives, coupled with other forces, have led to significant changes. Over 27,000 foster homes have been developed in the past 3 years and the number of children in institutions has been reduced

significantly. Although over 30,000 children remain in institutions in Romania today, many of these are older children with multiple handicaps who cannot be easily placed in family settings. As a comparison, as recently as 6 years ago, there were virtually no state-funded foster homes in Bucharest (ANPCA, 2004), and over 100,000 children living in orphanages. It is within this context that the Bucharest Early Intervention Project (BEIP) was conceived and initiated.

The Bucharest Early Intervention Project

The Bucharest Early Intervention Project was a randomized controlled trial of foster care as an alternative to institutional care for young children abandoned at birth and placed in institutions (Zeanah et al., 2003). The Bucharest Early Intervention Project (BEIP) started in 2000 with 136 children between 9 and 30 months of age living in Romanian orphanages, 69 of whom were randomly assigned to foster families in Bucharest. A group of 72 never-institutionalized Romanian children living with their biological families, and matched on age and gender with the institutionalized children, was recruited as a comparison group.

The BEIP began with comprehensive assessments of children and their caregiving environments prior to randomization, and then assessed their development serially at 9, 18, 30, 42 and 54 months. Because participants were 6-30 months of age at the beginning of the study, all children were seen for follow-up assessments at 30, 42 and 54 months. Assessments included measures of physical growth, cognitive function, social-emotional development and attachment, temperament, problem behaviors, language development, caregiving environment, and brain development.

Analyses thus far show that, in a number of domains, children placed in foster care (FCG) experience developmental gains that resemble the group of children who were never institutionalized (NIG); however, other domains prove relatively resistant to intervention. Here, we will discuss how institutionalized children differ from community children at baseline, and how children placed in foster care compare to both institutionalized and community children at 42 months of age.

When compared to typically developing children raised in families, children raised in institutions show deficits or abnormalities in:

i) physical growth (e.g., for height, weight, and head circumference, they fall at roughly the 10th percentile)
ii) cognitive development (whereas the IQ of children living with their parents is about 100, those in the institution is about 65)
iii) language development (like physical growth, institutionalized children's language performance is at about the 10th percentile)
iv) social communication (it is generally poorer among institutionalized vs. non-institutionalized children)
v) attachment (whereas all of our never institutionalized children have formed attachments, about 75% of which are secure attachments, 95% of the institutionalized children have formed incomplete attachments and many suffer from attachment disorders)
vi) brain development (based on our EEG data, our institutionalized group shows a vastly underpowered brain).

In contrast to our baseline findings, placement in foster care leads to at least some improvement in many, although not all, domains. For example, at 42 months, we are observing improvements in language, emotion recognition, emotion responsivity, joint attention, height and weight, and some aspects of attachment, as well as a reduction in the incidence of depression and anxiety. In contrast to those domains where we have observed improvement, there are other domains where little improvement is evident; for example, we are not observing definite improvements in EEG power, head circumference, or the incidence of attention deficit hyperactivity disorder or disruptive behavior disorders.

In the BEIP study, caregivers of never-institutionalized children were both more available and interacted more frequently with their children than did caregivers in institutional settings. Further, within the institutionalized group, quality of caregiving at baseline was strongly associated with cognitive development and with child competence, explaining variance over and above what was accounted for by large between group (institutionalized vs. never-institutionalized) differences. Among institutionalized children, quality of caregiving was related to signs of attachment disorder and to a more fully developed attachment to caregiver. Quality of caregiving also was the only significant factor associated with an institutionalized child having an organized (as opposed to disorganized or unclassifiable) attachment (Zeanah et al., 2005).

At follow-up, infants and toddlers randomized into foster care were observed to use speech-like vocalizations and to exhibit more positive interactions with caregivers significantly more frequently than children who had been randomized to continued institutional care. This pattern of findings, both in the NICHD child care studies, and in the findings from the infant and toddler BEIP suggest that quality of the caregiving environment, as measured by the ORCE, is an important construct in understanding child outcome.

To illustrate both baseline differences in institutionalized children and never-institutionalized children, we have selected five different domains to use illustratively. These are attachment, cognitive development (i.e., Bayley scores), EEG power and coherence, ERPs to facial recognition of emotion, and psychiatric disorders.

Attachment: At baseline, institutionalized children had substantially more disorganized (includes non-attached) attachment than children raised with their parents (78% v. 22%). Furthermore, 100% of never- institutionalized children were coded blindly as having fully developed attachments to their mothers, whereas only 4% of institutionalized children were coded as having fully developed attachments to their caregivers. In addition, caregivers reported significantly more signs of both emotionally withdrawn/inhibited Reactive Attachment Disorder (RAD) and indiscriminately social/disinhibited RAD in institutionalized compared to never-institutionalized children. At follow-up, signs of emotionally withdrawn/inhibited RAD were significantly lower in the foster care group than the institutionalized group, and indistinguishable from the never-institutionalized group. Indiscriminate sociability/disinhibited RAD, on the other hand, was significantly lower at follow-up in the foster care group than in the institutional group, but was still significantly higher than in the never-institutionalized group.

Cognitive Development: At baseline there were substantial differences in the institutionalized group and the never-institutionalized group. Mean scores on the Bayley MDI were 103 in the never-institutionalized group and 65 in the institutionalized group. The latter score was inflated since the lowest score assigned on the Bayley is <50. All children who received this score were assigned a score of 49. Following randomization, children in foster care demonstrated more significant gains in MDI scores than children in the institution group, although they did not attain levels of the never-institutionalized group at any follow-up point. Not surprisingly, gains in IQ vary as a function of length of time in the institution – thus, children who spent more time in the institution have lower IQs than those who spent less time.

EEG Power and Coherence: At each assessment in the original BEIP study, the EEG was recorded from 15 electrode sites during an episode designed to elicit quiet attention in infants and young children. Power in three frequency bands (3-5 Hz as theta, 6-9 Hz as alpha, 10-18 Hz as beta) was computed at each electrode site using both the absolute and relative power metrics. At the baseline assessment, the institutionalized group (IG) showed a higher level of relative theta power and a reduction in alpha and beta relative power compared with a group of never-institutionalized children (NIG) (Marshall et al., 2004). Recent analyses of the 42 month EEG data suggest that the foster care intervention has a specific effect on the development of relative alpha power in the EEG signal collected at rest. Correlational analyses examined the relation between relative alpha power at 42 months and the length of time that had elapsed since the original baseline assessment at the onset of the study. Significant positive correlations for the FCG were found bilaterally across frontal, central, parietal, and occipital scalp regions, and unilaterally in the temporal region. Comparable analyses for the IG showed significant correlations bilaterally in the occipital region and unilaterally in the temporal and parietal regions, with no significant correlations at frontal or central electrode sites. No significant correlations were found for the NIG.

Event Related Potentials: Event-related potentials (ERPs), in response to 4 facial expressions of fear, angry, happy, and sad, were collected from 72 institutionalized children (IG) and 33 never-institutionalized children (NIG), ranging in age from 7 to 32 months. The NIG and IG exhibited different patterns of responding in early latency components (e.g., N1, P1). Moreover, group differences in amplitude were evident across all components; specifically, for both early (e.g., N1, P1) and late (e.g., NC, PSW) components, and ERP amplitudes were dramatically reduced among IG compared to NIG infants. These findings are consistent with our EEG findings (Marshall et al., 2004), in which we demonstrated reduced EEG activity across several frequency bands.

Event-related potentials (ERPs) were recorded to brief images of caregivers' and strangers' faces from 72 institutionalized children (IG) and 33 never-institutionalized (NIG) children, aged 7 to 32 months. Prominent differences in 4 ERP components were observed: the N170, P250, NC, and PSW. For all but the P250, the amplitude of these components was larger in the NIG than the IG; this pattern was reversed for the P250. Typical effects of the NC (amplitude greater to stranger vs. caregiver) were observed in both groups; in contrast, the IG

group showed an atypical pattern in the PSW. Over both studies, our findings collectively point to the role of experience in influencing the neural circuitry putatively involved in recognizing familiar and novel faces and facial expressions.

We have only recently begun to examine our follow up data. Preliminary inspection reveals that the ERP amplitude of children placed in foster care appears to normalize and resemble that of our community controls, a finding that parallels our EEG data.

Psychiatric Disorders: Because of the age of the children, psychiatric disorders could not be assessed at baseline. Instead, we assessed psychiatric disorders as an outcome at 54 months. At that time, caregivers/parents were administered the Preschool Age Psychiatric Assessment ([PAPA] Egger & Angold, 2004), a structured psychiatric interview that represents a downward extension of the Child and Adolescent Psychiatric Assessment ([CAPA] Angold et al., 1995). To date, caregivers/parents of 41 children in institutions, 45 children in foster care, and 26 never institutionalized children have been interviewed. As shown in Table 1, preliminary results indicate a remarkable number of children with psychiatric disorders. Thus, 44% of children reared in institutions have a diagnosable psychiatric disorder, whereas only 14% of never institutionalized children meet criteria for a disorder. Both emotional and behavioral disorders are prevalent in children who are or who were previously institutionalized, also shown in Table 1. It should be noted that children who were deemed highly symptomatic as a result of this questionnaire, or whose parents/caregivers expressed concerns to staff, were referred to appropriate clinicians for services.

Table 1. Prevalence of Composite Disorders: Preschool Age Psychiatric Assessment (PAPA)

	PAPA TRT* N = 307	IG N = 41	FCG N = 45	NIG N = 21
Any emotional disorder	10.6%	43.9% (18)[a]	22.2% (10)	4.8 % (1)
Any behavioral disorder	11.3%	29.3% (12)[b]	31.1% (14)	14.3% (3)
Any disorder	17.4%	53.7% (22)[c]	37.8% (17)	14.3% (3)

a. IG versus FCG predicting internalizing disorder(s): OR=2.7 (1.1, 7.0); p = 0.03
b. IG versus FCG predicting externalizing disorder(s): OR=0.9 (0.4, 2.3); p = 0.9
c. IG versus FCG predicting any disorder(s): OR=1.9 (0.8, 4.5); p = 0.1

*PAPA TRT refers to a test retest study of the PAPA conducted by Egger and colleagues in Durham, North Carolina pediatric clinics with 3 year old children. This provides a basis for comparison to the 3 groups of Romanian children.

Interestingly, intervention effects differed for emotional and behavioral disorders. That is, at 54 months of age there are significantly more emotional disorders diagnosed in the institutional group (44%) compared to the foster care group (22%), but no differences in behavioral disorders between the groups (29% vs. 31%). Both institutional and foster care groups had higher levels of emotional and behavioral disorders compared to the never institutionalized group. Based on these preliminary results, it appears that there is a differential sensitivity in emotional and behavioral disorders to the intervention (i.e., foster care). The intervention reduced emotional disorders but appears to have had no effect on behavioral disorders in this group of children who shared the experience of early institutional care. Table 2 provides more detail about specific diagnoses.

Table 2. Prevalence of Specific Disorders: Preschool Age Psychiatric Assessment (PAPA)

	PAPA TRT* n = 307	IG n = 41	FCG n = 45	NIG n = 21
ADHD	5.1%	24.4% (10)	26.7% (12)	9.5% (2)

ODD	7.3%	2.4% (1)	13.3% (6)	0
CD	3.4%	4.9% (2)	11.1% (5)	4.8% (1)
Depression	2.0%	9.8% (4)	4.4% (2)	0
Any anxiety d/o	9.5%	36.6% (15)	20.0% (9)	4.8% (1)
SAD	2.4%	4.8% (2)	0	0
GAD	6.5%	19.5% (8)	17.8% (8)	0
Social phobia	2.2%	7.3% (3)	0	0
Simple phobia	2.3%	2.4% (1)	6.7% (3)	0
Selective mutism	0.6%	17.5% (7)	2.2% (1)	4.8% (1)
PTSD	0.6%	0	2.2% (1)	0

*PAPA TRT refers to a test retest study of the PAPA conducted by Egger and colleagues in Durham, North Carolina pediatric clinics with 3 year old children. This provides a basis for comparison to the 3 groups of Romanian children.

4. Design and Methods

a. Study Design

This investigation is a cross-sectional follow-up study of severely deprived young children who have been studied for 4-5 years in the Bucharest Early Intervention Project (BEIP) in Bucharest, Romania. These children were institutionalized at birth (or soon thereafter) and half were randomly assigned to foster care when they were between 6 and 31 months of age (siblings were randomized together so 69 children were placed in care and 67 were randomized to continued institutional care). The children were followed systematically through 54 months of age, and the development of children in foster care was assessed compared to the development of children in institutions and to another group of never institutionalized children (community controls). In the current study, the originally institutionalized children are being assessed at 8 years. Over the past 3.5 years, many children have changed from their original groupings, so that at the time of this submission, only 17/67 children remain institutionalized. This decrease is largely because Romania has made a policy commitment to de-institutionalize abandoned children, and a sample with a range of caregiving adversity from 6 to 96 months (such as this one) will not likely be available there in the foreseeable future. At the outset of the investigation, we determined that we would not interfere with any permanent plans that were developed for the children (Zeanah et al, in press). Therefore, 20 of the children originally randomized to institutional care have now been adopted by Romanian families or reunited with their biological families, and another 21 have been placed in government sponsored foster care that did not exist at the time the BEIP began.

As a result of these policy changes, ours is a proposed study of young children who have experienced varying degrees of adversity from caregiving environments that range from poor quality institutions to much better quality homes. Thus, this is not a follow-up of the existing cohort to evaluate the outcome of our randomized controlled trial (which would not be meaningful given the large number of "cross-overs"). Rather, it is a study of the effects of varying degrees of caregiving adversity on young children's development at age 8 years. We are interested in the details of the caregiving environment for each child (rather than merely contrasting institution-reared vs. home-reared children, for example), and therefore, we are proposing to examine 3 different variables believed to be associated with risk for poor psychosocial outcomes: (1) length of institutionalization (range will be 6 months to 8 years), (2) number of placement disruptions (e.g., changes from institution to foster care, from one foster home to another, from institution to institution, from unit to unit within an institution, or from institution or foster care to adoptive home [all adoptions were within Romania due to a ban on international adoptions]), and (3) a summary of quality of the early caregiving environment (measured in previous assessments by direct observation and coded from videotapes). Determining if length of institutionalization, number of disruptions, and quality of early caregiving environment contribute uniquely, additively or interactively to predict outcomes in children at age 8 years is the major aim of this investigation.

This sample and design include children with a continuum of caregiving adversity, ranging from 6/96 months (those who were randomized to foster care at 6 months of age) to (possibly) 96/96 months of institutional care (those who were randomized to institutional care and have remained there). This design will enhance our ability to ask important questions about timing/amount of adversity, since some children will have experienced more early adversity and others more continuous adversity. The sample is uniquely valuable because we have direct, observational measures of their early caregiving environments, as well as links to early brain and behavioral measures (which are beyond the space limitations of this application to describe in detail).

Genetics

Although previous studies have looked at genetic polymorphisms in non-clinical populations, there are advantages to examining them in this particular study population. That is, we can examine the contributions of both polymorphisms and a setting of social deprivation to undesirable outcomes. Furthermore, the experimental manipulation of the caregiving environment (placing half the children in foster care) allows for an evaluation of a potential interaction. This is important because simply looking for genetic predispositions to psychiatric conditions alone is often not illuminating. Rather, it is the combination of subtle changes in DNA in the context of environmental stressors that is important. For example, Caspi et al. (2003) found no impact of genotype on later depression unless previous life stressors were incorporated into the analysis. Additionally, in another study there was no impact of genotype on the development of psychopathology without the additional incorporation of abuse exposure (Kaufman et al., 2004).

Another feature of the current study population that makes it extremely valuable to examine from a genetic perspective is the wealth of physiologic data that has already been collected on these children. The ability to evaluate the influence of normal genetic population variants on physiologic markers will provide potentially valuable information about the underlying molecular basis of both normal and abnormal physiological responses.

Finally, risks to the children will be minimal, as all identifying information will be removed from the DNA samples, and the risk of buccal swabs on the children is minimal. We plan to explore functional polymorphisms in genes involved in the regulation of norepinephrine (NE), dopamine (DA), and serotonin (5HT) neurotransmission systems. These include the serotonin transporter gene length polymorphism (5HTTLPR), the catechol o-methlytransferase (COMT) val 158 met allele, val 66 met allele of the brain derived neurotrophic factor (BDNF) gene, the VNTR alleles of the dopamine receptor 4 (DRD4) gene, polymorphisms in the dopamine receptor 2 (DRD2) gene and polymorphisms in the dopamine transporter (DAT) gene. , Although there are many other potentially interesting polymorphisms that could be examined, these specific polymorphisms have the greatest amount of extant data related to our outcome measures. Additionally, they are all functional polymorphisms which alter the gene product and are all directly involved in the neurobiological pathways that are most likely implicated in the development of psychological differences in these children. The reasons for selecting these particular polymorphisms are detailed below.

The serotonin transporter gene (5HTT) is a critical regulator of serotonin function in the synapse. Located within the promoter region of this gene is a 44 base pair functional polymorphism (5HTTLPR) that alters the expression level of the 5HTT protein encoded by this gene (Lesch et al 1998). The variant without the 44 base pairs (the short variant) leads to decreased expression of 5HTT protein (Heil et al 1996, Frodl et al 2004). Caspi et al., 2003, demonstrated that the impact of stress on the development of major depression disorder (MDD) is moderated by this polymorphism and that childhood maltreatment predicted adult onset depression only in individuals who carried the short allele. As the short allele leads to dysregulation of 5HT which likely impacts the prefrontal cortex (PFC) regulation of the amygdala (Hariri et al 2002), the storage and recall of memories, and regulation of the HPA axis (Caspi et al 2003) it is likely that this allele is important in children's vulnerability to early social deprivation.

The catechol o-methlytransferase (COMT) gene encodes an enzyme important in the breakdown of DA in the prefrontal cortex (PFC), an area implicated in the expression of anxiety (Rotondo et al 2002, McGrath et al 2004). COMT also inhibits □-opiod mediated suppression of the hypothalamic pituitary axis (HPA) which is integral to the stress response and has important implications in the etiology of affective disorders. A functional "val to met" polymorphism exists in codon 158, and the met allele has a four fold decrease enzyme activity, resulting in slower breakdown of DA in the synaptic cleft selectively in the PFC (Lotta et al 1995, Sesack et al 1998). The met allele has been associated with bipolar disorder, panic disorder, schizophrenia, and impairments of cognitive performance and working memory tasks (Nolan KA et al 2004, Rotonado et al 2002, Diamond et al 2004). Children with the lower functioning met allele may have decreased □-opiod tonic inhibition of the HPA axis and a subsequent increased physiologic stress response. This elevated response would mediate the development of behavioral and social problems in children faced with the chronic stress of social deprivation and adverse early life events. We predict an increase in the met allele in children with lower baseline scores, less recovery with foster placement and more affective disturbances at the 54 month assessment.

BDNF is involved in long term potentiation of memory and is essential for neuronal survival and differentiation, is integral in neuronal plasticity (Egan et al 2003) and increases extracellular 5HT levels (Mossner et al 2000, Siuciak et al. 1998). Elevated levels of BDNF increase the growth of 5HT and NE neurons in the hippocampus and are neuroprotective to stress (Thoenen et al 1995). Maternal behavior has been shown to elevate BDNF expression in mice (Liu et al 2000). High levels of stress lowers BDNF levels and results in decreased survival of 5HT neurons. Chronic stress may lead to low levels of BDNF, resulting in decreased growth and protection of 5HT and NE neurons. This impact would be extremely important during the first three years of life when the most rapid neurodevelopment is occurring. In the BDNF coding region is a val66met polymorphism. The met allele produces a non-functional protein product that results in the reduction of hippocampal neuronal integrity and synaptic activity. Individuals with the met allele had inappropriate over-activation of the hippocampus during working memory tasks (Egan et al 2003). We hypothesize that institutionalized children will have lower BDNF levels due to the stressful nature of their environment. In children with the met allele, the functional amount of BDNF would be even lower and would accentuate the negative impact of decreased BDNF, influencing neuronal survival and synaptic efficiency. Children with the met allele, when placed in foster care, would be less able to correct this BDNF deficiency and would show decreased cognitive and social recovery. We predict an increase in the met allele in children with lower baseline scores, less recovery with foster placement, and increased affective disturbances at the 54 month assessment.

The dopamine receptor 4 (DRD4) is implicated in reinforcement behavior, emotional expression, modulation of working memory, and the integration of the neuronal signals controlling behavioral response (Falzone et al., 2002). Studies have found weak association between infant behavior, temperament, and disorganized attachment with several receptors and proteins including the 5HTTLPR polymorphism, the VNTR of DRD4, and the –521 T allele of DRD4 (Ebstein et al 1998, Auerbach et al 1999, Auerbach et al 2001, De Luca et al 2001), highlighting the importance of examining genetic risk factors for attachment disturbances. The 7 allele of the VNTR has also been shown to have a strong association with the development of ADHD. We predict an increase in the 7 allele of the VNTR in those children with the poorest baseline scores, least amount of recovery and increased amount of affective and ADHD symptoms at the 54 month assessment.

Although the impact of early social deprivation upon neurodevelopment is complex and multifactorial, genetic predisposition likely has a significant role. Importantly current results indicate that while foster care is leading to significant improvement in cognitive and behavioral aspects of these children the recovery is not uniform. The tremendous amount of data already collected in these children, when combined with the analysis of common genetic polymorphisms, presents an unprecedented opportunity in which to study gene:environment interactions, with virtually no additional risk to these children. Exploring the impact of these polymorphisms will not only help to explain variation in recovery, but also may lead to a clearer understanding of the roles these genes play in early development.

b. Patient Selection and Inclusion/Exclusion Criteria

Participant Selection

The first group of participants will be those children that participated in the BEIP by virtue of a history of institutional care and who have been followed since 2001. These children were recruited from all 6 institutions for young children in Bucharest, Romania between April and September of 2001. Eligibility requirements were that 1) they were institutionalized and had been for a substantial portion of their lives (children had been institutionalized for on average 90% of their lives), 2) were less than 32 months old in April 2001, and 3) did not have a severely handicapping condition (e.g., Fetal Alcohol Syndrome, Down syndrome). This yielded 136 children who ranged in age from 6 months to 32 months of age. We will try our best to recruit as many of the original 136 participants as possible. We have followed more than 100 of these children through assessments at 54 months, and we have remained in contact with the caregivers/foster families/parents of these children. We will make every effort to contact all participants from previous assessments and anticipate that at least 110/136 of these children will participate in the follow-up study.

The second group of participants will be 110 never-institutionalized children recruited from the Bucharest community. These children will comprise our comparison group and will be matched on age and gender to study participants described above.

Inclusion/Exclusion Criteria for Previous Participants

Upon initial phone contact with the parents/caregivers of **previous** participants, the researcher will give a description of the study. If the parent/caregiver indicates interest in participating in the study, the researcher will ask the following question to ascertain eligibility:

"Has your child experienced any neurological trauma in the past 12 months?" If parent/caregiver responds, "Yes" to this question, the researcher will ask the parent/caregiver to elaborate.

Only those children who have not experienced an open or closed head injury, viral or bacterial infection (MENINGITIS) within the past 12 months will be invited to take part in the study.

Inclusion/Exclusion Criteria for Additional Community Participants

PARENTS OF CHILDREN TO BE RECURITED FROM WITHIN THE COMMUNITY WILL BE ASKED THE FOLLOWING QUESTIONS TO ASCERTAIN ELIGIBILITY:

1. IS THIS YOUR BIOLOGICAL CHILD? (WE DO NOT WANT TO INCLUDE ANY INSTITUTIONALIZED OR FORMERLY-INSTITUTIONALIZED CHILDREN THAT DID NOT PARTICIPATE IN THE BEIP AS INFANTS OR TODDLERS AS PART OF OUR COMMUNITY COMPARISON SAMPLE).

2. HAS YOUR CHILD EVER ATTENDED A WEEKLY NURSERY? (WE DO NOT WANT TO INCLUDE CHILDREN RAISED IN FAMILIES IN ROMANIA WHO HAVE BEEN CARED FOR IN AN INSTITUTION-LIKE SETTING, SUCH AS A WEEKLY NURSERY). *A WEEKLY NURSERY REFERS TO A MON-FRI SLEEP OVER DAYCARE THAT THE CHILD MAY HAVE BEEN IN AS A TODDLER/PRESCHOOLER.*

3. DOES YOUR CHILD HAVE A HISTORY OF NEUROLOGICAL ABNORMALITY OR TRAUMA?

4. DOES YOUR CHILD HAVE UNCORRECTED VISION DIFFICULTIES (SUCH AS AMBLYOPIA, STRABISMUS OR CATERACTS)?

5. DID YOUR CHILD EXPERIENCE ANY PREGNANCY OR BIRTH RELATED COMPLICATIONS?

ONLY THOSE CHILDREN WHOSE PARENTS RESPOND "YES" TO QUESTION 1 AND "NO" TO QUESTION2 2 – 5 WILL BE INVITED TO TAKE PART IN THE STUDY.

ONLY THOSE CHILDREN WHOSE PARENTS RESPOND "YES" TO QUESTION 1 AND "NO" TO QUESTIONS 2 – 5 WILL BE INVITED TO TAKE PART IN THE STUDY.

Although an ideal group would be one that shares risk factors (particularly prenatal and genetic) with the institutionalized group, but who were never institutionalized, this is impossible, for several reasons. First, there will always be a difference between families who do and do not abandon their children, however similar they may appear in terms of demographic and other risk status variables. Second, the likelihood of identifying and obtaining the cooperation of a sample of families matching the demographics of the families of the children who were institutionalized is highly improbable. Therefore, we include a comparison group for purposes of determining how large and in which areas the deficits are in the children reared in institutions, fully aware that differences in early rearing experiences were not the only contributors to the expected deficits.

Rationale for inclusion of children

Young children are considered a vulnerable population and the rationale for including them in this study is that the potential benefits to be gained outweigh the anticipated risks. Children in this age group must master a variety of cognitive, socio-emotional, and adaptive skills that will serve as the basis for their performance in educational/work and social settings throughout the life span. Although children who have experienced early, severe social deprivation may experience some recovery when placed in more nurturing environments, the effects of timing and degree of deprivation and their impact on future functioning remains an important question. It seems imperative to study this age group as the consequences of deprivation may continue to have an impact long into childhood and beyond. Given the longitudinal nature of this study coupled with the developmental questions of interest, children previously enrolled in the study are the targeted population of interest.

Inclusion/Exclusion Criteria for Adult Participants for Stimuli Development
As in Adolphs et al., 1998, Romanian adult males with beards and mustaches will be excluded from the photograph stimuli development. Adults younger than 28 years or older than 60 years will be also be excluded as we want to match the age range of the caregivers/foster parents/parents of children in the sample.

c. Recruitment Methods

i. HOW, WHERE and WHEN will potential subjects be recruited?

Recruitment Methods for Previous Participants
Our Romanian research team has maintained contact with many of the children and their caregivers/families that have participated in previous assessments for the BEIP. Members of the BEIP Research Laboratory will contact the parents and caregivers of all previous participants by telephone to see if they will be interested in taking part in the follow-up study.

Recruitment Methods for Additional Community Participants To recruit additional children for the community comparison sample, we will employ recruitment methods established through scientific partnership with the Institute of Maternal and Child Health (IOMC), which assisted us by screening and recruiting a comparison group of community children for the original study. The IOMC has vested interest in pediatric research and has offered the study continued scientific collaboration and logistical support throughout the span of this research endeavor. ADDITIONALLY, OUR RESEARCH STAFF WILL REQUEST PERMISSION FROM TEACHERS OF SEVERAL ELEMENTARY SCHOOLS WITHIN BUCHAREST TO SEND LETTERS HOME TO FAMILIES WHOSE CHILDREN ARE THE APPROPRIATE AGE TO PARTICIPATE IN OUR STUDY. THE LETTER WILL DESCRIBE THE STUDY AND INVITES PARENTS TO CONTACT THE LAB IF THEY ARE INTERESTED IN LEARNING MORE ABOUT THE STUDY OR DECIDE THAT THEY WOULD LIKE TO PARTICIPATE.
Parents of potential participants will be approached by a social worker from the Institute of Maternal and Child Health (IOMC) at their child's routine clinic visit and invited to participate. If parents express interest in participating in the study with their child, AND RESPOND TO THE FIVE ELIGIBILITY QUESTIONS AS DESCRIBED ABOVE, the social worker will ask the parents for permission to be contacted by a member of the BEIP Research Laboratory staff. The social worker will provide our research staff with the contact information of the family and a member of the lab will contact the family within one week to schedule the first visit.

PARENTS OF CHILDREN WITHIN THE COMMUNITY WHO RECEIVE A LETTER FROM OUR RESEARCH LABORATORY WILL BE ASKED TO CONTACT THE LABORATORY IF THEY ARE INTERESTED IN PARTICIPATING IN THE STUDY OR IF THEY WOULD LIKE ADDITIONAL INFORMATION. IF PARENTS EXPRESS INTEREST IN PARTICIPATING IN THE STUDY WITH THEIR CHILD, A RESEARCH ASSISTANT WILL ASK THE FIVE ELIGIBILITY QUESTIONS DESRIBED ABOVE. IF THE PARENT RESPONDS TO THESE QUESTIONS AS DECRIBED ABOVE, THE RESEARCH ASSISTANT WILL SCHEDULE THE FIRST VISIT.

Recruitment Methods for Adult and Child Participants (Stimuli Development): Adult and child participants will be recruited from local elementary schools with which our research laboratory has already established relationships (see the attached recruitment letter). All participants will be asked to sign a photo release (see attached release), allowing researchers use of their pictures as research stimuli and in scientific meetings and presentations.

Recruitment Methods for Adult Participants (Stimuli Ratings): An independent group of 30 adults (15 M and 15 F) living in Romania will be recruited via an email circulated to faculty and students in the Psychology Department at Bucharest University. These participants will be asked to rate all stimuli to be used in the Social Group Preferences, Empathetic and Trustworthiness tasks. All participants will be asked to provide consent prior to completing the stimuli ratings. Copies of the recruitment materials and consent form are attached.

***It is crucial to note that the PI and his colleagues have carefully vetted their approach to recruiting in Romania through multiple levels of official and unofficial channels to be certain that the recruitment methods employed are culturally appropriate.*

ii. WHAT recruitment methods and materials (e.g. posters, fliers) will be used? - *attach all materials*

All recruitment will be through verbal communication with parents/caregivers. No written materials will be used for recruitment.

iii. WHO will be responsible for subject recruitment?

BEIP Research Laboratory staff and a social worker employed by the IOMC will be responsible for recruitment of all participants.

d. Description of Study Treatments or Exposures/Predictors

NA

e. Definition of Primary and Secondary Outcomes/Endpoints

NA

f. Data Collection Methods, Assessments and Schedule (what assessments performed, how often)

Administrative Details

Data collection for this study will consist of 6 sessions. Session 1 will occur within the first two months of protocol approval. The remaining 5 sessions will occur within ± 8 months of the participant's 8th birthday. We expect that data collection for the entire sample will take **36** months.

All sessions will be scheduled at times most convenient for the participant and their parents/caregivers. All sessions will be conducted in Romanian. Sessions 1 and 2 will take place in the home or placement center of the participant. Sessions 3, 4 AND 6 will take place at the BEIP Research Laboratory in Bucharest, Romania. Session 5, the MRI component of the protocol, will take place at the Medical Center UNIREA, a private center that offers a complete set of clinical medical services including high resolution imaging. We estimate that no one session will last more than 3.5 hours.

The tasks described in sessions 2-5 will be piloted on a small number of children recruited from the community prior to the enrollment of actual subjects to ensure that children understand task instructions and that the inclusion of these tasks will keep the total session time within our estimate.

Informed Consent

Consent to participate for children who are currently in the custody of Child Protection will be sought from the Director of Child Protection for each sector. Consent for children who are currently in foster care will be sought from the foster care parents. Consent for children who have been adopted will be sought from their adoptive parents. Consent for children who have been reunited with their biological families will be sought from their biological parents. For children in the community comparison (never-institutionalized) group, consent to participate will be sought from the child's biological parents.

A separate consent form will be created for each of the SIX sessions. With the exception of the consent form submitted with this protocol for the genetic component of the study (already approved at Tulane University and the University of Maryland), each consent form will indicate the purpose of the original study and the purpose of the follow-up study. Once approved by CCI, all consent forms will be translated in Romanian by our Romanian research staff.

Informed consent will be conducted in Romanian by a member of the BEIP Research Lab staff. Informed consent will be obtained prior to the start of each session. A detailed description of the procedure will be provided. Parents/caregivers will be given a copy of the consent form for their records. Parents/caregivers and children will be given the opportunity to ask any questions before, during or after the sessions. Parents/caregivers will sign a consent form in the presence of study personnel before any testing commences. In addition, families and children will be told that all information will be kept confidential and that they can stop the session at any time without penalty.

Due to the age of the children in this sample, and given how unfamiliar children are likely to be with child development research, we do not feel that it is feasible or culturally appropriate to obtain written assent from

our participants. However, the research staff will explain the details of each session to the child, taking into account the age and cognitive ability of the children. Research staff will always obtain their voluntary verbal agreement to proceed during the course of the procedures at each step of the way.

With the exception of the consent form submitted with this protocol for the genetic component of the study (already approved at Tulane University and the University of Maryland), each consent form will include a statement to be signed by the parent/caregiver indicating that the child was informed about the details of the session and gave their verbal assent to participate. No procedures will be administered to a child who is unwilling to participate or if any parent/caregiver feels their child is unable or unwilling to continue.

Session 1 – Genetic Sample Collection (Buccal Swabs)

A member of the BEIP Research Lab will contact the parent/caregiver of the child to schedule a visit to the home or placement center of the child in order to collect the DNA samples from the child. The session will be scheduled at a time that is most convenient for the child and parent/caregiver.

After informed consent is obtained from the parent/caregiver (and verbal assent is obtained from the child), one of our trained Romanian research assistants will place a cotton swab in the mouth of the child and will rub the swab against the child's cheek. The swab will be placed in a tube and labeled with the child's study number (Two buccal swabs will be obtained from each participant. All samples will be transported back to New Orleans, LA where the DNA will be extracted from the sample. All genotyping will be done blind to any clinical information. The DNA will solely be used for this study and remaining DNA will be destroyed at the completion of the study.

We will examine the effects of genotype on the development of psychiatric disorders, developmental outcomes (IQ, attention, executive functioning, and social cognition), and social relatedness (peer relations). We will also examine the effects of genotype on brain functioning (EEG, ERPs). To achieve these aims, we will genotype all participants in this study for four different genes: the serotonin transporter length polymorphism (5HTTLPR) alleles, the catechol o-methlytransferase (COMT) val 158 met allele, the brain-derived neurotrophic factor (BDNF) val 66 met allele, and the dopamine D4 receptor (DRD4) variable number tandem repeat (VNTR) allele. The genetic material will be obtained from swabs of the childrens' cheeks.

The investigators will not disclose genetic polymorphisms of clinical relevance to families. The polymorphisms we plan to study are risk and protective factors—not "disease" genes. Therefore the long term implications of any specific variant is uncertain. In addition, alleles believed to function as risk factors in one context may confer protection in another context (e.g., the short allele of the 5HTTLPR is protective with good maternal care from ETOH consumption in rhesus monkeys).

If there is a psychological disorder then that is what should be treated – not the individual's genotype. Genetic testing of children, even in cases of clearly defined outcomes associated with specific genes (e.g., Huntington's disease) is very limited and is usually only performed when the child is an adult and requests the testing. Because the relationship of the alleles in question and future psychiatric disorders is uncertain, it is arguably more harmful than not to inform the participants' families.

IMPORTANT NOTE: Dr. Charles Nelson has two co-PIs on this protocol, Dr. Nathan Fox at the University of Maryland and Dr. Charles Zeanah at Tulane University School of Medicine. Dr. Zeanah and one of his colleagues, Dr. Stacy Drury, also listed on this protocol, have received IRB approval from Tulane University School of Medicine to conduct the genetic aspect of this study. Dr. Drury has received funding from the American Academy of Child and Adolescent Psychiatry (AACAP) to conduct this portion of the research. We felt it necessary to include this aspect of the study in our protocol because the funding Dr. Nelson has received to conduct the study will pay the salary of the staff responsible for collecting the DNA samples. The funding Dr. Drury received from AACAP will pay for the supplies, transportation costs to homes/placement centers of the participants, and the genetic analysis. A consent form for this component of the study has already been approved by the IRBs at Tulane University and the University of Maryland (please see attached approval letters). We have included this consent form (Bucharest Early Intervention Project Protocol Addendum – Genetic Analysis) with this protocol and request that the CHB CCI approve this consent form so that we may expedite data collection for this component of the follow-up study.

Total Time to complete Session 1 will be 20 minutes or less.

Session 2 – Assessments of Social Behavior and Mental Health

A member of the BEIP Research Lab will contact the parent/caregiver of the child to schedule a visit to the home or placement center of the child. The session will be scheduled at a time that is most convenient for the child and parent/caregiver. During the scheduling phone call, the researcher will describe the procedures of the Stranger at the Door task detailed below and will obtain *verbal consent* from the parent/caregiver to conduct this task. Informed *written consent* will be obtained for session 2 after the debriefing of the Stranger at the Door task.

One of our trained Romanian research assistants will conduct all interviews with parents/caregivers of the participants**.** Scheduled breaks will be taken throughout the session and participants will be encouraged to request breaks whenever needed. With the exception of the PAPA, WISC-IV, and the Social Skills Rating System, copies of all questionnaires are included as appendices with this protocol.

Stranger at the Door (Zeanah et al., 2005)
In the literature on the effects of early institutionalization on child development, there is a long-standing and highly replicated finding that such children, *as late as adolescence*, show indiscriminately friendly behavior towards complete strangers. Such behavior represents a major risk factor, for obvious reasons (e.g., getting in the car of a stranger). For this reason we feel it is important to get some estimate of indiscriminate behavior in our sample of children, as we have done in our work to date. The *Stranger at the Door* task is an observational procedure designed to be an ecologically valid assessment of socially disinhibited or indiscriminate behavior in young children. This refers to a willingness of the child to approach, interact with, and even accompany unfamiliar adults. Clinically, this behavior has been linked to long-term social problems and is one of the most persistent findings in post-institutionalized children who are adopted. The procedure has been demonstrated to have both convergent and discriminant validity in the BEIP at participant age 54 months. Further, the children's behavior in this procedure clearly differentiated all three groups (institution, foster care, and community).

PARTICIPANTS: CHILD, PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE = LESS THAN 10 MINUTES

Procedure
A research assistant (RA) from the BEIP Research Lab will contact the parent/caregiver of the child to schedule the visit. The RA will describe the Stranger at the Door task to the parent/caregiver and will explain that we are including this task as an indicator of indiscriminate friendliness. The RA will ask the parent/caregiver to greet the RA at the door when the RA arrives for the session. When the door opens, the RA (unfamiliar to the child) will greet the parent/caregiver and the child. The RA will say to the child, "Come with me. I have something to show you." The RA will use a friendly tone of voice when speaking to the child and their parent/caregiver. The parent/caregiver will be instructed to look at the RA rather than the child during this exchange. If the child speaks to the parent/caregiver, the parent/caregiver may respond, but otherwise, the parent/caregiver simply looks in silence at the RA. If the child accompanies the RA, the RA will walk with the child a short distance out of the home/placement center/Leagan and will retrieve a stack of papers saying, "Look, here is something I need for the questions I am going to be asking your mom/caregiver [showing the child the stack of measures].

The RA will record the child's initial reaction to the stranger's invitation, also indicating whether the child hesitates (latency to respond), asks the parent's/caregiver's permission, looks to the parent/caregiver, or accompanies the stranger unhesitatingly.

Debriefing
The parents/caregivers will be informed ahead of time about this task and the RA will describe to the parent/caregiver that we are using this task as a measure of indiscriminate friendliness.

After completing the task, in the presence of both the parent/caregiver, the RA will debrief the child.

The RA will say, "My name is _____. When I came to the door a few minutes ago, I invited you to come with me. You didn't know who I was and I wanted to see if you would leave with me. We (looking toward parent/caregiver) think that it is a good idea if children make sure it is ok with their parents/caregivers before they go off with strangers. It's important that you tell your parents/caregivers that you're leaving the house/Center so they won't worry and it's important for you to be safe. It's not a good idea to go off with someone you don't know, no matter how nice they might be."

If the child refused to go with the stranger, or asked the parent's/caregiver's permission, the RA will say, "You were right not to go with me," or "It was good that you checked first."

If the child did leave with the stranger, the RA will say, "Next time it would be good if you checked with your parent/caregiver first."

At the conclusion of the debriefing, the RA will ask,"Do you have any questions for me?"

The Preschool Age Psychiatric Assessment (Egger & Angold, 2004; Egger, Ascher, & Angold, 1999)
The PAPA is an interviewer-based structured parental interview for the assessment of the full range of psychiatric symptoms and disorders in children ages 2 through 5 years old. The PAPA also assesses school/daycare functioning, family structure and functioning, parenting behaviors, and host of demographic variables including socioeconomic status. Although our participants will be beyond the age range included in this measure, we will use a modified version of this measure for several reasons. First, this measure was administered as part of earlier assessments in the BEIP and continued use of this tool will allow us to easily evaluate 'performance' over time. Second, the next version is designed to assess the full range of psychiatric symptoms in adults. There is no intermediary version of this assessment tool. Finally, this tool has already been translated into Romanian.

PARTICIPANT: PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE = 60 – 120 MINUTES

** The RA will ask the participant if they need to take a break at the mid-point of this interview. A scheduled break will be taken at the conclusion of the interview.*

MacArthur Health and Behavior Questionnaire - HBQ (Ablow et al., 1999; Essex, Boyce, Goldstein, Armstrong, Kraemer, & Kupfer, 2002; Luby et al., 2004)
The HBQ consists of 140 items regarding child functioning. This questionnaire is administered to the child's parent/caregiver and teacher. Items are scored on a three-point scale from 0 (not true) to 3 (very true). The questionnaire is scored on four domains: emotional and behavioral symptomatology, impairment, adaptive social functioning and physical health. *This questionnaire will be completed by the parent/caregiver and a teacher of the child. The consent form for this session will obtain consent from parent/caregiver to contact child's teacher.*

PARTICIPANT: PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE = 20 MINUTES

** A scheduled break will be taken at the conclusion of this interview.*

Disturbances of Attachment Interview – School Age (DAI-SA) (Smyke & Zeanah, 1999)
The DAI is a parent/caregiver interview designed to assess signs of attachment disorders and disturbances. It has been used in two different samples of institutionalized children, and has been sensitive to differences in caregiving. At 8 years, we will be most concerned with signs of indiscriminate/disinherited attachment.

The DAI-SA originally submitted with this application has been modified to include additional questions regarding risk-taking behaviors. Based on piloting on maltreated children in the US, we wanted to include more items examining self-endangering and impulsive behavior. Inclusion of these additional questions will allow us to assess social and interpersonal impulsivity in relation to various measures of cognitive impulsivity. The modified version has been included with the protocol amendment application submitted in June 2007.

PARTICIPANT: PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE = 10 MINUTES

The Bruininks-Oseretsky Test of Motor Proficiency, Second Edition, Short Form
This is an individually administered test that assesses the motor functioning of children from 4-1/2 to 14-1/2 years of age. The test provides a comprehensive index of motor proficiency as well as differentiated measures of gross and fine motor skills. The Complete Battery contains eight subtests comprised of 46 separate items. The Short Form consists of 14 items from the Complete Battery and provides a quick, brief survey. One score provides an index of general motor proficiency. Eight subtests assess these skills:

Fine Motor Precision (e.g., cutting out a circle, connecting dots)
Fine Motor Integration (e.g., copying a star, copying a square)
Manual Dexterity (e.g., sorting cards, stringing blocks)
Bilateral Coordination (e.g., tapping foot and finger, jumping jacks)
Balance (e.g., walking forward on a line, standing on one leg on a balance beam)
Running Speed and Agility (e.g., shuttle run, one-legged side hop)
Upper-Limb Coordination (e.g., throwing a ball at a target, catching a tossed ball)
Strength (e.g., standing long jump, sit-ups).

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 20 MINUTES

***IMPORTANT NOTE**:* The interviewers who will administer these questionnaires are all licensed psychologists and have received extensive training in research ethics and confidentiality. If our trained interviewers determine that there is immediate need for hospitalization, removal from the family or some other urgent measures to be applied, they will refer the family to a local mental health provider in Romania.

Total Time to complete Session 2 will be 3.5 hours or less.

Session 3 – Assessments of Social Interaction, IQ, Attention, and Executive Function
This session will be conducted at the BEIP Research Laboratory. Informed consent will be obtained prior to the start of the session. Scheduled breaks will be taken throughout the session and participants will be encouraged to request breaks whenever needed. The Peer Interaction Task will be audio-taped and videotaped for subsequent transcription, coding, and analysis. *The tasks described in this session will be piloted to ensure that children understand task instructions and that the inclusion of these tasks will keep the total session time under 3.5 hours.*

Peer Interaction Task (Fox et al., 2001)
One of the most prevalent findings in the research literature on post-institutionalized children is their inability to form competent social relationships, particularly with same age peers. We will assess their peer relationships by observing each child interaction in both unstructured and semi structured situations with an unfamiliar same sex peer. Each of our target children will be paired with a same age, same sex unfamiliar peer and will be introduced into a playroom in the laboratory. There will be a set of age-appropriate toys in the playroom. The first 10 minutes of the session will be unstructured free play. This will be followed by a clean up. Next, an experimenter will provide a structured game in which the children will have to interact together to plan a party. This interaction will include leading vs. following, dividing the work, negotiating, sharing of a rewarding toy and giving a gift.

Each participant will also be asked several questions about their best friend. For example, the RA will ask the participant if he/she spends a lot of time with his/her best friend, if the child and his/her best friend give one another advice and if the child and his/her best friend help each other when they can't figure something out. Inclusion of these questions will allow us to assess the quality of each participant's significant friendship.

The session will be audio-taped and videotaped for subsequent transcription and coding. Transcriptions of the dialog between the two children will be used to evaluate the vocabulary and language production of the target child. Videos will be coded for quality and maturity of play as well as verbal and non-verbal social interaction behaviors.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 40 MINUTES

** A short break will be taken at the end of this task.*

Weschler Intelligence Scale for Children-IV (WISC-IV)
The WISC-IV is a widely-used, individually administered, comprehensive test designed to measure intelligence of children from 6 to 16 years. It provides composite scores representing intellectual functioning in specified cognitive domains (verbal comprehension, perceptual reasoning, working memory, and processing speed). This will help us determine whether cognitive problems are generalized or in more specific areas (visual processing).

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 80 MINUTES

** A short break will be taken at the end of this task*

Social Skills Rating System (Gresham & Elliot, 2003)
The Social Skills Rating System allows one to obtain a more complete picture of social behaviors from teachers, parents, and even students themselves. It evaluates a broad range of socially validated behaviors-behaviors that affect teacher-student relationships, peer acceptance, and academic performance. It identifies children who have problems with behavior and interpersonal skills and detects the problems behind shyness, trouble initiating conversation, and difficulty making friends. Parents/Caregivers will be asked to complete this measure while their children are being administered the WISC.

PARTICIPANT: PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE = 10 MINUTES

Cambridge Neuropsychological Test Automated Battery (CANTAB)
CANTAB provides a unique test platform that utilizes touch-screen technology to assess a variety of cognitive functions and specific cognitive deficits associated with neuropsychological and psychiatric disorders. We will use a subset of the following CANTAB tests to evaluate cognitive domains including memory, attention, processing speed, visuospatial function and executive function. *A subset of tests to be included in the study will be determined by piloting.* The major advantage of the CANTAB is that it permits one to evaluate a variety of neuropsychological functions using an automated system that places minimum requirements on language; indeed, the PI on this protocol was one of the developers of the use of the CANTAB with children, and has reported that scores on the CANTAB among a sample of 5- and 6-year-old Hmong and Vietnamese children whose native language was not English was identical to that of a solidly middle class sample of Northern European stock.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE EACH TASK NOTED BELOW EACH TASK DESCRIPTION

1) MOTOR SCREENING (MS)
Motor screening is a screening task administered before other tests. It introduces the child to the touch-screen and acts as a training procedure to ensure that the child can touch the screen accurately. It simultaneously screens for visual and movement problems and ensures that the child can hear, understand and follow simple instructions. A series of crosses is shown in different locations on the screen. After a demonstration of the correct way to point using the forefinger of the dominant hand, the child must touch the crosses in turn.

ESTIMATED TIME TO COMPLETE = 5 MINUTES

2) DELAYED MATCHING TO SAMPLE (DMS)
This task presents the child with a complex visual pattern (the sample) and then, after a brief delay, four patterns between which she or he must choose. Each pattern is made up of four sub-elements, each of a different color. One of the choice patterns is identical to the sample, one is a novel distracter pattern, one has the shape of the sample and the colors of the distracter, and the fourth has the colors of the sample and

the shape of the distracter. To discourage strategies based on encoding single quadrants, all four choice patterns have a quadrant in common with the sample.

ESTIMATED TIME TO COMPLETE = 10 MINUTES

3) PAIRED ASSOCIATES LEARNING (PAL)
This task is a form of delayed response procedure, which tests two different aspects of the ability to form visuo-spatial associations. First, the number of patterns placed correctly on the first presentation of each trial gives an index of 'list memory.' Second, the number of repeat, reminder presentations needed for the child to learn all the associations provides a measure of 'list learning' (the task can also be conceived as a test of visuo-spatial conditional learning.

ESTIMATED TIME TO COMPLETE = 10 MINUTES

** A short break will be taken at the conclusion of this task.*

4) STOCKINGS OF CAMBRIDGE (SOC)
This is a spatial planning test based upon the 'Tower of London' test. The child is shown two displays containing three colored balls, presented so they can be perceived as stacks of colored balls in stockings. In each trial, the child must move the balls in the lower display to copy the pattern shown in the upper. A later motor control task, in which the child simply copies earlier moves, allows planning time (versus movement time) to be calculated and taken, relative to the number of moves required to complete each trial, as a measure of the child's planning ability.

ESTIMATED TIME TO COMPLETE = 10 MINUTES

5) SPATIAL WORKING MEMORY (SWM)
This is a test of spatial working memory and strategy performance. The aim of the test is that the child should find a blue 'token' in each of the boxes displayed and use them to fill up an empty column on the right hand side of the screen, whilst not returning to boxes where a blue token has previously been found. The color and position of the boxes used are changed from trial to trial to discourage the use of stereotyped search strategies.

ESTIMATED TIME TO COMPLETE = 10 MINUTES

** A short break will be taken at the conclusion of this task.*

Dot Probe Task (Mogg, Bradley, Miles, & Dixon, 2004)
The Dot Probe paradigm is a test of engagement and disengagement of attention. In this version of the computerized task (based on the work of Mogg & Bradley) participants are required to attend cue location while being primed with various pictures of emotional expressions. A trial begins with the presentation of two faces, one on the left side and the other on the right side of the screen. Each pair of faces consists of the same person, however, the pictures may differ in emotional expression. There are two possible expression combinations: angry-neutral, happy-neutral. Immediately following the presentation of faces the cue is presented on either the right or left side of the screen. The participant is asked to indicate, via button press, on which side of the screen the cue is located. The task consists of two blocks of 80 trials each for a total of 160 trials.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 15 MINUTES

Posner Cued-Attention Task
The Posner Task assesses orienting to sensory stimuli. Children are shown a fixation point appearing in the center of computer screen. Next they are presented with three boxes outlined in white arranged horizontally across the screen. Each trial begins with the presentation of a cue, which consists of one of the boxes turning from black to blue in color. Cues are distributed so that they appear in the central box in 20% of

trials, and equally in the right-most and left-most boxes for the remaining trials. The target, a small white box, then appears in either the left-most or right-most box (ISI = 200 ms). In a valid trial the cue and target appear in the same location. For invalid trials, the cue appears in the outer-most box opposite from where the target appears. Trials in which the cue appears in the center box served as controls. A total of 50 trials will be presented with a 20%, 40%, 40% distribution of control, valid, and invalid trials, respectively. Trial order will be randomized. Children are asked to indicate the location of the target by pressing a corresponding button as quickly as possible. Stimuli presentation (ITI = 4000 ms; time-out latency = 2000 ms) will be controlled by the STIM stimulus presentation system from the James Long Company (Caroga Lake, NY).

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 10 MINUTES

Total Time to complete Session 3 will be 3.5 hours or less.

Session 4 – Assessments of Physical Growth, Motor Functioning, Brain Functioning and Language

This session will be conducted at the BEIP Research Laboratory. Informed consent will be obtained prior to the start of the session. Scheduled breaks will be taken throughout the session and participants will be encouraged to request breaks whenever needed. In order to assess brain functioning in this sample, we will acquire EEGs and derive ERP measures to specific cognitive tasks. The tasks described in this session will be piloted to ensure that children understand task instructions and that the inclusion of these tasks will keep the total session time under 3.5 hours.

Height, Weight and Head Circumference
The participant's height, weight and head circumference will be measured by a member of the BEIP Research Lab.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 5 MINUTES

Behavioral Face Discrimination Task
Each participant will be asked to sort a series of cards that display faces with varying degrees of emotion (happy, sad, fearful/scared, and neutral). Previous research has shown that the ability to recognize facial expressions develops into adolescence (Kolb, Wilson, & Taylor, 1992). While children are quite good at recognizing intense expressions of emotion, they are less sensitive to more subtle portrayals of emotion and have particular difficulty recognizing negative emotions (Gao & Maurer, 2007). Research has also demonstrated that children who experience aberrant caregiving environments (e.g., abuse, neglect) early in life show abnormal processing of facial expressions of emotion (Pollak, Cicchetti, Hornung, & Reed, 2000). Based on this research, we expect that institutionalized children will show deficits on this task compared to never-institutionalized children.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 20 MINUTES

Electroencephalogram (EEG)
The EEG is a relatively inexpensive and non-invasive method of recording brain activity using individual electrodes distributed over the surface of the scalp. Several inherent properties of cortical circuits produce an ongoing rhythmicity in the EEG signal, which may be decomposed into oscillations occurring in different frequency bands with specific functional correlates and physiological origins (Niedermeyer & da Silva, 1993). For the quantification of electrical activity within each frequency band, the digitized EEG data are typically edited for motor and muscle artifact, and samples of artifact-free data are analyzed using a Fourier transform to quantify the spectral power in the EEG signal.

We will examine institutionalized, previously institutionalized (those now in foster care), and never-institutionalized children on measures of EEG absolute power and relative power in three frequency bands: 3-5 Hz (theta), 6-9 Hz (alpha), and 10-18 Hz (beta). Based on the literature relating specific patterns of EEG frequency distribution to cognitive deficits, behavioral problems, environmental risk factors, and

developmental delays, we predict that we will find a higher proportion of EEG power at lower frequencies and a corresponding reduction in EEG power at higher frequencies in children with a greater number of risk factors compared with the never-institutionalized group. We also plan to examine hemispheric asymmetries in the EEG signal, which have proved useful in the study of behavioral development in infancy and childhood (Segalowitz & Berge, 1995), particularly in the domains of individual differences in approach and withdrawal tendencies (e.g., Fox, Henderson, Rubin, Calkins, & Schmidt, 2001).

The EEG will be recorded from 12 scalp sites(F3, F4, Fz, C3, C4, P3, P4, Pz, O1, O2, T7 and T8) (this nomenclature simply represents the exact locations on the scalp over which we place electrodes) plus the left and right mastoids using a lycra Electro-Cap (Electro-Cap International, Eaton, OH) with sewn-in tin electrodes. An anterior midline site (AFz) will serve as the ground electrode, and the EEG will be collected referenced to the vertex.

After the cap has been correctly fitted, the scalp underlying each electrode site will be gently abraded before electrolytic conducting gel is inserted into the space between the scalp and the electrode. Impedances will be measured at each electrode site and will be considered acceptable if the are at or below 10 k ohms. All channels will be digitized at 512 Hz onto the hard drive of a PC using a 12-bit A/D converter ($\pm$ 2.5 V input range) and Snap-Master acquisition software (HEM Data Corporation, Southfiled, MI). One channel of vertical electrooculogram (EOG) will be recorded using tin electrodes placed above and below the left eye to record blinks and other eye movement. The EEG and EOG signals will be amplified by factors of 5000 and 2500, repectively, using custom bioelectric amplifiers from SA Instrumentation Company (San Diego, CA). Amplifier filter settings for all channels will be).1 Hz (high pass) and 100 Hz (low pass). Prior to the recording of EEG from each participant, a 50 uV 10-Hz signal will be input into each of the channels to and the amplified signal will be recorded for calibration purposes.

1) Alpha Baseline
The child will be asked to sit in a chair and a stretch lycra cap, with the electrodes sewn in, will be placed on the child's head in order to record EEG. Small amounts of gel will be inserted into each of the electrodes. Also, two small stickers will be placed near the child's eyes in order to record eye blinks and muscle movement. Baseline EEG will be recorded for six minutes, three one-minute periods of eyes open and three one-minute periods of eyes closed. The six one-minute segments will alternate between eyes open and eyes closed. The cap and electrodes will then be removed and the gels wiped from the child's head.

2) Theta Baseline
The Sternberg Paradigm (1966) assesses working memory. A modified version of this paradigm (Tesche & Karhu, 2000) will be used in which children are asked to remember a set of integers (i.e. 1,2, …., 9) which are presented sequentially on a computer monitor. Memory sets can vary in size from 1, 3, 5, or 7 items. Each integer within a memory set is presented individually at an interstimulus interval of 1-2 seconds. There is a 3 second delay after the presentation of the entire memory set and this delay is followed by the presentation of a probe digit. Children are instructed to raise their right index finger if the probe was in the memory set (in-set) or their left index finger is the probe was not part of the memory set (out-of-set). The integers and the sizes of the memory sets are randomized throughout the task.
The goal is to repeat our original observations by recording the EEG at rest, and examining different frequency across the scalp.

Please note that this task will not be administered.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE ALL EEG TASKS = 10 MINUTES

** A short break will be taken at the conclusion of this task.*

Event-Related Potential (ERP)
The event-related potential (ERP) represents the brain's response to the presentation of a discrete event. ERPs are simply a subset of the EEG, and have been extensively used in the study of a variety of perceptual and cognitive abilities in infants and children (see Nelson & Monk, 2001; DeBoer, Scott, & Nelson, 2004, for reviews). In the current case, we will examine both the peak amplitude and latency of various ERP

components whose functional significance has been established; moreover, we will also examine the topographic distribution of these components so as to develop hypotheses about underlying brain circuits (recognizing the limitations of doing so with relatively low-density arrays of electrodes). Further, the ERP has been used extensively to examine a variety of clinical disorders (for review, see Nelson & Luciana, 1998).

1) Facial Emotion Discrimination Task
We will record ERPs as children view static, color images of females posing the facial expressions of neutral, angry and fear. The images will be taken from the NimStim-MacBrain Face Stimulus Set (http://www.macbrain.org). These images were taken against a gray background while the women wore a gray scarf around their necks to conceal any clothing. As children view the images, they will be asked to press a button when they see a 'mad' face. The images will be presented such that neutral faces will appear on 50% of the trials, angry faces will appear on 25% of the trials and fearful faces will appear on 25 % of the trials. We anticipate that institutionalized children will show a larger amplitude N400 component of the ERP to the negative emotions than will never institutionalized children; children who have spent the most time in foster care (e.g., those placed under 18 months of age) will show a response virtually identical to never institutionalized children. In contrast, children who have spent the least time in foster care will show a response at the midpoint between the institutionalized group and never institutionalized group.

Each child will be tested individually while sitting in a chair facing a computer monitor approximately 60 cm away. The monitor will be surrounded by black panels that block the child's view of the room behind the screen and to his/her sides to limit distractions. A small hole in the screen is directly above the computer monitor, allowing the researcher to monitor the child's behavior. Parents/caregivers will be seated in the room with the child.

Trials will consist of a 100-ms baseline, followed by a 500 ms presentation of the visual stimulus, followed by 1,200 ms during which the screen will be a blue blank background. The intertrial interval will vary randomly between 500 and 1,000 ms, and during this time the screen will display the blue background.

The researcher behind the screen will watch the child through the hole and if the child looks away the observer will signal the computer via a button press to delete the trial; thus brain activity from the trials during which the child does not look will not be recorded or used in further analysis. The session will continue until the child observes the maximum number of trials (100) or until the child indicates that they no longer want to participate.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 20 MINUTES

2) The Go – No Go Paradigm (Inhibitory Control Task)
The Go-No Go tasks involve selective responding to target stimuli and response suppression to non-target stimuli. Children will be given a standard version of the task, in which they are instructed to respond via button press to any sequentially presented letter except for the letter X. There are two conditions, "Go" and "No go". The first condition—"Go"—is a control condition with trials consisting entirely of non-Xs. The second condition—"No go"—is a response inhibition condition with trials consisting of both go (70%) and no go stimuli (30%). For each condition, stimulus duration is 500 ms with an interstimulus interval of 1500 ms. Several dependent measures are collected online via computer software for later analysis including response accuracy (number of total correct responses), number of responses made to no go stimuli (false alarms), number of response omissions to go stimuli, and average reaction time in each condition.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 20 MINUTES

3) The Flanker Task (Error Monitoring Task)
The Flanker Task is a computer-based task that assesses an individual's ability to inhibit predominant response biases in the face of interfering stimuli. For the proposed study a modified flanker paradigm with four different stimulus arrays (each consisting of a five-arrow arrangement: >>>>>, <<<<<, >><>>, <<><<) will be used to assess participant's physiological and behavioral responses to the commission of errors. One

of the four arrays will appear on each trial and the participant's task will be to press a key corresponding to the central arrow in the array, either an < or an >. The paradigm consists of 480 trials presented in 3 blocks of 160 trials. Prior to the presentation of the actual test blocks subjects are given a short practice round so that they become accustomed to the task.

We will additionally record EEG and create ERP components based upon this task. In particular, one component, the Error Related Negativity (ERN), is obtained by examining specific neural activity patterns that correspond to the commission of an error. The ERN is defined as the negative most deflection in a 50 to 150 ms window of time after response execution (e.g. button press).

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 20 MINUTES

Language and Reading Tasks
To assess their language processing abilities, children will be administered two spoken language tasks, non-word repetition (NWR) and rapid automatic naming (RAN). The NWR task examines coding of phonological (speech sound) information in short term memory; the RAN task examines efficient retrieval of phonological information. These tasks de-emphasize experience, are used in the diagnosis of language delays, and are robust correlates of reading performance. Measures of reading decoding and comprehension will also be administered as complementary and sensitive measures of language change at this age. All four experimental tasks will be audio-recorded for subsequent coding and analysis. The tasks will be administered in Romanian by a member of the research staff.

1) Non-word Repetition Task (adapted for Romanian from Gathercole and Baddeley,1993)
Children will be asked to repeat 40 invented words of varying syllable lengths that follow the language principles of Romanian but are not real words (e.g., *co*, *lego*, *paluţă*, *guderoşa*). Words will be presented to children under headphones and their responses audio-taped. The main dependent variable is the percentage of consonant sounds produced correctly.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 10 MINUTES

2) Rapid Automatic Naming Task (adapted for Romanian from Wagner, Torgesen, & Rashotte, 1999)
Children will be asked to name series of 30 colors, digits, letters, and pictures of common objects as quickly as possible. Items will be presented in paper format. The main dependent variable is time in seconds taken to name the series of items. A stop-watch is used for timing.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 10 MINUTES

3) Word Identification Task (adapted for Romanian from Woodcock, 1987)
Children will be asked to read 50 single words of varying familiarity *(e.g., baine/bread, curăţătorie/laundry).* Words will be presented in paper format and responses scored for accuracy.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 10 MINUTES

4) Passage Comprehension (adapted for Romanian from Woodcock, 1987)
Children will be presented with short Romanian passages (3-4 sentences) describing an accompanying picture and asked to repeat the sentences. Passages will be presented in paper format. A comprehension question will be asked for each passage and responses scored for accuracy.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 10 MINUTES

Total time to complete Session 4 will be 3.5 hours or less

IMPORTANT NOTE: We have included the never-institutionalized group in this study because the measures we propose to use have not been normed on Romanian children. So far, never-institutionalized children have performed similarly to average American children (or perhaps a bit better) on measures used in previous assessments (e.g., PAPA). For practical purposes, using the never-institutionalized group as a reference group is the most reasonable approximation to detailed validity studies of every measure we are using. If our never-institutionalized group has "unusual" (by US standards) scores on the Woodcock or Weschler, then we will consider the implications of those responses for the validity of the measure.

We will work closely with our Romanian colleagues to review the proposed tasks for cultural appropriateness. The Social Skills Rating Scale has been used in the United States across multiple populations of students from varying ethnic and racial backgrounds and cultures, and the items have been found to be valid and non-biased (see references below).

Some of the measures we propose to use have been modified. For example, the frequency/familiarity of Rapid Automatic Naming task stimuli (high-frequency letters and objects, common color names, and familiar digits) was determined with reference to a corpus obtained from representative children's story books used in Romania. Similarly, the stimuli in the reading subtests adapted from the Woodcock Reading Mastery Tests are not a direct translation of English, but were adapted to include a continuum of high- and low-frequency Romanian words. This again was determined using text corpora and dictionary sources. The Non-Word Repetition task relies on nonsense words that follow the linguistic constraints of Romanian in that each stimulus item is a possible but non-occurring word in the language; these nonsense words also are relatively easy to pronounce in that they involve early developing Romanian speech sounds. To increase content validity and reduce item effects, each of the language tasks has been created so that basal and ceiling levels are not used. Raw rather than standard scores will be calculated, and no comparison to English test norms will be made, with the different linguistic structures of the two languages making this type of comparison uninformative. That is, the tasks have been adapted to be criterion-referenced rather than norm-referenced. Each task has been designed with the input of native speakers of Romanian to facilitate cultural and linguistic appropriateness as well as pilot tested with a small number of Romanian children living in the United States.

Feng, H., & Cartledge, G. (1996). Social skill assessment of inner city Asian, African, and European American students. *School Psychology Review*, *25*, 228-239

Powless, D. L., & Elliott, S. N. (1993). Assessment of social skills of Native American preschoolers: Teachers' and parents' ratings. *Journal of School Psychology*, *31*, 293-307.

Session 5 - Magnetic Resonance and Diffusion Tensor Imaging, Social Cognition Tasks and DNA

As valuable as our EEG measures are, they do not have the spatial resolution required to examine the anatomy of the brain. Given reports in the literature that early neglect or abuse can alter brain anatomy, it is imperative for us to acquire detailed MRI-based images from our sample of children. We will do whole-head scans and focus particular attention on structures that support memory, executive functions, face processing and emotion (specifically, the medial and inferior temporal lobe, prefrontal cortex and amygdala). We will also conduct DTI, a refinement of magnetic resonance imaging, that measures the flow of water and tracks the pathways of white matter in the brain. DTI is able to detect abnormalities in the brain that do not show up on standard MRI scans.

1) MRI and DTI scans: All MRI and DTI scans will take place at the Medical Center UNIREA, located in Bucharest, Romania. This facility is a private center that offers a complete set of clinical medical services. The MRI scanner to be used is a 1.5T system from Philips Intera Enterprise (please see attached MRI scanner parameters). All scans will be performed by Adina Chirita, MD, Ph.D., a neuroradiologist who will also provide a clinical read of all scans (please see attached CV). She will be assisted by a technician on staff at the Center and a research member from our Romanian laboratory who has worked with the children and families in the BEIP study since its inception. We feel that the presence of a familiar researcher will comfort the children during the session.
PARTICIPANT: CHILD

ESTIMATED TIME TO COMPLETE = 25 MINUTES

2) Social Group Preferences: A longstanding issue in the development of social group preferences concerns the role of familiarity vs. patterns of close social relationships in promoting in-group bias. For example, do children come to prefer members of their own racial group primarily because those individuals are most familiar to them (and familiarity breeds liking), or because those are the individuals with whom they have had rich and positive social relationships? This is a difficult question to investigate because the two factors are confounded over development for most children. Studying children raised in institutions – where social networks are impoverished and rich social relationships may be rare – may shed light on this question. If in-group preferences are primarily the result of familiarity, then children raised in institutions may show the same patterns of inter-group bias observed in home-reared children in the U.S. (e.g., Kinzler et al., under review; Shutts et al., under review; Shutts et al., in preparation-a) and elsewhere (e.g., Shutts et al., in preparation-b). If, however, social group preferences require rich social environments to develop, then children raised in institutions may fail to show preferences for members of their own group. Previous research suggests that by 8-9 years of age, children exhibit robust preferences for individuals of their own gender, race, and ethnicity (as conveyed by language, dialect, or physiognomy).

Method: Children will be presented with pairs of photographs of unfamiliar children (e.g., a boy and a girl) on a laptop screen, and will be asked to indicate (e.g., by pointing) whom they like more. Pairs of photos will be matched for attractiveness (as rated by a group of Romanian adults) and trials will be counterbalanced for both the positions of in-group vs. out-group faces and other relevant variables (race, gender, and ethnicity, as conveyed by language, dialect, or physiognomy). Gender trials will feature pairs consisting of one boy and one girl; some trials will show two Romanian children, some will show two Roma children, and some will show two other-race children (e.g., Black children from South Africa). Race trials will feature pairs consisting of one Romanian and one Black child or one Roma and one Black child; half the pairs will show two girls and half will show two boys. Ethnicity trials will feature pairs consisting of one Romanian and one Roma child; half the pairs will consist of two girls and half will consist of two boys (see description below for stimuli development).

Analyses: Data will be analyzed within each condition to determine if children show consistent social preferences by race, gender, and ethnicity. For gender analyses, we will test for effects of participant gender and ethnicity, as well as interactions with race and ethnicity of face pairs. For the race and ethnicity analyses, we will compare preferences of children of Roma vs. Romanian heritage. Finally, analyses will compare in-group preferences along each of these dimensions, both for the group as a whole and for children varying in peer relationships (as assessed by the peer interaction task).
PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 15 MINUTES

2a) **Affective Judgments**: Data from both behavioral and ERP studies of emotion perception suggest that children in the institutionalized group are relatively spared in their ability to perceive facial expressions of emotion. Is this sparing restricted to discrimination (e.g., this facial expression is different from that facial expression), or does it extend to finer judgments of affect, as well as to more complex emotional processes such as social preferences and as empathy?

Method: We will adapt the "who-do-you-like-more?" task described above to assess children's preferences based on affective cues in the face and on information about prior actions. In the *affective cue condition*, children will be presented with pairs of unfamiliar, computer-generated adult faces on a laptop screen. The pairs of faces will exhibit different emotions (e.g., happy vs. angry) and children will be asked to indicate whom they like more. Across the trials, face pairs will display graded emotions (e.g., a very angry face vs. a less angry face) so as to vary task difficulty. In the *action cue condition*, we will contrast positive and negative actions, as well as gradations of positive and negative actions. Children will see pairs of unfamiliar, computer-generated adult faces exhibiting a neutral expression and told a fact about each face. The facts will consist of happy/friendly actions or angry/threatening actions *committed* by each face (e.g., "this person helped someone today" vs. "this person pushed someone today"). Across the two faces and across trials, actions will vary in valence (good vs. bad) and intensity, parallel to the affective cue condition. On each trial, children will again choose the person they like more. Gender and ethnicity will be equated across each pair of faces and will vary orthogonally across trials. Comparisons of the visual and verbal conditions may allow

us to disentangle deficits in emotion understanding, as in Adolphs et al. (1998). Please see the description below for stimuli development.
PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 15 MINUTES

2b) **Empathy Judgments**
Method: We will again adapt the "who-do-you-like-more?" method to examine children's use of affective cues and actions to make empathic judgments about other individuals. In the ***affective cue condition***, children will be presented with pairs of unfamiliar, computer-generated adult faces exhibiting different emotions (e.g., happy vs. sad) on a laptop screen. Across the two faces and across trials, expressions will vary in valence (positive vs. negative) and intensity (e.g., very sad vs. moderately sad). Participants will be asked (1) which person feels sadder (judgment of another's affect); (2) which person they feel sadder for (empathic responding); and (3) which person they would like to give a sticker to (prosocial behavior). In the ***action cue condition,*** children will see pairs of computer-generated adult faces with neutral expressions, and told a fact about each face. The facts will consist of happy/friendly actions and angry/threatening actions ***received*** by each face (e.g., "this person's car was stolen."). Across the two faces and across trials, actions will vary in valence (positive vs. negative) and intensity, parallel to the affective cue condition. On each trial, children will again be asked (1) which person feels sadder; (2) which person they feel sadder for; and (3) which person they would like to give a sticker to (prosocial behavior). Please see the description below for stimuli development.
PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 15 MINUTES

3) **Trustworthiness/Approachability**: As Adolphs et al. (1998) and others (e.g., Engell et al., 2007) have reported, the ability to judge whether someone appears trustworthy depends critically on an intact amygdala. Patient S.M., for example, who has a greatly reduced amygdala due to a genetic syndrome, consistently rates faces others view as untrustworthy as trustworthy. Similarly, young adults with Williams Syndrome (WS) make comparable errors of judgment – i.e., they show an abnormal positive bias in their social judgments of unfamiliar people (Bellugi et al., 1999). Importantly, there are some autopsy data to suggest that some WS patients show a posterior curtailment of the amygdala (Galaburda et al., 1994) and perhaps a general reduction in amygdala volume (Galaburda et al., 1998).

Given our interest in indiscriminate behavior, and our hypothesis that the development of the amygdala is compromised among institutionalized children, it would seem critical to ascertain whether children with histories of institutionalization make errors in judging the trustworthiness of faces. Unfortunately, there is no developmental literature on making such social judgments, and thus, we have elected to modify a task used by Adolphs and colleagues with neurologically intact and impaired adults.

Method: The methods of Adophs et al al (1998) will be adapted to investigate children's ability to make trustworthiness and approachability judgments about other individuals. Children will be presented with 20 pairs of photographs of unfamiliar adults with a neutral expression on a laptop screen. Face pairs will be matched based on independent raters' judgments of trustworthiness/approachability (one face rated as trustworthy/approachable and one face rated as untrustworthy/unapproachable). All face pairs will be presented twice: once asking children to make trustworthiness judgments and once asking children to make approachability judgments. For trustworthiness trials, children will be asked, "Which of these people would you trust if you really needed help?" For approachability trials, children will be asked, "Which of these people would you rather talk to?" Trial order will be counterbalanced such that half the children will be asked first to make trustworthiness judgments and half the children will be asked first to make approachability judgments. Please see the description below for stimuli development.
PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE = 15 MINUTES

4) DNA Samples: Although we have obtained DNA from buccal swab samples for the participants during session 1, we would like to obtain a second DNA sample to extend the genetic analysis of those children enrolled in the Bucharest Early Intervention Project (BEIP). These samples will be collected during session 5 and will take less than 5 minutes to collect. The genetics section of the protocol has been updated in Part B on pages 27-28.

Over and above the effects of early psychosocial deprivation on brain development and function, early negative life events likely also affect physical health via the genes involved in the generation of free radicals and antioxidant defense. The balance between these two biological processes is called oxidative stress and has been implicated in cancer development, the aging process, neurodegenerative disorders, and most recently in morbidity and mortality associated with mood and anxiety disorders (Harris et al., 2007; Lung et al., 2007; Simon et al.., 2006).

We would like to extend the genetic analysis of those children enrolled in the Bucharest Early Intervention Project (BEIP) to include additional mechanisms through which genetic variation may influence recovery from early social deprivation.

While the BEIP sample characteristics make it difficult to explore directly in vivo measures of oxidative stress we will explore INDIRECTLY the cellular impact of oxidative stress IN TWO METHODS: 1) WE WILL EXAMINE the impact of early social deprivation on telomere length as an indicator of the biological impact of chronic stress in these children (Epel et al., 2004) and 2) WE WILL EXAMINE POLYMORPHIC VARIATIONS IN GENES INVOLVED IN THE OXIDATIVE STRESS PATHWAY.

While the sequence of DNA is static over time telomere length is not and additional time points at which DNA is collected would permit the evaluation of changes in telomere length over time and provide further insight into the continuing or time limited influence of early social deprivation on telomere length. WE WILL COLLECT A SECOND BUCCAL SWAB SAMPLE AT SESSION 5 TO EXAMINE CHANGES IN TELOMERE LENGTH OVER TIME IN THESE CHILDREN. An accurate estimate of telomere length using real time PCR, has been developed and validated (Cawthon, 2002) AND THUS, TELOMERE LENGTH CAN BE DETERMINED FROM BUCCAL SWAB SAMPLES. THE SALIVA SAMPLE PROPOSED IN THE PREVIOUS AMENDMENT WILL NOT BE COLLECTED. This assay uses telomere- to single-copy gene ratio (T/S) which is proportional to the average telmore length in a cell.

Assays will be performed in duplicate from each participant and a standard dilution will be incorporated into each reaction. In addition results will be compared with control samples where Southern blot analysis and (T/S) ratio can be obtained to confirm standardization. Appropriate data reduction strategies will be employed for all dependent variables. Analysis will be performed to determine the effects of chronic stress on telomere length. We will compare the change in telomere length as a function of time in children in institutional care and foster care as compared to non-institutionalized children.

References:

Harris, S., Fox, H., Wright, A., Hayward, C., Starr, J., Whalley, L., & Deary, I. (2007). A genetic association analysis of cognitive ability and cognitive ageing using 325 markers for 109 genes associated with oxidative stress or cognition. BMC Genetics, 8, 1-18.

Lung, F., Chen, N., & Shu, B. (2007). Genetic pathway of major depressive disorder in shortening telomeric length. Psychiatric Genetics, 17, 195-199.

Simon, N., Smoller, J., McNamara, K., Maser, R., Zalta, A., Pollack, M., Nierenberg, A., Fava, M., & Wong, K. (2006). Telomere shortening and mood disorders: Prelminary support for chronic stress model of accelerated aging. Biological Psychiatry, 60, 432-435.

Michelhaugh, S.K., Fiskerstrand, C., Lovejoy, E., Bannon, M.J., & Quinn, J.P. (2001). The dopamine transporter gene (SLC6A3) variable number of tandem repeats domain enhances transcription in dopamine neurons. Journal of Neurochemistry, 79(5), 1033-1038.

Hirvonen, M., Laasko, A., Nagren, K., Rinne, J.O., Pohjalainen, T., & Hietala, J. (2004). C957T polymorphism of the dopamine receptor (DRD2) gene affects striatal DRD2 availability in vivo. Molecular Psychiatry, 9, 1060-1061.

All telomere analyses will be conducted by Immaculata De Vivo, Ph.D. and Jason Wong (both affiliated with Harvard Medical School, Brigham and Women's Hospital) in consultation with Dr. Stacy Drury at Tulane University.

All MRI scanning will be scheduled at times convenient for the families. Because we do not have access to a mock scanner, we will reserve a 2 hour time slot on the scanner, and the first 30-60 minutes (or however long it takes) will be devoted to acclimating the child to the scanner. We anticipate the actual scan time will take approximately 15 minutes. Any child who becomes uncomfortable in the scanner will lead us to stop the scan; similarly, if the parents/caregivers are uncomfortable with the scan, we will stop the scan. As stated above, all scans will be read by Dr. Chirita, a trained neuroradiologist If anything concerning appears in the scan, the neuroradiologist will refer the child to the appropriate consultant for follow-up assessment. Dr. Nelson has extensive experience (dating back nearly 10 years) conducting MRI studies in children 6 and older and Dr. Fox has been conducting such work for the past 5 years. Drs. Nelson and Fox do not foresee children of this age having difficulty lying still in the scanner and thus, all scans will be performed without sedation.

Exclusionary Criteria
We anticipate that not all children will be eligible to be scanned; for example, those with physical handicaps will be excluded. Moreover, as is done here at CHB, any child who is contraindicated for MRI scanning (e.g., metal in the body, etc) will be excluded from this portion of the study. Any child meeting any of these exclusionary criteria will not eligible to participate in this session.

Total session time to complete Session 5 will be 2.0 hours or less.

SESSION 6 – PEDIATRIC AND NEUROLOGICAL EXAMINATIONS, FREE PLAY EPISODE AND ASSESSMENT OF SOCIAL COMMUNICATION SKILLS

This session will consist of well-child pediatric and neurological evaluations of study participants, including evaluation of the presence of autism symptoms as defined by DSM and a free play episode with the physician or research assistant.

The pediatric examination will include evaluation of the following: heart rate, respiratory rate, basic skin exam, height, weight, head circumference, blood pressure, reflexes in elbows, knees and feet, abdomen, back/spine, ears, eyes, nose, mouth and heart.

The neurological examination will include evaluation of the following: mental status, cranial nerves, motor system, tendon reflexes, cerebellar function, involuntary movements, gait, and sensory exam.

The mental status portion of this exam will include observation of the child's ability to interact with adults, language skills, and basic skills such as counting. Cranial nerve testing will include testing of smell, visual fields and visual acuity, extraocular movements, facial sensation, symmetry of facial movements, auditory acuity, ability to turn head and open jaw against the resistance of the examiner's hand, protrusion of the tongue, midline uvula and palate and intact voice. For motor function, we will observe the child walking, looking specifically for abnormalities such as asymmetries or unsteadiness. We will assess strength in all 4 extremities, proximally and distally, and will also assess muscle tone. We will evaluate the symmetry and magnitude of tendon reflexes for the following: biceps, triceps, brachioradialis, knee, ankle and feet. To evaluate cerebellar function, we will observe the child's ability to sit, balance, and walk, as well as maneuvers such as finger-to-nose, finger-to-finger, heel-shin, and rapid alternating movements of the hands. Throughout the exam, we will observe for any involuntary movements such as tremor, myoclonus, tics, or dystonia. The sensory exam will include sensation of touch and pain with a fingertip or prick, as well as vibration sense.

All pediatric and neurological examinations will take place in a private room in the BEIP Laboratory in Bucharest. The examinations will be conducted by Karen Bos and Dr. April Levin. Ms. Bos has completed 3 years of medical school and her MPH and Dr. Levin is a second-year resident at CHB in Pediatrics. Dr. Levin speaks Romanian and WILL SUPERVISE Ms. Bos. They will be assisted by one of our Romanian research assistants AS NEEDED for translation.

PARTICIPANT: CHILD
ESTIMATED TIME TO COMPLETE: 35 minutes

Free Play Episode: Autistic symptoms will be evaluated in the examination according to the current DSM criteria and by evaluation of a 10-minute, free play activity with Dr. Levin, Ms. Bos or a Romanian RA. Each free play episode will be videotaped for subsequent coding of behaviors including non-verbal communication, joint attention behaviors, requesting, sense of imagination, and repetitive and sensory oriented behaviors. A diagnosis of autism will not be given, rather Dr. Levin and Ms. Bos will note "clinical concern for autism" or "no clinical concern for autism." If clinical concern for autism is noted for any child, Dr. Levin or Ms. Bos will refer the participant to one of the pediatricians named below for follow-up evaluation.
PARTICIPANT: CHILD
ESIMATED TIME TO COMPLETE: 10 minutes

Health Questions and Social Communication Questionnaire: Parents/caregivers will be asked questions regarding the overall health of their child and will also be administered the SCQ. The SCQ is a 40-item questionnaire that evaluates a child's communication and social functioning.
PARTICIPANT: PARENT/CAREGIVER
ESTIMATED TIME TO COMPLETE: 25 minutes

Total time to complete Session 6 will be 60 minutes or less.

If medical problems or clinical concern for autism are noted, Dr. Levin and Ms. Bos will DISCUSS THEIR CONCERNS WITH PARENTS/CAREGIVERS AND WILL refer participants to one of the following Romanian physicians for follow-up evaluation and care:

1) Mihai Iordachescu, M.D.
2) Alin Stanescu, M.D.
3) Andrei Zamfirescu, M.D.
4) Carmen Burloiu, M.D.
5) Sanda Magureanu, M.D.

PHYSICIANS WILL RECEIVE A COPY OF THE FINDINGS FROM THIS SESSION IS FOLLOW-UP EVALUATION IS REQUIRED.

Subjects will not be compensated for their participation in this component of the study. We will provide transportation to/from the laboratory for all participants that agree to take part in this research session. UNLESS ADDITIONAL FUNDING IS SECURED, THIS SESSION WILL BE LIMITED TO CHILDREN IN THE INSTITUTIONALIZED AND FOSTER CARE GROUPS.

Stimuli Development
Photographs of 100 adults (all Romanian, 50 M and 50 F) and 48 children (24 Romanian, 12 M and 12 F and 24 Roma, 12 M and 12 F) living in the Bucharest community will be taken to develop stimuli for the tasks described above. Adults will be aged between 28 and 60 years; children will be aged between 7 and 10 years. Adult and child participants will be recruited from local elementary schools with which our research laboratory has already established relationships. Please see the attached recruitment letter. All participants will be asked to sign a photo release (attached), allowing researchers use of their pictures as research stimuli and in scientific meetings and presentations.

All adults and children will be photographed against a white background wearing a gray t-shirt provided by the research laboratory. Adult men with beards or mustaches will be excluded and individuals with earrings or eyeglasses will be asked to remove these items prior to having their photograph taken. Adults and children will be asked to depict a number of facial expressions including happy, neutral, disgust, angry, fear and sad. To help children elicit these expressions, the researcher will show the child a picture of another child (or adult) making the expression they want the child to express. The child will be asked to label the expression and then asked to copy the expression in the picture while the researcher takes their photograph.

This stimuli set will be used for the Social Preferences and Trustworthiness/Approachability tasks described above.

In addition to the set of photographs, a second set of stimuli will be developed using FaceGen, a parametric face modeling software that allows users to manipulate an individual face up to 150 ways. Faces generated with this software will be manipulated to vary the intensity of the target emotions (i.e., very happy to slightly happy) used in the Empathy and Affective Judgments task detailed above. Expressions included in this stimuli set will include varying intensities of happy, angry, sad, fear, disgust and neutral.

Stimuli Ratings
All stimuli will be rated by an independent group of 30 adults (15 M and 15 F) living in Romania. This group of adults will be recruited via an email circulated to faculty and students in the Psychology Department at Bucharest University. All participants will be asked to provide consent prior to completing the stimuli ratings. Copies of the recruitment materials and consent form are attached.

Using a 7-point scale, participants will be asked to rate each face for perceived age, perceived gender, perceived nationality/ethnicity/race, and attractiveness. Adults will also be asked to rate each adult photograph with respect to approachability and trustworthiness. For approachability, subjects will be asked to imagine meeting the person on the street, and to indicate how much they would want to walk up to that person and strike up a conversation. For trustworthiness, subjects imagined trusting that person with all their money, or with their life (as in Adophs et al., 1998).

g. Study Timeline (as applicable)

We expect that data collection for the entire sample will take 36 months. Piloting of the tasks described in sessions 2 – 5 will be conducted within the first two months of protocol approval. Session 1 will also be conducted in this timeframe, as age of participants is irrelevant for this session. The remaining 5 sessions will occur within $\pm$ 8 months of the participant's 8th birthday.

The oldest participants in the BEIP turned 8 years in July of 2006, and the youngest will turn 8 years in June of 2009.

h. Adverse Event Criteria and Reporting Procedures

The PI and his co-PIs communicate frequently with each other and with the staff at the BEIP Research Laboratory in Bucharest, Romania. Elizabeth Furtado communicates daily with the PI and the director of the BEIP Research Laboratory. Any adverse event will be reported immediately by the Romanian research staff to the PI and Elizabeth Furtado. The PI will notify his co-PIs of the adverse event and all investigators will report the event to their respective Institutional Review Board. The PI will work closely with the director of the BEIP Research Laboratory to ensure that all proper authorities in Romania are notified of any adverse event.

5. Data Management and Statistical Analysis

a. Data Management Methods

All data will be collected by trained Romanian research assistants and psychologists affiliated with the BEIP Research Lab. Files, audio-recordings, videotapes, and all other data will be kept in locked cabinets in the Bucharest Study lab and carried by hand, as needed, back to the United States, for coding. Some data (e.g., raw EEG data files) will be transmitted via FTP as a password-protected ZIP file for analysis in the United States. Children will be identified only by their subject identification numbers and not by their given names. Identifying information will not be used in publications or presentations. Subjects will be informed in the consent form at the beginning of the process that any newly discovered abuse of children must be reported by the investigator to the appropriate direction of child protection. All data will be overseen by a data manager. Data will be double-entered, read into SAS data files and stored on a secure server. Access will be provided only to staff directly involved in this project and the data manager will provide routine updates as to when new data is available for analysis.

b. Quality Control Method

All research staff responsible for collecting the data are extensively trained in the use of electrophysiological recording techniques (ERP, EEG) with human participants in this age range. Likewise, all assessments involving the use of standardized, clinical measures will be administered by a trained Romanian psychologist on our staff. The Romanian research staff will be responsible for sending monthly updates on recruitment, response rates and number of children scheduled for experimental sessions to help the US staff monitor progress of the study. This information will be incorporated into the progress and annual reports required by our funding agency and CCI.

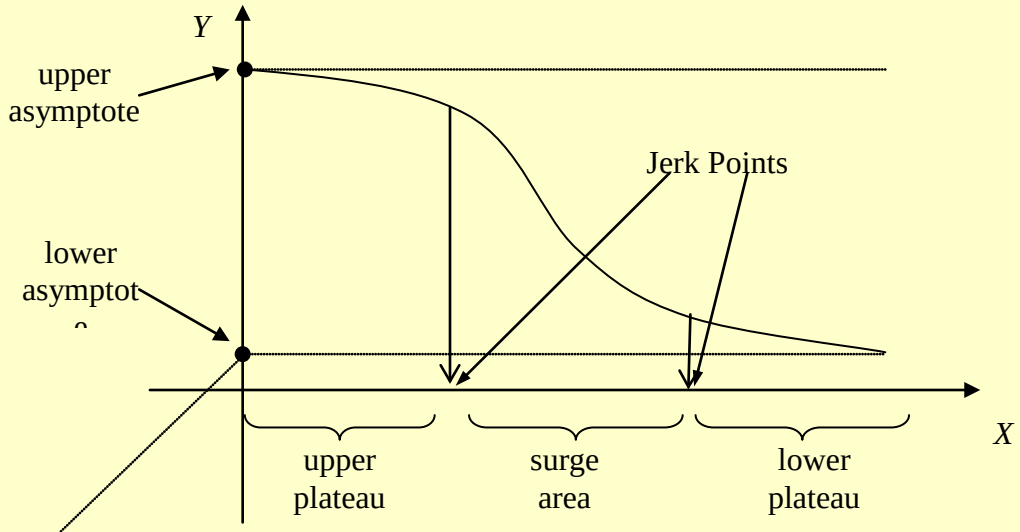
c. Data Analysis Plan

As data are collected, scored, and checked, they will be entered into a data base to be matched with the data previously obtained on this sample in the original BEIP project. Management, integrity, and security of new data from index sample, and of prior data from both index and community comparison samples, will be overseen by our current data manager.

Prior to addressing the specific aims of this investigation, all data will be examined for univariate and multivariate outliers, skewness, and other conditions that may impinge upon the effectiveness of the statistical methods described below. Some data may also require distributional transformations, the need for which cannot be predicted until data are available. Further, certain measures (e.g., EEGs) are multidimensional in nature and will require initial reduction to facilitate analysis. In the BEIP study, the application of principal component analysis generally yielded recognizable and interpretable factors; hence, similar preliminary procedures are planned here as well.

Finally, we should note special issues regarding the longitudinal nature of these data. One may characterize our overarching conceptual model as one of "course corrections." Specifically, for a wide variety of domains, children in institutional settings proceed along particular developmental trajectories believed to lead to generally less positive outcomes later. Placement in a foster care environment potentially alters these developmental trajectories, yielding, we predict, more positive subsequent outcomes. Meanwhile, variables such as the timing and quality of placement in families may have a bearing on the degree to which the course correction is ultimately successful at enhancing developmental functions into a more typical developmental range. Logically, earlier placements would be expected to provide great course corrections, while later placements may be less corrective; a shorter placement, however, could attenuate the magnitude of the placement's corrective benefit. These phenomena, further, will likely differ across different developmental domains.
That said, the purpose of the proposed investigation is <u>not</u> about capturing the corrective mechanisms as they occur throughout the longitudinal period studied. Rather, of key interest here is the long-term impact of children's residential experiences on specific developmental sequelae. Methodologically speaking, that is to say that although interesting latent growth curve models integrating the time dependent covariates associated with caregiving can be analyzed (and likely will be conducted separately), these models would not address the current proposal's objectives focusing on outcomes at age 8. In fact, the specific aims here are much more direct both in their focus as well as their implications for caregiving, although indeed not without their own potential for analytical complexity. Below, for each of the previously presented specific aims the proposed analyses will be described.

A major question we hope to address in detail concerns the presence or absence of sensitive periods. We are specifically interested in the extent to which specific domains of functioning are particularly sensitive to changes in variables associated with timing of institutionalization. Each 8-year outcome variable (here, *Y*) falls into one of five domains of functioning: IQ, attention and inhibitory control, social behavior, psychiatric disorders, and brain activity. Using each *Y* as dependent variable (following any necessary transformations), a nonlinear sigmoidal (S-shape) function will be fit using a given *X* variable (e.g., age at first placement in foster care). As seen in the illustrative figure below, such functions are characterized first by slow initial change in *Y* as a function of *X* (the *upper plateau*), which in the current example could reflect minimal differential long-term impact of immediate placement versus very early placement. Following an early slow decline, a functional *jerk point* is reached after which more rapid acceleration occurs on into a *surge area*. In the current example, this would reflect entrance into a developmentally sensitive area for which changes in *X* have greater impact on *Y*. After the surge area, another functional jerk point is reached and a *lower plateau* is entered. This would reflect another period in which changes in *X* yield relatively little change in *Y*, in this case because, for example, placement into foster care no longer has appreciable benefits in terms of the outcome *Y* being modeled.



So, as an example, let the *Y* axis represent age-8 IQ and the *X* axis represent age at first intervention. As illustrated in the figure above, one might theoretically expect that children securing placement closer to birth will reach typical or near typical developmental IQ levels by age 8. If so, their data should be scattered around the left side of the sigmoidal function shown. As children's age of placement becomes later, one would expect ultimate IQ levels to decrease; if, in fact, a critical developmental threshold is crossed, this decrease might become quite abrupt as a sensitive period is entered (at the function's upper jerk point). Here we would expect to observe children's data scattered around the function in the middle portion of the figure above. Finally, although the negative effect on IQ of later placement likely continue, it could level off as the end of a sensitivity zone is reached (at the function's lower jerk point). In the current context, it would constitute a point of no return, of sorts, beyond which changes in time of placement have little remaining differential impact upon IQ at age 8. Here the data points would be expected to scatter around the sigmoidal function on the right side of the figure. Thus, such an analysis could yield information about the parameters of a sensitive period where later IQ is most susceptible to changes in age of placement, before which children are relatively robust to expediency of placement and after which the majority of the adverse developmental impact has already been incurred.

Practically speaking, then, for each outcome the goal then is to estimate the upper and lower jerk points in terms of *X*, with a confidence interval for each. This will help to identify demarcation characteristics of a sensitivity area for each outcome as a function of each relevant *X* variable. It should be noted that one might expect the S-shape to be inverted in some cases, depending on the nature of the *X* variable. Also, and more importantly, jerk points may, in fact, occur outside the range of *X* values, yielding either nonsensical values (e.g., negative time) or values not examined (e.g., age 10). This latter result could occur when, in the *X* span available, the *X*-*Y* relation is nearly linear, or nonexistent. Similarly, if data have considerable variability around the sigmoidal function, yielding a relatively low model R^2, then confidence intervals for the jerk points, and hence for a potential sensitive area reflected in the function's surge area, may become too wide to be practically useful. Such results would, in and of themselves, be telling in terms of the given *X*-*Y* relation. Specifically, we would learn if the outcome variable remains sensitive to changes in variables related to caregiving, or if such sensitivity no longer exists.

The above initial analyses are univariate in nature, potentially defining for each developmental outcome variable in each domain the sensitive area associated with each individual *X* variable. One might expect very different periods for each *X* variable, depending upon the outcome variable's domain. For example, children might be more robust to later foster placement in one domain compared to another. Or, in some domains, children might have a much narrower sensitive area in which the outcome decreases (increases) more precipitously as compared to other domains where the decline (incline) is relatively more gradual.

What the current analyses do not address thus far is the potentially cumulative, and perhaps interactive relations of the multiple independent variables of interest toward understanding the domain-specific

outcomes. To deal with this multivariate question, extensions of the sigmoidal model will be examined (1) with multiple first-order *X* predictors in a pseudo-additive model (pseudo in the sense that the overall model is already nonlinear), as well as (2) with first-order variables and second-order interaction (product) terms in a model designed to assess both additive and multiplicative contributions. For additive models, the joint multivariate *X* boundaries may be used to define a multivariate surge area, yielding ranges of *X* variables that jointly define a sensitive zone (with associated joint confidence intervals). For models requiring multiplicative terms such zones are not as easily determined analytically; in this case a nonparametric bootstrapping approach will be employed.

Another critical question we hope to address concerns the impact of current and prior caregiving adversity on a variety of outcome variables across domains. For each outcome variable separately, the predictive contribution of current caregiving environment will be assessed using linear and nonlinear (potentially including sigmoidal) regression models. One cannot determine *a priori* which type of model will be optimum as the relations remain to be determined. Following the selection of the appropriate model for current caregiving environment, the marginal contribution of length of institutionalization, number of disruptions, and quality of early caregiving environment will be assessed. The models will examine both linear and nonlinear relations of these variables, as well as interactive terms of each with current caregiving adversity. Thus, using linear and nonlinear regression models, we will attempt to learn the impact of early conditions, as well as their potential moderating effect on the impact of current conditions.

An additional aim of our project is to contrast index children (with a history of institutional care) with community peers matched on relevant demographic characteristics. For each of the outcomes measures of interest, standard statistical methods such as t-tests (or their nonparametric analogs) and χ^2-tests will be used with the addition of demographic covariates as needed. A second, and more descriptive statistical approach will involve using the community data as a yardstick against which to gauge the index children. We will, after adjustment for covariates such as gender and ethnicity (if necessary), assign each BEIP child with a z-score representing where that child would fall among the community sample for each specific outcome variable. Naturally, we expect the preponderance of these z-scores to be negative, that is, to show impaired development.

Limitations: 1) Children entered the BEIP study anywhere from 6-30 months of age, so for some children, details about the quality of the caregiving environment before 30 months will be lacking. Variability in experiences prior to entry in the study may well relate in important ways to outcomes assessed. *On the other hand, this is a large sample with direct observational measures of caregiving—something never before included in studies of this kind.* 2) Ideally, a control group for this investigation would comprise children who share all other risk factors with the index group except for the experience of being institutionalized. Unfortunately, there are several reasons why this is impossible. Perhaps most important, it is unlikely that such a population actually exists, since families with many risk factors who abandon their children are, by definition, different in some psychologically important way from families who have many risk factors and do not abandon their children. Further, identifying such a population now, without having longitudinal data on their early experiences, would be extraordinarily difficult. *In any case, in this proposal, using comparison children is not to control for institutional experience, per se, but rather to calibrate the developmental outcomes in children who had extreme early experiences.* 3) We would like to be able to assess and evaluate the social behavior of the children in this study more extensively. Unfortunately, this would add so substantially to the costs of this project that we have limited our measures of social development to those described earlier. *Further, we believe that by emphasizing social cognition, we may accelerate efforts to understand brain functioning in children who have experienced severe deprivation.*

d. Statistical Power and Sample Considerations

Sample size and statistical power: We are fortunate to be able to collect, analyze and report on approximately 110 children who were assessed comprehensively and repeatedly during the BEIP. We feel that it is reasonable to recruit and collect data on an equal number of community children. This number will enable us to attain a good degree of matching of ages, genders and ethnicities. Regarding power analysis specifically, this is always a challenging endeavor, resting tenuously on the shoulders of considerable speculation. It is difficult to present precise power estimates given that we will have to wait until we have our data to see which statistical models can be used to interpret them, but as a guideline we would comment that a sample of 110 provides power of .80 to detect a simple correlation of roughly .25.

Similarly, for simple comparisons between the two groups, there would be power of .80 to detect a standardized effect size of about .38 standard deviations (between *small* and *medium* by common social science standards). These estimates are, of course, purely speculative and do not include consideration of possible covariates. They do, however, support our opinion that the sample of 110 index children is sufficient to provide good statistical insight into possible deviations in development attributable to early deprivation. Further, and critically important, is that outcome variables that are themselves well-established scales, or are derived herein as a result of data reduction (e.g., principal component analysis), will tend to have less error variance (i.e., higher reliability). This translates into stronger (more detectable) variable relations and larger (more detectable) effect sizes as the disattenuating nature of error is minimized through prior or current scale optimization.

IMPORTANT NOTE: As noted by one of the scientific reviewers, the planned analyses, as described above, do not state the necessity for statistical corrections or adjustments when multiple comparisons between groups are made. Our analyses are focused on specific hypotheses, and there is a primary outcome (dependent) variable identified for each hypothesis. As such, the testing of each hypothesis constitutes a separate analysis, and we feel that correction for multiple tests in not necessary. We are, however, quite aware that inconsistent findings among the outcome variables will have to be rationalized. Our approach would be to examine and report any inconsistencies carefully. Global correction for multiple comparisons, e.g. by using Bonferroni methods, would impose a drastic penalty on the statistical power of our study, and on the power to test each of the specific hypotheses.

The reviewer also asked how we will handle missing data in the event that some subjects may complete only 1 or 2 sessions. Our longitudinal analyses will be based on random effects hierarchical models. Under certain restrictive assumptions, that data are missing at random, these likelihood based models are valid when subjects drop out or are otherwise unavailable; if subjects drop out for reasons unrelated to our intervention this seems like a valid assumption. We expect that most of our dropouts will fall into this category. It will, however, be necessary to examine each lost case individually and to decide whether the case should be included. Last-observation-carried-forward (LOCF) methods are sometimes used to compensate for dropping out, but given that our subjects are in a developmental period, these seem inappropriate in our context. We see no alternative to dropping the few non-random dropouts from our sample and treating them anecdotally.
This reviewer also noted that there is no power calculation to address specifically the potential for Type II error regarding the genetic hypotheses given the underlying prevalence of such polymorphisms for DRD4, BDNF, 5HTT, and COMT in the Romanian population. Psychiatric genetic studies with these polymorphisms have similar or smaller sample sizes (e.g., Kaufman et al., 2004 the N=109, Gervai et al., 2005 N=95, Baker et al., 2005 N=50, Miller et al., 2004 N=32, and Romberg et al., 2005 N=16).

It is possible to do a power analysis for each allele with the outcome being prevalence of psychological disorders, but those outcomes will be guesses (as power analyses usually are) due to the lack of precise allele frequencies in the Romanian population for each of these alleles and the unknown outcomes regarding disorders and symptomatology. However, as this is a relatively homogenous genetic population we do not expect wide variations in allele frequencies. While we accept the possibility of type II error there are multiple statistical modeling approaches that can be used to minimize this and these alternatives will be explored as we analyze data. Additionally, should we find that our power is limited we can use temporal data to increase our observations and this will increase our power (i.e., look at the 54 month assessment and the 8 years assessment results). An additional approach to limiting the type II error would be to use ancestral proportion scores which can be generated from using unlinked additional genetic markers and Bayesian cluster analysis with the STRUCTURE software that can recognize cryptic population genetic patterns without prior information on population of origin.

e. Study Organization

The study organization is as follows: The Principal Investigator, Charles A. Nelson, Ph.D., and his co-PIs, Charles Zeanah, M.D. and Nathan A. Fox, Ph.D., will be responsible for overseeing data collection, processing, and analysis. Elizabeth Furtado (US) and Calin Gligorea (Romania) will be responsible for all administrative oversight of this research. Calin Gligorea will assist in testing of subjects on an as-needed basis. Members of the BEIP Research Laboratory are the primary study staff and will be responsible for recruitment, scheduling, data collection, transcription, and coding.

f. Data and Safety Monitoring Plan

6. Risks and Discomforts

None of the procedures described in this protocol are invasive or designed to be markedly distressing.

One of the potential risks is fatigue from the assessments. It is important to note that the proposed assessments have been conducted with a similar age group in United States research labs and both Drs. Nelson and Fox have had participants successfully complete sessions of this length. Regular breaks (and snacks) will be scheduled during each session and participants will be told that they may request additional breaks whenever needed.

Another potential risk is invasion of privacy and probing of personal or sensitive information. The interviews and questionnaires used in this study include questions that may be considered sensitive or personal in nature. All of the personnel who will conduct these interviews are trained psychologists or social workers and have completed the Course in the Protection of Human Research Subjects. They will ensure participants that any information they share will remain confidential and will be identified only by subject numbers.
Preparation for EEG/ERP data collection involves minimal electrode preparation and has been used successfully in many studies by the investigators, including the BEIP study. However, some children may become distressed by the physical sensation of wearing the EEG cap during the EEG or ERP procedures, although this is less likely with this age group than with younger children. Sufficient time will be included to orient the child to both procedures. If a child exhibits distress during EEG/ERP data collection, the assessments will be paused or stopped depending on the degree of distress which a child demonstrates.

There are also some risks associated with MRI. These potential risks very rarely cause harm when MRI is performed within established guidelines by people who are trained. MRI uses a powerful magnet to make images. Therefore, persons with metal implants, such as certain types of surgical clips or pacemakers should not have an MRI. Parents/caregivers and children will be advised to remove other metal objects such as keys, pocketknives, or some types of jewelry from their person prior to entrance to the magnet room. These objects can be pulled towards the magnet at very high speeds and can cause serious injury. In addition to a large magnet, the MRI scanner also uses radio frequency waves that can, on rare occasions, cause a mild warming sensation similar to what one might feel on a warm day at the beach. The MRI scanner makes loud banging noises during the scanning session. During the MRI study the children will be provided with earplugs to reduce the noise heard from the scanner. It is also possible that the magnetic fields in the scanner can cause mild nerve and muscle twitching in the arms and legs. Such effects are extremely rare but, however possible. Some people simply find it uncomfortable and/or claustrophobic to lie in the closely space of the MRI scanner. If during the MRI, the child gets nervous or upset, the procedure will be stopped.

7. Potential Benefits

Given the nature of this research and the vulnerability of our sample, the PI and his colleagues have worked mindfully and arduously to develop a research plan that combines scientific aims with substantial concern for the continued health of the children in the study.

From the scientific perspective, this will be the largest study ever conducted following children from early rearing in institutions through age 8 years. The only study that approaches it in scope is the study conducted by Barbara Tizard and her colleagues of children raised in residential settings in London more than 30 years ago. There are two major reasons why there have been few studies in the interim following children longitudinally from institution to long-term placement like the Tizard study. First, in the U.S. and the U.K, institutions are very rarely used to care for abandoned children anymore, as we rely almost exclusively on foster care. Second, in countries that use institutional care, the kinds of logistical, administrative, cultural, and ethical challenges to such a study are formidable. To have those barriers already successfully negotiated, as with this sample, makes this study rare indeed.

This study will make significant contributions beyond the Tizard study for a number of reasons: (1) we will include almost twice as many children (110 in this vs. 65 in Tizard with institutional rearing); (2) we have detailed observations of the caregiving environment in the institutions (the Tizard measures were descriptive); (3) we will use state of the art outcome measures that were not available 30 years ago; and (4) we will include

measures of brain functioning. This study also has distinct advantages over the follow-up studies of children adopted out of institutions (e.g., O'Connor & Rutter and Ames & Chisholm) in that: (1) we have detailed observations of the caregiving environment in the institutions (adoption studies have no such measures); (2) we chose our sample to be more representative of the population of institutionalized children so that we do not have the problem of selection bias inherent in adoption studies; (3) we have Romanian comparison children, thus eliminating the confound of ethnic differences in comparisons of post-institutionalized children; and (4) we will include measures of brain functioning, which have been lacking in all of these previous longitudinal studies.

For these reasons, results of this study should be able to contribute substantially to our knowledge of the effects of early experiences of deprivation. These results will be important from the standpoint of illuminating the impact of social and material neglect on young children's development, and therefore contribute to and raise questions to pursue about the more traditional types of neglect that we have in this country. In addition, these results will be important from the perspective of the thousands of families in the United States who have adopted children internationally who were raised in their early years in institutions.
Our most compelling humanitarian cause in conducting research of this nature was to ensure that children placed in foster care as a result of our 'intervention' would not be returned to an institutionalized setting at the conclusion of our original study. As such, we negotiated with local Romanian government authorities that no child would have to return to institutionalization. This effort was successful with all but one of the Leagans/sectors in Bucharest and we have agreed with our administrative partner, Solidarite Enfants Roumaines Abandonees (SERA) Romania, to assist them in supporting foster care for these children after the conclusion of the study. We have also agreed not to interfere with placement of *any* child in a family setting, if such a setting becomes available during the course of the study. These decisions will be decided by the various Commissions on Child Protection in Bucharest. Therefore, children in either the institutionalized or the foster care groups can return to their families or be adopted if the commission so directs.

Our team of collaborators in the United States has established and maintained partnerships with several organizations in Romania, all of which are making significant contributions to the national effort to deinstitutionalize children. One of these organizations, SERA Romania, works throughout the country to restructure residential facilities, develop community based child welfare services, strengthen local governments to protect children's rights, and to build an infrastructure of trained personnel to assist children in need. It is through this entity that we were able to employ the research assistants and foster families required for the project. SERA Romania also employs the team of social workers who developed and now maintain the 56 foster homes in Bucharest that this project supports. This team of social workers provides continuous support to the children and their foster families by maintaining regular contact with the foster parents.

We also partnered with the Institute of Maternal and Child Health (IOMC), an entity affiliated with the Ministry of Health. Through this scientific collaboration, we developed recruitment methods that were culturally appropriate and constructed a team of pediatricians who performed thorough medical examinations on all children to determine eligibility for participation in the original study. These physicians were qualified to identify any medical difficulties in these children that might otherwise have gone undetected, particularly those in institutionalized care. All children were given a hearing test, something that is not established as a national health standard in Romania.

Additionally, our subsequent, comprehensive assessment of these children has allowed us to track the developmental progress of each child. The opportunity to see these children repeatedly over the course of several years led to the diagnosis of cerebral palsy in one of our participants, a medical condition that is not (often) detectable during infancy. Although the child is no longer eligible to participate in the study, we appropriately referred this child to a qualified physician/clinician for specialized care. Although none of the BEIP staff are trained clinicians and our electrophysiological measures are not used as diagnostic tools, we suspected signs of seizure activity in the EEG of another participant. In this instance, we referred the child to the appropriate physician/clinician for follow-up. Indeed, approximately 50 children in the BEIP sample have been referred to date. These children have received intervention from the IDC social work staff or were referred to state run clinics because of our concerns, parent/caregiver concerns, or both. As for process, if children were highly symptomatic, or significantly impaired, our staff discussed referral as an option for parents/caregivers. If parents/caregivers were concerned -- even in the absence of the child being highly symptomatic or significantly impaired -- our staff discussed referral options with parents/caregivers.

8. Privacy Provisions

Consent and testing will occur in the participant's home, private rooms within the placement centers, and at the BEIP Research Laboratory.

9. Confidentiality Provisions

Signed consent forms will be kept in locked cabinets in the BEIP Research Laboratory. Files, audio-recordings, videotapes, and all other data will be kept in locked cabinets in the BEIP Research Laboratory and carried by hand, as needed, back to the United States, for coding. Some data (e.g., raw EEG data files) will be transmitted via FTP as a password-protected ZIP file for analysis in the United States. Children will be identified only by their subject identification numbers and not by their given names. Identifying information will not be used in publications or presentations. Any data shared among the investigators listed on this protocol will be identified only by subject number to ensure privacy and confidentiality of the participants.

10. References

Ablow, J. C., Measelle, J. R., Kraemer, H. C., Harrington, R., Luby, J., Smider, N., et al. (1999). The MacArthur three-city outcome study: Evaluating multi-informant measures of young children's symptomatology. *Journal of the American Academy of Child & Adolescent Psychiatry, 38,* 1580-1590.

Adolphs, R., Tranel, D., & Damasio, A.R. (1998). The human amygdala in social judgment. *Nature*, *393*, 470-474.

Albers, L. H., Johnson, D. E., Hostetter, M. K., Iverson, S., & Miller, L. C. (1997). Health of children adopted from the former Soviet Union and Eastern Europe: Comparison with pre-adoptive medical records. *Journal of the American Medical Association, 278,* 922 – 924.

Angold, A., Pendergrast, M., Cox, A., Harrington, R., Simonoff, E., & Rutter, M. (1995). The Child & Adolescent Psychiatric Assessment (CAPA). *Psychological Medicine, 25,* 739 – 753.

ANPCA (National Authority for Child Protection and Adoption), Government of Romania (2004). Protectia Copilului: Intre Rezultate Obtinute si Priritati pentru Viitor, page 43.

Ames, E. W. (1997). *The development of Romanian orphanage children adopted into Canada. Final report to human resources development, Canada.* Burnaby, Canada: Simon Fraser University.

Auerbach, J., Geller, V., Lezer, S., Shinwell, E., Belmaker, R. H., Levine, J., & Ebstein, R. P. (1999). Dopamine D4 receptor (DRD4) and serotonin transporter promoter (5-HTTLPR) polymorphisms in the determination of temperament in 2-month-old infants. *Molecular Psychiatry, 4*, 369-373.

Auerbach, J., & Faroy, M. (2001). The association of the dopamine D4 receptor gene (DRD4) and the serotonin transporter promoter gene (5-HTTLPR) with temperament in 12-month-old infants. *Journal of Child Psychology and Psychiatry, 42*, 777-783.

Bellugi, U., Adolphs, R., Cassady, C., & Chiles, M. Towards the neural basis for hypersociability in a genetic syndrome. *Neuroreport: For Rapid Communication of Neuroscience Research, Vol 10(8)*, 1653-1657 (1999).

Benoit, T. C., Jocelyn, L. J., Moddemann, D. M., & Embree, J. E. (1996). Romanian adoption: The Manitoba experience. *Archives of Pediatric & Adolescent Medicine, 150,* 1278 – 1282.

Caspi, A., Sugden, K., Moffitt, T. E., Taylor, A., Craig,I. W., Harrington, H., McClay, J., Mill,J., Martin, J., Braithwaite, A., & Poulton, R. (2003). Influence of life stress on depression: moderation by a polymorphism in the 5-HTT gene. *Science, 301*, 386-389.

Cawthon, R. (2002). Telomere measurement by quantitative PCR. *Nucleic Acids Research, 30,* e47- 53.

Cermak, S. A., & Daunhauer, L. A. (1997). Sensory processing in the postinstitutionalized child. *American Journal of Occupational Therapy, 51,* 500 – 507.

Chisholm, K. (1998). A three year follow-up of attachment and indiscriminate friendliness in children adopted from Romanian orphanages. *Child Development, 69,* 1092 – 1106.

Chisholm, K., Carter, M. C., Ames, E. W., & Morison, S. J. (1995). Attachment security and indiscriminately friendly behavior in children adopted from Romanian orphanages. *Development and Psychopathology, 7,* 283 – 294.

Chisholm, K., & Savoie, L. (1992, June). Behavior and attachment problems of Romanian orphanage children adopted to Canada. In E. W. Ames (Chair), Development of Romanian orphanage children adopted to Canada. Symposium conducted at the meeting of the Canadian Psychological Association, Quebec City, Canada.

DeBoer, T., Scott, L., & Nelson, C. A. (2004). Event-related potentials in developmental populations. In T. Handy (Ed.), *Event-Related Potentials: A Methods Handbook* (pp. 263 – 297). Cambridge, MA: MIT Press.

De Luca, A., Rizzardi, M., Torrente, I., Allasndroni, R., Salvioli, G., Filograsso, N., Dallapiccola, B., & Novelli, G. (2001). Dopamine D4 (DRD4) polymorphism and adaptability trait during infancy: a longitudinal study in 1- to 5- month old neonates. *Neurogenetics, 3*, 79-82.

Diamond, A., Briand, L. Fossella, J., & Gehlbach, L, (2004). Genetic and neurochemical modulation of prefrontal cognitive functions in children. *American Journal of Psychiatry, 161*, 125-132.

Ding, Y. C., Chi, H. C., Grady, D. L., Morishima, A., Kidd, J. R., Kidd, K. K., Floodman, P., Spence, M. A., Schuck, S., Swanson, J. M., Zhang, Y. P., Moyzis, R. K. (2002). Evidence of positive selection acting at the human dopamine receptor D4 gene locus. *Proceedings of the National Academy of Sciences of the United States of America, 99*, 309-314.

Ebstein, R. P., Levine, J., Geller, V., Auerbach, J., Gritsenko, I., Belmaker, R. H. (1998). Dopamine D4 receptor and serotonin transporter promoter in the determination of neonatal temperamnet. Molecular Psychiatry, 3, 238-246.

Egan, M. F., Kojima, M., Callicott, J. H., Goldberg, T. E., Kolachana, B. S., Bertolino, A., Zaitsev, E., Gold, B., Goldman, D., Dean, M., Lu, B., & Weinberger, D. R. (2003). The BDNF val66met polymorphisms affects activity-dependent secretion of BDNF and human memory and hippocampal function. *Cell, 112*, 257-269.

Egger, H. L., & Angold, A. (2004). The Preschool Age Psychiatric Assessment (PAPA): A structured parent interview for diagnosing psychiatric disorders in preschool children. In R. DelCarmen-Wiggins & A. Carter (Eds.), *Handbook of infant, toddler, and preschool mental assessment* (pp. 223–243). New York: Oxford University Press.

Egger, H. L., Ascher, B. H., & Angold, A. (1999). *The Preschool Age Psychiatric Assessment*: *Version 1.1.* Unpublished interview schedule, Center for Developmental Epidemiology, Department of Psychiatry and Behavioral Sciences, Duke University Medical Center, Durham, NC.

Engell, A.D., Haxby, J.V., & Todorov, A. Implicit trustworthiness decisions: Automatic coding of face properties in the human amygdala, *Journal of Cognitive Neuroscience*, *19*, 1508-1519 (2007).
Epel, E., Blackburn, E., Lin, J., Dhabhar, F., Adler, N., Morrow, J., & Cawthon, R. (2004). Accelerated telomere shortening in response to life stress. *Proceedings of the National Academy of Science, 101,* 17312-17315.

Eriksen, B.A., & Eriksen, C.W. (1974). Effects of noise letters upon the identification of the target letter in a nonsearch task. *Perception & Psychophysics, 16*, 143-149.

Falzone, T. L., Gelman, D. M., Young, J. I., Grandy, D. K., Low, M. J., & Rubinstein, M. (2002). Absence of dopamine D4 receptors results in enhanced reactivity to unconditioned, but not conditioned, fear. *European Journal of Neuroscience*, *15*, 158-164.

Federici, R.S. (1999). Help for the hopeless child: A guide for families. Alexandria, VA.

Fisher, L., Ames, E.W., Chisholm, K., & Savoie, L. (1997). Problems reported by parents of Romanian orphans adopted to British Columbia. *International Journal of Behavioral Development, 20,* 67 – 82.

Fox, N. A., Henderson, H. A., Rubin, K. H., Calkins, S. D., & Schmidt, L. A. (2001). Continuity and discontinuity of behavioral inhibition and exuberance: Psychophysiological and behavioral influences across the first four years of life. *Child Development, 72,* 1 – 21.

Frodl, T., Meisenzahl, E. M.,, Zill, P., Baghai, T.,, Rujescu, D., Leinsinger, G., Bottlender, R., Schule, C., Zwanzger, P., Engel, R. R., Rupprecht, R., Bondy, B., Reiser, M., & Moller, H. J. (2004). Reduced hippocampal volumes associated with the long variant of the serotonin transporter polymorphism in major depression. *Archives of General Psychiatry, 61*, 177-83.

Galaburda, A.M., Wang, P.P., Bellugi, U., & Rosen, M. Cytoarchitectonic anomalies in a genetically based disorder: Williams syndrome. *Neuroreport: An International Journal for the Rapid Communication of Research in Neuroscience, 5(7),* 753-757 (1994).

Gao, X., & Maurer, D. (2007, March). The influence of intensity on children's perception of facial expressions. Poster presented at the Biennial Meeting of the Society for Research in Child Development.

Gathercole, S., & Baddeley, A. (1993). *Working memory and language processing*. Hove, UK: Lawrence Erlbaum.

Groze, V., & Ileana, D. (1996). A follow-up study of adopted children from Romania. *Child & Adolescent Social Work Journal, 13,* 541 – 565.

Hacshi, T.A. (1997). *Second home: Orphan asylums and poor families in America.* Cambridge: Harvard University Press.

Hariri, A. R., Mattay, V. S., Tesssitore, A., Kolchana, B. S., Fera, F., Goldman, D., Egan, M. F., & Weinberger, D. R. (2002). Serotonin transporter genetic variation and the response of the human amygdala.*Science, 297*, 400.

Harris, S., Fox, H., Wright, A., Hayward, C., Starr, J., Whalley, L. & Deary, I. (2007). A genetic association analysis of cognitive ability and cognitive ageing using 325 markers for 109 genes associated with oxidative stress or cognition. *BMC Genetics, 8*, 1-18.

Heils, A., Teufel, A., Petri, S., Strober, G., Riederer, P., Bengel, D., & Lesch, K. P. (1996). Allelic variation of human serotonin transporter gene expression. *Journal of Neurochemistry, 66*, 2621-2624.

Iorga, N. (1970). *A history of Roumania: Land, people, civilization* (J. McCabe, Trans.). New York: Ams Press. (Original work published 1925).

Johnson, D. E. (2000). Medical and developmental sequelae of early childhood institutionalization in international adoptees from Romania and the Russian Federation. In C. Nelson (Ed.), *The Effects of Early Adversity on Neurobehavioral Development*. Mahwah, NJ: Lawrence Erlbaum Associates, Inc.

Johnson, D. E. (1997). Medical issues in international adoption: Factors that affect your child's pre-adoption health. *Adoptive Families, 30,* 18 – 20.

Johnson, D. E., Miller, L. C., Iverson, S., Thomas, W., Franchino, B., & Dole, K. (1992). The health of children adopted from Romania. *Journal of the American Medical Association, 268,* 2446 – 3451.

Kaufman, J., Yang, B. Z., Douglas-Palumberi, H., Houshyar, S., Lipschitz, D., Krystal, J. H., & Gelernter, J. (2004). Social supports and serotonin transporter gene moderate depression in maltreated children. *Proceedings of the National Academy of Sciences of the United States of America. 101(49*), 17316-17321.

Kolb, B., Wilson, B., & Taylor, L. (1992). Developmental changes in the recognition and comprehension of facial expression: Implications for frontal lobe function. Brain and Cognition, 20, 74-84.

Kreppner, J. M., O'Connor, T. G., & Rutter, M. (2001). English and Romanian Adoptees Study Team. Can inattention/overactivity be an institutional deprivation syndrome? *Journal of Abnormal Child Psychology. 29(6)*, 513-528.

Lesch, K., & Mossner, R. (1998). Genetically driven variation in serotonin uptake: is there a link to affective spectrum, neurodevelopmental, and neurodegenerative disoders? *Biological Psychiatry, 44*, 179-192.

Liu, D., Diorio, J., Day, J., Francis, D., & Meany, M. (2000). Maternal care, hippocampal synaptogenesis and cognitive development in rats. *Nature Neuroscience, 3*, 799-806.

Lotta, T., Vidgren, J., Tilgmann, C., Ulmanen, I., Melen, K., Julkunen, I., & Taskinen, J. (1995). Kinetics of himan soluble and membrane-bound catechol O-methyltransferase: a revised mechanism and description of the thermolabile variant of the enzyme. Biochemistry, 34, 4202-4210.

Luby, J. L., Heffelfinger, A., Koenig-McNaught, A. L., Brown, K., Spitznagel, E. (2004). The PreSchool Feelings Checklist: a brief and sensitive measure for depression in young children. Journal of American Academy of Child and Adolescent Psychiatry, 43(6), 708-717.

Lung, F., Chen, N., & Shu, B. (2007). Genetic pathway of major depressive disorder in shortening telomeric length. *Psychiatric Genetics, 17,* 195-199.

Marshall, P. J., Fox, N. A., & the Bucharest Early Intervention Project Core Group. (2004). A comparison of the electroencephalogram between institutionalized and community children in Romania. *Journal of Cognitive Neuroscience, 16,* 1327 – 1338.

McGrath, S. D., Kawachi, I., Ascherio, A., Colditz, G. A., Hunter, D. J., & DeVivo, I. (2004). Association between catechol-o-methyltransferase and phobic anxiety. *The American Journal of Psychiatry*, *161*, 1703-1705

Mogg, K., Bradley, B.P., Miles, F., & Dixon, R. (2004). Time course of attentional bias for threat scenes: Testing the vigilance-avoidance hypothesis. *Cognition and Emotion, 18*, 689-700.

Morison, S. J., Ames, E. W., & Chisholm, K. (1995). The development of children adopted from Romanian orphanages. *Merrill-Palmer Quarterly, 41,* 411 – 430.

Moskoff, W. (1980). Pronatalist policies in Romania. *Economic Development and Cultural Change, 28,* 597-614.

Mossner, R., Daniel, S., Albert, D., Heils, A., Okladnova, O., Schmitt, ,A., & Lesch, K.P. (2000). Serotonin transporter function is modulated by brain-derived neurotrophic factor (BDNF) but not nerve growth factor (NGF). *Neurochemistry International, 36*, 197-202.

Muhamedrahimov, R. (2000). New attitudes: Infant care facilities in Saint Petersburg, Russia. In J. Osofsky & H. Fitzgerald (Eds.), WAIMH Handbook of infant mental health. New York: Wiley.

Naatanen, R. & Alho, K. (1995). Generators of electrical and magnetic mismatch responses in humans. *Brain Topography, 7*, 315-320.

National Institute of Statistics, Romania. Census of Population and Residences 2002. http://www.insse.ro/rpl2002rezgen/rg2002.htm

Nelson, C. A., & Luciana, M. (1998). Electrophysiological studies II: Evoked potentials and event-related potentials. In C. E. Coffey & R. A. Brumback (Eds.), *Textbook of pediatric neuropsychiatry* (pp. 331 – 356). Washington, Dc: American Psychiatric Press.

Nelson, C. A., & Monk, C. S. (2001). The use of event-related potentials in the study of cognitive development. In C. A. Nelson & M. Luciana (Eds.), Handbook of developmental cognitive neuroscience (pp. 125-136). Cambridge, MA: MIT Press.

Niedermeyer, E., & da Silva, F. (1993). *Electroencephalography: Basic principles, clinical applications, and related fields*. Baltimore, MD: Williams and Wilkins.

Nolan, K. A., Bilder, R. M., Lachman, H. M., Volavka, J. (2004). Catechol O-methyltransferase Val158Met polymorphism in schizophrenia: differential effects of Val and Met alleles on cognitive stability and flexibility. *American Journal of Psychiatry, 161(2)*, 359-61

O'Connor, T. G., Brendenkamp, D., & Rutter, M. (1999). Attachment disturbances and disorders in children exposed to early severe deprivation. *Infant Mental Health Journal, 20,* 10 – 29.

Pollak, S. D., Cicchetti, D., Hornung, K., & Reed, A. (2000). Recognizing emotion in faces: Developmental effects of child abuse and neglect. Developmental Psychology, 36, 679-688.

Posner, M. I. & Cohen, Y. (1984). Components of performance. In H. Bouma & D. Bowhuis (Eds.), *Attention and Performance X*. Hillsdale, NJ: Erlbaum.

Ribases, M., Gratacos,M., Armengol, L., de-Cid, R., Badia, A., Jimenez, L., Solano, R., Vallejo, J., Fernandez, F., & Estivill, X. (2003). Met66 in the brain-derived neurotrophic factor (BDNF) precursor is associated with anorexia nervosa restrictive type. *Molecular Psychiatry, 8*, 745-751.

Rosapepe, J. C. (2001). *Half way home: Romania's abandoned children ten years after the revolution.* Report to Americans from the U.S. Embassy, Bucharest, Romania.

Rotondo, A., Mazzanti, C., Dell'Osso, L., Rucci, P., Sullivan, P., Bouanani, S., Gonnelli, C., Goldman, D., Cassano, G. B. (2002). Catechol o-methyltransferase, serotonin transporter, and tryptophan hydroxylase gene polymorphisms in bipolar disorder patients with and without comorbid panic disorder. *American Journal of Psychiatry, 159*, 23-29

Rutter, M. (1999). Psychosocial adversity and child psychopathology. *British Journal of Psychiatry, 174,* 480 – 493.

Rutter, M., Anderson-Wood, L., Beckett, C., Brendenkamp, D., Castle, J., & The English and Romanian Adoptees Study Team. (1999). Quasi-autistic patterns following severe and early global privation. *Journal of Child Psychology and Psychiatry, 40,* 537 – 549.

Rutter, M., & The English and Romanian Adoptees Study Team. (1998). Developmental catch-up, and deficit, following adoption after severe global early privation. *Journal of Child Psychology and Psychiatry, 39,* 465 – 476.

Segalowitz, S. J., & Berge, B. E. (1995). Functional asymmetries in infancy and early childhood: A review of electrophsiologic studies and their implications. In R. J. Davidson & K. Hugdahl (Eds.), Brain asymmetry (pp. 579 – 615). Cambridge: MIT Press.

Sesack, S. R., Hawrylak, V. A., Melchitzky, D. S., & Lewis, D. A. (1998). Dopamine innervation of a subclass of local circuit neurons in monkey prefrontal cortex: ultrastructural analysis of tyrosine hydroxylase and parvalbumin immunoreactive structures. *Cerebral Cortex, 8(7)*, 614-22.

Siuciak, J. A., Clark, M. S., Rind, H. B., Whittemore, S. R., & Russo, A. F. (1998). BDNF induction of tryptophan hydroxylase mRNA levels in the rat brain. *Journal of Neuroscience Research*, *52(2)*, 149-158.

Simon, N., Smoller, J., McNamara, K., Maser, R., Zalta, A., Pollack, M., Nierenberg, A., Fava, M., & Wong, K. (2006). Telomere shortening and mood disorders: Prelminary support for chronic stress model of accelerated aging. *Biological Psychiatry, 60,* 432-435.

Smyke, A. T., & Zeanah, C. H. (1999). *Disturbances of Attachment Interview.* Unpublished manuscript, Tulane University School of Medicine.

Sternberg, S. (1966). High-speed scanning in human memory. *Science, 153*, 652-654.

Tesche, C. D., & Karhu, J. (2000). Theta oscillations index human hippocampal activation during a working memory task. *Proceedings of the National Academy of Sciences, 97*, 919-924.

Thoenen H. (1995) Neurotrophins and neuronal plasticity. Science 270:593-8

Tucker, P., Ruwe, W., Masters, B., Parker, D. E., Hossain, A., Trautman, R. P., & Wyatt, D. B. (2004). Neuroimmune and cortisol changes in SSRI and placebo treatment of chronic PTSD. *Biological Psychiatry, 56,* 121-128.

Wagner, R. K., Torgesen, J, K., & Rashotte, C. A. (1999). *Comprehensive Test of Phonological Processing*. Austin, TX: Pro-Ed.

Wand, G. S., McCaul, M., Yang, X., Reynolds, J., Gotjen, D., Lee, S., & Ali, A. (2002). The Mu-Opiod receptor gene polymorphism (A188G) alters HPA axis activation induced by opiod receptor blockade. *Neuropsychcopharmacology, 26(1),* 106-114.

Wei, J., Ramchand, C., & Hemmings, G. (1997). Possible control of dopamine b-hydroxlase via a codominant mechanism associated with the polymorphic (GT)n repeat at its gene locus in healthy individuals. *Human Genetics, 99*, 52-55.

Woodcock, R. (1987). *Woodcock Reading Mastery Tests-Revised*. Circle Pines, MN: American Guidance Service.

Zeanah, C. H., Koga, S. F., Simion, B., Stanescu, A., Tabacaru, C. L., Fox, N. A., et al (in press). Ethical considerations in international research collaboration: The Bucharest Early Intervention Project. *Infant Mental Health.*

Zeanah, C. H. (2006). Placement in Families Prevents Psychiatric Disorders in Institutionalized Young Children. Symposium paper presented at the annual meeting of the American Association for the Advancement of Science, Minneapolis, MN.

Zeanah, C. H., Nelson, C. A., Fox, N. A., Smyke, A. T, Marshall, P., & Parker, S. W. (2003). Designing research to study the effects of institutionalization on brain and behavioral development: The Bucharest Early Intervention Project. *Development and Psychopathology, 15,* 885 – 907.

Zeanah, C. H., Smyke, A. T., Koga, S. F., Carlson, E. and the Bucharest Early Intervention Project Core Group (2005). Attachement in instituitionalized and community children in Romania. *Child Development, 76,* 1015 – 1028.

11. Appendix Materials – please check off as appropriate if included with submission.

- ☐ Sponsor's Protocol
- ☐ Investigator brochure
- ☐ Flow charts, schemas
- ☒ Other – See list below
- ☐ Federal grant application (please submit 3 copies)
- ☒ Survey, questionnaires, assessments
- ☐ Recruitment letters, postings, flyers

1) IRB approval letters from Tulane University and the University of Maryland for genetic component of this study

a. IRB approval letter from Tulane University dated July 31, 2006
b. Letter of support from Drs. Stanescu and Nanu in Romania regarding genetic component of the study
c. Consent form (Bucharest Early Intervention Project Protocol Adddendum: Genetic Analysis) most recently approved by Tulane University (stamped by Tulane on 4/21/06; approved 7/25/06). PLEASE NOTE THAT THIS IS THE VERSION OF THE CONSENT FORM FOR WHICH WE ARE REQUESTING CHB CCI APPROVAL.
d. IRB approval letter from the University of Maryland dated May 9, 2006.
e. Consent form approved by University of Maryland (stamped and approved until April 14, 2007)

2) Surveys/Questionnaires/Assessments

a. Preschool Age Psychiatric Assessment (submitted electronically)
b. MacArthur Health and Behavior Questionnaire (parent and teacher versions)
c. Vineland Adaptive Behavior Scales
d. Disturbances of Attachment Interview
e. Sample Stimuli to be used in Language and Reading Tasks

Please note that all other questionnaires to be used in this study have not yet been purchased.

3) Parameters for MRI scanner to be used in Session 5

4) CV of Dr. Adina Chirita

5) Relevant papers

6) Consent Forms

a. Bucharest Early Intervention Project Protocol Addendum: Genetic Analysis (Session 1 Consent Form)
b. Session 2 Consent Form
c. Session 3 Consent Form
d. Session 4 Consent Form
e. Session 5 Consent Form
f. Session 6 Consent Form