Part B: Experimental Design and Protocol – ALL APPLICANTS MUST COMPLETE THIS FORM
AGE 8 FOLLOW-UP

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.

Further information may be obtained by referring to **the polices and procedures on the CCI website**

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 ± 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

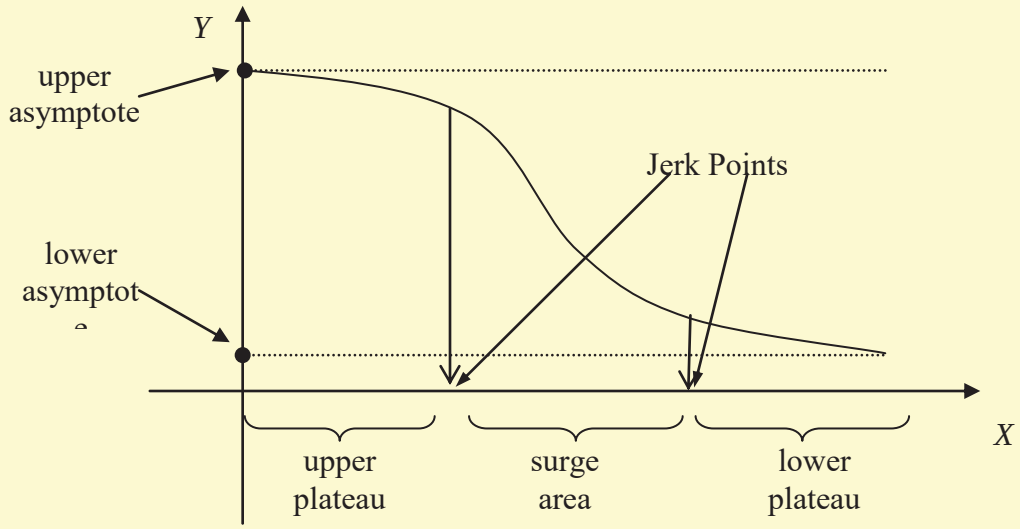
As data are collected, scored, and checked, they will be entered into a data base to be matched with the data previously obtained on this sample in the original BEIP project. Management, integrity, and security of new data from index sample, and of prior data from both index and community comparison 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 not 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.

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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

Part B: Experimental Design and Protocol – ALL APPLICANTS MUST COMPLETE THIS FORM
AGE 12 FOLLOW-UP

All investigators must submit a completed Part B with their New Protocol or Continuing Review application. If a protocol from a corporate sponsor or cooperative group is available, this must also be submitted.

Each question in Part B should be answered thoroughly with answers that are specific to how the research will be conducted at Children's Hospital, Boston.

Do not cut and paste from the protocol or from a grant application to complete Part B. Instead, complete each question in Part B by referencing the applicable page and section number of the protocol which answers the questions in Part B. For some questions in Part B, such as those regarding recruitment methods, confidentiality provisions, and adverse event reporting, you will need to provide complete answers rather than references to the protocol, since the protocol will not address these items as they apply specifically to how the research will be conducted at CHB.
Further information may be obtained by referring to **the polices and procedures on the CCI website**

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

Adolescence is a period of significant development and transition in which there are changes across multiple domains including the biological, interpersonal and cognitive (Goodnow, 1995; Grotevant, 1998; Masten, et al., 1995). This is also a period when risk for psychopathology, especially externalizing disorders, is exacerbated (Walker, 2002; Shulenburg & Maggs, 2002) due to both biological and social changes that come with the advent of puberty (e.g., Walker, 2002; Walker & Bollini, 2002). The proposed study includes follow-up of a sample of children with a history of institutionalization as they enter into early adolescence (ages 12-13 years). These children have participated in the Bucharest Early Intervention Project (BEIP) since infancy and have been followed up through age 8 years. We will evaluate psychiatric symptomatology, risk taking behaviors, physiological reactivity to stress, peer relationships, cognition, brain behavior and pubertal status in these children.

In the current proposal, we aim to predict mental health outcomes and risk taking behaviors in two groups of children: those originally assigned to our foster care intervention (Foster Care Group [FCG]) and those originally randomized to remain in the institution (Care as Usual Group [CAUG]) when they are 12-13 years of age, and we will compare their functioning to typically developing age-matched Romanian children (Never Institutionalized Group [NIG]).

We will specifically examine whether intervention with children exposed to early severe psychosocial deprivation has lasting effects, whether the timing of intervention suggests there are sensitive periods in their recovery, whether the number of placement changes/disruptions impacts their recovery and whether early caregiving quality predicts later outcomes. In addition, we will attempt to identify mechanisms linking early life deprivation to the onset of mental health problems, as well as identify mechanisms associated with recovery in the cognitive, social, and psychological development in these children.

a) Specific Aims /Objectives

Sensitive periods are defined as a time in development during which the brain is particularly responsive to experiences in the form of patterns of activity [1]. This time point may be termed a "critical" period if the presence or absence of an experience results in irreversible change [2,3]. Those factors that allow a circuit underlying development to be plastic – or render it unchangeable – are becoming increasingly well understood in a small number of domains; for example, a great deal is known about human sensitive periods for visual and auditory perceptual development and for language development. However, there is a paucity of data on sensitive periods for normative social and emotional development in human infants and children. And we know little about the effects of aberrant experiences during early development on the emergence of psychopathology. Clear experimental models of the effects of early deprivation and recovery

are, for ethical and practical reasons, lacking in the human psychological literature. However, the case of infants abandoned since birth into institutions provides an opportunity to examine the effects of severe psychosocial deprivation and sensitive periods in typical neural, social-emotional and cognitive development, as well as in trajectories that lead to psychopathology. Over the past 10 years we have conducted just such a study in which infants, abandoned since birth and raised in institutions in Bucharest, Romania, were randomly assigned either to be removed from the institution and placed into a family/foster care intervention or to be left in the institutions to care as usual. We have followed these children through 8 years of age and have found in data collected on children through 54 months of age that a) early institutionalization leads to perturbations in brain electrical activity, profound deficits and delays in cognitive and socio-emotional behaviors, and a greatly elevated incidence of psychiatric disorders and impairment, b) our intervention was broadly effective in enhancing children's development, but c) for specific domains of neural activity, language, cognition and social-emotional functioning there appear to be sensitive periods regulating recovery. In the current proposal, we will extend these analyses with the aim to predict mental health outcomes and risk taking behaviors in two groups of children: those originally assigned to our foster care intervention (Foster Care Group [FCG]) and those originally randomized to remain in the institution (Care as Usual Group [CAUG] when they are 12-13 years of age, and we will compare their functioning to typically developing age-matched Romanian children (Never Institutionalized Group [NIG]).

We will specifically examine whether intervention with children exposed to early severe psychosocial deprivation has lasting effects, whether the timing of intervention suggests there are sensitive periods in their recovery, and we will attempt to identify mechanisms associated with such recovery in the cognitive, social, and psychological development in these children.

These questions will be addressed within the following five specific aims:

Specific Aim 1: Examine the impact of a randomized clinical trial of foster care on brain development, cognitive and social-affective development, and how psychopathology at ages 12-13 may be mediated by disturbances in cognitive and social-affective development among children who experienced, as infants and toddlers, severe social deprivation in institutions in Romania.

Hypothesis 1: *Children removed from institutions and placed into foster/family care (FCG) will display enhanced behavioral and neural patterns of attention, cognition and social cognition, which in turn will be associated with lower rates of psychiatric disorder/impairment and risk-taking behaviors compared to children randomized to care as usual (CAUG)*. This hypothesis will be assessed using an intent-to-treat (ITT) approach. Specifically, we will compare two groups of children, those originally assigned to foster care placement and those originally assigned to remain in institutional care (care as usual) on measures of social behavior, social cognition, psychiatric status, EEG and ERP measures of attention, and cognition. This hypothesis will test whether an enhanced environment (i.e., living in a family compared to an institution) provided to institutionalized children early in life conferred protection against the development of psychiatric disorders and impairment in early adolescence as mediated by enhanced cognitive and social-cognitive development.

Hypothesis 2: We hypothesize that institutionalization is associated with a pattern of hypothalamic-pituitary-adrenal (HPA) axis reactivity characterized by a blunted initial response to a stressor followed by a delayed, and potentially incomplete, return to baseline and an autonomic nervous system (ANS) characterized by elevated sympathetic activation without concomitant increases in cardiac output efficiency (a "threat" response; Mendes et al., 2001; Mendes et al., 2008). In addition, we hypothesize that children randomized to foster care exhibit a pattern of physiological reactivity that is more similar to community-reared children and that elevated physiological reactivity mediates the association between institutionalization and psychopathology.

Hypothesis 3: As there is clear evidence of epigenetic alternations resulting in differential gene expression following early adverse caregiving, it is critical to examine the combined impact of genetic and epigenetic factors in this population. Given our previously demonstrated modification of the impact of foster care intervention by genetic factors, we hypothesize that gene regulation by epigenetic mechanisms will further

contribute to this differential impact. Specifically we predict that response to the foster care intervention will be correlated with methylation status and differential gene expression (RNA) measured peripherally in salivary samples.

Specific Aim 2: **Determine how the *dose* of institutionalization influences brain, cognitive and social-affective development which in turn influences psychopathology. We will specifically examine whether length of time spent in an institution (from birth to age 12-13) impacts long term mental health outcomes in early adolescence, as mediated by changes in cognitive and social-affective development. Here we offer two competing hypotheses:**

Hypothesis 3a: *The more time a child spends in an institution, the worse their developmental outcome (e.g., higher prevalence of psychiatric symptoms and disorders).* Here we posit a cumulative dose model (vs. a sensitive period model; see Specific Aim 3), in which the more time a child spends in an institution, the greater the negative impact on brain development (e.g., EEG, ERP), cognitive and social affective development, which in turn will mediate an increase in mental health disorders and symptoms and risk-taking behaviors.

Hypothesis 3b*: What most determines long-term outcome will not be the overall, cumulative dose of institutionalization but rather, whether children spend more than 2 years in an institution.* Here we adopt an early experience model. For children who spent more than 2 years in an institution, we expect brain, cognitive and social affective development to be negatively compromised, which in turn will mediate negative outcomes in early adolescence in terms of psychopathology. Children who spent less than 2 years in an institution will have more favorable mental health outcomes at ages 12-13, as mediated by better cognitive and social affective development.

Hypothesis 4: Clear timing and duration effects have been identified with regard to the development of physiological stress reactivity and early life deprivation in the animal literature.[4] Although the degree of exposure to adversity is critically important in determining the long-term effects of early deprivation in animal studies, human studies that can rigorously examine degree of exposure are largely lacking. *We anticipate that greater duration of institutionalization will be associated with greater dysregulation in physiological responses to stress.*

Specific Aim 3: Determine how the *timing* of placement in foster care influences mental health status at ages 12-13, as mediated by changes in brain, cognitive and social-affective development.

Hypothesis 5: In keeping with our previous findings regarding timing of intervention, *children randomized to the FCG who were removed from the institution prior to 24 months of age will show enhanced performance on measures of brain function, cognition and social cognition, and correspondingly, lower rates of psychiatric symptoms compared to children randomized to the FCG group who were removed after they were 24 months of age*. This hypothesis will be assessed within the FCG group and will examine whether earlier vs. later placement affects behavioral performance in early adolescence. As we have previously, if we find an effect at 24 months, we will assess other possible cut points, at two month intervals, from 18 to 30 months to determine the age at which the sensitive period ends.

Hypothesis 6: We will assess a developmental domain that, in animals, is sensitive to adverse rearing environments, has a clearly documented sensitive period, and involves physiological systems known to respond to environmental intervention (Francis et al., 2002; Sanchez, Ladd, & Plotsky, 2001; Liu et al., 1997; Suomi, 1997; Plotsky & Meaney, 1993; Ladd, Owens, & Nemeroff, 1996). Based on the animal literature we hypothesize that the timing of placement into foster care will influence long-term physiological reactivity to stress. We expect to observe a sensitive period, after which the foster care intervention has little beneficial effect on physiological stress reactivity.

Specific Aim 4: Explore the association between telomere length and a history of institutional care in young children.

Hypothesis 7a: We predict that children with a history of institutional care will have shorter average telomere length than age matched children with no history of institutional care.

Hypothesis 8b: We predict that, over time, due to the lasting neurobiological impact of early severe social deprivation, children with a history of institutional care will have a faster rate of telomere length decline than those with no history of institutional care.

Hypothesis 8c: We will also explore whether telomere length and rate of telomere change is associated with negative health outcomes including cognitive, psychiatric, and risk behavior measures.

Specific Aim 5: Determine the association between catch-up growth (in height), psychopathology, IQ and risk-taking behaviors (specific to foster care children).

Hypothesis 9a: We hypothesize that children with catch-up growth in height during the first 12 months in foster care will have higher IQ than other FCG children and CAUG children.

Hypothesis 9b: We hypothesize that catch-up growth in height will be associated with less psychopathology, fewer behavioral problems and fewer risk-taking behaviors.

2. Background and Significance

Background to a World-Wide Public Health Concern

Institutional rearing of young children is now and has been a common practice throughout many parts of the world. UNICEF estimates that approximately 1.5 million children in Central and Eastern Europe live in public care (orphanages, group homes, psychiatric units). These include children who have been abandoned by their parents, whose parents have died, who live in hospitals because of chronic illness (e.g., AIDS), and who live in penal institutions. Tragically, UNICEF also estimates that there are currently 50 to 70 *million* orphaned children living in Sub-Saharan Africa, and 1.9 million in Afghanistan. Although African nations have historically not institutionalized children (rather, children frequently live in small households headed by an older child), it is not clear that this trend will continue, given the magnitude of the problem.

Effects of Early Institutionalization on Development

Examining the deleterious effects of institutional rearing on the development of young children has a long history. Initially, many of these studies were poorly designed, but more rigorous, recent investigations have confirmed earlier findings that institutional care is often associated with a variety of deleterious outcomes (for recent review, see [5,6]). Taken together, previous studies suggest that although early psychosocial deprivation may be associated with deficits later in life across a range of developmental domains, some domains are affected more than others; for example, long-term outcome appears worse in the psychosocial domains compared to the cognitive domains. Thus, in the English Romanian Adoption Study (ERAS), mean IQ at age 12 years was 101 for children adopted prior to 6 months, 86 for children adopted between 6-24 months and 83 for those adopted between 24 and 42 months [7]. Long-term impairments were more evident for children who were adopted later, *but the vast majority of the sample was in the average range*. In contrast, in the same sample, risk of pervasive psychosocial impairment (6/7 indicators of impairment were psychosocial variables) was significantly increased in children adopted after 6 months of age, with only a minority within this group showing no impairment at age 12 years [8]. In fact, a significant proportion of these children had abnormally high levels of indiscriminate behavior. Furthermore, studies that show only mild cognitive impairments in institutionalized children have found that the same children also have profound attachment disturbances [9,10]. What remains unknown is the degree to which early adversity may be overcome in children who experience subsequent change in caregiving environment. Because these studies are limited by potential selection bias about who is adopted, as well as by lack of data on individual differences in institutional experiences and pre-adoption status and by lack of adequate comparison groups (i.e., children living in the same country/city/town who have never been institutionalized), conclusions derived from them have limitations that the current study can address.

Why Institutional Rearing is Bad for the Brain: A Conceptual Framework for this Proposal

It is now well established that early psychosocial deprivation can lead to mental problems including anxiety, depression, ADHD (inattention/overactivity) and externalizing disorders. It is also increasingly clear that the deficits and developmental delays that result from such institutional rearing have their origins in compromised or perturbed neural circuits that are built during early development [11]. Greenough and colleagues have argued that brain development reflects a combination of experience-expectant and experience-dependent mechanisms [12]. The former refers to features of the environment that are (or at least, should be) common to all members of the species, whereas the latter refers to features of the environment that are unique to the individual. Thus, having access to patterned light information or a caregiver are features of the environment common to the species, whereas individual differences in environmental challenges (e.g., quality and quantity of stimulation) are unique to the individual.

A short list of experience-expectant features of the environment might include access to a caregiver, adequate nutrition, sensory (e.g., visual, auditory, tactile) and cognitive stimulation and linguistic input. This list is far from exhaustive, but by inference, it illustrates a key point: many forms of institutional life lack most elements of an "expectable" environment. As a result, the immature nervous system, which actively awaits and seeks out environmental input, and does so during sensitive periods of development, is deprived of such input. This lack of input may lead to underspecification and miswiring of circuits.

An additional point must be made about the effects of early experience, which is that domains vary in *when* experience is required to facilitate a typical developmental trajectory. Thus, the long-term development of children with histories of early institutionalization will depend on a) the age they were institutionalized, b) how long they were institutionalized, and importantly, the age at which they left the institution and were placed into family care.. Moreover, these three dimensions must be set against a backdrop of a child's genetic makeup and his or her prenatal experience (e.g., was the mother adequately nourished? was the fetus exposed to alcohol or other teratogens?). Unfortunately these last two dimensions are rarely known in most studies of post-institutionalized children because genetic information was not obtained and because no reports exist about prenatal development. However, the combination of these three factors—prenatal experience, postnatal experience, and genetic makeup—likely lead to developmental programming effects that may well set the stage for years to come (see [13], for elaboration).

Drawing on data from the Bucharest Early Intervention Project (BEIP), we have argued that the developmental outcomes of children who have ever been institutionalized depend largely on the age at which they *left* the institution and were placed into a family, and less on how much time they spent in an institution; indeed, this hypothesis has been born out in several of the domains we have examined, including IQ [14], language [15], EEG [16], and attachment [17]. These studies all point to the fact that children removed from institutions prior to 24 months of age appear to recover function, while those removed after 24 months of age do not (see [8] for similar findings in an adoption study). In the current proposed work, we intend to examine whether this sensitive period in exposure to psychosocial deprivation predicts psychiatric outcomes in our sample during early adolescence.

In this follow up study, we seek to examine the link between exposure to psychosocial deprivation, age at which a child is removed from the institution, physiological reactivity and specific cognitive and social processes (e.g., attachment) that may create the conditions for the onset of specific mental health outcomes. We draw upon a rich literature in developmental psychopathology that has argued for examining underlying cognitive or social mechanisms that place a child at risk for development of maladaptive behavior. Our reasoning and this literature argues that early psychosocial deprivation perturbs the neural circuits underlying basic cognitive processes such as attention and executive function or basic social processes such as attachment formation and social cognition, and that deficits in these processes lead to development of maladaptive behaviors [11,18]. For example, we predict that later age of removal from the institution will affect those brain circuits underlying basic attention and executive function processes, leading to the emergence of ADHD and emotion regulatory disorders such as externalizing problems. Similarly, length of psychosocial deprivation will affect brain circuits underlying attachment formation and social cognition, leading to emergence of internalizing disorders such as anxiety and depression. Furthermore, we expect that the magnitude of deficits in these tasks will be a function of age at which children are removed from the institution and hence a direct result of sensitive periods in exposure to psychosocial neglect.

Physiological Reactivity

The effects of early-life adversity on the development, functioning, and reactivity of physiological stress response systems has been well characterized in animals. In rodent and non-human primate models the primary method used to experimentally induce early life adversity has been forced separation of the animal from its mother for an extended period. Exposure to this type of early life adversity is associated with decreased hippocampal volume, disrupted hippocampal function and hyper-reactivity of the hypothalamic-pituitary-adrenal (HPA) axis and the autonomic nervous system (ANS) in adulthood; these physiological disruptions are accompanied by elevations in anxiety and fearful behaviors (Sanchez et al., 2001; Liu, et al., 1997; Suomi, 1997; Plotsky & Meaney, 1993; Ladd et al., 1996). Similar lasting changes in physiological systems are not observed in adult animals exposed to chronic stress. In studies of mature rodents, chronic stress exposure is associated with immediate, but not long-term neurobiological deficits (Conrad et al., 1999; van Hasselt et al., in press). The animal literature thus clearly demonstrates a sensitive period during which exposure to an adverse environment results in long term changes in the functioning of physiological stress response systems.

Critically, physiological hyper-reactivity to stress and anxiety behaviors induced by exposure to early life adversity can be ameliorated by placing animals in an enriched environment during puberty (Francis et al., 2002). These findings suggest that the biological consequences of early life adversity may be reversed, at least in part, through improvements to the later environment. Together, these lines of research demonstrate clearly that early-life adversity alters the development of physiological stress responses systems, including the HPA axis and the ANS. These alterations, in turn, engender long-term changes in reactivity to later environments and to stress (Eiland & McEwen, in press). The timing of stress exposure matters a great deal, with early exposure to adverse environments conferring far greater risk for long-term alterations in physiological regulatory systems and anxiety behaviors. The deleterious effects of early life deprivation can be mitigated, however, by later improvements in the environment.

The consistency of findings regarding the effects of early-life adversity on physiological stress response systems in rodents and primates contrasts sharply with the lack of knowledge regarding these associations in humans.[5] Although dysregulation in reactivity of the HPA axis and ANS have been documented in children exposed to abuse, neglect, and other serious adversities (Essex et al., 2002; Hart, Gunnar & Cicchetti, 1995;Fries, Shirtcliff, & Pollak, 2008), including institutional rearing (Gunnar et al., 2001; van der Vegt, 2009), prior studies have not yielded a clear picture of the nature, timing or duration of early-life adversity that leads to physiological dysregulation. Although the timing of exposure is critically important in determining the long-term effects of psychosocial deprivation in animal studies, human studies that can rigorously examine the timing of exposure are largely lacking. It is likely that the long term physiological consequences of exposure to adverse environments vary depending on the developmental timing of exposure, similar to the timing effects observed in animals. Human development is extended relative to rodent development, and the specific developmental stages during which humans are most sensitive to adverse environments remain unknown.

Gaps between the animal and human literatures also exist regarding the benefits of environmental intervention. Despite strong evidence suggesting that improvements in the environment can mitigate the effects of early-life exposure to adversity among animals, we are unaware of a single experimental study that has examined whether random assignment to an improved environment alters physiological reactivity in humans. A recent study found that children whose families were provided with a poverty reduction intervention involving cash transfers had lower basal cortisol than children whose families did not receive this intervention.[9] This study suggests that improvements in the environment might influence human physiological stress response systems, although the study did not assess physiological reactivity directly.

Differences between animal and human research extend to the nature of adverse environments studied and the techniques used to characterize stress responses. In animals, separation from the primary caregiver is typically used to create an adverse early environment (Plotsky & Meaney, 1993; Ladd, Owens, & Nemeroff, 1996). Human studies, in contrast, have examined adverse environments with considerable heterogeneity in the type, frequency, severity, and co-occurrence of exposure across studies (Fisher & Gunnar, 2010; Hart, Gunnar & Cicchetti, 1996; Heim et al., 2001; Heim et al., 2008). Moreover, physiological reactivity in animals is assessed following exposure to an uncontrollable stressor, such as restraint or shock (Conrad et al., 1999), for which the animal has limited response options. In humans, strategies used to elicit a stress typically involve social situations that can elicit nuanced physiological responses related to the phenomenological experience of the stressor itself—including appraisal of the significance of the task, the situational demands, and the personal resources available to respond to those demands—as well as motivation and performance. These

complexities create challenges for differentiating adaptive from maladaptive physiological responses. These inconsistencies in human as compared to animal studies are also due, at least in part, to the lack of experimental data to examine *true causal effects* of the environment on physiological reactivity. Together, these gaps in the literature have impeded progress in understanding the mechanisms linking adverse environments to psychopathology and determining how, when, and where to intervene with regard to preventing the deleterious physical and mental health consequences of exposure to early-life adversity and deprivation.

Summary
Data from the BEIP has convincingly demonstrated that children institutionalized since birth are at risk for developing impairments and delays in a variety of domains. We also have demonstrated that early intervention appears to remediate some but not all of these deficits. Further, the data from assessments of the children in early childhood suggests that if children are removed from the institution and placed in families prior to age two, their development is redirected along a more typical course. This preliminary evidence for sensitive periods in the effects of early experience motivates the current proposal. We propose to examine whether these initial effects persist into late childhood/early adolescence, and whether sensitive periods in early experience continue to exert an influence on development.

3. Preliminary Studies/Progress Report

The Bucharest Early Intervention Project (BEIP) was a randomized controlled trial of foster care as an intervention for children abandoned at or around the time of birth and placed in one of six institutions for young children in Bucharest, Romania [19]. The BEIP began with an initial assessment of 187 children who were screened via pediatric exam and history; 136 children were then selected from the larger group, and all were deemed to be free of neurological or genetic disorders and to show no overt signs of fetal alcohol or any other syndrome. A comprehensive baseline assessment of these children and their caregiving environments was conducted, after which half were randomly assigned to high-quality foster care and the other half to remain in institutional care (care a usual). The average age at entry into foster care was 22 months (range=6-31 months). All children were initially seen for follow-up assessments at 30, 42 and 54 months, and again at 8 years, and the development of children in foster care was compared to the development of children randomized to care as usual and to a group of never institutionalized children (community controls).

Findings Through age 54 Months
Attachment: The Strange Situation Procedure (SSP; [20] was used to assess attachment in the BEIP sample at the baseline and 42-month evaluations. Institutionalized children were assessed with their "favorite" caregiver as determined by staff consensus. If a favorite caregiver could not be identified, a child was assessed with a caregiver who worked regularly with the child and knew the child well. Children living in the community were assessed with their mothers. Infant behavior in each episode was coded and attachment styles were classified as indicated in Tables 1 and 2 below.

Table 1. Attachment Prior to Randomization (i.e., baseline)

	Secure	Avoidant	Resistant	Disorganized	Unclassifiable
Institution	18.9%	3.2%	0%	65.3%	12.6%
Community	74.0%	4.0%	0%	22.0%	0%

Table 2. Attachment at 42 Months (intervention effects)

	Secure	Avoidant	Resistant	Disorg/Contl	Insecure/Other
CAUG	17.5%	24.6%	12.3%	5.3%	40.4%
FCG	49.2%	19.7%	8.2%	13.1%	9.8%
NIG	64.7%	11.8%	13.7%	9.8%	0%

The intervention favorably impacted attachment, as measured by the SSP. Strong timing effects were evident both for security and organization of attachment. Children placed in foster care before 24 months were more likely to be securely attached than children placed in foster care after 24 months—this was not evident for

children dichotomized at ages 22, 20 or 18 months, however. Similarly, the younger they were placed, the more likely they were to have an organized (typical) attachment (Smyke et al, in press).

Relevant Publications:
Zeanah, C., Smyke, A, Koga, S., & Carlson, E. Attachment in institutionalized and community children in Romania. *Child Development, 76(*5), 1015-1028 (2005).

Smyke, A.T., Zeanah, C.H., Fox, N.A., Nelson, C.A., & Guthrie, D. Placement in foster care enhances quality of attachment among young institutionalized children. *Child Development* (in press).

Attachment Disorders (Emotionally Withdrawn/Inhibited and Indiscriminate Behavior): Responses of caregivers to the Disturbances of Attachment Interview (DAI) were coded to derive continuous scores of Emotionally Withdrawn/Inhibited (EW/I) and Indiscriminately Social/Disinhibited (IS/D) Reactive Attachment Disorder (RAD). Results indicated large differences at baseline between institutionalized and never institutionalized children on both variables ($p < .001$ for each). Following randomization to foster care, the FCG had large reductions in signs of EW/I which became indistinguishable from the NIG (community) children at 30, 42 and 54 months of age. The reductions in signs of IS/D were less dramatic. Only at 42 months was the FCG significantly lower than the CAUG. Preliminary analyses reveals that for children placed in foster care at 22 months of age (median split) or younger, reductions occurred early and were large. For these children, IS/D were indistinguishable from the NIG at 30, 42 and 54 months of age. On the other hand, those children placed into foster care *after* 22 months of age had IS/D scores that were indistinguishable from the CAUG at all ages (Smyke, et al., in preparation).

Relevant Publications:
Smyke, A.T., Zeanah, C.H., Fox, N.A., & Nelson, C.A. (in preparation). A randomized controlled trial of foster care vs institutional care for children with signs of reactive attachment disorder.

The ***Stranger at the Door*** task is an observational procedure we developed to assess indiscriminate behavior in young children. Briefly, children were greeted at the door by a complete stranger, who asked the child to take a walk with him/her. Children were classified as those who left with the stranger and those who did not. Fifty-five percent of children in the CAUG accompanied the stranger compared to 28% of children in the FCG and only 5% of children in the NIG. Furthermore, the kappa coefficient of the child's behavior in the Stranger at the Door Procedure and the caregiver's report of indiscriminate behavior was 0.72. Children removed earlier (before 22 months) had lower scores of indiscriminate behavior [21]. Taken together, these data are most compatible with a sensitive period for indiscriminate behavior. That is, young children who failed to experience more species-typical caregiving environments by 22 months of age may not possess adequate experience in social relatedness to foster subsequent typical development in social cognition. It is crucial that we test this hypothesis formally in the proposed investigation.

Relevant Publications:
Gleason, M.M., Smyke, A.T., Egger, H.L., Drury, S.S, Nelson, C.A., Fox, N.A. & Zeanah, C.H. (submitted). Towards the validation of reactive attachment disorder II: Indiscriminately social/disinhibited subtype. *Journal of the American Academy of Child and Adolescent Psychiatry.*

Psychiatric Disorders/Symptomatology/Impairment: Because of the age of the children at the start of the study, psychiatric disorders could not be assessed at baseline. However, at 54 months, caregivers/parents were administered the Preschool Age Psychiatric Assessment ([PAPA]; [22,23], a structured psychiatric interview that represents a downward extension of the Child and Adolescent Psychiatric Assessment ([CAPA]; [24]). As can be seen in Table 3, results indicate a substantial number of children with psychiatric disorders.

Table 3. Rates of Psychiatric Disorders by Group

	CAUG	FCG	NIG	CAUG + FCG vs NIG	CAUG vs FCG
	Percentage			OR (95% CI)	
Any Disorder	61.5%	45.8%	22.0%	**4.0 (2.0, 8.2); p=0.001**	1.9 (0.9, 4.0); p=0.10
Any Externalizing	30.2%	25.4%	6.8%	**5.1 (1.7, 15); p=0.004**	1.2 (0.5, 2.8); p=0.69
Any Internalizing	44.2%	22.0%	13.6%	**3.1 (1.3, 7.1); p=0.01**	**2.8 (1.2, 6.4); p=0.01**

ADHD	23.1%	18.6%	3.4%	**7.4 (1.7, 33); p =0.008**	1.3 (0.5, 3.3); p=0.57
ODD +/- CD	11.4%	15.3%	3.4%	**4.5 (1.0, 20); p=0.05**	0.7 (0.2, 2.2); p=0.57
Depression	3.9%	1.7%	0	No cases in NIG	2.3 (0.2, 26); p=0.50
Any Anxiety	42.3%	20.3%	13.6%	**2.8 (1.2, 6.6); p=0.02**	**2.9 (1.2, 6.6); p=0.01**
Abbreviations: OR=odds ratio; CI=confidence interval; CAUG=Care as Usual Group; FCG=Foster Care Group; NIG=Never Institutionalized Group					

Interestingly, intervention effects differed for emotional and behavioral disorders. Specifically, at 54 months of age there was a definite intervention effect of reducing internalizing disorders, such as anxiety (20% in the FCG vs. 42% in the CAUG). On the other hand, there was no effect on behavioral disorders such as ADHD, which were comparable between the groups (23% in the CAUG vs. 19% in the FCG). There were no timing effects of placement on psychiatric outcomes [25].

Relevant Publications:
Zeanah, C.H., Egger, H.L., Smyke, A.T., Nelson, C.A., Fox, N.A., Marshall, P.J. & Guthrie, D. Altering early deprived psychosocial experience reduces psychiatric disorders among institutionalized Romanian preschool children. *American Journal of Psychiatry*, *166,* 777-785 (2009).

Cognitive Development/Intellectual Functioning: Cognitive *development* was assessed at baseline (age prior to randomization), 30 and 42 months using the Bayley Scales of Infant Development (BSID-II; [26], and at 54 months with the Wechsler Preschool Primary Scales of Intelligence [27]. Results indicate that children reared in institutions showed greatly diminished intellectual performance compared to children reared in their families of origin at baseline. Mean Bayley Mental Developmental Index (MDI) scores were 66 for children in the institutionalized group and 103 for children in the never institutionalized group [28].

As predicted, children randomly assigned to the foster care group experienced significant gains in cognitive function. For example, at 54 months the mean IQ was 81 for children in the FCG compared to 73 for children in the CAUG. What these findings disguise, however, is the role of timing of intervention in subsequent outcome. For example, as seen in Table 4, children placed before 24 months had far better outcomes than those placed after 24 months of age, *even after controlling for duration of time in foster* care. This suggests that there may be a sensitive period for optimizing intellectual functioning [14].

Table 4: IQ of FCG by Entry Age Group

	54 Months (WPPSI)			
	N	Mean	Std Dev	Std Err
0-18	14	85	16.0	4.3
18-24	15	87	14.8	3.8
24-30	22	78	19.5	4.2
30+	8	72	23.8	8.4

Relevant Publications:
Smyke, A.T., Koga, S.F., Johnson, D.E., Fox, N.A., Marshall, P.J., Nelson, C.A., Zeanah, C.Z., & the BEIP Core Group. The caregiving context in institution reared and family reared infants and toddlers in Romania. *Journal of Child Psychology and Psychiatry, 48(2),* 210-218 (2007).

Nelson, C.A., Zeanah, C.H., Fox, N.A., Marshall, P. J., Smyke, A.T., & Guthrie, D. Cognitive recovery in socially deprived young children: The Bucharest Early Intervention Project. *Science, 318,* 1937-1940 (2007).

Brain Functioning: One of the unique characteristics of the BEIP is the measurement of brain activity in infants and young children at the onset of the study and over the course of the foster care intervention. Brain activity was measured by recording the electroencephalogram (EEG), and event-related potentials (ERP) to specific emotion face stimuli.

EEG Power and Coherence: The EEG was recorded 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. Findings from the baseline assessment indicate that the institutionalized group showed a higher level of relative theta power

(indicating a lag in brain development) and a reduction in alpha and relative beta power compared to the group of age-matched children living in the community [29].

Additional analyses suggest that there were minimal group differences between the FCG and CAUG in EEG power and coherence across all measured frequency bands at 42 months of age. Of note, however, similar to our IQ findings, earlier age at foster care placement was associated with increased alpha power and decreased short-distance EEG coherence. Further analyses separating age at placement from duration of intervention suggested that this effect may be more robust for EEG coherence than EEG band power [16].

Relevant Publications:

Marshall, P.J., Fox, N.A., & the BEIP Core Group. A comparison of the electroencephalogram between institutionalized and community children in Romania. *Journal of Cognitive Neuroscience 16(8),* 1327–1338 (2004).

Marshall, P.J., Reeb, B.C., Fox, N.A., Nelson, C.A., & Zeanah, C.H. Effects of early intervention on EEG power and coherence in previously institutionalized children in Romania. *Development and Psychopathology, 20,* 861-880 (2008).

EEG and Mental Health Outcomes at 54 Months

Although the effects of institutionalization on social, emotional and cognitive functioning are clearly documented, the mechanisms that underlie these associations remain poorly understood. As recent evidence suggests that institutional deprivation is associated with neurobiological abnormalities, it is possible that deviations in brain development may be responsible for the association between institutionalization and the host of physical and mental health problems with which it is associated. Evidence to support this claim, however, is lacking. We provide novel evidence suggesting that atypical brain development resulting from institutionalization explains the association between institutional rearing and the development of symptoms of attention deficit and hyperactivity disorder (ADHD), a disorder that afflicts approximately 20% of our ever institutionalized children in BEIP.

To provide evidence for mediation, four criteria must be met [30,31]. First, an association between the exposure (i.e., institutionalization) and outcome of interest (i.e., ADHD symptomatology) must be established. In the BEIP, institutionalization predicts symptoms of inattention, $F=19.2$, $p<.001$, hyperactivity, $F=10.9$, $p<.001$, and impulsivity, $F=15.0$,$p<.001$, at 54 months. Second, the exposure must be associated with the putative mediator (i.e., brain functioning, as indexed by EEG power). We find significant associations between institutionalization and several indices of brain functioning at baseline. Institutionalization predicts increased low-frequency EEG activity (theta relative power) in frontal, $F=4.7$, $p=.032$, temporal, $F=5.9$, $p=.016$, and occipital regions, $F=4.7$, $p=.032$, and decreased high-frequency EEG activity (alpha relative power) in frontal, $F=4.8$, $p=.029$, parietal, $F=8.6$, $p=.004$, and occipital regions, $F=7.8$, $p=.006$. Third, the putative mediator must be associated with the outcome. Here, EEG power at baseline predicts ADHD symptoms at 54 months. Specifically, relative theta power in the temporal region ($\beta=0.2$-0.3, $p=.034$-$.007$) and relative alpha power in the frontal and parietal regions ($\beta=-0.2$, $p=.03$-$.008$) is associated with symptoms of hyperactivity and impulsivity. Relative theta power in frontal and occipital regions also is associated with impulsivity ($\beta=-0.2$, $p=.023$-$.013$), and relative alpha power in the parietal region is associated with inattention, $\beta=-0.3$, $p=.004$).

The final critical test of mediation requires examination of the association between the exposure and outcome in a model that includes the mediator. Here, we evaluated the hypothesis that deficits in brain functioning related to institutionalization explain the relationship between institutional rearing and ADHD symptomatology. We tested the significance of the mediator using a bootstrapping approach that provides bias corrected confidence intervals and allows multiple mediators (i.e., multiple EEG components) to be tested in the same model [32]. Confidence intervals that do not include zero indicate a significant indirect effect. The mediation model was examined for symptoms of inattention, hyperactivity, and impulsivity separately to determine whether the mediating effects of brain functioning were differentially related to specific types of ADHD symptoms.

We find evidence for a significant indirect effect of institutionalization on hyperactivity (CI of indirect effect: 0.06,0.8) and impulsivity (CI: 0.03,0.4) through EEG power. The association between institutionalization and hyperactivity is no longer significant when EEG components are added the model, $\beta=0.8$, $p=.06$. This finding

indicates that differences in the prevalence of hyperactivity among children reared in institutions relative to community controls is explained entirely by differences in brain functioning between these groups that are attributable to institutionalization. The association between institutionalization and impulsivity is attenuated but remains significant when EEG components are added the model, β=0.6, p=.006. This finding indicates that differences in brain functioning between children reared in institutions versus the community partly explain differences in the rate of impulsivity between these groups but that other mechanisms are also involved. Birth weight and head circumference were included as covariates in all analyses. Importantly, these mediating effects were specific to ADHD, and did not, for example, relate to symptoms of depression or anxiety.

Relevant Publications:

McLaughlin, K., Zeanah, C.H., Fox, N.A., & Nelson, C.A. (in preparation). Neurodevelopmental mechanism links early deprivation to symptoms of Attention-Deficit/Hyperactivity Disorder (ADHD).

Summary

Through 54 months our data convincingly demonstrate that a) spending any time in an institution exerts deleterious effects on virtually every domain of development we have studied; b) the earlier in life one leaves an institution and is placed in a family, the greater the chances of recovery; and c) the sensitive period for recovery varies by domain, with some domains showing earlier sensitive periods for recovery (e.g., growth, language) than others (IQ, EEG) and still others (e.g., mental health) not showing any sensitive period.

Preliminary Findings at 8 Year Follow Up

Behavioral Dot Probe Task: Children's responses to social cues were examined using a dot probe paradigm that assesses attention biases to happy or threatening faces. Participants were presented with a pair of faces, one affective (happy or angry) and one neutral, followed by the presentation of the probe. Participants were instructed to press the button matching the side of the screen on which the probe appeared (i.e. right or left). For congruent trials the probe appeared on the same side of the screen as the affective face and for incongruent trials the probe appeared on the side opposite of the affective face. Responses were examined separately for the emotion conditions (angry and happy) and attention bias scores were calculated by subtracting reaction times (RTs) on congruent trials from RTs on incongruent trials for all correct responses. Larger, more positive bias scores indicate an attention pattern of vigilance toward the emotion faces whereas smaller, more negative scores reflect an avoidance of the emotion faces.

Analyses of the reaction time data revealed that children in the CAUG had an attention bias toward threatening faces ($t(33)=2.01$, $p=.053$). In contrast, children in the FCG exhibited a bias towards happy faces ($t(43)=5.30$, $p=.001$). This positive bias was significantly larger in the FCG ($F(2,125)= 5.41$, $p=.006$) than in the CAUG and NIG (p's<.05). No attention biases emerged for children in the NIG.

Sensitive period effects for the intervention also emerged. Within the FCG, participants were grouped based upon duration of time spent in the institution prior to placement in foster care. Children who spent less than 21 months in the institution had a greater attention bias to happy faces than children who spent a longer period of time in the institution ($t(41)=2.53$, $p= .015$).

Flanker Task: A modified flanker task with a stimulus array of arrows was used to assess children's behavioral and physiological responses to the commission of errors. Children were seated in front of a computer monitor and asked to hold a small box with two pushbuttons that were located on the upper portion of the box. Subjects were asked to respond to the central target arrow by pressing the corresponding button (right or left) regardless of the direction of the flanking arrows. Trial blocks contained both congruent trials and incongruent trials. In the congruent trials the target was flanked by identical stimuli and in the incongruent trials the flanking stimuli were facing the opposite direction of the target.

All groups displayed the typical flanker interference effect of more accurate responding on congruent as compared to incongruent trials ($F(1,115) = 181.81$, $p = .000$); however, overall performance accuracy varied between the groups ($F(2,117) = 7.34$, $p = .001$). The CAUG committed more errors than the NIG ($p = .011$) whereas the performance of the FCG did not differ significantly from either the NIG or CAUG. Group differences also emerged in physiological reactivity to errors across frontal (Fz) and central (Cz) scalp sites (F's$(2,115) \geq 3.15$, p's <.05). Specifically, the NIG exhibited a larger error-related negativity (ERN) response

than the CAUG across frontal and central sites (*p*'s < .02). The FCG also displayed a significantly larger ERN then the CAUG at Cz (*p* = .006). The NIG and FCG did not differ in the magnitude of their ERN response at either site. Unlike our dot probe task, no timing effects were evident.

Memory and Executive Function: Memory and executive functioning were assessed using the Cambridge Neuropsychological Test and Automated Battery (CANTAB). As expected, children with a history of early institutional care performed worse on measures of both visual memory and executive functioning compared to their peers without a history of institutional care. In addition, there were no significant differences between children in the FCG and children in the CAUG. However, after controlling for birth weight, head circumference, and duration of time spent in early institutional care, the foster care intervention was a significant predictor of Spatial Working Memory Strategy scores. No significant timing effects were noted [33].

Relevant Publications:

Bos, K , Fox, N.A., Zeanah, C.H., & Nelson, C.A. The effects of early psychosocial deprivation on the development of memory and executive function. *Frontiers in Behavioral Neuroscience, 3*, 1-7. (2009).

Intellectual Quotient: Children's IQ was assessed at 8 years using the Wechsler Intelligence Scale for Children (WISC-IV; [27]. IQ data from 30, 42, and 54 months and 8 years were subjected to a latent profile analysis in order to examine the longitudinal patterns in the Care as Usual and Foster Care Groups. Three profiles were identified in the model (BIC=3105.67): low, typical, and declining. The low profile included children with consistently very low IQ scores across development, the typical profile included children with consistently average IQ scores, and the declining profile included children who had average IQ scores initially, followed by lower scores over time. Children in the CAUG were significantly more likely to be members of the low profile group, $F(1,102)= 9.62$, $p=.002$, while children in the FCG were significantly more likely to be members of the typical profile group, $F(1,102) = 15.96$, $p=.000$. In addition, for children in the FCG, those placed in foster care before the age of 25 months were significantly less likely to be in the low profile group, $F(1,52)=4.87$, $p=.032$. Separate analyses using the "intent to treat" approach found no significant differences between children in the FCG and CAUG on measures of verbal comprehension, processing speed, working memory, perceptual reasoning, or full scale IQ, nor were there any significant timing effects.

Attachment: At age 8, signs of reactive attachment disorder declined slightly from 54 months, but the children in the CAUG continued to show more signs of the emotionally withdrawn inhibited type than the FCG or NIG. The ever institutionalized group (EIG) showed more signs of indiscriminately social RAD than the NIG, but there were no significant differences between the FCG and the CAUG. There were no timing effects for either type.

Summary

Our 8 year findings, although still in a preliminary stage, largely support our earlier findings. First, we continue to observe profound delays in nearly all domains among children assigned to the CAUG. Here it is important to stress that at age 8 only 14 children were still living in an institution, yet the data from these children differed little from the remaining children who were assigned to the CAUG yet left for a family setting after 24 months of age. This reinforces the finding that it is early experience that accounts for the majority of the delays and deviance in their behavior. Second, in a few domains we are now seeing that children assigned to the foster care group (FCG) look very similar to the children in the never institutionalized group (NIG) – that is, they are showing complete "recovery." For example, all signs of emotionally withdrawn/inhibited reactive attachment disorders are gone by 30 months of age and do not reappear at any subsequent assessment. Third, in other domains this is not the case – that is, children in the FCG are situated at the midpoint between the CAUG and the NIG, much as they did at 54 months. This is true for attachment, emotional responsiveness, anxiety disorders, and IQ. This suggests that in these domains their recovery has hit a ceiling, possibly due to later (vs. earlier) restoration of an adequate environment.

4. Design and Methods

a. Study Design

This investigation is a follow-up study of severely deprived young children who have participated in the Bucharest Early Intervention Project (BEIP) in Bucharest, Romania since early 2001. 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 8 years 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).

Over the past 3.5 years, many children have changed from their original groupings, so that at the time of this submission, only 14/67 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 months to 12 years (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, 2003). 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 12-13 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 12-13 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 12-13 years is the major aim of this investigation.

This sample and design include children with a continuum of caregiving adversity, ranging from those who were randomized to foster care as young as 6 months of age to those who were randomized to institutional care and have remained there since early infancy. 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.

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 31 months of age. We will try our best to recruit as many of the original 136 participants as possible. We have followed more than 105 of these children through assessments at 8 years, 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 105 of the original 136 children will participate in the follow-up study.

The second group of participants will include an equal number of never institutionalized children, all of whom will be matched on age, gender and ethnicity to children in the group described above. We will retain community children from the 8 year follow-up and supplement as necessary by recruiting from the same local elementary schools in Bucharest where FCG and non-institutionalized CAUG attend. These schools are attended by children from all 6 sectors of Bucharest and are representative of the city's population and socioeconomic groups.

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 recruited 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/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 Mon-Fri sleepover daycare).
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?
6. Does your child have any serious handicapping conditions (e.g., genetic syndromes, Fetal Alcohol Syndrome, cerebral palsy)?

Only those children whose parents respond "YES" to question 1 and "NO" to questions 2 – 6 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.

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 are interested in taking part in the proposed study.

Recruitment Methods for Additional Community Participants
To recruit additional children for the community comparison sample, our research staff will request permission from principals and teachers of several elementary schools within Bucharest to send letters home to families whose children are the appropriate age to participate in this study. We used this same method to recruit additional community children for the follow-up study at 8 years and had very good response.

The recruitment letter (see attached) will describe the study and invite parents to contact the research lab if they are interested in learning more about the study or decide that they would like to participate. If parents express an interest in participating in the study, a research assistant will ask the five eligibility questions listed above. If the parent responds to these questions as described above, the research assistant will schedule the research session at a time convenient for the family.

***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.*

Recruitment of Participants for Peer Evaluation Stimuli
To recruit additional children to develop the peer rating stimuli, our research staff will request permission from principals and teachers of several elementary schools and directors of community programs within Bucharest to send letters home to families whose children are the appropriate age to participate in this study. The recruitment letter (see attached) will describe the photographs needed for the study and invite parents to contact the research lab if they are interested in learning more about the study or decide that they would like to participate. If parents express an interest in participating in stimuli development, a research assistant will schedule a time convenient for the family to visit the laboratory for the photos.

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

Please see the attached recruitment letter to be used for recruiting additional community control subjects.

iii. WHO will be responsible for subject recruitment?

Staff in the BEIP Research Laboratory will be responsible for subject recruitment.

d. Description of Study Treatments or Exposures/Predictors

Predictors include group status (CAUG vs FCG vs NIG), age at which children entered foster care, length of time spent in an institution, quality of early caregiving environment and number of placement changes.

e. Definition of Primary and Secondary Outcomes/Endpoints

Outcomes include psychiatric status (total number of symptoms, as well as disorders based on self-report and parent report), risk-taking behavior, IQ, self-reported emotional responses, and physiological responses to the active and passive tasks proposed in Session 4, all of which are measured continuously. Physiological response includes measures of autonomic nervous system reactivity (heart rate, systolic and diastolic blood pressure, total peripheral resistance, cardiac output) and HPA axis reactivity (cortisol, DHEA). Reactivity is defined as change in each of these parameters from baseline to each task.

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

Administrative Details

Data collection for this study will consist of 5 research sessions. We expect that data collection for the entire sample will take 4 years.

The research sessions will be scheduled at a time most convenient for the participant and their parents/caregivers, including weekends if families prefer. The research sessions will be conducted in Romanian and will take place at the BEIP Research Laboratory in Bucharest, Romania. We estimate that each research session will last no more than 4 hours.* A minimum of three scheduled breaks will be taken throughout each session and participants will be told that they can request additional breaks, as needed. Snacks and refreshments will be provided to participants during breaks. All proposed tasks will be piloted on a small number of children (no more than 15) recruited from the community prior to the enrollment of actual subjects to evaluate the measures for cultural and age-appropriateness, ensure that children understand task instructions and confirm that the inclusion of these tasks will keep the total session time within our estimate.

If a participant is unable to read or has difficulty reading, one of our trained research assistants will read aloud all questionnaires to the participant so they may respond.

** The follow-up conducted at age 8 consisted of research sessions lasting between 3-4 hours. All participants successfully completed the tasks requested of them within this time and no complaints about session length were made on anonymous session feedback forms completed by parents/caregivers of the participants.*

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, as well as the legal representative of each child. Depending on the case, this could be the biological parents, the sector Mayor, the President of the local sector, adoptive parents or foster parents. If the legal representative of the child gives consent, the child will be enrolled in the study. If the legal representative of the child is the biological parents, and they cannot be located or do not give consent, the child will not be enrolled. 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 consent form will be created for each research session and will indicate the purpose of the original study and the purpose of the proposed adolescent follow-up. Once approved by CCI, the consent form will be translated to Romanian by our Romanian research staff. Informed consent will be conducted in Romanian by a member of the BEIP Research Lab staff and will be obtained prior to the start of each research session.

Upon arrival to the BEIP Research Laboratory, participants and their parents/caregivers will be greeted by two members of the research team and escorted to a private room in the laboratory. Research Assistant 1 will provide a summary of the research activities scheduled for that day and will review the session consent form with the parent/caregiver.

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 each session. Parents/caregivers will sign the consent form in the presence of study personnel before any testing commences. In addition, the consent form will also contain a statement obtaining consent from the parent/caregiver to contact the child's teacher to complete two questionnaires described below. 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.

If the parent/caregiver is unable to read/write, the consent form will be read aloud and Research Assistant 2 will indicate that informed consent was obtained by signing the Witness section of the consent form.

Once the parent/caregiver has provided consent, Research Assistant 2 will escort the parent/caregiver to the reception area of the laboratory to confirm/update their contact information (address, phone, email address,

etc.) Meanwhile, Research Assistant 1 will remain in the private office with the child and will review the written assent form (see *Written Assent* section below).

Written Assent

Written assent will be obtained for participants in the community control group.

Written assent for children in the foster care and care as usual (institutionalized) groups will be attempted with all participants and dependent upon the cognitive ability of the child. If a participant in the foster care or care as usual group is unable to give written assent, verbal assent will be attempted. The research staff will explain the details of each session to the child, taking into account cognitive ability. Research staff will always obtain their voluntary verbal agreement to proceed during the course of the research session. Assent will be obtained in a private room away from parents and caregivers.

In the event that written/verbal assent cannot be obtained, parental consent will be considered sufficient in conjunction with the child's reaction to the research sessions.

In the event that a child in any of the three groups chooses not to participate, the researcher will inform the parent/caregiver of the child's decision and will complete only those activities relevant to the parent/caregiver. No procedures will be administered to a child who is unwilling to participate or if any parent/caregiver fees their child is unable or unwilling to continue.

A separate assent document will be developed for each research session. The assent documents include a description of the activities scheduled for a given research session and:

a. A statement to the effect that a subject can stop that day's session, and also stop being in the study altogether;
b. A statement to the effect that no will be disappointed with – or mad at- a child who makes a decision to withdraw;
c. A statement to the effect that they can skip any questions they do not wish to answer;
d. An explanation of which analyses/instruments their parents/caregivers will be present for;
e. An explanation of what types of answers to which the researchers will need to alert parents or caregivers;
f. Information regarding what payments will be given to subjects;
g. Contact information for the study team and the IRB.

Conditional Assurance of Confidentiality

The consent and assent forms provide information regarding conditional assurance of confidentiality. This section on each form indicates the steps the study team will take if they have reason to believe the participant or others are at risk for self-harm or harm (consent and assent), what kinds of referrals or interventions are possible (consent only), and the fact that families will be responsible for payment if they elect to receive private, rather than universal care (consent only).

If confidentiality must be breached for a participant in the care as usual (institutionalized) group, Anca Radulescu will inform the caregiver attending the research session, the Director of the institutional setting, the Director of the General Direction for Social Assistance and Child Protection (DGASPC) and the legal representative of the child to ensure that everyone involved in the adolescent's care is appropriately informed of the situation.

In addition, a resources list will be provided to all families regardless of how their child responds to the Youth Risk Behavior Survey and Diagnostic Interview Schedule for Children (described below). This list of resources will include information about hotlines and support groups for adolescents experiencing mental health or substance abuse issues. There will also be resources listed for parents (support groups, etc.).

MEASURES TO BE INCLUDED

Many of these measures listed below were chosen because they have been used with typically-developing children of a similar age range in the United States. For example, Dr. Nathan Fox, co-PI on this project, has used the Friendship Qualities Questionnaire, the Self-Report Coping Measure and the Interpersonal Competence Questionnaire extensively with children in this age range in his research laboratory at the University of Maryland. The PI has consulted extensively with colleagues whose research focuses on pre-adolescents/adolescents on the measures described below. The HBQ, DAI and SSRS have been used in

previous assessments of the BEIP. Although a new version of the HBQ will require additional translation, we feel compelled to maintain consistency in measures used across assessments as much as we can. The DISC was chosen over the CAPA for its ease of interviewing and lower cost. The BART and BIRD have been used extensively with children in this age range by Lejuez and colleagues at the University of Maryland. Drs. McLaughlin and Sheridan currently use the TSST-C and the computer game task with adolescents who have a wide range of adverse early-life and current family circumstances and mental health problems (IRB-P00000200), All measures will be translated to Romanian and back-translated to English by our research team.

Session 1

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 6-16 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: 90 minutes
Present during Administration: Research Assistant and Child

CDC Youth Risk Behavior Survey (CDC, 2001)
To examine lifetime (baseline assessment period only) and past year (all assessment periods including baseline) prevalence of real world risk behaviors, we will use a shortened version of the CDC Youth Risk Behavior Survey (CDC, 2001). In total, the shortened measure will assess different risk taking behaviors across the domains of drug and alcohol use, safety compromising behaviors (e.g., not wearing a seat belt, provoking packs of wild dogs), and delinquency related behaviors (e.g., gambling, stealing, bringing a weapon to school) relevant to the Romanian youth population. In addition to the examination of individual behaviors, we also will examine an aggregate measure of risk including behaviors that pose a direct (e.g., injecting drugs) and indirect (e.g., alcohol use) risk for HIV infection. A similar aggregate will be examined for risk-behaviors unrelated to HIV infection. This strategy has been used successfully in previous research with youth ranging from early through late adolescence (Aklin, Lejuez, Zvolensky, Kahler, & Gwadz, 2005; Lejuez et al., 2002; 2003). In these studies, the composites have shown good reliability (all α's > .7), and have been shown to be significantly related to other self-report measures of risk related constructs including sensation seeking and impulsivity. This measure was chosen over more extensive measures such as the Global Appraisal of Individual Needs (GAIN-I) measure (Dennis et al., 2002; Tims et al., 2002) because the level of risk behavior at this age can be captured in a shorter more general measure which limits participant burden. This is in line with other similar research (e.g., Lejuez et al., 2007).

Participant: CHILD
Estimated time to complete: 20 min
Present during Administration: Research Assistant and Child

The Brief Sensation Seeking Scale (BSSS; Hoyle, Stephenson, Palmgreen, Lorch, & Donohew, 2002)
The BSSS is an 8-item self-report measure of sensation seeking designed specifically for use with child and adolescent populations, and has been used extensively for this purpose (e.g., Stephenson, Velez, Chalela, Ramirez, Hoyle, 2007). Example items include, "I would love to have new and exciting experiences, even if they are illegal" and "I like wild parties." Participants are asked to rate each item according to the extent to which it accurately describes their experience using a 5-point Likert scale (1 = strongly disagree; 5 = strongly agree). The BSSS has been found to be associated with well-established measures of other aspects of disinhibition, such as impulsivity (see Stephenson, Hoyle, Palmgreen, & Slater, 2003), and is predictive of impulsive-like behaviors, such as substance use (Hoyle et al., 2002; Stephenson et al., 2003). Items are summed to create a total sensation seeking score.

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Interpersonal Competence Questionnaire (Buhrmester, et al. (1988).
This questionnaire assesses social competence skills in a variety of situations and includes five subscales: Initiation, Negative Assertion, Disclosure, Emotional Support, and Conflict Management within the child's peer relationships.

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Friendship Quality Questionnaire (Parker & Asher, 1993)
This questionnaire assesses friendship quality amongst target child and self-nominated close friends and includes six sub-scales: Validation & Caring, Conflict Resolution, Conflict & Betrayal, Help & Guidance, Companionship & Recreation, and Intimate Exchange.

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Expressed Emotion
Expressed Emotion (EE) is a qualitative measure of the 'amount' of emotion displayed, typically in the family setting, by a family or care takers. EE, as originally assessed using the Camberwell Family Interview, is considered a measure of the patient-relative relationship and is a highly valid and reliable predictor of poor clinical outcomes among patients with major psychopathology (as reviewed by Hooley &Parker, 2006). Parents/caregivers will be asked to describe their child as if the interviewer has never met the child. This assessment will be videotaped/audiotapted for subsequent coding and analysis. If parents fail to give a meaningful response to the open-ended question, the interviewer will follow-up with more specific probes.

Participant: PARENT/CAREGIVER
Estimated time to complete: 5-10 minutes
Present during Administration: Research Assistant and Parent/Caregiver

DNA Collection
The impact of early experience on stress reactivity over the course of the lifespan is not yet fully understood. Specifically, it is unclear if early stress exposure creates a permanent increased vulnerability to stress, similar to a scar, that results in lifelong increased stress responsiveness or whether instead, proximal life events are more relevant to current stress reactivity. Two putative measures which may reflect the lasting impact of early stress exposure are telomere length and epigenetic modifications including methylation and histone acetylation. In our initial sample, we noted that percent time spent in an institution at 30, 42 and 54 months of age was predictive of telomere length at age 8, indicating that these early experiences were indeed relevant to one putative measure of cumulative stress exposure, telomere length. However, to differentiate between these two hypotheses, it is important to determine whether the influence of early experience, compared to more proximal life stressors, is more determinant of telomere length. More precisely, we seek to determine if early experience is more predictive of telomere length over time, or if telomere length is more related to life stressors during the past year. This longitudinal analysis will also help us determine if changing the caregiving environment and presumably decreasing stress early in life prevents further telomere length decline or if the early experience results in lasting alterations in stress reactivity that continues to be reflected in decreased telomere length, regardless of more proximal life stressors. Evidence demonstrating early experience related epigenetic changes points to another putative mechanism by which early adversity results in lasting neurobiological changes. In order to explore these hypotheses, we will collect annual DNA samples and examine the change in telomere length over time.

To further assess the influence of proximal life events, we will also collect annual measures of stressful life events. In addition to determining telomere length we will also explore methylation status and putative

patterns of histone acetylation as key alternative regulators of gene expression that can be modified by early experience.

Additionally, as recent evidence suggests that methylation patterns are not as static as originally expected, and because we have DNA samples collected at multiple time points for the telomere analysis, it is expected that comparison of methylation patterns longitudinally will provide greater insight into the underlying biological mechanisms. We will examine methylation patterns in DNA collected at age 12, as well as examine methylation patterns in DNA already collected at earlier times points (as approved in protocol 06-10-0455) to determine if alterations in methylation are more predictive of outcome than methylation status at age 12.

DNA samples will be collected **annually** (maximum of 3 collection points) from the participants in our study using buccal swab kits. The first sample (2 swabs) will be collected when the participant visits the research laboratory to complete Session 1. Two additional DNA samples (2 swabs at each collection) will be collected annually in the BEIP Research Laboratory or each child's home/living situation each year by one of our research assistants. We will collect DNA samples annually from this point forward in order to be able to explore the trajectory of telomere length as a function of both early experience and life events in the intervening year (see Session 5).

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 at each annual visit). All samples will be transported back to New Orleans, LA where the DNA will be extracted from the sample. Telomere, methylation and histone studies will all be done blind to all other measures. Telomere and histone studies will be completed by Stacy Drury, MD, Ph.D., at Tulane University School of Medicine and methylation analyses will be completed by Amy Non, Ph.D., MPH at Vanderbilt University.

The DNA will solely be used for this study. DNA samples are coded when they are collected with the participant's study number and sent without any other identifying information. DNA will be destroyed at the completion of data analysis.

Participant: CHILD
Estimated time to complete: 5 min
Present during Administration: Research Assistant, Child and Parent/Caregiver

Session 2

Balloon Analogue Risk Task, Youth Version (BART; Lejuez, 2002; 2007)
Lejuez et al., (2002) developed the Balloon Analogue Risk Task (BART) to model risk taking in the laboratory. Based on this measure which has been well-validated in adult samples, an adolescent-appropriate version was developed and published (Lejuez et al., 2007). Before starting the BART, the task will be thoroughly explained using a visual of the task accompanied by directions provided below: "On the screen, you will see 30 balloons, one after another. For each balloon, you will use the mouse to click on the box that will pump up the balloon. The bigger you pump up the balloon, the more points you will build up on that balloon. If you stop pumping a balloon before it pops and you click on the button labeled "Collect", your points will be added to the prize meter on the left. The bigger you make up the balloon when you press "Collect", the more the prize meter will fill up. At the end of the game, the size of your prize will equal the number of points you have saved into your prize meter, which will determine if you get a small, medium, large, or bonus prize. Good Luck!"

Once the subject presses a button agreeing that he/she understands the task, the computer screen will show a small simulated balloon accompanied by a balloon pump, a reset button labeled "Collect", and the prize meter. Each click on the pump inflates the balloon one degree (about .125" in all directions). With each pump, 1 point will be accrued in a temporary reserve (the number of points in this reserve is never indicated to the subject). When a balloon is pumped past its individual explosion point, a "pop" sound effect emanates from the computer. When a balloon explodes, all points in the temporary bank are lost and the next un-inflated balloon is shown. At any point during each balloon, the subject can stop pumping the balloon and click the "Collect" button. Clicking this button transfers all points from the temporary bank to the prize meter,

during which the new total earned is incrementally updated on the prize meter, point by point with a "bells" sound-effect playing.

After each balloon explosion or point collection, the subject's exposure to that particular balloon ends and a new balloon appears until 30 balloons (i.e., trials) have been completed. The probability that a balloon will explode is arranged by constructing an array of N numbers. The number "1" is designated as indicating a balloon explosion. Upon each pump of the balloon, a number is selected without replacement from the array. The balloon explodes if the number 1 is selected. The array contains the integers 1 through 128. With this system, the expected amount of money earned and the expected frequency of explosions can be predicted as a function of the number of pumps. The probability that the balloon will explode on the first pump is 1/128. If the balloon does not explode after the first pump the probability that the balloon will explode is 1/127 on the second pump, 1/126 on the third pump and so on up until the eighth pump at which the probability of an explosion is 1/1 (i.e., 100%). A great strength of the task is the wide age range for which strong validity data exist, covering both the lower age here (see Lejuez et al., 2003) through the upper age of 18 (Lejuez et al., 2007), thereby allowing for the advantage of keeping the same measure across years.

Participant: CHILD
Estimated time to complete: 30 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Behavioral Indicator of Resiliency to Distress (BIRD; Daughters et al., 2009)
The BIRD, which was developed based upon the well-validated version for adults, the PASAT, measures distress tolerance by determining how long a participant persists on a challenging task that increases in difficulty until success on the task is virtually impossible. Specifically, participants are asked to use the computer's mouse to click a green dot that appears above a number (with numbers ranging from 1-10) before the green dot disappears. If the number is clicked before the dot disappears, then a bird picture on the screen sitting in a cage is let out of the cage and the computer makes a chirping sound. Alternatively, if the green dot disappears before the child clicks on the number, then a loud noise is made and the bird remains in its cage. A participant receives one point each time the bird is let out of its cage. There are no points for missed green dots. There are three levels of difficulty. The first level of the BIRD lasts 3 minutes. This level begins with a 5-second latency in between dot presentations and titrates this latency based upon performance (correct answers reduce the latency by 0.5 seconds whereas incorrect answers or nonresponses increase the latency 0.5 seconds). The second level is more difficult, beginning with the average latency from the previous level for four minutes and then reducing the latency in half for the final minute making the task extremely difficult (i.e., challenge latency). Following a brief rest period, the final level lasts up to 5 minutes and utilizes the extremely difficult challenge latency. At all points in the final level, the adolescent has an escape option. Specifically, the participants is informed prior to beginning the task that once the final level has begun, the task can be quit by clicking the 'quit game' button on the computer screen, however the magnitude of their prize is dependent on how well they do on the task. Throughout the task, a point meter remains visible on the screen that indicates how many points the adolescent has earned. Distress tolerance is indicated by persistence on the task and can be examined as a continuous variable (latency in seconds to terminate) or a categorical variable (whether or not they terminated the task). Notably, persistence on the task is unrelated to change in levels of distress in response to the task (Daughters et al., 2009; MacPherson et al., under review). Data from our progress report above support the use of the BIRD in our younger sample, and pilot data using the BIRD with an older adolescent sample support our extending the age range (R21 DA 22741; PI: Daughters).

Participant: CHILD
Estimated time to complete: 20 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Neural Correlates of Attention – EEG/ERP Tasks

Electrophysiological recording:
Event-related potentials (ERPs) represent transient changes in the electrical activity of the brain in response to a discrete stimulus event. They are recorded from electrodes placed on the scalp, and provide a tool for

evaluating the timing of mental events. In the current study, ERPs will be recorded using a 64-channel net (Electrical Geodesics Inc., EGI) and data acquisition software (NetStation 4.0, EGI). The Geodesic Sensor Net consists of an array of electrodes arranged in an elastic tension structure. The net contains small plastic pedestals, distributed evenly across the head surface. Each pedestal contains a small sponge, inside which there is an Ag/AgCl electrode.

Net application:
The participant's head will be measured, and a small mark will be made (with a grease pencil) at the very top of the participant's head to allow proper placement of the net. Before the net is placed over the participant's head, the sponges will be soaked in a salt water solution (distilled water + KCl + baby shampoo) until warmed to body temperature. The net is held in place by a chin strap. The Geodesic Sensor Net provides a very user-friendly system for recording EEG and ERPs. The elastic tension structure allows the net to be quickly and easily stretched over the participant's head. No cleaning or abrasion of the scalp is necessary, and there are no gels or creams to clean up afterwards. After removing the net, a small amount of baby oil is used to remove the grease mark. In general, these procedures minimize both the electrode application time and any discomfort to the participant.

Baseline EEG (EEG measure)
We will examine participants 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 [34], particularly in the domains of individual differences in approach and withdrawal tendencies e.g., [35].

Baseline EEG will be collected at the start of the EEG/ERP tasks in order to assess relative EEG power. Findings from the baseline BEIP assessment indicated that children in the institutionalized group showed a higher level of relative theta power and a reduction in alpha and beta power (suggesting delay) compared to age-matched community controls. We continued to see similar patterns at 42 months and 8 years, with children in the foster care group demonstrating higher alpha power than children in the institutionalized group. It will be important to determine if these effects are long-lasting and if the timing effects observed at 42 months and 8 years continue into early adolescence.

The addition of this measure will add approximately 10 minutes to Session 2. This measure addresses study aims 1, 2 and 3 and the data will be incorporated into analyses exploring the impact and timing of the randomization on brain development, as well as the association with psychiatric impairment.

- **Alpha Baseline**

Once fitted with the net, the child will be asked to sit in a chair.
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.

Participant: CHILD
Estimated time to complete: 6 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Flanker Task (EEG/ERP task)
We will employ the Flanker Paradigm, which is a computer-based task that assesses an individual's ability to inhibit a predominant response in the face of interfering stimuli. The stimuli consist of four different arrays of arrows which compose either congruent (>>>>> or <<<<<) or incongruent (>><>> or <<><<) trials. One of the four arrow arrays will appear on each trial and the participant's goals is to press a key corresponding to the central arrow in the array. The ability to resist the distracting stimuli and exert cognitive control is measured by comparing accuracy and reaction time differences between congruent and incongruent trials.

The flanker task also assesses behavioral and physiological correlates of error monitoring (i.e. post-error slowing in reaction time and the error related negativity; ERN). The ERN is defined as the negative most deflection in a 50-150 ms time window after response execution (button press). The task consists of 480 test trials presented in three blocks of 160 trials each. Prior to presentation of the test blocks subjects are given a short practice round to become accustomed to the task. Reaction time and accuracy on each trial will be recorded along with ongoing EEG for creation of ERP components (i.e. the ERN).

Participant: CHILD
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

The Go – No Go Paradigm (Inhibitory Control Task) EEG/ERP 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 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Emotional Intensity Task (EEG/ERP task)
Participants will be presented with visual stimuli consisting of two sets of emotional facial expressions that were used in protocol 06-10-0455 (Bucharest Early Intervention Project: Developmental Follow-Up, under the "Behavioral Face Discrimination Task"). Each set consists of a neutral facial expression and three series of facial expressions (one for each of three emotions: happy, angry and fearful) of increasing intensity (including from 20%, 40% and 60% intensity of emotional expression). Therefore, each set consists of a neutral and nine emotional expressions (three emotions X three levels of intensity).

The collection of event-related potentials requires the repetition of stimuli in order to obtain an averaged brain response across multiple trials. Therefore, during the session, each face (1 neutral face + 9 emotional faces = 10 faces) will be repeated 25 times for a total of 250 trials (10 faces x 25 trials = 250 trials). Images of faces will be shown on the screen in random order so as to control for the effects of image order. Children will not be asked to make any response to the images on the screen.

Participant: CHILD
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Dot Probe Task (behavioral task only)
This task was first designed by MacLeod et al. [36]. In this task, two stimuli, one threat-related and one neutral, are shown briefly together and their offset is followed by a small probe in the location just occupied by one stimulus. Participants are required to respond as fast as possible to the probe without compromising accuracy. Response latencies on the task provide a "snap-shot" of the distribution of the subject's attention, with faster responses to probes presented in the attended relative to the unattended location. Attention bias to threat is evident when participants are faster to respond to probes that replace threat-related rather than neutral stimuli.

The face stimuli will be photographs of 16 different individuals (8 male, 8 female) taken from the NimStim stimulus set [37]. Previous work with these stimuli performed by Fox and colleagues demonstrates that these

face stimuli effectively elicit threat bias. Three different pictures of each individual will be selected depicting angry, happy, and neutral expressions. Participants will be presented with pairs of faces (neutral-angry, neutral-happy, or neutral-neutral). Each face pair will comprise of pictures of the same person. Following the parameters of Mogg and Bradley [38] and our own prior experimental work, each trial will begin with the presentation of a fixation display (white cross 2*2 cm at the center of the screen), on which the participants will be requested to focus their gaze. The fixation display will last 1000 ms and will be followed by a face pair display for 500 ms.

Each face photograph will subtend 55 mm in width and 80 mm in height. The face photographs will be presented with equal distance to the left and right of the fixation cross, with a distance of 16.5 cm from the center of one face to center of the other. Immediately following the faces display a target probe will appear for 100 ms, after which the screen will go blank. The target-probe display will consist of two dots distant from each other by 5 mm center to center. Each dot will subtend 2 mm in diameter. The dot pair is oriented either horizontally (..) or vertically (:) and will appear at a distance of 8.5 cm either to left or to the right from fixation (center to center), that is, at the location of the center of either the left or the right photograph of each pair. Participants will be required to determine the orientation of the dots (horizontal or vertical) by pressing one of two pre-specified buttons on a response box. A new trial will begin 1,400 ms after target probe offset. We selected to use a probe-discrimination task instead of a probe side location task because it allows better control of the Simon effect. The Simon effect states that reaction times are usually faster when stimulus and response occur at the same location than when they do not, even if the stimulus location is irrelevant to the task. Thus, to neutralize this effect in the dot-probe task one needs to counterbalance the configuration of the responding fingers/hands to left/right target's location. However, if the response is based on probe side location, counterbalancing could create an interference condition for half the participants (e.g., responding with the left hand to a right side target). This problem is resolved if subjects are asked to discriminate (:) from (..) instead. Across trials, the angry or happy face will equally likely be on the left or on the right, and the dots orientation will equally likely be horizontal or vertical. These two variables will be randomly mixed in presentation.

There will be a total of 320 trials, presented in four equal blocks of 80 trials each. Participants will be presented with 128 Angry/Neutral, 128 Happy/Neutral, and 64 Neutral/Neutral pairs. The attention task will be presented on a 17 inch computer monitor controlled by the E-Prime software package. Trials will be counterbalanced across emotion face location, probe location, probe orientation, gender of face, and type of emotion.

Participant: CHILD
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Growth Measurements
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 min
Present during Administration: Research Assistant, Child and Parent/Caregiver

Disturbances of Attachment Interview (Early Adolescent Version) (DAI-12 Early Adolescent Version; Smyke & Zeanah, 2011)
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 12-13 years, we will be most concerned with signs of indiscriminate/disinherited attachment and risk-taking behaviors.
The wrong version of the DAI was submitted originally. It is important that the correct version of the interview for the child's age be administered. The Early Adolescent version of the DAI differs from the School Age version of the DAI in Parts IV and V. The School Age version included questions regarding risk-taking behaviors. These questions are omitted in the Early Adolescent version and replaced with questions

assessing the child's emotional regulation/expression and actions toward others. These questions have implications for psychiatric impairment and it is important to include parental report of these behaviors.

The Early Adolescent version of the DAI is not much longer than the School Age version of the DAI and will add approximately 5 minutes to parent/caregiver activities in Session 2. This measure addresses study aims 1, 2, and 3 and data from this measure will be incorporated into analyses exploring the impact of the randomization on social-affective development, as well as the association with psychiatric impairment.

Participant: PARENT/CAREGIVER
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Parent/Caregiver

Life Experiences Interview
The Life Experiences Interview will be administered during Session 2 to assess each child's life history. This interview is roughly analogous to the 5 minute speech sample completed by parents and will be used as a measure of the parent child relationship and best friend relationship from the perspective of the child. Because many of the children in this sample have had atypical caregiving histories, it will be important to know how early placed children versus later placed children respond to this measure. We developed this measure specifically for this study and will begin to finalize our coding scheme once piloting of this task is complete. The interview will be coded for negative comments, positive comments, warmth, negativity and attachment and social relationships.

The addition of this measure will add approximately 5 minutes to Session 2. This measure addresses study aims 1 and 3 and the data will be incorporated into analyses exploring the impact of the randomization on social-affective development, as well as the association with psychiatric impairment.

Children will be asked to describe all the places they have lived, as well as to describe their best friend and primary caregiver. All questions will be presented as open-ended questions and researchers will ask the child if they can add any additional information before proceeding to the next question. The interview will be video/audiotaped for subsequent coding and analysis.

Participant: CHILD
Estimated time to complete: 5 min
Present during Administration: Research Assistant and Child

This Is My Child Interview – Revised
Whereas the Expressed Emotion interview administered in Session 1 measures warmth, criticism and emotional involvement, the This Is My Child Interview measures commitment, acceptance and degree of influence.

The addition of this measure will add approximately 10 minutes to the parent measures administered in Session 2. This measure addresses study aims 1 and 3 and the data from this measure will be used to determine if parental/caregiver commitment mediates the effect of early placement on outcomes.
The TIMC-R interview is a semi-structured interview lasting approximately 10 minutes. The interview consists of six basic questions relating to the mother-child relationship, as well as a seventh question regarding the mother's experience as an adoptive parent/foster parent/caregiver.

This interview will be administered by certified clinicians on our research team. Both clinicians have been thoroughly trained on this measure by Charles H. Zeanah, Jr., M.D., a child psychiatrist and co-PI on this investigation.

Dr. Zeanah will monitor interviewer fidelity and competency via biweekly skype discussions with the Romanian research team during piloting and data collection. Dr. Zeanah and the research team will review 2-3 videotaped interviews for pilot subjects who have completed the session and will discuss any issues. As data collection for this task begins, Dr. Zeanah will continue skype discussions with the research team and will periodically review videotapes of the interviews with the two administering clinicians in the lab.

All interviews will be videotaped and coded by two trained research assistants to establish inter-rater reliability. The demands of administering and coding this interview are roughly comparable to the DAI which we have used at every assessment age during the past 10 years. We have had no difficulty with fidelity of reliability with that measure.

Participant: PARENT/CAREGIVER
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Parent/Caregiver

Fostering Experience Interview
This interview will be administered to all foster caregivers participating in the study. We are interested in obtaining descriptive qualitative data about the experience of fostering to increase our understanding of the motivations, challenges and rewards of fostering children in a setting in which this is an unusual thing to do. The interview will take 15 minutes to adminster and includes questions such as, "How quickly did (child's name) adapt to your family?" The interview will be videotaped for subsequent transcription and coding.

Participant: FOSTER PARENTS ONLY
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Parent/Caregiver

Session 3

Diagnostic Interview Schedule for Children (DISC-IV)
The DISC is a fully structured diagnostic instrument that assesses 34 common psychiatric diagnoses of children and adolescents. The DISC is designed for interviewer administration – either by lay interviewers or by clinicians or by self-completion.

The DISC-IV has been designed to obtain information about Diagnostic and Statistical Manual - IV (DSM-IV) diagnoses, essentially by ascertaining the presence or absence of symptoms. The instrument uses the diagnostic criteria as specified in DSM-IV (with DSM-III-R, and ICD-10 in development. In addition, the DISC is DSM-IV loyal and all symptom criteria must be met to meet the diagnosis. Both diagnoses in past month (embedded current) and in the past year are coded and recorded. Past year, current (past 4 weeks) & Whole Life (optional). The DISC-IV assesses diagnoses that have occurred anytime in last 12 months. Continuous symptom scales and impairment (6 impairment domains and 3 levels of severity) are also coded.

In clinical populations, the DISC requires 90-120 minutes to administer; in community samples, it requires about 70 minutes. Time of administration, of course, is dependent upon number of diagnostic modules administered and number of symptoms endorsed.

The validity of the DISC-IV was originally established in large-scale epidemiological surveys of children and adolescents, but it has since being used in many clinical studies, screening projects, and service settings.

For the purposes of this research, we will administer 13 sections of the interview (including components from anxiety disorders, miscellaneous disorders, mood disorders, disruptive behavior disorders and the Whole Life module) to both the parent/caregiver and child. An electronic copy of the child version of the DISC has been submitted to the CCI office.

Anca Radulescu, Sr. Research Assistant, will oversee all administered DISC interviews. Ms. Radulescu has received training on the DISC by Dr. Prudence Fisher at Columbia University. She will train the RAs who administer the parent and child versions of this measures (blind to participant status) to note and report to her at the completion of the interview (with parent/caregiver or child) any of the following: suicidal ideation, self-injurious behavior, threatening to harm others, serious risk taking behavior, significant impairment in any domain, or any child or parent who wishes assistance with the child's emotions or behaviors.

A referral will be made to an appropriate outpatient clinic in Bucharest. Any report to Anca Radulescu of a child with an identified concern will also be reported within 24 hours to Charles H. Zeanah, M.D., Co-PI, for review of findings and disposition.

Participant: PARENT/CAREGIVER and CHILD

Estimated time to complete: 120 min
Present during Administration: RA 1 and Child (Private Room 1) and RA 2 and Parent/Caregiver (Private Room 2)

Cambridge Neuropsychological Test and Automated Battery (CANTAB)

As we did at 8 years, we will again perform a number of tests from the CANTAB, focusing most on executive functions and memory. All CANTAB tasks run on a touch screen monitor and require minimal language and motor coordination skills (for reviews, see [39-41]. We wish to employ this task in order to examine associations between EFs in particular and specific forms of psychopathology (e.g., poorer planning behavior as indexed on the Stockings of Cambridge and poorer set shifting on the ID/ED shift task should be associated with poorer executive control and thus a higher prevalence of externalizing problems).

Motor Screening Test. The first subtest, a motor screening task, screens for visual, movement, and comprehension difficulties. A flashing cross is displayed on the screen in various locations, and subjects are instructed to touch it as quickly as possible.

Delayed Matching to Sample. This subtest assesses forced choice recognition memory for patterns. The subject is shown a pattern and then must choose out of four similar patterns which one exactly matches the original pattern. In some of the trials, the original pattern is obscured before the choices appear, or there is a brief delay between these steps.

Paired Associates Learning. This subtest assesses visual memory and new learning. A number of boxes are displayed, some with patterns inside, and after a brief delay the subject must identify where each individual pattern was displayed. If the subject does not identify each location correctly, the trial is repeated. As the subject progresses through the task, an increasing number of boxes and patterns are displayed.

Stockings of Cambridge. This version of the Tower of London planning task is a spatial planning task in which the subject must copy a pattern displayed on the screen by moving colored circles one at a time, using the fewest number of moves possible.

Spatial Working Memory. This subtest assesses the subject's ability to retain spatial information and to manipulate remembered items in working memory by locating tokens hidden in boxes. The subject is instructed that after a token has been found in a box, that box will not contain any tokens in the future. Subsequent stages include increasing numbers of boxes and tokens.

Intradimensional/extradimensional Shift Task (ID/ED Shift). This task (which was not employed at age 8) assesses discrimination and reversal learning under conditions in which the subject is required to shift attention to changing patterns of visual stimuli. A functional dissociation exists between the dorsolateral prefrontal cortex in between-category set shifting and the orbitofrontal cortex in within-category reversal shifts using this task. This task progresses along a series of stages of increasing difficulty. In the first stage (termed the 'simple discrimination stage), the child views two lined patterns on the computer screen. The child is told that one of them is correct and that the other is incorrect and that s/he must determine which the correct pattern is by touching one or the other. If correct, the computer will flash green; if incorrect, the computer will flash red. Hence, this stage requires the child to learn a two-alternative forced-choice discrimination of two lined drawings using immediate feedback provided by the computer. The child is told that as s/he works, a rule will become apparent that will guide the selection of subsequent choices. However, once the computer has determined that the child knows the rule, the rule will change. The child is told that despite these changes, s/he should try to make as many correct choices as possible. Learning criterion is six consecutive correct responses. After achieving criterion on the Simple Discrimination stage, the feedback provided to each stimulus is reversed so that the one first correct is now incorrect, and the one first incorrect is now correct (Simple Reversal). Again, there are six trials to criterion. At the third stage, a second dimension (purple shapes) is introduced together with the lined drawings so that each stimulus now contains two drawings--one lined and one shaped. To succeed on this Compound Discrimination (CD) condition, the subject must continue to respond to the previously relevant lined drawing while ignoring the presence of the new irrelevant dimension (purple shape.). Two CD conditions are administered, one where the lined and shaped drawings are distinct and one where the same stimuli are superimposed upon one another. Following successful completion of these two conditions, there is a CD reversal. Through the CD reversal stage, the subject is viewing the same stimuli over and over again, trial-by-trial. The next (6th) stage

involves the first demand for an attentional shift. Termed the intradimensional (ID) shift stage, novel or never-seen exemplars of each of the two dimensions (line and shape) are introduced, and the subject must continue to respond to the previously relevant dimension (lined drawing). Success on this stage requires that the subject generalize previous learning (e.g., "lined drawings are correct") to new stimuli. Following another feedback reversal shift (IDR), the second demand for an attentional shift is required. This stage is termed the extradimensional shift (EDS). Once again, novel exemplars of each stimulus dimension are presented. In order to succeed at the ED stage, the subject must shift response set from the previously relevant dimension (lined drawing) to the previously irrelevant dimension (purple shape). This shift requires that the subject learns and responds to a new rule (e.g., "Lines are no longer correct--Shapes are correct"). This stage is presumably analogous to the types of category shifts that are required by standard neuropsychological tests of set-shifting ability such as the Wisconsin Card Sort. The final task stage is a reversal of the ED shift. Each response made by the subject is presumably influenced by the feedback (correct versus incorrect) that s/he receives on the previous trial.

Participant: CHILD
Estimated time to complete: 45 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Facial Emotion Identification Task

This task will evaluate children's sensitivity in detecting different facial emotions. Previous research has shown that the ability to recognize facial expressions develops into adolescence [42]. 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 [43]. 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 [44]. For this task, children will be presented with morphed images created by Gao and Maurer (in press) using the MacBrain Stimulus Set [45]. These stimuli have been morphed to produce linear continua of images between a neutral face and extreme versions of four emotions – happy, sad, fearful, and angry, creating continua that vary in emotional intensity. Images from the four facial emotion continua (Neutral-Happy, Neutral-Sad, Neutral-Fearful, Neutral-Angry) will be presented to children one at a time, in random order, and they will be asked to sort the images into containers that represent the five different emotions (Neutral, Happy, Sad, Fearful, Angry). Children have as long as they want to decide which emotion the image is portraying and place it into the correct container. Each continuum is comprised of 11 images, so there are 44 images in total for children to sort.

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Security Scale Interview [46,47] This is a self-report measure designed to assess children's perceptions of security in parent-child relationships in middle childhood and early adolescence. This measure consists of 15 items that evaluates the degree to which children believe an attachment figure is responsive and available, their tendency to rely on the attachment figure in times of stress, and their reported ease and interest in communicating with the attachment figure. We will administer this measure as an interview (read to the child) to control for variable reading skills in the sample. The 15 items will be scored from 1-4, with higher scores indicating a more secure attachment. The measure requires 5-10 minutes to complete.

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Assessment of Pubertal Status (Morris & Udry, 1980)
This puberty assessment scale (18 questions for boys; 20 for girls) asks adolescents to answer a variety of questions on a five-point scale. Examples include, "How often do you shave?" and "Does your voice crack?" Children will also be shown a series of pictures depicting different stages of physical development in breasts,

genitalia, and pubic hair (Tanner, 1962) and asked to circle the picture that best resembles their current appearance. The instrument also assesses the individual's social-emotional reaction to puberty through items such as "How happy are you with your weight?" and "How happy are you with your overall body build?"

Participant: CHILD
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Self-Report Coping Measure (Causey & Dubow, 1992)
This questionnaire assesses children's coping skills in two stressful situations (one social, one academic) and includes five subscales: Seeking Social Support (16 items; e.g., "Get help from a friend"), Self-Reliance/Problem-Solving (16 items; e.g., "Decide on a way to deal with a problem and do it"), Internalizing (14 items; e.g., "Just feel sorry for myself"), Externalizing (8 items; e.g., "Get mad and throw or hit something"), and Distancing (14 items; e.g., "Forget the whole thing"). Relevant items will be averaged for each individual subscale.

Participant: CHILD
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Child

MacArthur Health and Behavior Questionnaire (HBQ version 2.1 (for late childhood and adolescence); Ablow et al., 1999; Essex, Boyce, Goldstein, Armstrong, Kraemer, & Kupfer, 2002; Luby et al., 2004)

The HBQ consists of approximately 140 items regarding child functioning. This questionnaire is administered to the child's 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.

The parent version of this measure should have been included in the original protocol. While this questionnaire was not completed by parents/caregivers at age 8, we felt it was important to include at age 12 so we may assess adaptive social functioning and physical health in children who do not attend school and/or those children whose parents do not want teachers to complete the measure. (Note that as much as we would welcome the opportunity to have a more direct measure of child health [either through pediatric exam or chart review], it will prove impractical to do so and thus we must resort to parent report.)

The addition of this measure will add approximately 25 minutes to the parent/caregiver measures in Session 2. This measure addresses study aims 1, 2, and 3 and data from this measure will be incorporated into analyses exploring the impact of the randomization on social-affective development, as well as the association with psychiatric impairment.

Participant: TEACHER and PARENT/CAREGIVER of child
Estimated time to complete: 25 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER, Teacher

Social Skills Rating System (SSRS; 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.

The parent version of this measure should have been included in the original protocol. We administered this measure to parents/caregivers and teachers at age 8 and felt it was important to ask parents/caregivers to complete this measure at age 12 so we can obtain a more complete and longitudinal picture of each child's social behaviors and academic performance.

The addition of this measure will add approximately 10 minutes to the parent/caregiver measures in Session 2. This measure addresses study aims 1, 2, and 3 and data from this measure will be incorporated into

analyses exploring the impact of the randomization on social-affective development, as well as the association with psychiatric impairment.

Participant: TEACHER and PARENT/CAREGIVER of child
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER, Teacher

Demographic Questionnaire
Because a number of children in the BEIP sample have experienced numerous placement transitions since the initial randomization, we will administer a new demographic questionnaire. This questionnaire consists of 20 items and includes questions about family income, marital status, length of time the participant has lived with the adult completing the form and number of other individuals living in the household.

Participant: PARENT/CAREGIVER of child
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER

Resistance to Peer Influence Questionnaire
We felt it was important to add this measure to the protocol because the importance of peer influence increases during early adolescence and is a hallmark of adolescent psychosocial functioning.

The addition of this measure will add approximately 5 minutes to Session 2. This measure addresses study aims 1 and 3 and the data will be incorporated into analyses exploring the impact of the randomization on aspects of social-affective development, as well as the association with psychiatric impairment.

This questionnaire consists of 10 items in which the participant is presented with two scenarios and asked to select which scenario best matches themselves. For example, "Some people think it's better to be an individual even if people will be angry at you for going against the crowd BUT other people think it's better to go along with the crowd than to make people angry at you." Items will be summed for a total score.

The Resistance to Peer Influence Scale was generated by a group of developmental psychologists with expertise in adolescent psychosocial development and pilot tested with small samples of high school students and college undergraduates and has been used with individuals aged 10 to 30 years (Steinberg & Monahan, 2007).

Participant: CHILD
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and CHILD

Preparation for Peer Evaluation Task in Session 4:
Before Session 3 ends, the Research Assistant will tell the child briefly about the Peer Rating Task that will take place in Session 4 (see description in 'Session 4' tasks below). Each participant will be told that when they return to the lab for their final visit, they will look at pictures of children their age, learn a little about each child they see and decide which of the children they would like to meet. The RA will ask each participant if it is okay to take their picture and ask them several questions about things they like so that their picture can be shown to other kids in the study. Children who agree to have their picture taken will be asked several questions such as, "Do you like to play sports? What is your favorite color? Do you like to play video games?"

Session 4

During this session, we will use 3 tasks designed to elicit physiological reactivity. These tasks fit within a passive/active and a non-social/social crossed two-by-two design. This design is as follows: One passive/social task (Peer Evaluation Task), one active/non-social task (Computer Game Task), and one active/social task (Trier Social Stress Test for Children; TSST-C).

	Social	Non-Social

Passive	Peer Evaluation Task	No task that is age/population appropriate
Active	Trier Social Stress Test for Children (TSST-C)	Computer Game Task

The goal of assessing both passive, active, social, and non-social tasks is to determine how early adversity shapes children's physiologial responses across a variety of situations. Although children are faced with both social and non-social challenges in their day-to-day lives, some of which require an active response (e.g., answering a question in class) and some of which do not (e.g., being excluded by peers), prior research on early adversity and physiological reactivity has tended to focus on either social or non-social tasks without consideration of the active or passive nature of the task. For example, many studies have administered the TSST-C to children exposed to adversity. However, because this task is both social and requires an active physiological response to perform the task (i.e., mobilizing physiological resources to deliver a speech), reactivity to this task provides no information about how children respond to a variety of other kinds of social situations that are more commonly experienced in daily life, including peer interactions and family relationships. In addition, to capture the full spectrum of physiological reactivity, it is important to include tasks for which mobilizing a physiological response is not necessary to complete the task (e.g., peer evaluation, playing a computer game).

Although these sorts of tasks have been widely used in pediatric research, they have rarely been examined in previous research on early adversity and physiological reactivity, despite the ubiquity of these situations in children's lives (e.g., taking a test, learning about a bad grade, waiting in line, etc.). The use of both social and non-social, as well as active and passive tasks together in the same study represents one of the primary innovations of the study design proposed to the National Insitute of Health. We have explicitly excluded a non-social, passive task. Tasks of this sort exist (e.g., the cold pressor task which asks children to hold their arm in cold water for as long as possible). We did not include this kind of task, because we did not think there was a task that was age-appropriate or population-appropriate. We also have strived to reduce participant burden to the greatest extent possible.

Health Assessment

After consent is obtained, caregivers/parents will complete a brief health assessment of their children. Because of the hormone measures we will be obtaining we need to ascertain the presence of medical problems that may affect the physiological and hormone measures.

The research assistant will ask whether the child has had mononucleosis, malaria, or surgical procedures that required anesthesia in the past 3 months. The research assistant will record the yes or no answer. Regardless of the answer, participants will continue with the study, and for participants who replied affirmatively we will forgo the hormone sample assays. Participants' parents will also be asked if the child is currently taking any medications or if they have a pacemaker. Children with a pacemaker will be excluded from the autonomic recording portion of the tasks.

Participant: PARENT/CAREGIVER
Estimated time to complete: 3 minutes
Present during Administration: Research Assistant and Parent/Caregiver

Autonomic Measures

Participants will be connected to the physiological recording equipment after completing the sorting portion of the Peer Evaluation Task and completion of the Baseline Affect Questionnaire (see below). Autonomic nervous system (ANS) responses will be measured throughout the duration of the session using a variety of instrumentation including: electrocardiograph (Standard Lead II configuration, right arm, left leg, right leg ground), impedance cardiograph (four tape sensors: inner sensors are placed at the xiphisternal and base of the neck, outer sensors are placed 3 cm distally to the inner sensors), skin conductance (obtained from two fingers of the non-dominant hand, skin temperature (obtained from one finger of the non-dominant hand) and continuous blood pressure monitoring through an upper arm cuff. The sensors and tape are non-invasive and feel like band-aids when pulled from the skin. Participants' ANS responses will be obtained for the duration of all studies. Drs. McLaughlin and

Sheridan will train all Romanian research assistants who handle ANS equipment on physiological recording, monitoring, and safety. Immediately following each participant any reusable ANS equipment (BP arm and wrist cuff, skin conductance and skin temperature) are cleaned thoroughly. The skin sensors are removed from the electrical leads and immersed in rubbing alcohol. We then use small Q-tips to remove any skin conductance cream residue from the lead. Following the rubbing alcohol we clean off the leads with soapy distilled water. All participants are given hand sanitizer immediately following removing the sensors.

Initial Baseline Recording
Prior to connection to the electrophysiological equipment, participants will complete the Baseline Affect Questionnaire to rate their overall cognitive appraisals of the situation, mood and emotional reactions. Once connected to the physiological recording equipment, participants will provide a saliva sample (see ***Saliva Collection*** below) and complete a five-minute baseline period to record autonomic activity.

Participant: CHILD
Estimated time to complete: 30 minutes (includes time for baseline affect questionnaire, saliva sample collection, connection to physiological equipment and baseline recording)
Present during Administration: Research Assistant and Child

Saliva Collection
We will measure saliva samples four times during the experiment. Research assistants wear latex gloves during the collection and storage of saliva samples. The measurement of these salivary hormones is pain free and non-invasive. We will use the drool method to collect saliva for cortisol and hormone assays, as the cotton in cotton swabs can interfere with measurement of anabolic steroid hormones. Participants will spit, or drool, into IBL tubes. Saliva will be assayed to assess levels of cortisol and other hormones and proteins in your body that are influenced by stress. Cortisol levels rise immediately with heightened arousal, but peak in the saliva after 20 minutes. Because we would like to measure levels before, during, and after various lab activities, we will take saliva samples from the participants at several points during the study: 1) baseline and 2) 20 minutes following each of the tasks (Peer Evaluation Task, TSST-C and Computer Game Task).

Participant: CHILD
Estimated Time to Complete: less than 2-3 minutes per sample collection
Present during Administration: Research Assistant and CHILD

Tasks
Each task described below includes a baseline period followed by a task period, allowing us to examine changes in physiological responses during the task as compared to baseline. The baseline period involves the participant sitting quietly for several minutes. The inclusion of a baseline meets two goals: 1) it allows us to examine changes in physiological responses during the task as compared to the participant's typical level of physiological activity; and 2) it provides time for the participant to return to their physiolgical baseline (i.e., their typical level of activity) before introducing a new task.

In our own experience and in previous studies using similar tasks, a return to baseline typically occurs within 1-2 minutes after each task. To ensure that each participant has returned to their physiological baseline we will confirm that their systolic blood pressure is within 5 points of the initial value acquired on their arrival in the laboratory before moving from the baseline period to the task.

Following the initial Baseline Recording Period, participants will complete the three tasks (one passive, two active) described below. Physiological recordings will be conducted continuously throughout these tasks. Following the conclusion of each task, participants will be asked to complete the Post-Task Questionnaire (1-2 mins to complete) to indicate their emotional and cognitive reaction to the task. Additionally, saliva samples will be obtained 20 minutes after the conclusion of each task to measure cortisol response across the session.

Peer Evaluation Task (passive/social task)

Peer evaluation will be manipulated using a previously validated task used with children aged 5-17. Participants will be told that children from all over Romania are taking part in a study examining how children make friends. The participants will then be told that they are going to look at pictures of children to decide who they would like to spend time getting to know. They will be told that if they are a 'match', that is they both want to meet with each other, then they will have an opportunity to get to know each other at the end of the session. A research assistant will take the participant's picture and the participant will be asked 3 short yes/no questions about their interests (for example: What is your favorite color? Do you like to play sports? Do you like to play video games?). During the process of piloting and in discussion with the research assistants in Romania, we will identify three questions that are most appropriate for Romanian children. They will be told that the picture and the answers to the questions will be sent to the other children and that they will later find out if those other children wanted to get to know them.

The children will then be given thirty laminated cards showing color photographs of children around age 12 (half male, half female) in smiling poses. "Answers" to the 3 questions asked of the participants will be displayed on the back of the cards. The participants will then be shown two large poster boards (Boards A & B) that they will use to organize their ratings of the pictured children. Board A will be red and have room for 20 pictures, and Board B will be green and have room for 10 pictures. The participants will be told that they should choose which of the pictured children they are interested in getting to know. If they would like to get to know a pictured child (Interested), the participant places the picture on Board B (the green board). If they do not wish to get to know a pictured child (Not Interested), they place the picture on Board A (the red board). During this process, children will be given five cards at a time and will be allowed to move pictures across categories if they want. Participants will be required to designate 10 of the pictures as Interested and 20 as Not Interested. Participants will be told that the other children are rating them in the same way and their answers will be sent in an email to the experimenter during the session.

Participants will complete the sorting portion of the task at the beginning of the study. They will then be hooked up to the physiology equipment so that their heart rate and cardiac impedance can be recorded. An initial 5 minute baseline will be acquired. After this, the participants will see each of the pictured children again on the boards they used to sort their preferences: Board A (Not interested in meeting them; red), and Board B (Interested in meeting them; green). Next the participant will be told that the experimenter is looking at data from each of these other children to see how they rated the participant. The experimenter will remind the participant that if they have a 'match' (i.e., a child who they are interested in meeting is also interested in meeting them), that they will meet the other child at the end of the session.

A set of two additional boards (Boards C & D) will be brought out. These boards will also be red and green and be labeled "YOU" at the top. The participant will be told that the pictures are now going to be sorted to show how each of the pictured children rated the participant. The experimenter will go through each of the pictures one-by-one, moving them from the red and green boards the participant used to rate the pictures to the new boards that reflect the supposed ratings of the participant by the pictured children. The primary psychophysiology measurement will occur for the first minute of the sorting. During this minute, the research assistant will move 5 of the 10 pictured children that the participant was interested in meeting on the green board (Board B) to the red board (Board C), indicating that children were not interested in speaking with the participant. The research assistant will then move pictures of 10 of the children that the participant was not interested in meeting (Board A). 6 out of 10 of these children will be moved to the red board (Board C), indicating they were also uninterested in meeting the participant. 4 out of 10 of these children will be moved to the green board (Board D), indicating that they are interested in meeting the participant. The research assistant will then move back to the children that the participant was interested in meeting (Board B), and move the remaining 5 pictures to Board C, indicating that none of these children were interested in meeting the participant. Psychophysiology will also be measured during this portion of the task. Finally, the research assistant will move the remaining 10 children who the participant was not interested in meeting (Board A). 6 out of 10 of these children will be moved to the red board (Board C), and 4 out of 10 will be moved to the green board (Board D). The participant will be told that there were no 'matches;' that is there were no children who the participant wanted to meet who also wanted to meet the participant.

Participant: CHILD
Estimated Time to Complete: 30 minutes (includes time for baseline recording, task completion, post-task questionnaire and recovery),

Present during Administration: Research Assistant and CHILD

Stimuli Development for Peer Evaluation Task: To create stimuli for this task, research staff in Romania will take photographs of 40 children (20 male, 20 female) in Romania between the ages of 12-13. Because of differences in facial features, clothing, and hairstyles between children in the United States and Romania, pictures of similarly aged children in Romania will be used to ensure cultural sensitivity and relevance of the stimuli for the Romanian participants in the study. Pictures will be taken from the shoulders upwards to create the stimuli to be used in this task.

Participants for the stimuli development will be recruited from local elementary schools and community programs with which our research laboratory has already established relationships. A recruitment letter has been included with this submission. All participants will be asked to sign a photo release (also included), allowing researchers use of their pictures as research stimuli and in scientific meetings and presentations.

All children will be photographed against a white background wearing a gray t-shirt provided by the research laboratory. 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.

A break will be taken between completion

Trier Social Stress Task for Children (TSST-C; active/social task)**
The TSST-C is the most widely used task to elicit physiological responses from children [48]. This task has been used in numerous studies with children and adolescents, including children as young as 6 [49-65]. The task involves delivering a verbal performance (either a speech or completion of a story) and a subtraction task completed out loud in front of two evaluators. The task reliably elicits a response in measures of autonomic nervous system activity (heart rate, blood pressure, skin conductance) and in measures of the HPA axis (cortisol, DHEA). Importantly, the task has frequently been used to examine physiological reactivity in vulnerable populations. These have included children exposed to physical and sexual abuse, including children who have been placed in the child welfare system following removal from parental custody [60,61], children who were born pre-term [65], and children with mental disorders—including depression, anxiety, externalizing disorders, and autism—[51,52,55,57,58,61-64]. This task has also been used in a sample of previously institutionalized children (aged 8-10) from Romanian orphanages who were later adopted by families in the United States [59].

The TSST-C takes 15 minutes to complete, and consists of a speech delivery task and a mental arithmetic task in the presence of evaluators. The TSST-C formally begins by having the participant read along while the research assistant reads the instructions of the speech task aloud. Participants will be instructed that they will be delivering a speech to two "evaluators" (research assistants). They will further be instructed that the evaluators have been trained to pay attention to their speech as well as their delivery and body language. Prior to meeting the evaluators, the research assistant will confirm with the participant that they want to go forward with the experiment at this time. If participants are willing to go forward, two research assistants (one male and one female) will enter the room. The evaluators will reiterate the instructions just provided by the research assistant. The speech involves discussing their positive and negative qualities and the qualities of a good friend for 5 minutes. For example, participants will be asked to describe why they make a good friend, the qualities they have that make them a good friend, and the qualities they have that don't make them a good friend. After the evaluators inquire as to whether the participant has any questions, they will leave the room while the participant prepares for their speech. The participant will be allowed 5 minutes to prepare.

Once the questions are complete, the evaluators will re-enter the room and take seats across from the participant behind a table. A timer will be set for 5 minutes. The evaluators will then instruct the participant that he/she can begin the speech. The evaluators will be trained so as to display a neutral disposition toward participants. Specifically, they will not smile nor nod, instead they will display flat affect. If the participant stops talking before the 5 minutes is over, the evaluators will ask pre-specified questions regarding the respondents' positive and negative qualities and why they believe they make a good friend.

After five minutes the timer will sound indicating that the participant is to move on to the mental arithmetic task, which will be described by the evaluator. The participant will be instructed to count backwards as quickly as possible from 758 in steps of seven. The participant will be instructed to count backwards as quickly and accurately as possible. If the participant makes a mistake one of the evaluators will interrupt them and have them begin again at the original number. After five minutes of this task, the evaluators will instruct the participant that they have completed the task and the evaluators will leave the room. If participants make more than five errors in the first minute and are unable to get 5 numbers in a row correctly, the task will be modified to make it easier for the participant. Specifically, they will be asked to count backwards from 307 in steps of 3.

Following completion of the speech and math tasks, participants will be asked to complete the Post-Task Questionnaire and Attributions for Evaluation Questionnaire. This task will be videotaped for subsequent coding of task performance (speech = body movements, eye contact, speech disfluencies (um, ah, etc.) and math = correct responses).

Participant: CHILD
Estimated Time to Complete: 30 minutes (includes time for pre-task questionnaire, task completion, saliva sample collection post-task questionnaire, attributes of evaluation questionnaire and recovery)
Present during Administration: Research Assistant and CHILD

Pre- and Post-TSST-C RNA Collection

Clear evidence exists that early experiences can lead to changes in stress reactivity which may be the result of altered gene expression due to alteration in epigenetic mechanisms including methylation, acetylation and alterations in chromatin structure. To examine the influence of early adversity on stress reactivity we will explore differential gene expression in salivary samples of children with a history of institutional care compared to normal controls. We wish to look at the acute alterations in gene expression in children with a history of early adversity to begin to understand if there are lasting changes in the stress reactivity pathway are reflected in differential gene expression when these children are exposed to mildly stressful normative experiences. While examining gene expression changes is challenging, recent technological advances now permit the exploration of mRNA levels in saliva. Further, these studies will complement our studies examining epigenetic and genetic factors to provide a comprehensive pattern of the genetic involvement in physiological reactivity.

We predict that the TSST-C will elevate cortisol levels the most of these procedures and will collect two additional saliva samples using Oragene RNA kits. One sample will be collected at the initial baseline recording and the second sample will be collected 15 minutes after the completion of the mental arithmetic task in the TSST-C. The second sample collection will be coordinated with the collection of the cortisol sample. The sample collection is non-invasive and takes less than 5 minutes to complete. Each child will be asked to spit 1-2 mls into two separate Oragene collection containers. Capping the container releases a preserving fluid which then mixes with the saliva, making the sample stable for long-term storage. Samples will be mailed to Stacy Drury at Tulane University Medical School for analysis.

Computer Game Task (active/non-social) :**

Subjects also will participate in a task designed to be mildly frustrating but which does not require academic knowledge. In this task they will view numbers on a computer screen and are asked to press the corresponding number after each number appears on the screen. The participant has 500 milliseconds to respond correctly. On "correct" trials subjects will see a green smiling face and hear a positive noise and on "incorrect" trials they will see a red frowning face and a negative noise. After a three minute period with accurate feedback about performance, task difficulty will be increased by making the accuracy feedback inconsistent. 70% of the time feedback will be random or negative, making the task more frustrating.

Participant: CHILD
Estimated Time to Complete: 15 minutes (includes task completion, post-task questionnaire, and recovery)
Present during Administration: Research Assistant and CHILD

** In an ongoing study in Boston adolescents being conducted by Drs. McLaughlin and Sheridan, which is currently approved by the CHB IRB (IRB-P00000200), we use the TSST-C and the computer game task with

adolescents who have a wide range of adverse early-life and current family circumstances and mental health problems. These range from current parasuicidal behavior and recent hospitalization for suicidality to histories of exposure to sexual and physical abuse. In this study we have not yet experienced an adverse event. Most participants find the tasks challenging or mildly annoying or distressing. No participant or parent/guardian has complained about the experimental procedures at the end of the study or demonstrated undue or inconsolable distress during the procedures. Of the 95 participants we have run in Boston, 94 have provided us with contact information to call them for follow up visits, some of these visits have already been completed. Thus both of the proposed active tasks—which, by definition, are expected to elicit stronger physiological responses than the passive tasks—have been used without problem or undue participant burden in an ongoing CHB study of early adversity and adolescent mental health.

Self-Report Measures

Throughout the experimental tasks, participants' cognitive appraisals of the situation, mood and emotional reactions will be assessed. These measures will be administered after each of the study tasks. All of the study measures have been validated in previous research and have been used with adolescents aged 12-13. All measures have been used without problem in the ongoing study approved by the CHB IRB for adolescents exposed to maltreatment and violence in the Boston area (IRB-P00000200).

1) Baseline Affect Questionnaire (completed at baseline)
2) Post-Task Questionnaire (completed during recovery after each tasks)
3) Pre-Speech and Math Questionnaire (completed prior to Trier Social Stress Task)
4) Attributions for Evaluation (completed after Trier Social Stress Task)

Participant: CHILD
Estimated Time to Complete: the time to complete each of these questionnaires is included in the total time for each task described above
Present during Administration: Research Assistant and CHILD

Piñata Game

Following the study tasks, participants will engage in a fun and engaging game that is designed to ensure that they master the task and receive positive feedback at the end of the session. The task was developed by Dr. Nathan Fox, one of the primary investigators of the BEIP and is based on widely used reward anticipation tasks (Haber & Knutson, 2010; Scheres, Milham, Knutson, & Castellanos, 2007; Samanez-Larkin, Kuhnen, Yoo, & Knutson, 2010).

The task is a reward processing task that involves making a speeded response to a target in order to receive a reward. Each trial is composed of three stages: anticipation, response, and outcome. In the anticipation stage, subjects see a cue indicating the size of the potential reward for that trial; in the response stage, subjects have the opportunity to make a response; in the outcome stage, subjects see feedback indicating whether or not their response was fast enough to receive the reward. These three stages are presented in the context of a piñata whacking game. Subjects are told to whack at piñatas as quickly as possible to earn the stars inside, and that the number of stars they earn during the task will determine the size of the reward they receive at the end. In the anticipation stage, subjects see the piñata partially revealed at the top of the screen—the number of stars inside the piñata is visible, but subjects cannot yet hit it. In the response stage, the piñata drops to the middle of the screen and the subject has the opportunity to make a speeded button press response. In the outcome stage, subjects either see the piñata cracked open and the stars in a basket at the bottom of the screen, indicating a hit, or they see the intact piñata swinging off to the side of the screen, indicating a miss. The task was designed to be visually appealing and engaging for children. All stimuli were drawn in a colorful cartoon style, with piñatas of many different shapes and sizes. The task is also easy enough that all children are expected to perform well and receive positive feedback throughout.

Participant: CHILD
Estimated Time to Complete: 15 minutes
Present during Administration: Research Assistant and CHILD

The entire session is expected to take less than 2.5 hours, including at least one break. All participants will take a break after completing the Peer Evaluation Task and may request breaks at any other time during the

session. We will ensure that each participant has returned to their physiological baseline and is not experiencing distress before leaving the study.

Session Debriefing
A detailed debriefing session will occur at the end of the session to ensure that participants are aware of the purpose of the study tasks, understand that the feedback they received during the peer evaluation, speech, and math tasks was not based on their performance, and are not experiencing physiological arousal distress.

The debriefing pays specific attention to explaining that the peer evaluation task did not provide accurate feedback about whether other children wanted to meet the participant and that the evaluators were instructed to provide neutral feedback toward the participant during the speech and math tasks. During the debriefing, the evaluators return for a "reunion," which allows the participant to meet the evaluators in a very informal matter. Participants feel much better after meeting the evaluators. The evaluators each provide at least one type of positive feedback to the participant about their performance (e.g., I really liked the part of your speech when you said…"). Drs. McLaughlin and Sheridan have found this reunion to be a very important part of the study design. Participants are universally happy to receive positive feedback about their performance and to learn that they were not being evaluated during the study tasks.

Participants will be asked how they are feeling at the end of the debriefing to ensure that they are not experiencing any distress or arousal. We are confident that the study staff will be able to accurately assess the presence of distress in participants. The staff who will be running the study and conducting the debriefing have know they children for more than 10 years, and are quite adept at discerning how they are feeling. Most importantly, the children trust the study staff and feel comfortable disclosing personal information to them. Thus, we are confident that no child will leave the study with any lingering negative feelings.

We will also debrief the parents at the end of the study. The research assistant will provide a detailed debriefing regarding the purpose of the study and the tasks their child completed during the study. Parents will be given the opportunity to ask any questions they have about the study.

We will also tell parents about their child's resting heart rate and blood pressure. If there are any children who meet the clinical cut-off for hypertension (based on the baseline blood pressure assessment, prior to completing study tasks), we will inform the parents of this. Parents will be encourgaed to take their child to a physician to have their hypertension evaluated.

The debriefing scripts are based on the script being used in the previously mentioned Boston study (IRB-P00000200).

Participant: CHILD (and PARENT/CAREGIVER)
Estimated Time to Complete: 5-7 minutes
Present during Administration: Research Assistant and CHILD (and PARENT/CAREGIVER)

Session 5 – Annual DNA Collection

DNA samples will be collected **annually** (maximum of 3 collection points) from the participants in our study using buccal swab kits. The first sample (2 swabs) will be collected when the participant visits the research laboratory to complete Session 1. Two additional DNA samples (2 swabs at each collection) will be collected in the participant's home/living situation or in the BEIP Research Laboratory (whichever is most convenient for participants) each year by one of our research assistants. We will collect DNA samples annually from this point forward in order to be able to explore the trajectory of telomere length as a function of both early experience and life events in the intervening year.

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 at each annual visit).

All samples will be transported back to New Orleans, LA where the DNA will be extracted from the sample. Telomere, methylation and histone studies will all be done blind to all other measures. Telomere and histone

studies will be completed by Stacy Drury, MD, Ph.D., at Tulane University School of Medicine and methylation analyses will be completed by Amy Non, Ph.D., MPH at Vanderbilt University.

The DNA will solely be used for this study. DNA samples are coded when they are collected with the participant's study number and sent without any other identifying information. DNA will be destroyed at the completion of data analysis.

Each child will also be asked to complete the Life Events Scale at the time of each annual DNA collection. Children are asked to indicate if any of the events occurred in their lives within the last 12 months, and if yes, how stressful the event was and to what extent they felt they had control over the event. The questionnaire includes 30 items like dating, moving, death of a loved one, and changing schools. We will also ask children to complete the pubertal status questionnaire administered in Session 3 if their responses at the time of the first administration indicated that they had not yet entered/reached the final stage of pubertal development.

Additionally, parents/caregivers will be asked to provide a copy of their child's report card for the current academic year and complete a brief questionnaire related to their child's health and dental hygiene. These measures will allow us to better approximate school performance and overall health. We will also request parent/caregiver permission to obtain school attendance records (absences, suspensions, expulsions) from each participant's school.

Finally, we will photograph each child's face to complete a facial dysmorphology assessment to confirm findings at the study outset that no participants appeared to show signs of Fetal Alcohol Syndrome. These findings were based on a physical examination completed prior to each participant's baseline assessment. It is important for us to determine if any of the children currently enrolled in the study demonstrate facial features suggestive of prenatal alcohol exposure, and potentially meet the criteria for an alcohol related disorder (e.g., FAS (fetal alcohol syndrome) or partial-FAS. Per the diagnostic criteria of the Revised Institute of Medicine, in the absence of confirmed maternal alcohol use in pregnancy, a diagnosis of FAS can be rendered if the child demonstrates all three of the following features: ie, facial dysmorphic features, growth restriction, and microcephaly. A diagnosis of partial FAS can be provided if the children demonstrate typical facial dysmorphic features and abnormalities in 1 of the other domains (growth or central nervous system structure or function)(Hoyme, May et al. 2005). Should the children meet the diagnostic criteria for FAS or partial FAS, we will need to determine whether we need to account for this in our analyses.

Dysmorphic FAS Facial features are characterized by the presence of 2/3 of the following facial features as described by the Revised Institute of Medicine Criteria for FASD (Hoyme, May et al. 2005): (1) Palpebral fissure length (PFL)<10% compared with normative values; (2) Lip: scored 4 or 5 on the University of Washington Lip and Philtrum Scale; (3) Philtrum: scored 4 or 5 on the University of Washington Lip and Philtrum Scale.

Digital Photography Protocol: Digital photos will be taken, and the presence of FAS facial features will be ascertained using specialized digital photographic analysis software. Children will be photographed in a series of **4 photos** as follows:

1. Place ¾" (19.05 mm) sticker on forehead between eyebrows.
2. Eyes wide open, no glasses.
3. Lips gently closed, no smile
4. In **Photo 1 (frontal)**, child looking straight ahead.
5. In **Photo 2**, take a **¾ view** (45 degree) photo for better visualization of the philtrum
6. In **Photo 3**, take a **lateral view** of the face
7. In **Photo 4**, take a frontal photo **with the child looking UP:**

Photographic Analysis: FAS Facial Photographic Analysis Software(www.fasdpn.org) will be used to measure the PFL, and assess the morphology of the lip and philtrum from the digital photograph. The software automatically computes FAS facial features. Facial features will be graded on a 0-3 Likert scale quantifying number of facial features present (0,1,2,3), and demonstration of "Full FAS facies" will be characterized by the presence of >2 facial features (PFL <10%, or Lip, score of 4 or 5, or Philtrum score of 4 or 5, with Lip and philtrum morphology graded on the on the University of Washington Lip and Philtrum Scale)(Hoyme, May et al. 2005).

Participant: CHILD
Estimated time to complete: 30 min
Present during Administration: Research Assistant, Child and Parent/Caregiver

Session 6 (for participants meeting SCQ criteria)

During the BEIP Developmental Follow-Up (06-10-0455), our research staff expressed concern about some of the children in the sample and suspected that they had autism or an autism spectrum disorder. There were also several children that the staff attempted to evaluate but these children were so low functioning they could not complete all of the tasks. As a result, we requested and received permission to perform pediatric and neurological examinations on the children in the sample, as well as to administer the Social Communication Questionnaire (SCQ) to parents/caregivers. Following these examinations, we talked with our staff so appropriate referrals could be made for those children identified as needing follow-up care.

We will conduct follow-up clinical autism and neurological evaluations on those children whose age 8 SCQ total score was equal to or greater than 12. We chose this cut-off score because it is used to distinguish PDDs from other diagnoses (Berument et al., 1999). Children meeting this SCQ score criterion will be evaluated by April Levin, M.D, in the BEIP Research Laboratory in Bucharest, Romania. Dr. Levin accompanied us to Romania during the BEIP Developmental Follow-Up and currently serves as Chief Resident in Pediatric Neurology at CHB. She is also fluent in Romanian, making her an ideal candidate to administer the evaluations. We estimate that Dr. Levin will evaluate 20-25 children.

The purpose of this additional visit is to provide a clinical service to those participants who meet the SCQ criteria. However, we may use the results of the examination to determine if any of these participants should be excluded from subsequent data analysis.

The examination will begin with a brief general examination (listening to the heart and lungs, examining the abdomen for tenderness or organomegaly, and checking the skin for any birthmarks), to look for any abnormalities that can be associated with autism (GI abnormalities, ash leaf spots as in tuberous sclerosis, etc). 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 visual fields and visual acuity, fundoscopic examination, extraocular movements, facial sensation, symmetry of facial movements, auditory acuity, ability to shrug shoulders against the resistance of the examiner's hand, protrusion of the tongue, midline uvula and palate and intact voice. For motor function, Dr. Levin will observe the child walking, looking specifically for abnormalities such as asymmetries or unsteadiness. She will assess strength in all 4 extremities, proximally and distally, and will also assess muscle tone. She will also evaluate the symmetry and magnitude of tendon reflexes for the following: biceps, triceps, brachioradialis, knees, and ankles. She will also test the Babinski reflexes in the feet. To evaluate cerebellar function, Dr. Levin 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, she 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. The sensory examination may also involve position sense and temperature sense, if the children are able to cooperate. Evaluating sensation to pinprick does not involve any puncturing of the skin, and Dr. Levin will omit this from the examination if children appear fearful at all.

Dr. Levin will also take a history from each child's current caregiver. Autistic symptoms will be evaluated both by history and physical examination and based on current DSM criteria. We will videotape all examinations to code for more sublte behaviors of the child that occur in the examination. These videos will be viewed by a US collaborator to address questions related to borderline cases. Participation in this session is voluntary and the total time to complete this session will be 2 hours or less. All examinations will be scheduled at a time convenient for the child and parent/caregiver.

If medical problems or autism is noted, Dr. Levin will discuss these concerns with parents/caregivers, our Romanian research staff, and if necessary, the appropriate child protective authority serving as legal guardian of the child, so that appropriate follow-up evaluation and care can be provided in Bucharest.

Subjects will be compensated 40 RON for their participation in this component of the study. We will also provide transportation to/from the laboratory for all participants that agree to take part in this research session. We view participation in this research session as beneficial to each participant given that very few of the subjects receive well-child examinations on a regular basis.

g. Study Timeline (as applicable)

Piloting for this study will begin in July/August 2010. We anticipate that data collection will take 4 years to complete.

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 Busko communicates daily with the PI and staff at the BEIP Research Laboratory. Any adverse event will be reported immediately by the Romanian research staff to the PI and Elizabeth Furtado Busko. 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 staff of the BEIP Research Laboratory to ensure that all proper authorities in Romania are notified of any adverse event.

i. <u>If the Investigator is the Sponsor/Assignee (IND or IDE-holder)</u>, he/she is responsible for selecting a qualified monitor who will monitor the progress of all clinical investigations conducted under the IND or IDE. Please describe the monitoring plan for this protocol below:

↳ Note: the EQuIP office provides monitoring services and advice. For info, contact EQuIP @ 5-7052.

☒ Not applicable

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 BEIP Research Laboratory and carried by hand, as needed, back to the United States, for coding. Some data (e.g., psychopathology raw data) 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 BEIP project manager, Elizabeth Furtado Busko. All data will be coded by Romanian research staff and reliability checks will be performed before data entry takes place. All data will be double-entered into Excel files, read into SAS data files by the BEIP data manager and stored on a secure server dedicated to the project. 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 assistants responsible for collecting the proposed data have worked extensively with human participants in this age range. The research staff will receive additional training by a licensed psychologist on the administration of the proposed measures that are new to our repertoire (WISC, BART and BIRD). All assessments involving the use of standardized, clinical measures will be administered by trained Romanian psychologists on our staff. The Romanian research staff will be responsible for sending weekly 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

The Clinical Research Information Technology Core will be used as a central location for data processing and management. Children's Hospital Boston, with collaboration from a consortium of institutional partners, has developed a software toolset and workflow methodology for electronic collection and management of research and clinical trial data. REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the Clinical Research Program. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap servers are housed in a local data center at Children's and all web-based information transmission is encrypted. REDCap has been disseminated for use locally at other institutions and currently supports > 200 academic/non-profit consortium partners on six continents and 13,000 research end-users (www.project-redcap.org).

As data are collected, scored, and checked, they will be entered into the REDCap database to be matched with the data previously obtained on this sample in the original BEIP project. Management, integrity, and security of new data from the index sample, and of prior data from both index and community comparison samples, will be overseen by Jisun Jang(CHB), Matt Gregas (CHB) and Dr. Donald Guthrie (formerly of UCLA, now retired and a private consultant), the BEIP statistician since the inception of the project. They will provide close supervision over the statistical analyses described below, and will work closely with Elizabeth Furtado Busko, BEIP project manager, who has worked for the PI for 4 years.

The BEIP has established a practice of holding data in a central secure data base in both SAS and SPSS formats. These data are subjected to extensive quality testing to enhance their accuracy. Investigators download data from this data base for analysis of specific hypotheses. These steps help assure that all investigators use the same data values. The data manager has authority to make documented changes in data values if they are found necessary by users. Since we are working with a modest sample and there are a large number of measured outcomes, statistical methods will be limited to simple and direct approaches. Smaller samples preclude application of complex longitudinal analyses, but most of the hypotheses appearing with the Specific Aims can be addressed directly. It will, however, be impossible to develop large scale statistical models involving comparisons across measurement domains. Most of our hypotheses may be evaluated using t and chi-squared tests for two-sample comparisons, analyses of variance/covariance and logistic regression for higher order analyses, and simple and multiple correlations for measures of association. In cases where distributional assumptions are questionable, we will use either transformation or rank-based distribution-free approaches. The most frequent comparisons will be between subjects in the CAUG and FCG. Inference from these analyses will be supported by the randomization that took place at the outset of our study and thus these comparisons will involve an intent-to-treat rationale and any significant findings may be subjected to causal interpretation. We will conduct similar analyses involving the NIG for comparison purposes. These would be outside the randomized trial context.

It is not feasible within the limited space to project detailed statistical methods for every one of our hypotheses. However for each specific aim we provide a detailed analysis for one of the primary outcome measures as an example. Statistical analysis for the other outcome measures described above will follow the same broad outline. Specific statistical methodology will vary depending upon the nature of the measure (categorical or continuous, symmetrically or skewed distribution). Power analyses are provided below using the example measure.

Primary Analysis for Specific Aim 1: The primary hypothesis of this aim is that subjects in the FCG group will display lower levels of internalizing and externalizing problems then subjects in the CAUG. Internalizing problems are measured by the DISC anxiety symptom score and externalizing problems will be measured by DISC aggression symptom score. These scores are continuous. Since this is a randomized controlled trial we will analyze the data for

this aim using the Intent to Treat (ITT) principle. Under the randomization framework, subjects in each group would have the same characteristics except for the intervention. We may therefore test the hypothesis for this aim using a two-sample t-test. A separate test will be run for each outcome, DISC aggression symptom score and each DISC anxiety symptom score. The significance level will be set at 0.05.

Power Analysis for Specific Aim 1: This power analysis is based on the two-sample t-test. The alternative hypothesis is one-sided. The statistical hypothesis test is one-sided because the hypothesis is that subjects in the FCG group will display lower levels of internalizing and externalizing problems. The table below gives the power we have to detect a difference in standard deviation units (effect size). Equivalently the pooled standard deviation is set at one. We are assuming 60 subjects in each group which represents a loss of 8 subjects from each group. The table below lists the power needed to detect the effect size given in the first column. The significance level is set at 0.05.

Effect Size	Power
0.345	60
0.398	70
0.457	80
0.537	90

Primary Analysis for Specific Aim 2: In this aim we examine the "dose" effect of institutionalization on psychiatric outcomes. The dose will be measured as percent of life spent in an institution. The hypothesis is that higher the percentage of life spent in an institution will result in greater psychiatric symptoms. The psychiatric outcomes are internalizing problems (DISC anxiety symptom score) and externalizing problems (DISC aggression symptom score). These relationships will be examined through regression/correlation analysis. The DISC symptom score is the response and percent of time spent in an institution is the predictor. The ITT principle is no longer applicable in this analysis and we may need to consider the presence of confounders as covariates in a regression model including IQ, pubertal status, gender, birth weight, and head circumference. We will build models that include all marginally significant effects simultaneously but excluding nonsignificant effects. Model selection will be based on likelihood ratio tests and Akaike's Information Criterion (AIC). A positive partial regression coefficient for the percentage of life spent in an institution would be evidence in favor of our hypothesis.

Primary Analysis for Specific Aim 3: Subjects differed in age at the time of randomization, and we will examine the effect of the timing of placement into foster care on psychiatric symptoms. The hypothesis for this aim is that subjects placed into foster care at a later age will have higher level of psychiatric symptoms. Note that only the subjects who were randomized into foster care will be included in this analysis. As with the previous two aims the DISC symptom score is our primary outcome. The statistical analysis, including will proceed as described for specific aim 2. .

For all of the aims there are other measures of psychiatric symptoms that we will consider but are not part of our primary analysis. One example of such a measure would be a diagnosis of ADHD or number of symptoms of ADHD. All significant relationships will need to be confirmed in the presence of other meaningful relationships. Significant relationships found in isolation will be considered with caution.
Primary Analysis for Specific Aim 4: In this aim, we will examine the effect of institutionalization on telomere length. Our hypothesis is that children with a history of institutionalization (CAUG and FCG) will display shorter average telomere lengths and a faster rate of telomere length than children in the NIG. Both these hypotheses can be accessed in the same model. Telomere length will be the response and group as well as age of measurement will be the primary predictors of interest. To account for the longitudinal nature of the data we will fit a mixed model with subject specific intercepts and slopes for age. A significant main effect for group or a group by age interaction will be evidence of a group effect for hypothesis 4a. The direction of the interaction term and its significance will be evidence in favor of a decline.

Primary Analysis for Specific Aim 5: The primary hypothesis of this aim is that subjects in the FCG who demonstrated catch up growth in the first 12 months of foster care placement will have higher IQ, less psychopathology (externalizing/internalizing behaviors) and fewer risk-taking behaviors than other children in the FCG. Like the analyses discussed for specific aim 3, only subjects randomized to foster care will be included in this analysis. IQ will be measured by the WASI Full Scale IQ score. Internalizing problems are measured by the DISC anxiety symptom score and externalizing problems will be measured by DISC aggression symptom score. Risk-taking behaviors will be measured by the BART (sensitivity to rewards) and BIRD (distress tolerance). This may be modeled by regression equations with IQ (for example) as the response and growth during first 12 months of foster care as the primary predictor of interest. A positive value of the coefficient will be taken as evidence in favor of our hypothesis. Other

confounders or covariates might need to be controlled for. Model selection will be made on the basis of Likelihood Ratio Tests and AIC.
The analysis strategy for each of the specific aims related to physiological reactivity is outlined below:

We hypothesize that institutionalization is associated with:

a) a pattern of hypothalamic-pituitary-adrenal (HPA) axis reactivity characterized by a blunted initial response to a stressor followed by a delayed, and potentially incomplete, return to baseline

b) an autonomic nervous system (ANS) characterized by elevated sympathetic activation without concomitant increases in cardiac output efficiency (a "threat" response; [66,67].

c) In addition, we hypothesize that children randomized to foster care will exhibit a pattern of physiological reactivity that is more similar to community-reared children and

d) that elevated physiological reactivity mediates the association between institutionalization and psychopathology.

e) We anticipate that greater duration of institutionalization will be associated with greater dysregulation in physiological responses to stress.

f) We hypothesize that the timing of placement into foster care will influence long-term physiological reactivity to stress. We expect to observe a sensitive period, after which the foster care intervention has little beneficial effect on physiological stress reactivity.

Analysis Strategy:

Cortisol responses to the study tasks will be examined using multilevel modeling to allow us to characterize the cortisol response across the entire study. This approach will be used because cortisol responses habituate slowly (on the order on 20 minutes to one hour); thus, we will need to examine the magnitude and shape of the response across the entire study period. This approach is standard in the literature [56]. A series of two-level models (observations over time nested within persons) will be estimated. This approach will allow us to simultaneously estimate the variance in cortisol reactivity both within and between individuals over time [68]. We will first estimate an unconditional growth model that predicts cortisol (measured in nmol/L), with the initial value at the beginning of the study coded as zero, and subsequent time points coded as the number of minutes from baseline. This model will be specified as follows:

Level 1: $Cortisol_{ti}=\pi_{0i}+\pi_{1i}Time+e_{ti}$

Level 2: $\pi_{0i}=\beta_{00}+r_{0i}$
$\pi_{1i}=\beta_{10}+r_{1i}$

In this model, π_{0i} (the intercept) represents the level of cortisol at baseline, π_{1i} is the effect of time, and e_{ti} represents time-specific residual variance in cortisol for child *i* at time *t*. β_{00} represents the average cortisol at baseline across children, β_{10} represents the average slope (rate of change over time across the study), and r_{0i} and r_{1i} represent the random effects, or individual deviations from these mean values. We will add a quadratic term for time to the model to determine the functional form of the growth trajectory and test the difference in model fit between the linear and quadratic model using a chi-square test.

Next, we will examine the association between institutional rearing and cortisol reactivity. Specifically, we examine the association of institutional rearing with cortisol intercepts (value at baseline) and slopes (linear change over time during the study). This model will be specified as follows:

Level 1: $Cortisol_{ti}=\pi_{0i}+\pi_{1i}Time+\pi_{2i}Time^2+e_{ti}$
Level 2: $\pi_{0i}=\beta_{00}+\beta_{01}Institutionalization+r_{0i}$
$\pi_{1i}=\beta_{10}+\beta_{11}Institutionalization+r_{1i}$
$\pi_{2i}=\beta_{20}$

In this model, β_{01} represents the effect of institutionalization on cortisol at baseline, and β_{11} represents the effect of institutionalization on the rate of linear change in cortisol over time.

Multilevel modeling will be conducted using the Hierarchical Linear Modeling (HLM 6.0) software system[69] using full maximum likelihood estimation with robust standard errors and heteroscedastic Level 1 time-specific residuals.

b) Autonomic responses to the study tasks will be examined separately for each task. Unlike cortisol responses, the autonomic nervous system returns to baseline quite quickly following an arousing task (typically on the order of 1-2 minutes). Thus, autonomic reactivity will be examined by calculating change scores for each task: task value minus baseline value. This is standard in the literature [67]. Differences in autonomic reactivity will thus be examined using straightforward linear regression and univariate ANOVAs with institutional rearing as a between-subjects factor. We expect to see differences in total peripheral resistance and cardiac output reactivity as a function of institutional rearing.

c) To determine whether the foster care intervention had ameliorative effects on physiological reactivity, analyses will be performed only among children exposed to institutional rearing. We will evaluate whether, among the institutionalized group, the foster care intervention predicts cortisol slopes (i.e., reactivity to study tasks) using the same multi-level modeling approach described in point a above. We will evaluate whether the foster care intervention influenced autonomic reactivity using simple linear regression and univariate ANOVAs with foster care placement as a between-subjects factor among the institutionalized group.

d) A set of analyses will evaluate the hypothesis that autonomic reactivity mediates the association between early adversity and psychopathology. This hypothesis will be evaluated using standard tests of statistical mediation. Analyses will determine whether: a) institutional rearing is associated with adolescent psychopathology; 2) exposure to institutional rearing is associated with elevated autonomic reactivity to the laboratory-based stressors (described in point b above); 3) autonomic reactivity is associated with adolescent psychopathology (evaluated using linear regression for symptom counts and logistic regression for diagnoses); and 4) the association between institutional rearing and adolescent psychopathology is attenuated significantly when autonomic reactivity is added to the model. Mediation effects will be examined first for each aspect of autonomic reactivity independently (e.g., blood pressure, cardiac output). Sobel's standard error approximation will be used to test the significance of the intervening variable effect [70]. The product of coefficients approach is associated with low bias and Type 1 error rate, accurate standard errors, and adequate power to detect small effects [71]. To determine autonomic reactivity measures, together, mediate the adversity-psychopathology association a final model will be estimated which tests the significance of the mediator using a bootstrapping approach that provides bias corrected confidence intervals and allows multiple mediators to be examined in one model [72]. Confidence intervals that do not include zero indicate significant mediation.

e-f) Duration and timing effects will be examined only among the institutionalized group. Duration of institutionalization will be added as a continuous Level 2 covariate in multi-level models predicting cortisol response to the study session (outlined in point a above) and as a continuous covariate in linear regression models predicting autonomic reactivity (outlined in point b above). Sensitive periods will be examined by creating dummy variables coding different cut-points for timing of placement (e.g., 6, 12, and 24 months). These variables will be added one at a time to the models predicting cortisol and autonomic reactivity to determine whether timing of placement is associated with physiological reactivity.

Comments on Data Analytic Plan

Intent-to-Treat (ITT). Consistent with all RCTs, we have adopted an intent-to-treat approach to our data analyses thus far. Doing so preserves the strengths of randomization and protects against sample bias. However, we also recognize that only 14 children originally assigned to the care-as-usual group (CAUG) still live in an institution, and that only 30 children originally assigned to our foster care group (FCG) still reside in their original foster placement. In other words, as with many RCTs, there has been drift out of the original group assignments. However, it is also important to note that we are in contact with many of the children in the sample. Thus, on the one hand, our attrition is very low and on the other, there has been considerable change in group assignment. By adopting an ITT approach, we are in the strongest position possible to examine sensitive periods and more generally, the effects of early experience on brain and behavioral development. On the other hand, an ITT approach fails to recognize the reality of our children's current living conditions. Thus, as described above, we will violate ITT in many of our analyses which will permit us to test the competing hypothesis that it is not early experience that most matters in long-term outcome but rather, where children

are living at the time of the current assessment. *Note that in our preliminary analyses of our 8 year data, violating ITT makes no difference in the outcome in some domains whereas in others it does.*

d. Statistical Power and Sample Considerations

See above.

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. Katie McLaughlin, Ph.D., and Margaret Sheridan, Ph.D,, will be responsible for training the Romanian research team on all aspects of data collection related to physiological reactivity and will oversee data collection, processing and analysis of these data. Elizabeth Furtado Busko (US) will be responsible for all administrative oversight of this research and will work in closely with Jisun Jang, Donald Guthrie and Matt Gregas on data management, integrity, and security of new data and . 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

The CCI required that we establish a DSMB for a previous research study conducted with the same sample (protocol 06-10-0455) and this DSMB remains intact. The board consists of three individuals. The members include Dr. Alin Stanescu, Dr. Mihai Iordachescu, Jr., and Mihai Anitei, Ph.D. Dr. Stanescu currently serves as Senior Pediatrician at the Ministry of Health, Institute for Mother and Child Care (IOMC). He previously served as Deputy General Director and has extensive experience in clinical pediatrics, pediatric public health and research. He is committed to improving the current structure of the child protection system and quality of care for children in Romania. Dr. Iordachescu, Jr. is a pediatrician and is also based at the IOMC. He also practices at MedLife Hospital, a private hospital in Bucharest. He has served as a pediatric consultant to NGOs involved in child protection in Bucharest and has evaluated many children in foster care and/or institutional care. Dr. Anitei is Chair of the Faculty of Psychology and Educational Sciences and Director of the Center for Applied Psychology at the University of Bucharest. Each member of the DSMB lives and works in Bucharest, and is familiar with the rights of children and with children who are in care of the child protection system.

The group will meet at a minimum of every 6 months. Meetings will be organized by Elizabeth Furtado Busko and also attended by one member of the BEIP Research Laboratory to ensure that the group meets on schedule. Ms. Busko will distribute a comprehensive summary of all research activities that have transpired since the last DSMB meeting, including the number of families that have agreed to participate, dropped out or refused participation. The DSMB will have access to all BEIP research assistants and may review any data collected that is relevant to their roles. Minutes of each DSMB meeting will be recorded and shared with the PI and co-PIs of this study.

The group will be asked to do the following:

1) Review the research protocol, informed consent documents and plans for data and safety analysis. If any DSMB members have concerns about the research protocol, they will be asked to submit these concerns in writing to the PI within one week of their meeting.
2) Evaluate the progress of the study, including periodic assessment of data quality and timelines, participant recruitment, accrual and retention and other factors that may affect study outcome. This information will be provided to the DSMB by BEIP project staff on site.
3) Make recommendations to the PI and co-PIs concerning continuation, termination or modification of the protocol based on the observed beneficial or adverse effects of the research. These recommendations will be based on responses to questions on the session evaluation form (see attached). This form will be completed anonymously by parents/caregivers of the study participants at the conclusion of the study session. If the DSMB notes that a single adverse events has occurred or more than 5% of participants have dropped out of the study within a 6-month time period, they will contact the PI and co-PIs of the

study and the CCI to request an immediate suspension of the protocol in order to complete a comprehensive review.

4) Monitor rate of reported risk behaviors. Each time the DSMB convenes, the attending Research Assistant will report the number of times that confidentiality has been breached since the last DSMB meeting. If this number is greater than 15% of the number of participants that have completed the DISC since the last DSMB meeting the DSMB will notify the PI and Co-PIs of the study and the CCI and will ask our research team to contact the appropriate adults (parents, foster parents, DGASPC, etc) responsible for these participants to ensure that follow-up care has been scheduled.
5) Consider factors external to the study when relevant, such as scientific or therapeutic developments that may have an impact on the safety of the subjects or the ethics of the study.

Additionally, once approved, this protocol will be submitted to the institutional IRBs of the co-PIs (Fox and Zeanah) and the Ethics committee at the University of Bucharest for approval.

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. It should also be noted that this sample successfully completed sessions of this length for protocol 06-10-0455. Regular breaks (and snacks) will be scheduled during the 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.

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 9-12 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 (120 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 have measures of brain functioning from previous assessments. 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 have included 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 private rooms within the BEIP Research Laboratory.

9. Confidentiality Provisions

Signed consent and assent forms will be kept in locked cabinets in the BEIP Research Laboratory. All 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., DISC 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.

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

- ☐ Sponsor's Protocol
- ☐ Investigator brochure (3 copies)
- ☐ Flow charts, schemas
- ☒ Other (session evaluation form)
- ☐ Federal grant application (3 copies)
- ☒ Survey, questionnaires, assessments **
- ☒ Recruitment letters, postings, flyers
- ☐ Materials given to subjects (reminders, letters, thank-you, etc.)*

** see instructions for further information*

** Questionnaires

1) Modified Youth Risk Behavior Survey (YRBS) – see attached
2) Brief Sensation Seeking Scale (BSSS) – see attached
3) Friendship Qualities Questionnaire (FQQ) – see attached
4) Diagnostic Interview Schedule for Children (DISC) – electronic copy submitted to CCI office
5) Disturbances of Attachment Interview (DAI) – see attached
6) Security Scale Interview – see attached
7) Health Behavior Questionnaire (HBQ) – see attached
8) Social Skills Rating System (SSRS) – see attached
9) Pubertal Status Questionnaire – see attached
10) Interpersonal Competence Questionnaire – see attached
11) Self-Report Coping Questionnaire – see attached
12) Baseline Affect Questionnaire – see attached
13) Post Task Questionnaire – see attached
14) Pre Speech and Math Questionnaire – see attached
15) Attributions for Evaluation – see attached
16) Fostering Experience Interview – see attached

Part B: Experimental Design and Protocol – ALL APPLICANTS MUST COMPLETE THIS FORM
AGE 16 FOLLOW-UP

Title of Protocol: Bucharest Early Intervention Project (BEP): Effects of early psychosocial deprivation on mental health in adolescence

Name of PI: Charles A. Nelson, Ph.D.

1. Specific Aims /Objectives

The Bucharest Early Intervention Project (BEIP) is the first randomized controlled trial (RCT) comparing foster care to institutional care in young children with histories of profoundly adverse early experiences. Major findings demonstrate brain and behavioral abnormalities associated with institutional rearing (compared to no institutional rearing), advantages of foster care over institutional care for most developmental domains, and evidence of sensitive periods in many but not all developmental domains, suggesting that during the first 24 months of life, early adversity can profoundly influence brain development, executive functioning, mental health, and physiological stress responses; however, these effects can be reversed if placement into a family occurs within this time period.

The proposed study will assess the original BEIP sample when they are 15-16 years of age in order to test the long-term effects of our intervention during the adolescent period, and to examine how our sample will make the transition to adolescence generally. We hypothesize that children originally assigned to care as usual (institutional care) will be poorly prepared to face the challenges of adolescence and thus, will evince higher rates of psychopathology, risk taking, and substance use compared to never institutionalized children; by contrast, children receiving our intervention will evince lower rates of psychopathology, risk taking, and substance use than institutionalized children. Our specific aims are:

Specific Aim 1: To examine the long-term effects of early institutionalization on neural, psychiatric, cognitive, socio-emotional outcomes and immune, inflammatory, and metabolic risk biomarkers at age 15-16. We will compare children with any history of institutionalization to a comparison group of typically developing, never institutionalized children. Children at age 15-16 who have experienced psychosocial deprivation associated with early institutionalization will display elevated symptoms of psychiatric problems, impaired cognitive and executive functioning, less white matter volume and a less mature pattern of EEG power, and maladaptive socio-emotional outcomes, including heightened reward sensitivity and risk taking, increased negative emotional learning, greater stress-emotional reactivity, and social relationships difficulties. We also predict that children at age 16 who have experienced psychosocial deprivation associated with early institutional rearing will display elevated levels of C-reactive protein (CRP), interleukin (IL)-6, IL-8, tumor necrosis factor- α (TNF- α), leptin, glycosylated hemoglobin A1c (HbA1c), and Epstein Barr Virus (EBV) antibody titers and lower levels of IL-10.
Given that our previous work (Drury et al., 2010; Drury et al., 2012) has demonstrated genetic modifications of these effects we will further assess whether genetic and epigenetic factors moderate long-term outcomes in this sample.

Specific Aim 2: To examine the long-term effects of a family/foster care intervention and establish whether sensitive periods with regard to the efficacy of the intervention continue to impact brain and behavioral development at age 15-16. We will compare those children randomized to foster care at the start of our study to those randomized to care as usual. We predict that:

a) Children randomized to foster care will display fewer symptoms of psychiatric problems, improvements in cognitive and executive functioning, a more mature pattern of EEG power and greater white matter volume, and better socio-emotional outcomes, including for example, improvements in stress-emotion regulation, reduced negative emotional learning less reward sensitivity and risk taking behavior, and positive social relationships. Children randomized to foster care will also display lower levels of CRP, IL-6, IL-8, TNF-α, leptin, HbA1c, and EBV antibody titers and higher levels of IL-10 compared to controls.

b) Children's earlier placement in foster care (e.g., <24 months), thus decreasing their percent time in an institution, will lead to better outcomes across all domains.

Specific Aim 3: To examine the mediating effects of stress-emotion regulation, reward sensitivity and executive functioning on the relations between early adversity and both internalizing and externalizing symptoms and risk for substance use at age 15-16 among children with histories of institutionalization. We will use longitudinal data collected on our entire sample of children with a history of institutionalization to model the meditational effects of these constructs on the relations between early experience and age 15-16 psychiatric outcomes and heightened risk of substance use. We predict that:

a) Percent time of a child's life in an institution will be related to symptoms of internalizing disorders at age 15-16 years. Specifically, children with a smaller percent time will display fewer internalizing symptoms. We will test attachment security (42 months), reward sensitivity (12 years), and stress-emotion regulation (12 years) as mediators of these relations.

b) Percent time of a child's life in an institution will be related to fewer externalizing symptoms. Specifically children with a smaller percent time will have fewer symptoms of externalizing disorders and less risk of substance use at age 16 years. We will test EEG power (12 years), white matter volume (8 years), executive functioning (12 years), stress-emotion regulation (12 years), and risk taking (12 years) as mediators of these relations.

2. Background and Significance

Exposure to early life adversity is a major public health concern. National surveys estimate that 25-50% of children are exposed to significant psychosocial deprivation or neglect (Finkelhor, et al., 2005; Green, et al., 2010; Kessler, 1997). There is a strong association between adverse childhood experiences and adolescent /adult psychopathology; for example, across numerous studies, individuals with a history of childhood adversities are at least twice as likely to develop a mental health problem as those with no exposure (Cohen, Brown, & Smaile, 2001; Fergusson & Lynskey, 1996; Molnar, Buka, & Kessler, 2001). Finally, childhood adversities account for a substantial proportion of mental health disorders in the population. Recent evidence suggests that the onset of over 30% of lifetime mental disorders in the U.S. are directly attributable to exposure to childhood adversities (Green, et al., 2010; Affifi, 2008; McLaughlin, Sheridan, & Nelson, in press).

Understanding the biological and neural mechanisms that mediate poor health outcomes later in life among individuals experiencing early life adversity has been challenging. Often such children are exposed to a variety of stressors (e.g., physical abuse/neglect), and the ability to parse the effects of early versus cumulative experiences is lacking. In the *Bucharest Early Intervention Project* (BEIP) we have been able to address these challenges by conducting a Randomized Controlled Trial (RCT) of foster care as an intervention for children who were abandoned to institutions early in life. Extending our current follow up to age 15-16 will provide much needed information about the long-term effects of early life adversity on adolescent psychosocial outcomes including psychopathology, risk taking, and substance use.

Deficits in the development of self-regulation skills play an important role in the onset of adolescent mental health disorders and risk-taking. A central task of adolescence is to learn to regulate affect and behavior in adaptive ways, increasingly without the aid of adults. Changes in biological, cognitive, and social systems present innumerable affectively-laden situations in which emotions and behavior must be successfully managed to ensure adaptive functioning. For some adolescents, the biological and psychosocial changes of adolescence come well before they have experienced the cognitive maturation that would facilitate good self-regulation. Indeed, areas of the brain that facilitate executive functioning, reward sensitivity, and emotion regulation undergo substantial remodeling during adolescence. The disjunction between the demands imposed by these environmental and social changes and the incomplete development of self-regulation skills needed to navigate these challenges is a major risk factor for the development of psychopathology in adolescence. Thus, adolescents with deficits in core aspects of self-regulation – including poor executive control, increased reward sensitivity, and diminished emotion regulation, all deficits that we have identified in children with a history of institutionalization – are more likely to develop internalizing and externalizing psychopathology as well as risk behaviors.

The BEIP began in 2000, with support from the MacArthur Foundation, and continues to the present time with primary support from the NIMH (MH091363). It is highly innovative for the following reasons: **First**, it is one of the few studies to assess infants and young children while they were living in institutions, prior to placement in families. **Second**, the measurement battery has been comprehensive and sweeping, ranging from performance on cognitive tests to social interactions, to stress response patterns and brain functioning to caregiver and child reports of

psychopathology. **Third**, its RCT design provides experimental power to assess the effects of the intervention as well as avoiding potential sample bias. **Fourth**, the study assessed children at multiple time points across early and middle childhood, thus providing an opportunity to examine longitudinal trajectories in predicting adolescent outcomes. **Fifth**, the study has employed brain imaging measures, including EEG/ERP and MRI. As such, we are in a position to examine neural mechanisms underlying the effects of early experience, as well as their possible mediating role in poor or positive outcomes among children who experienced institutionalization early in life. **Sixth**, the experimental design permits us, within the bounds of our sample, to examine sensitive periods in development, and as such, ascertain which domains are experience-dependent and when such experiences ought to occur in order to maximize developmental outcomes.

There are currently more than 70 million orphaned or abandoned children throughout the world. A common societal response to caring for such children is to house them in institutions. Children with histories of early institutionalization are at risk for developing a variety of mental health problems that have their origin in abnormal social behavior. The current application will examine the long term effects of early institutionalization on mental health outcomes in two groups of children with a history of institutionalization, as well as explore sensitive periods in recovery from institutionalization.

3. Preliminary Studies/Progress Report

The Bucharest Early Intervention Project (BEIP) was a randomized controlled trial of foster care as an intervention for children abandoned at or around the time of birth and placed in one of six institutions for young children in Bucharest, Romania (Zeanah, et al., 2003). The BEIP began with an initial assessment of 187 children who were screened via pediatric exam and history; 136 children were then selected from the larger group, and all were deemed to be free of neurological or genetic disorders and to show no overt signs of fetal alcohol or any other syndrome. A comprehensive baseline assessment of these children and their caregiving environments was conducted, after which half were randomly assigned to high-quality foster care (created, monitored and financially supported by the study team, since there was essentially no government foster care available at the time the study began) and the other half to remain in institutional care (care a usual). The average age at entry into foster care was 22 months (range=6-31 months). All children were initially seen for follow-up assessments at 30, 42 and 54 months, 8 years, and most recently at 12 years, and the development of children in foster care was compared to the development of children randomized to care as usual and to a group of never institutionalized children (community controls).

Intellectual Quotient: At baseline, institutionalized children's scores on the Bayley Scales of Mental Development were 30 points below same age community children. After placement, at 42 and 54 months, there were significant intervention and timing effects on IQ. Infants removed from institutions prior to 24 months of age and placed into our foster care intervention had higher scores compared to either those in the CAUG or infants removed after 24 months of age (Nelson et al., 2007). At age 8, there was an effect of intervention on verbal IQ (FCG > CAUG) (Fox, et al., 2011). Results continue to reveal a significant intervention effect for verbal IQ and a trend for full scale IQ with children in the FCG > CAUG.

Attachment: Prior to randomization, each infant's attachment behavior to a favorite or familiar caregiver was assessed using the Strange Situation Procedure. In addition to marked reductions in secure attachment and large increases in aberrant classifications, we also demonstrated incompletely developed attachments between institutionalized children and their caregivers.100% of community children were rated as having fully formed attachments, while only 3% of the institutionalized children had fully formed attachments (Zeanah, et al., 2005). At 42 months of age, we repeated the assessment of attachment and found that the children placed in foster care displayed dramatic improvements in their security of attachment (Smyke, et al., 2010). Almost half (49%) were securely attached, whereas only 18% of the care as usual children were securely attached. There also was evidence of a sensitive period, with children taken out of the institution and placed into foster care before the age of 24 months significantly more likely to have formed more secure attachments compared to children placed after 24 months. In addition, we have published a series of papers demonstrating the power of attachment security at 42 months to mediate relations between early childhood adversity and psychopathology, functional impairment, as well as social outcomes. For example, in a recent paper (Almas, et al., 2012) we reported that FCG children placed before 24 months of age displayed better social skills compared to either FCG children taken out after 24 months or children randomized to the CAUG. A significant mediating factor in this outcome was 42 month security of attachment. Children with secure attachments had better social skills at 8 years.

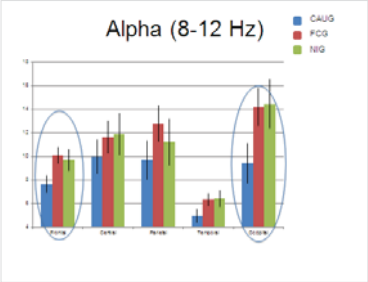

Figure 1 – EEG Alpha

Brain Activity: We assessed brain activity by examining the resting EEG initially at baseline (Marshall et al., 2004), and then at 42 months (Marshall et al., 2008), 8 years (Vanderwert, et al., 2010) and now at 12 years of age. We first reported that infants living in institutions had significant reductions in typical EEG activity (in the Alpha band) and heightened activity in slow wave EEG frequencies (e.g., Theta activity) compared to never institutionalized children. When children were 8 years of age we observed significant intervention and timing effects in that children placed in foster care < 24 months of age were not statistically different from the never institutionalized children whereas those placed >24 months looked indistinguishable from the CAUG. We are currently acquiring EEG at age 12 and have examined data on more than half of the sample (Figure 1). Again, we find significant intervention effects, with children in the FCG showing a pattern of alpha power identical to typical same age community children while children randomized to the institution continuing to show heightened slow wave power and reduced alpha power.

Executive Functions: At the 8 year assessment we used a subset of measures derived from the Cambridge Automated Neuropsychological Test and Battery (CANTAB), focusing most on memory and executive functions. Overall, we found that both children in foster care and those randomized to an institution performed worse on measures of both memory and executive functions than typical same age children from the community (Bos, et al., 2009). Preliminary analyses at age 12 (using the CANTAB), indicate that the CAUG and FCG are not performing as well as typical same age community children. Overall, this is one of the few domains where we have not observed intervention effects, perhaps the intervention did not target executive functions specifically.

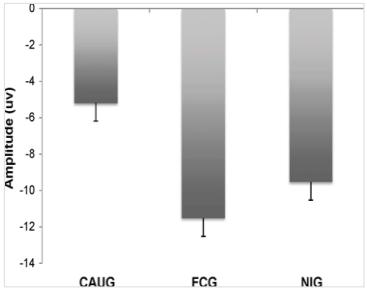

Figure 2 – N2 Amplitude

In addition to assessment of memory executive functions with the CANTAB at age 8, we administered two computerized tasks that have been used to examine inhibitory and cognitive control in children and adults. The Go-No-Go (see *Methods*) is designed to examine a subject's ability to inhibit an incorrect response to an infrequently occurring stimulus (letter or picture) while responding continuously to a set of frequently occurring stimuli. We recorded reaction time, accuracy and ongoing EEG, which was time locked to the "go" and "no go" stimuli. We computed a N2 ERP, the amplitude of which reflects inhibitory control (greater amplitude, greater inhibitory control). As can be seen in Figure 2 the children in the FCG and typical community controls had significantly greater N2 amplitudes compared to children in the CAUG suggesting a deficit in this latter group in inhibitory control. We also administered a cognitive control/error monitoring task (Flanker). Children were presented with a standard arrow flanker task. EEG was recorded during the task and the child's button press (as to whether the center stimulus arrow was pointing left or right) was linked to EEG. We then computed an ERN (Error Related Negativity), which reflects the neural activity during error monitoring (McDermott, et al., 2012). As can be seen in Figure 3 we found that both CAUG and FCG subjects displayed lower amplitude ERNs compared to the NIG (typical community children). These data together with the go no go data are striking for identifying specific attention processes that are either remediated or perturbed in the sample as a function of intervention. It appears that inhibitory control measured in the go no go is affected by the intervention but cognitive monitoring measured in the Flanker is not. We intend to repeat these tasks at age 16 to assess how the transition to adolescence impacts executive functioning.

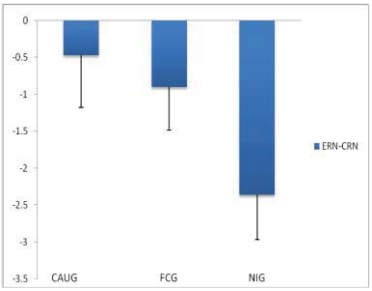

Figure 3 – ERN Amplitude

Brain Structure: When children in the study were 8-10 years of age, we collaborated with a Bucharest-based neuroradiologist to complete structural scanning. Children with a history of institutional rearing (CAUG, FCG) had reduced grey matter compared to community children (Sheridan, et al., 2012). However, the foster care intervention did have a beneficial effect on white matter – in regions of the corpus callosum the FCG showed white matter volume intermediate between the CAUG and the NIG. Importantly, we also found that differences in white matter between groups of children accounted for individual differences in EEG amplitude in the alpha band.

Psychopathology: Using a structured psychiatric interview (PAPA) when children were 54 months of age, we found 53% of the children who had ever been institutionalized had a psychiatric diagnosis, compared to 20% of the community children (Zeanah, et al., 2009). The effects of the intervention were marked for internalizing disorders (anxiety, depression), reducing them by half, but foster care had no impact on reducing externalizing disorders (e.g., ADHD, conduct disorder). No sensitive period was evident. We have also reported that security of attachment mediated internalizing outcomes, such that the more securely attached the child was to the foster parent at 42 months, the greater probability of the child having fewer internalizing symptoms. Preliminary findings at age 12 years indicate the pattern of psychopathology is similar to that reported when children were 54 months of age, but the rates are even higher. Using the DISC, there are significant intervention effects for any psychiatric disorder (81% in the CAUG vs. 59% in the FCG), and differences between these two groups are larger for internalizing (47% vs. 30%) compared to externalizing (56% vs. 46%) disorders. Importantly, at age 12 both FCG and CAUG have significantly higher rates of both externalizing and internalizing disorders compared to typical same age community children, similar to our findings at 54 months.

Stress Responsivity: Both the Trier Stress Test (TSST-C), a frustration task, and a reward sensitivity task are being administered to participants at 12 years with measures of Autonomic Nervous System (ANS) and HPA axis reactivity being collected. Preliminary ANS data from 51 participants (CAUG=23; FCG=21; NIG=7) reveal that 1) CAUG children exhibit **blunted** ANS reactivity (reduced Heart Rate (HR), increases in Blood Pressure (BP), and smaller reductions in Pre Ejection Period (PEP) and Respiratory Sinus Arrhythmia (RSA)) to social (vs. non social) tasks (TSST-C), indicating blunted sympathetic activation and less vagal withdrawal (greater vagal withdrawal is a marker of better emotion regulation capabilities (Bornstein & Suess, 2000; Porges, et al., 1996); 2) CAUG children exhibit **exaggerated** ANS responses (greater BP increase and PEP reductions) compared to NIG and FCG children to non-social stressors (frustration task; reward sensitivity task), 3) Both CAUG and FCG children exhibit **blunted** vagal rebound following a social stressor (TSST-C), indicating incomplete ANS recovery; and 4) FCG children exhibit an ANS reactivity profile similar to the CAUG children during the social task (TSST-C) and similar to the NIG children during the non-social tasks (frustration; reward sensitivity). The latter finding suggests some amelioration of stress reactivity dysfunction in FCG children, particularly to non-social stressors. Given the marked remodeling of physiological stress response systems that occurs during adolescence (Zuckerman, et al., 1978) particularly to social stressors, we anticipate that FCG children will more closely resemble NIG children by 16 years in their TSST performance. At the 16-year follow-up we include measures of negative emotional learning, one aspect disregulated physiological and emotional responding not previously captured in this study. Inclusion of these measures will increase the sensitivity with which we can assess the evolving physiological system across development.

Summary: Exposure to early adversity is associated with pervasive deficits in brain activity and structure, executive functioning, alterations in reward sensitivity and processing, and disruptions in emotion regulation,
all of which may contribute to subsequent psychopathology. On the other hand, placing infants and young children who have experienced psychosocial neglect into families, and providing the opportunities to form secure social relationships with adult caregivers may have a lasting protective influence on adolescent outcome. The five-year research plan we propose will examine executive functioning, reward processing and sensitivity, and stress-emotion regulation as mechanisms in the association between early-life adversity and adolescent mental disorders and risk behaviors and evaluate the role of early intervention in shaping the development of both self-regulation skills and mental disorders into adolescence.

4. Design and Methods

a. Study Design

The Bucharest Early Intervention Project (BEIP) was a randomized controlled trial of foster care as an intervention for children abandoned at or around the time of birth and placed in one of six institutions for young children in Bucharest, Romania (Zeanah, et al., 2003). The BEIP began with an initial assessment of 187 children who were screened via pediatric exam and history; 136 children were then selected from the larger group, and all were deemed to be free of neurological or genetic disorders and to show no overt signs of fetal alcohol or any other syndrome. A comprehensive baseline assessment of these children and their caregiving environments was conducted, after which half were randomly assigned to high-quality foster care (created, monitored and financially supported by the study team, since there was essentially no government foster care available at the time the study began) and the other half to remain in institutional care (care a usual). The average age at entry into foster care was 22 months (range=6-31 months). All children were initially seen for follow-up assessments at 30, 42 and 54 months, 8 years, and most recently at 12 years, and the development of children in foster care was compared to

the development of children randomized to care as usual and to a group of never institutionalized children (community controls).

Each child taking part in the proposed study will participate in five research sessions. Three sessions will take place in the BEIP Research Laboratory and one session will take place in a private medical center, and the final session may occur at the BEIP Laboratory or at the participant's place of residence. Both entities are located in Bucharest, Romania. Scheduled breaks will be taken throughout the sessions to ensure that the child does not become fatigued and no one session will take longer than 4 hours to complete. Session 1 will be scheduled +/- 6 months of the child's 16th birthday and all remaining sessions will be completed within 3-6 months of Session 1. Transportation to/from the laboratory will be provided for all participants. Based on the ages of the children in our sample, we plan to evaluate a total of 370 children: 100 children in Year 1, 90 children in Year 2, 90 children in Year 3, and 90 children in Year 4).

b. Patient Selection and Inclusion/Exclusion Criteria

Participant Selection

We will recruit the following participants for the study:

Foster Care Group (FCG): This group will consist of children who have previously participated in the BEIP by virtue of a history of institutional care, were randomized to placement in foster care at the outset of the study 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 group originally consisted of 68 children who met eligibility criteria and were randomized to placement in foster care. We will make every effort to contact all FCG participants from previous assessments and anticipate that at least 60 of the original 68 children will participate in the follow-up study at age 16 years. **Anticipated recruitment: 60 participants**

Care As Usual Group (CAUG): This group will consist of children who have previously participated in the BEIP by virtue of a history of institutional care, were randomized to remain in care as usual at the outset of the study and 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 group originally consisted of 68 children who met eligibility criteria and were randomized to placement in foster care. We will make every effort to contact all CAUG participants from previous assessments and anticipate that at least 60 of the original 68 children will participate in the follow-up study at age 16 years. **Anticipated recruitment: 60 participants**

Never-Institutionalized Group (NIG): This group will consist of community children who have participated in previous assessments of the BEIP at ages 8 and/or 12 years. These children are matched on age, gender and ethnicity to the children described in the FCG and CAUG groups above. If we are unable to successfully enroll 60 previous NIG participants, we will recruit additional community children from local elementary schools in Bucharest, Romania to complete the NIG sample (see attached recruitment letter). **Anticipated recruitment: 60 participants**

Nominated Peers Group (NPG): This group will consist of children who will be invited to participate after being nominated by the existing FCG, CAUG and NIG groups described above. The proposed protocol includes a research session in which we will evaluate interactions between target participants and a same-sex, age-matched close friend. Target participants will be asked to tell us the names of 3 of their closest friends at the end of research session 1. **Anticipated recruitment: 180 participants**

Pilot Participants Group (PPG): This group will consist of 10 community children who will be recruited from local elementary schools in Bucharest, Romania (see BEIP_Recruitment_Letter_NIG). They will be matched in age, gender and ethnicity to children described in the groups above.

Pilot participants will be asked to complete all proposed measures detailed in the protocol to ensure that estimated session completion times are accurate and that all measures are culturally appropriate and can be

understood/completed as translated. Pilot participants will complete these measures during 4 research sessions (as described later in the protocol) and will receive the same compensation and completion bonus as study participants (see Administrative Details below).

At the conclusion of each task, pilot participants will be asked to indicate how easy or difficult they find each task, as well as how much they enjoyed each task. Additionally, the experimenter responsible for administration of each task will indicate whether the pilot participant needed simplified/modified instructions in order to complete the task, needed re-direction during the task or seemed not to understand the task.
Anticipated recruitment: 10 children

Total anticipated recruitment: 370 children

We will try our best to recruit as many of the original 136 FCG and CAUG participants as possible. To date, we have followed 114 of these children through assessments at 12 years (enrollment is still ongoing), and have remained in contact with the caregivers/foster families/parents of these children. We anticipate that at least 120 of the original 136 children will participate in the follow-up study.

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 Pilot Subjects and Additional Community Participants
Parents of children to be recruited from local elementary school will be asked the following questions to ascertain eligibility:

7. 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/toddlers as part of our community comparison sample).
8. 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 Mon-Fri sleepover daycare).
9. Does your child have a history of neurological abnormality or trauma?
10. Does your child have uncorrected vision difficulties, such as amblyopia, strabismus or cateracts?
11. Did your child experience any pregnancy or birth-related complications?

Does your child have any serious handicapping conditions (e.g., genetic syndromes, Fetal Alcohol Syndrome, cerebral palsy)?

Only those children whose parents respond "YES" to question 1 and "NO" to questions 2 – 6 will be invited to take part in the study.

Our research team will attempt to talk with adolescents about the study during the time we are scheduling the study visits. The research assistant will briefly explain the follow-up study, provide some basic information about the study visit and will advise the adolescent that if they decide they want to continue, there will be a more detailed discussion when they come to the research laboratory. The adolescent will also be reminded that they can decide not to participate or may withdraw from the study at any time, even after coming to the research lab. Once the adolescent and parent/caregiver/legal guardian arrive at the laboratory, the consent/assent process will take place as described below. The research team will make it clear to the parent/caregiver/legal guardian and adolescent that they may withdraw from the study at any time.

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.

c. Description of Study Treatments or Exposures/Predictors

Predictors include group status (CAUG vs FCG vs NIG), age at which children entered foster care, length of time spent in an institution, quality of early caregiving environment, and number of placement changes.

d. Definition of Primary and Secondary Outcomes/Endpoints

Outcomes include psychiatric status (total number of symptoms, as well as disorders based on self-report and parent report), EEG power, ERP amplitude/latency, brain volume, risk-taking behaviors, RAD-inhibited and RAD-disinhibited behavior, IQ, self-reported emotional responses, and physiological responses to the tasks proposed in Session 3, all of which are measured continuously. Physiological response includes measures of autonomic nervous system reactivity (heart rate, systolic and diastolic blood pressure, total peripheral resistance, cardiac output) and HPA axis reactivity (cortisol, DHEA). Reactivity is defined as change in each of these parameters from baseline to each task. Genetic variation will be a moderator of long-term trajectories. Longitudinal changes in epigenetics is a secondary outcome. Blood-based inflammatory (CRP, IL-6, IL-8, IL-10, TNF-alpha), immune (EBV antibody titers), and metabolic (leptin, HbA1c) biomarkers will also be secondary outcomes.

e. Data Collection Methods, Assessments, Interventions and Schedule

Administrative Details

Data collection for this study will consist of 4 research sessions. Based on previous assessments, as well as the age range of our participants, we expect that data collection for the entire sample will take 4 years.

The research sessions will be scheduled at a time most convenient for the participant and their parents/caregivers, including weekends if families prefer. Sessions 1-3 will take place at the BEIP Research Laboratory and Session 4 will take place at a private medical clinic, both of which are located in Bucharest, Romania. All research sessions will be conducted in Romanian. We estimate that sessions 1-3 will last no more than 4 hours.* Session 4 will last no more than 1.5 hrs. Session 5 will last no more than 3 hours and will take place at the BEIP Research Laboratory or at the particpant's place of residence, if travel is difficult for the participant. Scheduled breaks will be taken throughout each session and participants will be told that they can request additional breaks, as needed. Snacks and refreshments will be provided to participants during breaks.

All proposed tasks will be piloted on a small number of children (no more than 20) recruited from the community prior to the enrollment of actual subjects to evaluate the measures for cultural and age-appropriateness, ensure that children understand task instructions and confirm that the inclusion of these tasks will keep the total session time within our estimate. All questionnaires will be read aloud to participants to control for reading ability.

All participants, including pilot participants, will receive a cash payment (100 RON or $30 USD) at each research session as a token of our appreciation. Additionally, a cash incentive (100 RON or $30 USD) will be provided as a completion bonus to all participants, including pilot participants, who complete 4 of the 5 research sessions. A cash reimbursement (100 RON or $30 USD) will be provided for transportation to all research sessions.

**** The follow-ups conducted at ages 8 and 12 consisted of research sessions lasting between 3-4 hours. All participants successfully completed the tasks requested of them within this time and no complaints about session length were made on anonymous session feedback forms completed by parents/caregivers of the participants.***

Informed Consent

Consent to participate for children who are currently considered wards of the state (in the custody of Child Protection) will be obtained from the legal guardian of each child. Depending on the case, the legal guardian could be the sector Director of Child Protection and Social Assistance (DGASPC); the biological parents, the sector Mayor, the foster parent(s), or another family member. If the legal guardian of the child gives consent, the child will be enrolled in the study. If the legal guardian of the child does not give consent, the child will not be enrolled in the study.

If a child who is considered a ward of the state DOES NOT reside with their legal guardian, consent will be obtained from the legal guardian prior to enrollment. Consent will also be obtained by the adult (i.e., foster parent, caregiver, other family member, etc) who accompanies the child to the research laboratory or medical center for each research session. If the legal guardian of the child cannot be located or does not give consent, the child will not be enrolled in the study.

Consent for children who have been reintegrated with their biological families will be sought from their biological parents. Consent for children who have been adopted will be sought from their adoptive parents. Consent for children in the community-comparison sample (NIG) will be sought from their biological parents.

A consent form will be created for each research session and will indicate the purpose of the original study and the purpose of the proposed adolescent follow-up. All consent forms will be translated into Romanian by our Romanian research staff. Informed consent will be conducted in Romanian by a member of the BEIP Research Team and obtained prior to the start of each research session.

Upon arrival to the BEIP Research Laboratory, participants and their parents/caregivers will be greeted by two members of the research team and escorted to a private room in the laboratory. Research Assistant 1 will provide a summary of the research activities scheduled for that day and will review the session consent form with the parent/caregiver.

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 each session. Parents/caregivers will sign the consent form in the presence of study personnel before any testing commences.

The consent form for session 1 will contain a statement obtaining consent from the parent/caregiver to contact the child's teacher to complete two questionnaires described below. 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.

If the parent/caregiver is unable to read/write, the consent form will be read aloud and Research Assistant 2 will indicate that informed consent was obtained by signing the Witness section of the consent form.

Once the parent/caregiver has provided consent, Research Assistant 2 will escort the parent/caregiver to the reception area of the laboratory to confirm/update their contact information (address, phone, email address, etc.). Meanwhile, Research Assistant 1 will remain in the private office with the child and will review the written assent form (see *Written Assent* section below).

Written Assent

Written assent will be obtained for participants in the never-institutionalized, community control group (NIG). Written assent for children in the foster care (FCG) and care as usual (institutionalized; CAUG) groups will be attempted with participants whose full-scale IQ (as determined at the age 12 assessment) is 61 or greater. If a participant meeting the IQ criteria is unable to give provide written assent, verbal assent will be attempted (see below).

Verbal Assent

Verbal assent will be attempted with participants in the foster care (FCG) and care as usual (CAUG) groups whose full-scale IQ is 60 or lower, or with FCG and CAUG participants who were unable to provide written assent. An independent observer (selected MA students from the Department of Psychology and Educational Sciences at the University of Bucharest) will observe the assent process and research sessions for children meeting this IQ criteria.

Upon arrival at the research laboratory, these participants will be shown still photographs depicting an adolescent their age participating in specific tasks for that session. Each photo will be accompanied by a standardized script using simple language to describe the research activity depicted in the photo (i.e., "In this session, you will be asked to play some games with the researcher. You will be asked to build with blocks, repeat numbers and tell the researchers the meaning of certain words.").

After each photograph is displayed, the participant will be asked the following questions:

1) Do you have any questions about what you just saw in the photo?
2) Do you understand what will take place/happen?
3) Are you willing to complete this portion of the research session?

After all photographs have been shown, the participant will be asked if the following:
1) Do you understand that you can stop at any time?

Assent (written or verbal) will be obtained in a private room away from parents and caregivers. The research staff will explain the details of each session to the child, taking into account cognitive ability.

If verbal assent is obtained, administration of all measures will be attempted. Research staff will always obtain a participant's voluntary verbal agreement to proceed during the course of the research session. If it becomes clear to our research team, the parent/caregiver, or the independent observer that a participant is unable to understand or complete a task, administration will be stopped, and only parent/caregiver and teacher measures will be administered.

If a participant in the foster care of care as usual group is unable to provide written or verbal assent, only parent/caregiver and teacher measures will be administered.

In the event that a child in any of the three groups chooses not to participate, the researcher will inform the parent/caregiver of the child's decision and will complete only those activities relevant to the parent/caregiver and teacher. 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.

A separate assent document will be developed for each research session. The assent documents include a description of the activities scheduled for a given research session and:

h. A statement to the effect that a subject can stop that day's session, and also stop being in the study altogether;
i. A statement to the effect that no will be disappointed with – or mad at- a child who makes a decision to withdraw;
j. A statement to the effect that they can skip any questions they do not wish to answer;
k. An explanation of which analyses/instruments their parents/caregivers will be present for;
l. An explanation of what types of answers to which the researchers will need to alert parents or caregivers;
m. Information regarding what payments will be given to subjects;
n. Contact information for the study team and the IRB.

In the event that a participant reaches the age of majority while still actively involved in the study, he/she will be asked to sign the approved age 18 consent forms, rather than the approved assent forms, for any remaining research sessions they complete.

We have consulted with our social workers, members of the DSMB, Bogdan Simion, M.D., and a Romanian lawyer regarding consent of participants who reach the age of majority while participating in the study.

Currently, there is no legislation mandating that wards of the state be given/assigned a legal representative when they reach age 18, regardless of their decision-making ability.

If any participant is a ward of the state and resides in a placement center or social apartment when they turn age 18, he or she can continue to reside in that setting until age 26 if they attend school. If any participant is a ward of the state and resides in a placement center or social apartment when they turn age 18, he or she may be reintegrated with their biological family (if possible) or transferred to an adult placement center if they are not enrolled in school.

If a participant is still considered a ward of the state and they reach the age of majority before completion of all research sessions associated with the protocol, we will obtain written consent from local child protective authorities and the participant following the procedures outlined below.

Written Consent

Written consent will be obtained for participants in the never-institutionalized, community control group (NIG). Written consent for children in the foster care (FCG) and care as usual (institutionalized; CAUG) groups will be attempted with participants whose full-scale IQ (as determined at the age 12 assessment) is 61 or greater. If a participant meeting the IQ criteria is unable to provide written consent, verbal consent will be attempted (see below).

Verbal Consent

Verbal consent will be attempted with participants in the foster care (FCG) and care as usual (CAUG) groups whose full-scale IQ is 60 or lower, or with FCG and CAUG participants who were unable to provide written consent. An independent observer (selected MA students from the Department of Psychology and Educational Sciences at the University of Bucharest) will observe the assent process and research sessions for children meeting this IQ criteria.

No measures will be administered to the now-adult participant, their parents/caregivers or teachers without their written or verbal consent. Additionally**,** members of the research team will not access data collected from parents/caregivers or teachers during any other session associated with the current study prior to the participant turning 18 years if written or verbal consent is not obtained from the now-adult participant**.**

Upon arrival at the research laboratory, these participants will be shown still photographs depicting an adolescent their age participating in specific tasks for that session. Each photo will be accompanied by a standardized script using simple language to describe the research activity depicted in the photo (i.e., "In this session, you will be asked to play some games with the researcher. You will be asked to build with blocks, repeat numbers and tell the researchers the meaning of certain words.").

After each photograph is displayed, the participant will be asked the following questions:

1) Do you have any questions about what you just saw in the photo?
2) Do you understand what will take place/happen?
3) Are you willing to complete this portion of the research session?

After all photographs have been shown, the participant will be asked if the following:

1) Are you willing to help us learn about you?
2) Do you understand that you can stop at any time?

Consent (written or verbal) will be obtained in a private room away from parents and caregivers. The research staff will explain the details of each session to the child, taking into account cognitive ability.

If verbal consent is obtained, administration of all measures will be attempted. Research staff will always obtain a participant's voluntary verbal agreement to proceed during the course of the research session. If it becomes clear to our research team or the independent observer that a participant is unable to understand or complete a task, administration will be stopped. No measures will be administered to the participant, their parent/caregiver or teacher without the participant's written or verbal consent.

Conditional Assurance of Confidentiality
The consent and assent forms provide information regarding conditional assurance of confidentiality. This section on each form indicates the steps the study team will take if they have reason to believe the participant or others are at risk for self-harm or harm (consent and assent), what kinds of referrals or interventions are possible (consent only), and the fact that families will be responsible for payment if they elect to receive private, rather than universal care (consent only).

If confidentiality must be breached for any participant, the BEIP Research Team will follow the actions detailed in the Safety Reporting Protocol to ensure that everyone involved in the adolescent's care is appropriately informed of the situation.

In addition, a resources list will be provided to all families regardless of how their child responds to the Youth Risk Behavior Survey and Diagnostic Interview Schedule for Children (described below). This list of resources will include information about hotlines and support groups for adolescents experiencing mental health or substance abuse issues. There will also be resources listed for parents (support groups, etc.).

Selection of measures
Many of these measures listed below were chosen because they have been used with typically-developing children of a similar age range in the United States. For example, Dr. Nathan Fox, co-PI on this project, has used the Resistance to Peer Influence, Network of Relationships Inventory, and Interpersonal Competence Questionnaire extensively with children in this age range in his research laboratory at the University of Maryland. Many of the proposed measures (BART, BIRD, HBQ, DAI, SSRS, WISC and DISC) have been used in previous assessments of the BEIP. The TSST-C and the computer game task were also used in the assessment completed at age 12 and Drs. McLaughlin and Sheridan have used this measure with adolescents who have a wide range of adverse early-life and current family circumstances and mental health problems (IRB-P00000200). Additionally, the PI and co-PIs have consulted extensively with colleagues whose research focuses on pre-adolescents/adolescents on several risk-taking measures/questionnaires described below (Probabilistic Gambling Task, Stoplight Task, Barratt Impulsiveness Scale, and Future Orientation Inventory). All measures will be translated to Romanian and back-translated to English by our research team. **A table indicating all measures to be administered is included with the submission and we indicate which measures were administered as part of the assessments completed at ages 8 (protocol 06-10-0455) and 12 years (protocol 10-04-0185).**

Research Session 1

Wechsler Intelligence Scales for Children (WISC-IV) We have learned that the Wechsler Intelligence Scale for Children - Fourth Edition (WISC-IV) is now available in Romanian. The ability to obtain a cognitive assessment using the native language of the majority of our participants is preferable in terms of both validity (the test has been standardized on a Romanian population), and ease of staff use (reducing potential staff error) to measures that must be translated from English. Additionally, in order to reduce participant burden, we have reduced the number of WISC-IV subtests from 10 to 3 in order to obtain an estimated full scale IQ score. The chosen subtests are Block Design, Digit Span, and Vocabulary, and this combination was selected given evidence that this "short form" of the WISC-IV is a both reliable and valid estimate of the full scale IQ score obtained from the full 10 subtest battery.

Participant: CHILD
Estimated time to complete: 40 minutes
Present during Administration: Research Assistant and Child

Zuckerman Sensation Seeking Scale
Self-reported sensation seeking will be assessed using a subset of 6 items (Steinberg et al., 2008) from the Sensation Seeking Scale (Zuckerman et al., 1978). Many of the items on the full 19-item Zuckerman scale appear to measure impulsivity, not sensation seeking (e.g., "I often do things on impulse." "I usually think about what I am going to do before doing it."). In view of our interest in distinguishing between impulsivity and sensation-seeking, we will use only the six Zuckerman items that clearly index thrill- or novelty-seeking ("I like to have new and exciting experiences and sensations even if they are a little frightening."; "I like doing

things just for the thrill of it."; "I sometimes like to do things that are a little frightening."; "I'll try anything once."; "I sometimes do 'crazy' things just for fun."; and "I like wild and uninhibited parties."). All items will be answered as either True (coded 1) or False (coded 0). Item scores will be averaged.

Participant: CHILD
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant and Child

Barratt Impulsivity Scale

Self-reported impulsivity was assessed using a slightly modified widely-used self-report measure of impulsivity, the Barratt Impulsiveness Scale for adolescents (Fossati et al., 2002). Based on inspection of the full list of items (the scale has 6 subscales comprising 30 items) and some exploratory factor analyses, we opted to use only 18 items from three 6-item subscales: motor impulsivity (e.g., "I act on the spur of the moment"), inability to delay gratification (e.g., "I spend more money than I should"), and lack of perseverance (e.g., "It's hard for me to think about two different things at the same time. Each item is scored on a 4-point scale (Rarely/Never, Occasionally, Often, Almost Always/Always), with higher scores indicative of greater impulsivity. The three subscales we elected not to use measure attention (e.g., "I am restless at movies or when I have to listen to people"), cognitive complexity ("I am a great thinker"), and self-control ("I plan for my future"), which the instrument developers describe as assessing "planning and thinking carefully" (Patton et al., 1995, p. 770). We concluded that scales measuring attention, cognitive complexity, and "planning and thinking carefully" were not components of impulsivity as we conceptualized the construct (see Steinberg et al., 2008).

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child

Balloon Analogue Risk Task, Youth Version (BART)

Lejuez et al., (2002) developed the Balloon Analogue Risk Task (BART) to model risk taking in the laboratory. Based on this measure which has been well-validated in adult samples, an adolescent-appropriate version was developed and published (Lejuez et al., 2007). Before starting the BART, the task will be thoroughly explained using a visual of the task accompanied by directions provided below: "On the screen, you will see 30 balloons, one after another. For each balloon, you will use the mouse to click on the box that will pump up the balloon. The bigger you pump up the balloon, the more points you will build up on that balloon. If you stop pumping a balloon before it pops and you click on the button labeled "Collect", your points will be added to the prize meter on the left. The bigger you make up the balloon when you press "Collect", the more the prize meter will fill up. At the end of the game, the size of your prize will equal the number of points you have saved into your prize meter, which will determine if you get a small, medium, large, or bonus prize. Good Luck!"

Once the subject presses a button agreeing that he/she understands the task, the computer screen will show a small simulated balloon accompanied by a balloon pump, a reset button labeled "Collect", and the prize meter. Each click on the pump inflates the balloon one degree (about .125" in all directions). With each pump, 1 point will be accrued in a temporary reserve (the number of points in this reserve is never indicated to the subject). When a balloon is pumped past its individual explosion point, a "pop" sound effect emanates from the computer. When a balloon explodes, all points in the temporary bank are lost and the next un-inflated balloon is shown. At any point during each balloon, the subject can stop pumping the balloon and click the "Collect" button. Clicking this button transfers all points from the temporary bank to the prize meter, during which the new total earned is incrementally updated on the prize meter, point by point with a "bells" sound-effect playing.

After each balloon explosion or point collection, the subject's exposure to that particular balloon ends and a new balloon appears until 30 balloons (i.e., trials) have been completed. The probability that a balloon will explode is arranged by constructing an array of N numbers. The number "1" is designated as indicating a balloon explosion. Upon each pump of the balloon, a number is selected without replacement from the array. The balloon explodes if the number 1 is selected. The array contains the integers 1 through 128. With this system, the expected amount of money earned and the expected frequency of explosions can be predicted

as a function of the number of pumps. The probability that the balloon will explode on the first pump is 1/128. If the balloon does not explode after the first pump the probability that the balloon will explode is 1/127 on the second pump, 1/126 on the third pump and so on up until the eighth pump at which the probability of an explosion is 1/1 (i.e., 100%). A great strength of the task is the wide age range for which strong validity data exist, covering both the lower age here (see Lejuez et al., 2003) through the upper age of 18 (Lejuez et al., 2007), thereby allowing for the advantage of keeping the same measure across years.

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Behavioral Indicator of Resiliency to Distress (BIRD)

The BIRD, which was developed based upon the well-validated version for adults, the PASAT, measures distress tolerance by determining how long a participant persists on a challenging task that increases in difficulty until success on the task is virtually impossible. Specifically, participants are asked to use the computer's mouse to click a green dot that appears above a number (with numbers ranging from 1-10) before the green dot disappears. If the number is clicked before the dot disappears, then a bird picture on the screen sitting in a cage is let out of the cage and the computer makes a chirping sound. Alternatively, if the green dot disappears before the child clicks on the number, then a loud noise is made and the bird remains in its cage. A participant receives one point each time the bird is let out of its cage. There are no points for missed green dots. There are three levels of difficulty. The first level of the BIRD lasts 3 minutes. This level begins with a 5-second latency in between dot presentations and titrates this latency based upon performance (correct answers reduce the latency by 0.5 seconds whereas incorrect answers or nonresponses increase the latency 0.5 seconds). The second level is more difficult, beginning with the average latency from the previous level for four minutes and then reducing the latency in half for the final minute making the task extremely difficult (i.e., challenge latency). Following a brief rest period, the final level lasts up to 5 minutes and utilizes the extremely difficult challenge latency. At all points in the final level, the adolescent has an escape option. Specifically, the participants is informed prior to beginning the task that once the final level has begun, the task can be quit by clicking the 'quit game' button on the computer screen, however the magnitude of their prize is dependent on how well they do on the task. Throughout the task, a point meter remains visible on the screen that indicates how many points the adolescent has earned. Distress tolerance is indicated by persistence on the task and can be examined as a continuous variable (latency in seconds to terminate) or a categorical variable (whether or not they terminated the task). Notably, persistence on the task is unrelated to change in levels of distress in response to the task (Daughters et al., 2009; MacPherson et al., under review). Data from our progress report above support the use of the BIRD in our younger sample, and pilot data using the BIRD with an older adolescent sample support our extending the age range (R21 DA 22741; PI: Daughters).

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Secure Base Script Test (SBST)

In order to assess participants' knowledge of and access to the secure base script, each child will be asked to produce 5 stories from sets of prompt-word outlines (developed by H. Waters and Rodrigues (2001). Three types of stories will be used: stories with attachment-related content that emphasize parent-child interactions (*Robin's Math Test* and *Robin's Accident*) and stores that emphasize child-child interactions (*Robin Has Troubles At School* and *Robin Moves Away*). A neutral theme story (*Robin's Birthday*) will be used as a warm-up exercise.

Each word-prompt outline consists of 12 words, organized in three columns, presenting the story premise and its main characters, suggesting a crisis involving the main character, and implying interactions with caregivers/friend and resolution. The neutral theme story (*Robin's Birthday*) will be used to familiarize children with the test procedure and put them at ease. If necessary, during this warm-up, the researcher will provide children with suggestions and help them through co-construction (e.g., asking an open-ended question, starting a sentence, encouraging the child to continue). Participants will be presented with the

remaining word prompts and asked to tell the best stories they can using the 12 words in each list. The task will be videotaped for subsequent transcription, translation and coding.

Narratives will be scored on a seven-point scriptedness scale reflecting how much they resemble a protypical SBS, according to a scoring manual (Psouni & Apetroaia, 2009). Narratives characterized by rich SBS content are scored as 6 or 7 depending on degree of relevant elaboration. Narratives containing some elements of the SBS but restricted in elaboration are scored as 5. Narratives with minimal elements of the SBS are scored as 4. Stories focused on actions or events, with no acknowledgement of emotional states or interactions are scored as 3. Stories lacking secure base content, and additionally being so short so as to be disjointed, are scored as 2. Finally, stories that include almost exclusively odd content or a different theme entirely are scored as 1.

Participant: CHILD
Estimated time to complete: 30 minutes
Present during Administration: Research Assistant and Child

Diagnostic Interview Schedule for Children (DISC-IV)
The DISC is a fully structured diagnostic instrument that assesses 34 common psychiatric diagnoses of children and adolescents. The DISC is designed for interviewer administration – either by lay interviewers or by clinicians or by self-completion.

The DISC-IV has been designed to obtain information about Diagnostic and Statistical Manual - IV (DSM-IV) diagnoses, essentially by ascertaining the presence or absence of symptoms. The instrument uses the diagnostic criteria as specified in DSM-IV (with DSM-III-R, and ICD-10 in development. In addition, the DISC is DSM-IV loyal and all symptom criteria must be met to meet the diagnosis. Both diagnoses in past month (embedded current) and in the past year are coded and recorded. Past year, current (past 4 weeks) & Whole Life (optional). The DISC-IV assesses diagnoses that have occurred anytime in last 12 months. Continuous symptom scales and impairment (6 impairment domains and 3 levels of severity) are also coded.

In clinical populations, the DISC requires 90-120 minutes to administer; in community samples, it requires about 70 minutes. Time of administration, of course, is dependent upon number of diagnostic modules administered and number of symptoms endorsed.

The validity of the DISC-IV was originally established in large-scale epidemiological surveys of children and adolescents, but it has since being used in many clinical studies, screening projects, and service settings.
For the purposes of this research, we will administer 13 sections of the interview (including components from anxiety disorders, miscellaneous disorders, mood disorders, disruptive behavior disorders and the Whole Life module) to both the parent/caregiver and child. An electronic copy of the child version of the DISC has been submitted to the CCI office under protocol 10-04-0185.

Anca Radulescu, Ph.D., will oversee all administered DISC interviews. Ms. Radulescu has received training on the DISC by Dr. Prudence Fisher at Columbia University. She will train the RAs who administer the parent and child versions of this measures (blind to participant status) to note and report to her at the completion of the interview (with parent/caregiver or child) any of the following: suicidal ideation, self-injurious behavior, threatening to harm others, serious risk taking behavior, significant impairment in any domain, or any child or parent who wishes assistance with the child's emotions or behaviors.

If such behaviors are reported, Dr. Radulescu will follow the actions detailed in the Safety Reporting Protocol.

The consent and assent forms indicate that if the child reports engaging in danger to self or danger to others, confidentiality will be breached, and the parent/guardian will be made aware of the child's report.

Participant: CHILD
Estimated time to complete: 60 minutes
Present during Administration: RA and Child

Inventory of Callous Unemotional Traits

Callous Unemotional Traits have been used to define a particularly virulent and treatment resistant subtype of conduct disorder. They were described in children in institutions more than 50 years ago, but there has been little systematic research ever since. Because of our interest in psychopathology, we have a uniquely valuable sample to determine prevalence, association with deprivation (percent time institutionalized), and especially co-occurrence with conduct disorder. This 24-item questionnaire is the gold standard for assessing this construct, and it is standard practice to obtain reports from multiple informants.

Participant: CHILD. PARENT/CAREGIVER, and TEACHER
Estimated time to complete: 10 minutes
Present during Administration: RA and Child (Private Room 1) and RA2 and Parent/Caregiver (Private Room 2)

DNA Collection

The impact of early experience on stress reactivity over the course of the lifespan is not yet fully understood. Specifically, it is unclear if early stress exposure creates a permanent increased vulnerability to stress, similar to a scar, that results in lifelong increased stress responsiveness or whether instead, proximal life events are more relevant to current stress reactivity. Two putative measures which may reflect the lasting impact of early stress exposure are telomere length and epigenetic modifications including methylation and histone acetylation. In our initial sample, we noted that percent time spent in an institution at 30, 42 and 54 months of age was predictive of telomere length at age 8, indicating that these early experiences were indeed relevant to one putative measure of cumulative stress exposure, telomere length. However, to differentiate between these two hypotheses, it is important to determine whether the influence of early experience, compared to more proximal life stressors, is more determinant of telomere length. More precisely, we seek to determine if early experience is more predictive of telomere length over time, or if telomere length is more related to life stressors during the past year. This longitudinal analysis will also help us determine if changing the caregiving environment and presumably decreasing stress early in life prevents further telomere length decline or if the early experience results in lasting alterations in stress reactivity that continues to be reflected in decreased telomere length, regardless of more proximal life stressors. Evidence demonstrating early experience related epigenetic changes points to another putative mechanism by which early adversity results in lasting neurobiological changes. In order to explore these hypotheses, we will collect DNA samples and examine the change in telomere length over time.

In addition to determining telomere length we will also explore methylation status and putative patterns of histone acetylation as key alternative regulators of gene expression that can be modified by early experience. Additionally, as recent evidence suggests that methylation patterns are not as static as originally expected, and because we have DNA samples collected at multiple time points for the telomere analysis, it is expected that comparison of methylation patterns longitudinally will provide greater insight into the underlying biological mechanisms. We will examine methylation patterns in DNA collected at age 15-16, as well as examine methylation patterns in DNA already collected at earlier times points (as approved in protocol 06-10-0455 and 10-04-0185) to determine if alterations in methylation are more predictive of outcome than methylation status at age 15-16. All samples will be shipped to New Orleans, Louisiana and DNA extracted. Telomere, methylation and histone studies will all be done blind to all other measures and under the direction of Stacy Drury, MD, Ph.D., at Tulane University School of Medicine.

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 participant number and date of collection (two buccal swabs will be obtained from each participant at each visit). The DNA samples collected during this session will solely be used for this study. All DNA samples are labelled with the participant number and date of collection. No other identifying information is included. All DNA samples will be destroyed 7 years after the project is completed.

Participant: CHILD
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant, Child and Parent/Caregiver

Life Events Scale

To further assess the influence of proximal life events, we will also administer the Life Events Scale. This 30-item questionnaire asks participants to indicate whether or not they experienced a specific life event and if so, how stressful the event was for them and *the extent of control they believe they had over the event.*

Participant: CHILD
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant, Child and Parent/Caregiver

Peer Nomination (Prep for Research Session 2 - Peer Interaction Task)
At the conclusion of research session 1, the researcher will ask the participant to nominate their 3 closest friends. The researcher will then tell the participant that one of these friends will be invited to come to the research laboratory to play some games with the participant when s/he returns to complete research session 2. The researcher will then ask the parent/caregiver to provide the parents/caregivers of the nominated peers a copy of the Nominated Peers Recruitment Letter (see attached). The letter will describe the research session and will ask interested parents/caregivers to contact the BEIP Research Team if they are willing to participate.

Participant: CHILD
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant, Child and Parent/Caregiver

BEIP Demographic Questionnaire
This questionnaire consists of 20 items and includes questions about family income, marital status, length of time the participant has lived with the adult completing the form and number of other individuals living in the household.

Participant: PARENT/CAREGIVER
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant and Parent/Caregiver

MacArthur Health and Behavior Questionnaire ** (HBQ-Teacher; version 2.1 (late childhood and adolescence)) The HBQ consists of approximately 140 items regarding child functioning Ablow et al., 1999; Essex, Boyce, Goldstein, Armstrong, Kraemer, & Kupfer, 2002; Luby et al., 2004). This questionnaire will be administered to the child's parent/caregiver and if consent is obtained, from the child's 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.

Participant: -TEACHER of child
Estimated time to complete: 25 minutes
Present during Administration: TEACHER

Social Skills Rating System (SSRS) **
The Social Skills Rating System (Gresham & Elliot, 2003) 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.
We administered this measure to parents/caregivers and teachers at ages 8 and 12 and feel it is important to ask parents/caregivers to complete this measure at age 15-16 so we can obtain a more complete and longitudinal picture of each child's social behaviors and academic performance.

Participant: TEACHER and PARENT/CAREGIVER of child
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER, Teacher

** The teacher versions of the HBQ and SSRS will include a cover note that indicates that if the teacher has any concerns about the child and would like to discuss or to obtain help, s/he may contact Anca Radulescu, Ph.D, or Nicoleta Corlan, Ph.D., both licensed clinical psychologists affiliated with the BEIP Research Team.

Contact information for the research laboratory and email addresses for Dr. Radulescu and Dr. Corlan will be provided.

Additionally, the following items from the HBQ-T and SSRS-T will be reviewed within 24 hours of receipt by a member of the research team:

HBQ-T: items 12 (pg. 3), 13k, 13l and 13m (pg. 4), 14 (pg. 10), 28 and 38 (pg. 11), 74 (pg. 14), 78 and 87 (pg. 15).

SSRS-T: items 32 and 35 (pg. 3)

If positive (worrying) responses are recorded for any of these items, the teacher will be contacted by Dr. Radulescu or Dr. Corlan, encouraged to discuss his/her concerns with the school psychologist and offered information about bullying.

Expressed Emotion
Expressed Emotion (EE) is a qualitative measure of the 'amount' of emotion displayed, typically in the family setting, by a family or care takers. EE, as originally assessed using the Camberwell Family Interview, is considered a measure of the patient-relative relationship and is a highly valid and reliable predictor of poor clinical outcomes among patients with major psychopathology (as reviewed by Hooley & Parker, 2006). Parents/caregivers will be asked to describe their child as if the interviewer has never met the child. This assessment will be videotaped/audiotapted for subsequent coding and analysis. If parents fail to give a meaningful response to the open-ended question, the interviewer will follow-up with more specific probes.

Participant: PARENT/CAREGIVER
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant and Parent/Caregiver

Disturbances of Attachment Interview (Adolescent Version)
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 15-16 years, we will be most concerned with signs of indiscriminate/disinhibited behavior and risk-taking behaviors. The Adolescent version includes questions assessing the child's emotional regulation/expression and actions toward others. These questions have implications for psychiatric impairment and it is important to include parental report of these behaviors.

Participant: PARENT/CAREGIVER
Estimated time to complete: 20 minutes
Present during Administration: Research Assistant and Parent/Caregiver

Screen for Child Anxiety and Related Disorders (SCARED)
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Participant: PARENT/CAREGIVER
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Parent/Caregiver

ESTIMATED TOTAL TIME TO COMPLETE RESEARCH SESSION 1:
Participant: 210 minutes
Parent/Caregiver: 90 minutes

Teacher: 45 minutes

* The proposed changes reduce the estimated completion time for the participant by 15 minutes.

Research Session 2

Growth Measurements
The participant's height, weight and head circumference will be measured by a member of the BEIP Research Team.

Participant: CHILD
Estimated time to complete: 5 minutes
Present during Administration: Research Assistant, Child and Parent/Caregiver

CDC Youth Risk Behavior Survey
To examine lifetime (baseline assessment period only) and past year (all assessment periods including baseline) prevalence of real world risk behaviors, we will use a shortened version of the CDC Youth Risk Behavior Survey (CDC, 2001). In total, the shortened measure will assess different risk taking behaviors across the domains of drug and alcohol use, safety compromising behaviors (e.g., not wearing a seat belt, provoking packs of wild dogs), sexual activity, delinquency related behaviors (e.g., gambling, stealing, bringing a weapon to school) relevant to the Romanian youth population. In addition to the examination of individual behaviors, we also will examine an aggregate measure of risk including behaviors that pose a direct (e.g., injecting drugs) and indirect (e.g., alcohol use) risk for HIV infection. A similar aggregate will be examined for risk-behaviors unrelated to HIV infection. This strategy has been used successfully in previous research with youth ranging from early through late adolescence (Aklin, Lejuez, Zvolensky, Kahler, & Gwadz, 2005; Lejuez et al., 2002; 2003). In these studies, the composites have shown good reliability (all α's > .7), and have been shown to be significantly related to other self-report measures of risk related constructs including sensation seeking and impulsivity. This measure was chosen over more extensive measures such as the Global Appraisal of Individual Needs (GAIN-I) measure (Dennis et al., 2002; Tims et al., 2002) because the level of risk behavior at this age can be captured in a shorter more general measure which limits participant burden. This is in line with other similar research (e.g., Lejuez et al., 2007).

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child

EEG/ERP Tasks
Electrophysiological recording: Event-related potentials (ERPs) represent transient changes in the electrical activity of the brain in response to a discrete stimulus event. They are recorded from electrodes placed on the scalp, and provide a tool for evaluating the timing of mental events. In the current study, ERPs will be recorded using a 64-channel net (Electrical Geodesics Inc., EGI) and data acquisition software (NetStation 4.0, EGI). The Geodesic Sensor Net consists of an array of electrodes arranged in an elastic tension structure. The net contains small plastic pedestals, distributed evenly across the head surface. Each pedestal contains a small sponge, inside which there is an Ag/AgCl electrode.
Net application: The participant's head will be measured, and a small mark will be made (with a grease pencil) at the very top of the participant's head to allow proper placement of the net. Before the net is placed over the participant's head, the sponges will be soaked in a salt water solution (distilled water + KCl + baby shampoo) until warmed to body temperature. The net is held in place by a chin strap. The Geodesic Sensor Net provides a very user-friendly system for recording EEG and ERPs. The elastic tension structure allows the net to be quickly and easily stretched over the participant's head. No cleaning or abrasion of the scalp is necessary, and there are no gels or creams to clean up afterwards. After removing the net, a small amount of baby oil is used to remove the grease mark. In general, these procedures minimize both the electrode application time and any discomfort to the participant.

Baseline EEG (EEG task)
Baseline EEG will be collected at the start of the EEG/ERP tasks in order to assess relative EEG power. Findings from the baseline BEIP assessment indicated that children in the institutionalized group showed a

higher level of relative theta power and a reduction in alpha and beta power (suggesting delay) compared to age-matched community controls. We continued to see similar patterns at 42 months and 8 years, with children in the foster care group demonstrating higher alpha power than children in the institutionalized group. It will be important to determine if these effects are long-lasting and if the timing effects observed at 42 months and 8 years continue into adolescence.

We will examine participants 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, particularly in the domains of individual differences in approach and withdrawal tendencies.

Once fitted with the net, the child will be asked to sit in a chair. 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.

Participant: CHILD
Estimated time to complete: 6 minutes
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Flanker Task (EEG/ERP task)

We will employ the Flanker Paradigm, which is a computer-based task that assesses an individual's ability to inhibit a predominant response in the face of interfering stimuli. The stimuli consist of four different arrays of arrows which compose either congruent (>>>>> or <<<<<) or incongruent (>><>> or <<><<) trials. One of the four arrow arrays will appear on each trial and the participant's goals is to press a key corresponding to the central arrow in the array. The ability to resist the distracting stimuli and exert cognitive control is measured by comparing accuracy and reaction time differences between congruent and incongruent trials. The flanker task also assesses behavioral and physiological correlates of error monitoring (i.e. post-error slowing in reaction time and the error related negativity; ERN). The ERN is defined as the negative most deflection in a 50-150 ms time window after response execution (button press). The task consists of 480 test trials presented in three blocks of 160 trials each. Prior to presentation of the test blocks subjects are given a short practice round to become accustomed to the task. Reaction time and accuracy on each trial will be recorded along with ongoing EEG for creation of ERP components (i.e. the ERN).

Participant: CHILD
Estimated time to complete: 20 minutes
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Go – No Go Task (EEG/ERP 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: 10 minutes

Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Dot Probe Task (behavioral only)
In this task, two stimuli, one threat-related and one neutral, are shown briefly together and their offset is followed by a small probe in the location just occupied by one stimulus. Participants are required to respond as fast as possible to the probe without compromising accuracy. Response latencies on the task provide a "snap-shot" of the distribution of the subject's attention, with faster responses to probes presented in the attended relative to the unattended location. Attention bias to threat is evident when participants are faster to respond to probes that replace threat-related rather than neutral stimuli.

The face stimuli will be photographs of 16 different individuals (8 male, 8 female) taken from the NimStim stimulus set [37]. Previous work with these stimuli performed by Fox and colleagues demonstrates that these face stimuli effectively elicit threat bias. Three different pictures of each individual will be selected depicting angry, happy, and neutral expressions. Participants will be presented with pairs of faces (neutral-angry, neutral-happy, or neutral-neutral). Each face pair will comprise of pictures of the same person. Each trial will begin with the presentation of a fixation display (white cross 2*2 cm at the center of the screen), on which the participants will be requested to focus their gaze. The fixation display will last 1000 ms and will be followed by a face pair display for 500 ms.

Each face photograph will subtend 55 mm in width and 80 mm in height. The face photographs will be presented with equal distance to the left and right of the fixation cross, with a distance of 16.5 cm from the center of one face to center of the other. Immediately following the faces display a target probe will appear for 100 ms, after which the screen will go blank. The target-probe display will consist of two dots distant from each other by 5 mm center to center. Each dot will subtend 2 mm in diameter. The dot pair is oriented either horizontally (..) or vertically (:) and will appear at a distance of 8.5 cm either to left or to the right from fixation (center to center), that is, at the location of the center of either the left or the right photograph of each pair. Participants will be required to determine the orientation of the dots (horizontal or vertical) by pressing one of two pre-specified buttons on a response box. A new trial will begin 1,400 ms after target probe offset. We selected to use a probe-discrimination task instead of a probe side location task because it allows better control of the Simon effect. The Simon effect states that reaction times are usually faster when stimulus and response occur at the same location than when they do not, even if the stimulus location is irrelevant to the task. Thus, to neutralize this effect in the dot-probe task one needs to counterbalance the configuration of the responding fingers/hands to left/right target's location. However, if the response is based on probe side location, counterbalancing could create an interference condition for half the participants (e.g., responding with the left hand to a right side target). This problem is resolved if subjects are asked to discriminate (:) from (..) instead. Across trials, the angry or happy face will equally likely be on the left or on the right, and the dots orientation will equally likely be horizontal or vertical. These two variables will be randomly mixed in presentation.

There will be a total of 320 trials, presented in four equal blocks of 80 trials each. Participants will be presented with 128 Angry/Neutral, 128 Happy/Neutral, and 64 Neutral/Neutral pairs. The attention task will be presented on a 17 inch computer monitor controlled by the E-Prime software package. Trials will be counterbalanced across emotion face location, probe location, probe orientation, gender of face, and type of emotion.

Participant: CHILD
Estimated time to complete: 15 min
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Screen for Child Anxiety and Related Disorders (SCARED)
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responses. The SCARED does not involve "skip outs", providing better standardization. Additionally, the SCARED is far more widely used than the DISC; as a result, having data on the SCARED will facilitate attempts to relate our findings to a broader array of studies, not all of which will have the DISC.

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child

Peer Interaction Task

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 engage in several semi-structured activities a familiar, same-sex, age-matched peer nominated by the child in research session 1. The session will be videotaped for subsequent transcription, translation and coding.

Planning Task: The participant and their nominated peer will be asked to planning an outing together. The researcher will offer outings they can plan such as going to the movies, going to a park, or going shopping downtown. The nominated peer will be asked to write down the plan after their discussion has ended.

Building Task: The participant and their nominated peer will be given pieces of Legos and asked to assemble an intricate model that they will be shown by the researcher.

Discuss an Issue: The participant and their nominated peer will be asked to discuss a current issue (Is it OK for children to smoke cigarettes? At what age is it okay for children to go off on their own?).

Stoplight Game: In the "Stoplight" game, which is used to generate a behavioral measure of *sensation seeking* (Steinberg et al., 2008), the player is asked to "drive" a car before time runs out to a distant location, where a party is taking place. The participant's vantage point is that of someone behind the wheel, with the road and roadside scenery visible and changing as the car travels down the road. Also shown on the screen is a clock counting down the time; the clock is initially set to 5 minutes. The participant hears the clock ticking down and "party" music, which grows increasing louder as the car approaches the destination. In order to reach the destination, the driver must pass through 20 intersections, each marked by a traffic signal.

Before playing the game, participants see a demonstration that is accompanied by instructions read by the researcher. They are informed that when they are approaching an intersection, the traffic signal will turn yellow, and that when this happens, they must decide whether to stop the car (by using the space bar) and either wait for the light to cycle from yellow to red to green, or attempt to cross through the intersection; participants are told that they cannot control the speed of the car, and that the only time the brake works is after the traffic light has turned yellow. Participants are told that if the car is driven through the intersection and the light turns red, there is a chance that it may crash into another vehicle that is driving through at the same time. The researcher then explains that one of three things may happen depending on the participant's decision (each scenario is illustrated with video simultaneously with the narrated instructions): (1) if the brakes are not applied and the car makes it through the intersection without crashing, no time is lost; (2) if the brakes are applied before the light turns red, the car will stop safely, but time will be lost waiting for the light to cycle back to green (approximately 3 seconds); (3) if the brakes are not applied or are applied too late, and the car crashes into the crossing vehicle (this is accompanied by squealing tires and a loud crash, as well as the image of a shattered windshield), even more time will be lost (approximately 6 seconds) than had the participant decided to brake. Thus, participants must decide whether to try to drive through the intersection in order to save time and risk losing twice as much time if a crash occurs, or to stop and wait (and willingly lose a smaller amount of time). The basic outcome variable of interest is the proportion of intersections the participant entered without braking. Performance on the task is correlated with self-reported sensation-seeking, but not with self-reported impulsivity (Steinberg et al., 2008).

Probabilistic Gambling Task: On each trial of the probabilistic gambling task (PGT), the participant is presented with a wheel divided into three distinct wheel-shaped sections, colored red, green, and gray. The green section indicates the participants' opportunity to win tokens (reward, +10 tokens), red indicates the opportunity to lose tokens (loss, -10 tokens), and grey indicates the chance of neither winning nor losing tokens (neutral outcome, 0 tokens). The experimenter explains that they will be shown a series of wheels

that would spin and come to rest with an indicator pointing to one of the three sections, and that the relative size of each section is exactly indicative of the chance of landing on that section and achieving the specified outcome. Each participant begins the task with a "bank" of 100 tokens. Participants are shown a wheel and asked to think for 2 seconds about whether they would like to "play" (and accept the outcome of the wheel's spin) or "pass" (move on to the next wheel). Next, the words "Play or Pass?" appeared above the wheel and participants were required to make their selection, within 1500 ms. Participants are not asked to decide how much to wager. If the participant chooses to play on a wheel, the wheel begins spinning and comes to rest with an indicator pointing to one of the three sections. If the participant chooses to pass, a screen indicating "no play" appears. After each trial, a feedback screen appears, and participants are shown the trial's outcome (+10 for a win, -10 for a loss, and 0 for a neutral or pass) and their overall game earnings up to and including that wheel. All participants undergo an instructional session with the researcher, followed by a practice session consisting of 10 wheels.

The BEIP research participant (target child) will complete the Stoplight Task as their nominated peer observes. The target child will then complete the Probabilistic Gambling Task as the nominated peer completes the Stoplight Task. The target child will then repeat the Stoplight Task as their nominated peer completes the Probabilistic Gambling Task. Performance on the Stoplight Task will be compared across the two conditions: playing while observed and playing alone. Previous research suggests that adolescents tend to engage in riskier behavior during the Stoplight Task when they are observed by a peer.
Participant: CHILD and NOMINATED PEER
Estimated time to complete: 60 minutes
Present during Administration: Research Assistant and Children

Network of Relationships Inventory (NRI)
The NRI (Furman & Buhrmester, 1992) assesses a broad range of social network qualities in adolescents and adults, such as number, age range, gender, and ethnic variability of close friends. Both the child and their nominated peer will be asked to complete this brief questionnaire following the conclusion of the peer interaction tasks.

Participant: CHILD and NOMINATED PEER
Estimated time to complete: 10 minutes
Present during Administration: RA 1 and Child (Private Room 1) and RA 2 and Nominated Peer (Private Room 2)

Friendship Quality Questionnaire (FQQ)
This questionnaire was administered at the 12-year assessment and will be repeated at age 16 to assess friendship quality amongst target participants and their nominated peers and includes six sub-scales: Validation & Caring, Conflict Resolution, Conflict & Betrayal, Help & Guidance, Companionship & Recreation, and Intimate Exchange.

Participant: CHILD and NOMINATED PEER
Estimated time to complete: 10 min
Present during Administration: Research Assistant and Child

Additional Questionnaires for Nominated Peers
In addition to the NRI and FQQ described above, nominated peers will also be asked to complete the Barratt Impulsivity Scale, Zuckerman Sensation Seeking Scale and the WISC-IV (see descriptions in Research Session 1). Given the association/relation between several outcome measures and IQ, we will assess the IQ of all nominated peers who participate in the peer interaction session. We will administer the same 3 subtests of the WISC-IV as children in the FCG, CAUG and NIG (Block Design, Digit Span, and Vocabulary) in order to estimate full scale IQ scores of nominated peers who participate in the study.

Participant: NOMINATED PEER
Estimated time to complete: 60 minutes
Present during Administration: Research Assistant and Nominated Peer

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The validity of the DISC-IV was originally established in large-scale epidemiological surveys of children and adolescents, but it has since being used in many clinical studies, screening projects, and service settings.
For the purposes of this research, we will administer 13 sections of the interview (including components from anxiety disorders, miscellaneous disorders, mood disorders, disruptive behavior disorders and the Whole Life module) to both the parent/caregiver and child. An electronic copy of the child version of the DISC has been submitted to the CCI office under protocol 10-04-0185.

Anca Radulescu, Ph.D., will oversee all administered DISC interviews. Ms. Radulescu has received training on the DISC by Dr. Prudence Fisher at Columbia University. She will train the RAs who administer the parent and child versions of this measures (blind to participant status) to note and report to her at the completion of the interview (with parent/caregiver or child) any of the following: suicidal ideation, self-injurious behavior, threatening to harm others, serious risk taking behavior, significant impairment in any domain, or any child or parent who wishes assistance with the child's emotions or behaviors.

If such behaviors are reported, Dr. Radulescu will follow the actions detailed in the Safety Reporting Protocol.

The consent and assent forms indicate that if the child reports engaging in danger to self or danger to others, confidentiality will be breached, and the parent/guardian will be made aware of the child's report.

Participant: PARENT/CAREGIVER Estimated time to complete: 60 minutes
Present during Administration: RA and Parent/Caregiver (Private Room)

BEIP Health Questionnaire
Parents/caregivers will be asked to respond to questions about their child's health, including questions about asthma, allergies, and oral hygiene.

Participant: PARENT/CAREGIVER
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and Parent/Caregiver

This Is My Child Interview – Revised (TIMC-R)
The TIMC-R interview is a semi-structured interview lasting approximately 10 minutes. The interview consists of six basic questions relating to the mother-child relationship, as well as a seventh question regarding the mother's experience as an adoptive parent/foster parent/caregiver. Whereas the Expressed Emotion interview measures warmth, criticism and emotional involvement, the TIMC-R measures commitment, acceptance and degree of influence. The data from this measure will be used to determine if parental/caregiver commitment mediates the effect of early placement on outcomes.

This interview will be administered by certified clinicians on our research team. Both clinicians have been thoroughly trained on this measure by Charles H. Zeanah, Jr., M.D., a child psychiatrist and co-PI on this investigation.

Dr. Zeanah will monitor interviewer fidelity and competency via biweekly skype discussions with the Romanian research team during piloting and data collection. Dr. Zeanah and the research team will review 2-3 videotaped interviews for pilot subjects who have completed the session and will discuss any issues. As data collection for this task begins, Dr. Zeanah will continue skype discussions with the research team and will periodically review videotapes of the interviews with the two administering clinicians in the lab.

All interviews will be videotaped and coded by two trained research assistants to establish inter-rater reliability. The demands of administering and coding this interview are roughly comparable to the DAI which we have used at every assessment age during the past 10 years. We have had no difficulty with fidelity of reliability with that measure.

Participant: PARENT/CAREGIVER
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and Parent/Caregiver

Secure Script Assessment
In order to assess parents'/caregivers' knowledge of and access to the secure base script, each parent/caregiver will be asked to produce six stories from sets of prompt-word outlines developed by H. Waters and Rodrigues (2001), each consisting of 12 words that frame an implied story line. Three types of stories will be used: stories with attachment-related content that emphasize mother-child interactions (*The Doctor's Office* and *Baby's Morning*), stories that emphasize adult-adult interactions (*The Accident* and *Jane & Bob's Camping Trip*), and stories without attachment content (*Trip to Park* and *An Afternoon Shopping*). For the purposes of this study, the overall attachment script scores (all four attachment narratives) will be used in the data analyses. Neutral stories serve the goal of introducing some variability and keeping participants from developing a particular mind-set. Each outline consists of twelve words that suggest a story line and enough content to result in a story of approximately one-half to a full page length when written. Each story takes less than three minutes to produce. Parents/caregivers will be given a list of word prompts and asked to use the columns of words to generate a story, going from left to right. After reviewing each outline, the participant will indicate when they are ready. The session will be videotaped for subsequent transcription, translation and coding. The measure was piloted on a sample of 25 Romanian mothers for a cross-cultural study of representations of attachment and was used subsequently on a different sample of 59 Romanian mothers.

The measure was validated against the AAI on a sample of adult women, showing high correlations with AAI coherence scores (.58, $p < .01$). AAI coherence is the scale most predictive of adult attachment security, and it refers to the participants' ability to tell an organized and believable story about their early experiences. In subsequent studies, the Attachment Script Assessment was found to be stable over a one year period (Vaughn et al., 2006), was correlated with maternal sensitivity (Coppola et al., 2006), with children's strange situation classification (Tini, Corcoran, Rodrigues-Doolabh, & Waters, 2003), and with children's attachment behavior in naturalistic settings, as measured with the Attachment Q-Sort (Bost et al., 2006; Verissimo & Salvaterra, 2006). The predictive value of the Attachment Script Assessment is comparable to that of the AAI (76% according to Tini et al., 2003), and the relationship is preserved in both biological (Bost et al., 2006) and adoptive families (Verissimo & Salvaterra, 2006). These studies show that "mothers with well-scripted secure base knowledge have children who treat them as a secure base for exploration at home" (Vaughn et al., 2006).

Narratives will be scored on a seven-point scriptedness scale reflecting how much they resemble a protypical SBS, according to a scoring manual (Psouni & Apetroaia, 2009). Narratives characterized by rich SBS content are scored as 6 or 7 depending on degree of relevant elaboration. Narratives containing some elements of the SBS but restricted in elaboration are scored as 5. Narratives with minimal elements of the SBS are scored as 4. Stories focused on actions or events, with no acknowledgement of emotional states or interactions are scored as 3. Stories lacking secure base content, and additionally being so short so as to be disjointed, are scored as 2. Finally, stories that include almost exclusively odd content or a different theme entirely are scored as 1.

Participant: PARENT/CAREGIVER
Estimated time to complete: 30 minutes

Present during Administration: Research Assistant and Parent/Caregiver

ESTIMATED TOTAL TIME TO COMPLETE RESEARCH SESSION 2:
Participant: 171 minutes
Nominated Peer: 120 minutes
Parent/Caregiver: 110 minutes
-

Research Session 3

Cambridge Neuropsychological Test and Automated Battery (CANTAB)
As we did at 12 years, we will again perform a number of tests from the CANTAB, focusing most on executive functions and memory. All CANTAB tasks run on a touch screen monitor and require minimal language and motor coordination skills (for reviews, see Luciana & Nelson, 1998; 2002). We wish to employ this task in order to examine associations between EFs in particular and specific forms of psychopathology (e.g., poorer planning behavior as indexed on the Stockings of Cambridge and poorer set shifting on the ID/ED shift task should be associated with poorer executive control and thus a higher prevalence of externalizing problems).

Motor Screening Test. The first subtest, a motor screening task, screens for visual, movement, and comprehension difficulties. A flashing cross is displayed on the screen in various locations, and subjects are instructed to touch it as quickly as possible.

Delayed Matching to Sample. This subtest assesses forced choice recognition memory for patterns. The subject is shown a pattern and then must choose out of four similar patterns which one exactly matches the original pattern. In some of the trials, the original pattern is obscured before the choices appear, or there is a brief delay between these steps.

Paired Associates Learning. This subtest assesses visual memory and new learning. A number of boxes are displayed, some with patterns inside, and after a brief delay the subject must identify where each individual pattern was displayed. If the subject does not identify each location correctly, the trial is repeated. As the subject progresses through the task, an increasing number of boxes and patterns are displayed.

Stockings of Cambridge. This version of the Tower of London planning task is a spatial planning task in which the subject must copy a pattern displayed on the screen by moving colored circles one at a time, using the fewest number of moves possible.

Spatial Working Memory. This subtest assesses the subject's ability to retain spatial information and to manipulate remembered items in working memory by locating tokens hidden in boxes. The subject is instructed that after a token has been found in a box, that box will not contain any tokens in the future. Subsequent stages include increasing numbers of boxes and tokens.

Intradimensional/extradimensional Shift Task (ID/ED Shift). This task (which was not employed at age 8) assesses discrimination and reversal learning under conditions in which the subject is required to shift attention to changing patterns of visual stimuli. A functional dissociation exists between the dorsolateral prefrontal cortex in between-category set shifting and the orbitofrontal cortex in within-category reversal shifts using this task. This task progresses along a series of stages of increasing difficulty. In the first stage (termed the 'simple discrimination stage), the child views two lined patterns on the computer screen. The child is told that one of them is correct and that the other is incorrect and that s/he must determine which the correct pattern is by touching one or the other. If correct, the computer will flash green; if incorrect, the computer will flash red. Hence, this stage requires the child to learn a two-alternative forced-choice discrimination of two lined drawings using immediate feedback provided by the computer. The child is told that as s/he works, a rule will become apparent that will guide the selection of subsequent choices. However, once the computer has determined that the child knows the rule, the rule will change. The child is told that despite these changes, s/he should try to make as many correct choices as possible. Learning criterion is six consecutive correct responses. After achieving criterion on the Simple Discrimination stage, the feedback provided to each stimulus is reversed so that the one first correct is now incorrect, and the one first incorrect is now correct (Simple Reversal). Again, there are six trials to criterion. At the third stage, a

second dimension (purple shapes) is introduced together with the lined drawings so that each stimulus now contains two drawings--one lined and one shaped. To succeed on this Compound Discrimination (CD) condition, the subject must continue to respond to the previously relevant lined drawing while ignoring the presence of the new irrelevant dimension (purple shape.). Two CD conditions are administered, one where the lined and shaped drawings are distinct and one where the same stimuli are superimposed upon one another. Following successful completion of these two conditions, there is a CD reversal. Through the CD reversal stage, the subject is viewing the same stimuli over and over again, trial-by-trial. The next (6th) stage involves the first demand for an attentional shift. Termed the intradimensional (ID) shift stage, novel or never-seen exemplars of each of the two dimensions (line and shape) are introduced, and the subject must continue to respond to the previously relevant dimension (lined drawing). Success on this stage requires that the subject generalize previous learning (e.g., "lined drawings are correct") to new stimuli. Following another feedback reversal shift (IDR), the second demand for an attentional shift is required. This stage is termed the extradimensional shift (EDS). Once again, novel exemplars of each stimulus dimension are presented. In order to succeed at the ED stage, the subject must shift response set from the previously relevant dimension (lined drawing) to the previously irrelevant dimension (purple shape). This shift requires that the subject learns and responds to a new rule (e.g., "Lines are no longer correct--Shapes are correct"). This stage is presumably analogous to the types of category shifts that are required by standard neuropsychological tests of set-shifting ability such as the Wisconsin Card Sort. The final task stage is a reversal of the ED shift. Each response made by the subject is presumably influenced by the feedback (correct versus incorrect) that s/he receives on the previous trial.

Participant: CHILD
Estimated time to complete: 45 minutes
Present during Administration: Research Assistant and Child (Parent/Caregiver will be given the opportunity to observe from another room)

Assessment of Pubertal Status (Morris & Udry, 1980)
This puberty assessment scale (18 questions for boys; 20 for girls) asks adolescents to answer a variety of questions on a five-point scale. Examples include, "How often do you shave?" and "Does your voice crack?" Children will also be shown a series of pictures depicting different stages of physical development in breasts, genitalia, and pubic hair (Tanner, 1962) and asked to circle the picture that best resembles their current appearance. The instrument also assesses the individual's social-emotional reaction to puberty through items such as "How happy are you with your weight?" and "How happy are you with your overall body build?"

Participant: CHILD
Estimated time to complete: 10 minutes
Present during Administration: Research Assistant and Child

Autonomic Measures
Participants will be connected to the physiological recording equipment after completing the Pubertal Status Questionnaire and completion of the Baseline Affect Questionnaire (see below). Autonomic nervous system (ANS) responses will be measured throughout the duration of the session using a variety of instrumentation including: electrocardiograph (Standard Lead II configuration, right arm, left leg, right leg ground), impedance cardiograph (four tape sensors: inner sensors are placed at the xiphisternal and base of the neck, outer sensors are placed 3 cm distally to the inner sensors), and blood pressure monitored through an upper arm cuff. Using these tools, we will examine changes in the following ANS measures from baseline to each task: heart rate (HR), blood pressure (BP), skin conductance, pre-ejection period (PEP; a measure of pure sympathetic nervous system activation), respiratory sinus arrhythmia (RSA; a measure of pure parasympathetic nervous system activation) and cardiac output (a measure of cardiac efficiency).

The sensors and tape are non-invasive and feel like band-aids when pulled from the skin. Participants' ANS responses will be obtained for the duration of all studies. Drs. McLaughlin and Sheridan will train all Romanian research assistants who handle ANS equipment on physiological recording, monitoring, and safety. Immediately following each participant any reusable ANS equipment (BP arm and wrist cuff, skin conductance and skin temperature) are cleaned thoroughly. The skin sensors are removed from the electrical leads and immersed in rubbing alcohol. We then use small Q-tips to remove any skin conductance cream residue from the lead. Following the rubbing alcohol we clean off the leads with soapy distilled water. All participants are given hand sanitizer immediately following removing the sensors.

Initial Baseline Recording
Prior to connection to the electrophysiological equipment, participants will complete the Baseline Affect Questionnaire to rate their overall cognitive appraisals of the situation, mood and emotional reactions. Once connected to the physiological recording equipment, participants will provide a saliva sample (see ***Saliva Collection*** below) and complete a five-minute baseline period to record autonomic activity.

Participant: CHILD
Estimated time to complete: 5 minutes Present during Administration: Research Assistant and Child

Saliva Collection
We will collect six saliva samples during the stress physiology tasks. Research assistants wear latex gloves during the collection and storage of saliva samples. The measurement of these salivary hormones is pain free and non-invasive. We will use the drool method to collect saliva for cortisol and hormone assays, as the cotton in cotton swabs can interfere with measurement of anabolic steroid hormones. Participants will spit, or drool, into IBL tubes.

Saliva will be assayed to assess levels of cortisol and other hormones and proteins in your body that are influenced by stress (dehydropepiandrosterone (DHEA) and testosterone). Cortisol levels rise immediately with heightened arousal, but peak in the saliva after 20 minutes. Accordingly, saliva will be assessed at the following points during the study: 1) baseline (3 samples obtained at least 20 minutes after arriving to the lab), 2) 20 minutes following the TSST-C (1 sample), 3) 20 minutes following the Number Task (1 sample) and 4) 20 minutes following the Pinata Task (1 sample).

Participant: CHILD
Estimated Time to Complete: 20 minutes for all samples
Present during Administration: Research Assistant and CHILD

Trier Social Stress Test for Children (TSST-C)
At the 12-year assessment, participants completed the TSST-C, and we will repeat the task at the 16-year assessment to determine whether participants habituate to the task (i.e., no longer show a physiological response) or exhibit sensitization (i.e., a stronger physiological response at the second administration). Sensitization occurs in about 20% of adolescents and adults, and is associated with risk for anxiety (Rohleder et al., 2012). The TSST-C is the most widely used task to elicit physiological responses from children (Gunnar, Talge, & Herrera, 2009). This task has been used in numerous studies with children and adolescents, including children as young as 6 (Buske-Kirschbaum et al., 1997; Buske-Kirschbaum et al., 2007; Buske-Kirschbaum et al., 2003; Corbett, Mendoza, Abdullah, Wegelin, & Levine, 2006; Dorn et al., 2003; Gerra et al., 2000; Gunnar, Frenn, Wewerka, & Van Ryzin, 2009; Jansen et al., 2000; Jones et al., 2006). The task involves delivering a verbal performance (either a speech or completion of a story) and a subtraction task completed out loud in front of two evaluators. The task reliably elicits a response in measures of autonomic nervous system activity (heart rate, blood pressure, skin conductance) and in measures of the HPA axis (cortisol, DHEA). Importantly, the task has frequently been used to examine physiological reactivity in vulnerable populations. These have included children exposed to physical and sexual abuse, including children who have been placed in the child welfare system following removal from parental custody (MacMillan, et al., 2009; Rao, et al., 2008), children who were born pre-term (Buske-Kirschbaum, et al., 2007), and children with mental disorders—including depression, anxiety, externalizing disorders, and autism—(Corbett, et al., 2006; Dorn, et al., 2003; Gerra, et al., 2000; Jansen, et al., 2000; Martel, et al., 1999; Popma, et al., 2006; Rao, et al., 2008; van West, et al., 2008; Zonnevylle-Bender, et al., 2005). This task has also been used in a sample of previously institutionalized children (aged 8-10) from Romanian orphanages who were later adopted by families in the United States (Gunnar, Frenn, et al., 2009) as well as in children in the BEIP at age 12. No adverse events occurred in this previous assessment.

Participant: CHILD
Estimated Time to Complete: 35 minutes (includes task completion and recovery)
Present during Administration: Research Assistant and CHILD

Bells Task

When confronted with emotional events, humans can develop emotional responses to specific cues associated with these events through a process called classical conditioning. The Bells Task was designed to assess this type of learning in children and adolescents through a conditioning procedure that relies on four stages. First a five-minute baseline is acquired during which participants sit quietly without moving (this part of the procedure is identical to the first stage of the Number Task). The task then involves preacquisition, acquisition, and extinction phases. During the preacquisition phase, participants are presented with 2 neutral stimuli – specifically, images of two different colored bells. They view each of these bells several times. During the acquisition portion of the task, participants view these same two bells, but one bell is paired with a loud ringing noise (a 95 db alarm sound) while the other is not paired with the alarm sound. During the final extinction phase, particpants see the bells again with no alarm sound. Physiological data (specifically, skin conductance responses and heart rate) will be acquired to measure responses to the task. In addition, participants will be asked the following questions about each bell (blue bell used as example) after the pre-acquisition, acquisition, and extinction phases: 1) how scared are you of the blue bell; 2) how anxious were you when you saw the blue bell; 3) how unpleasant was the blue bell; 4) how much did you like the blue bell? Responses will be on a 10-point likert scale. Together, these measures assess how quickly the child learned to associate the blue bell with the alarm sound.

This task has previously been used in children as young as age 5 in Nathan Fox's lab at the University of Maryland and in children as young as age 6 with a history of exposure to physical and/or sexual abuse in Katie McLaughlin's lab at the University of Washington (both of whom are collaborators on the project). Children are able to perform the task and, importantly, physiological responses to the bell that predicts the alarm sound provide a robust measure of emotional learning. The Bells Task was recently validated in children and adolescents (Schechner et al., in press), and has now been validated for use with children and adolescents with exposure to serious forms of adversity.

Participant: CHILD
Estimated Time to Complete: 20 minutes (includes task completion and recovery)
Present during Administration: Research Assistant and CHILD

** In an ongoing study in Boston adolescents being conducted by Drs. McLaughlin and Sheridan, which is currently approved by the CHB IRB (IRB-P00000200), we use the TSST-C and the computer game task with adolescents who have a wide range of adverse early-life and current family circumstances and mental health problems. These range from current parasuicidal behavior and recent hospitalization for suicidality to histories of exposure to sexual and physical abuse. In this study we have not yet experienced an adverse event. Most participants find the tasks challenging or mildly annoying or distressing. No participant or parent/guardian has complained about the experimental procedures at the end of the study or demonstrated undue or inconsolable distress during the procedures. Of the 95 participants we have run in Boston, 94 have provided us with contact information to call them for follow up visits, some of these visits have already been completed. Thus both of the proposed active tasks—which, by definition, are expected to elicit stronger physiological responses than the passive tasks—have been used without problem or undue participant burden in an ongoing CHB study of early adversity and adolescent mental health.

Pre-Post Task Questionnaires

Throughout the experimental tasks, participants' cognitive appraisals of the situation, mood and emotional reactions will be assessed. These measures will be administered after each of the study tasks. All of the study measures have been validated in previous research and have been used with adolescents aged 12-13. All measures have been used without problem in the ongoing study approved by the CHB IRB for adolescents exposed to maltreatment and violence in the Boston area (IRB-P00000200).

5) Baseline Affect Questionnaire (completed at baseline)
6) Post-Task Questionnaire (completed during recovery after each task)
7) Pre-Speech and Math Questionnaire (completed prior to Trier Social Stress Task)
8) Attributions for Evaluation (completed after Trier Social Stress Task)

Participant: CHILD
Estimated Time to Complete: 20 minutes
Present during Administration: Research Assistant and CHILD

Piñata Task

Following the study tasks, participants will engage in a fun and engaging game that is designed to ensure that they master the task and receive positive feedback at the end of the session. The task was developed by Dr. Nathan Fox, one of the primary investigators of the BEIP and is based on widely used reward anticipation tasks (Haber & Knutson, 2010; Scheres, Milham, Knutson, & Castellanos, 2007; Samanez-Larkin, Kuhnen, Yoo, & Knutson, 2010).

The task is a reward processing task that involves making a speeded response to a target in order to receive a reward. Each trial is composed of three stages: anticipation, response, and outcome. In the anticipation stage, subjects see a cue indicating the size of the potential reward for that trial; in the response stage, subjects have the opportunity to make a response; in the outcome stage, subjects see feedback indicating whether or not their response was fast enough to receive the reward. These three stages are presented in the context of a piñata whacking game. Subjects are told to whack at piñatas as quickly as possible to earn the stars inside, and that the number of stars they earn during the task will determine the size of the reward they receive at the end. In the anticipation stage, subjects see the piñata partially revealed at the top of the screen—the number of stars inside the piñata is visible, but subjects cannot yet hit it. In the response stage, the piñata drops to the middle of the screen and the subject has the opportunity to make a speeded button press response. In the outcome stage, subjects either see the piñata cracked open and the stars in a basket at the bottom of the screen, indicating a hit, or they see the intact piñata swinging off to the side of the screen, indicating a miss. The task was designed to be visually appealing and engaging for children. All stimuli were drawn in a colorful cartoon style, with piñatas of many different shapes and sizes. The task is also easy enough that all children are expected to perform well and receive positive feedback throughout.

Participant: CHILD
Estimated Time to Complete: 15 minutes
Present during Administration: Research Assistant and CHILD

DNA Collection

See previous description in Research Session 1.

Life Events Scale

See previous description in Research Session 1.

Dried Blood Spot (DBS) Collection

Blood spot collection involves pricking the middle or ring finger with a contact-activated micro-lancet that only triggers when positioned and pressed against the skin. A controlled uniform puncture that stimulates capillary blood flow but minimizes the possibility of injury will be delivered. Instant and automatic retraction of the lancet into the device will prevent accidental reuse.

Following standard procedures, the first drop of blood will be wiped away with gauze. The next drop will be collected using a microcuvette and will be analyzed immediately using point of care devices for glycolated hemoglobin (HbA1c;Siemens DCA Vantage Analyzer; 1 uL of blood) and hemoglobin levels (HemoCue Hb 201 analyzer; 10 µL of blood). Participants will be given immediate feedback regarding these levels. If either reading falls outside the normal range, the legal guardian of the participant will be notified and the participant will be referred to a medical professional for follow-up evaluation.

Subsequent drops (four, each approximately 50 µL) will be applied to filter paper. A second finger stick may be necessary if the first stick does not yield enough blood to perform the analyses. The blood drops will saturate the paper and will be air-dried for a minimum of 3 hours. After drying, DBS specimens will be stored in a -24C laboratory-grade freezer until they are shipped by courier service to Thomas McDade (Consultant) in the Laboratory for Human Biology Research in Evanston, IL for processing.

DBS specimens will be assayed according to procedures specifically developed and validated for use with DBS specimens for inflammatory (CRP, IL-6, IL-8, IL-10, TNF-α), immune (EBV antibody titers), and metabolic (leptin) biomarkers.

Measurement of height, weight, temperature, blood pressure, and self-reported health information (Recent Health Behaviors Survey) will be obtained at the time of DBS collection in order to ascertain essential information on covariates for analysis.

Participant: CHILD
Estimated time to complete: 20 minutes
Present during Administration: Research Assistant and CHILD
*** Data collection associated with the DBS component of the protocol was completed in January 2017. The description of these research activities will remain in the current protocol (last updated Feb 2017) for historical purposes.

MacArthur Health and Behavior Questionnaire (HBQ-Parent; version 2.1 (late childhood and adolescence)) The HBQ consists of approximately 140 items regarding child functioning Ablow et al., 1999; Essex, Boyce, Goldstein, Armstrong, Kraemer, & Kupfer, 2002; Luby et al., 2004). This questionnaire will be administered to the child's parent/caregiver and if consent is obtained, from the child's 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. **

Participant: PARENT/CAREGIVER of child
Estimated time to complete: 25 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER

Session Debriefing

A detailed debriefing session will occur at the end of the session to ensure that participants are aware of the purpose of the study tasks and understand that the feedback they received during the speech and math tasks was not based on their performance, and are not experiencing physiological arousal distress.

The debriefing pays specific attention to explaining that the evaluators were instructed to provide neutral feedback toward the participant during the speech and math tasks. During the debriefing, the evaluators return for a "reunion," which allows the participant to meet the evaluators in a very informal matter. Participants feel much better after meeting the evaluators. The evaluators each provide at least one type of positive feedback to the participant about their performance (e.g., I really liked the part of your speech when you said…"). Drs. McLaughlin and Sheridan have found this reunion to be a very important part of the study design. Participants are universally happy to receive positive feedback about their performance and to learn that they were not being evaluated during the study tasks.

Participants will be asked how they are feeling at the end of the debriefing to ensure that they are not experiencing any distress or arousal. We are confident that the study staff will be able to accurately assess the presence of distress in participants. The staff who will be running the study and conducting the debriefing have know they children for more than 12 years, and are quite adept at discerning how they are feeling. Most importantly, the children trust the study staff and feel comfortable disclosing personal information to them. Thus, we are confident that no child will leave the study with any lingering negative feelings.

We will also debrief the parents at the end of the study. The research assistant will provide a detailed debriefing regarding the purpose of the study and the tasks their child completed during the study. Parents will be given the opportunity to ask any questions they have about the study.

We will also tell parents about their child's resting heart rate and blood pressure. If there are any children who meet the clinical cut-off for hypertension (based on the baseline blood pressure assessment, prior to completing study tasks), we will inform the parents of this. Parents will be encourgaed to take their child to a physician to have their hypertension evaluated.

The debriefing scripts are based on the script being used in the previously mentioned Boston study (IRB-P00000200).

Participant: CHILD (and PARENT/CAREGIVER)
Estimated Time to Complete: 5 minutes
Present during Administration: Research Assistant and CHILD (and PARENT/CAREGIVER) 2hrs total

ESTIMATED TOTAL TIME TO COMPLETE RESEARCH SESSION 3:
Participant: 210 minutes
Parent/Caregiver: 25 minutes

**The proposed changes add an additional 20 minutes to the estimated completion time for the participant and 25 minutes to the estimated completion time for parent/caregiver.
All DBS-related measures must be collected within one year, as indicated in the text associated with the administrative supplement announcement (DO WE NEED TO REFERENCE THE PA?). As such, all DBS-related measures will be conducted in the home/living situation of the participant for those participants who have already completed Research Session 3 or who are not yet of age to complete the additional measures associated with the parent study.

Parents/caregivers who have already completed the HBQ-P in Research Session 1 will be asked to complete selected sections of the HBQ-P (INSERT SECTIONS) at the time of DBS collection in order to ascertain essential information on covariates for analysis that is concurrent with collection of the blood samples.

Research Session 4

Magnetic Resonance and Diffusion Tensor Imaging
As we did at age 8 years, we will do whole-head scans on all consenting participants, focusing 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.

All MRI and DTI scans will take place at a private medical clinic 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 Siemens. All scans will be performed by the clinic's neuroradiologist who will also provide a clinical read of all scans. Prior to the start of the scan, the neuroradiologist will screen all participants for contradindications. The neuroradiologist will be assisted by a technician on staff at the medical center and a member of the BEIP Research Team 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: 45 minutes
Present during Administration: Research Assistant and CHILD (Parent/Caregiver may observe from another room).

Waisman Activities of Daily Living (WADL)
The WADL is a modified set of ADL items that has been used in several longitudinal studies of adolescents and adults with substantial impairments, including intellectual disability. This 17-item questionnaire will be administered to parents/caregivers of participants in the study.

Participant: PARENT/CAREGIVER
Estimated Time to Complete: 5 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER

Social Communication Questionnaire (SCQ)
The SCQ is a 40-item questionnaire that evaluates a child's communication and social functioning. This measure was administered as part of the assessment completed at age 8 and will be administered again as a screening tool for autism spectrum disorders.

Participant: PARENT/CAREGIVER
Estimated Time to Complete: 10 minutes

Present during Administration: Research Assistant and PARENT/CAREGIVER

ESTIMATED TOTAL TIME TO COMPLETE RESEARCH SESSION 4:
Participant: 45 minutes
Parent/Caregiver: 15 minutes

RESEARCH SESSION 5
Wechsler Intelligence Scales for Children (WISC-IV) The WISC-IV is a widely-used, individually administered, comprehensive test designed to measure intelligence of children from 6-16 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: 90 minutes
Present during Administration: Research Assistant and Child

Peabody Picture Vocabulary Test (PPVT). The PPVT measures receptive vocabulary for ages 7-90. Administered individually, the researcher presents sets of 4 pictures to the participant. The researcher states a word and then asks the participant to point to the picture of that word.

Participant: CHILD
Estimated time to complete: 15 minutes
Present during Administration: Research Assistant and Child

Vineland Adaptive Behavior Scales (Vineland-3, Parent/Caregiver Form). This instrument is used to assist in the diagnosis and evaluation of special needs, including intellectual and developmental disabilities, of children and adults (birth to 90 years).

Participant: PARENT/CAREGIVER
Estimated Time to Complete: 10-15 minutes
Present during Administration: Research Assistant and PARENT/CAREGIVER

f. Study Timeline (as applicable)

We expect piloting for the study to begin in May-June 2014. Pilot participants will be asked to complete all proposed measures in the protocol to ensure that estimated session completion times are accurate and that all measures are culturally appropriate and can be understood/completed as translated. Data collection will take 4 years to complete.

5. 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. BEIP Project Manager communicates daily with the PI and staff at the BEIP Research Laboratory. The Project Manager has weekly Skype calls with the BEIP Research Team and monthly phone calls with the PI and co-PI.

All adverse events and incidental findings will be reported according to the attached Safety Reporting Protocol. Romanian research staff to the PI, co-PIs and Project Manager. All investigators will report the event to their respective Institutional Review Board. The PI will work closely with the staff of the BEIP Research Laboratory to ensure that all proper authorities in Romania are notified of any adverse event.

6. Data Management Methods

The Clinical Research Information Technology Core will be used as a central location for data processing and management. The management, integrity, and security of new data from the index sample, and of prior data from both index and community comparison samples, will be overseen by the PI, Project Manager and Data Manager. The Project Manager will provide monthly updates to project collaborators about the status of data collection, data entry and access to final data sets. Additionally, Kate Degnan, a consulting project statistician (Univ. of Maryland), will provide close supervision over the statistical analyses detailed in the *Data Analysis Plan* below.

Data Storage in Romania

All data will be collected by trained Romanian research assistants and psychologists affiliated with the BEIP Research Laboratory (the vast majority of whom have worked on this project since its inception). Participants 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. Participants 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 consent forms, assent forms, questionnaire data and DVD recordings of any research activities will be kept in locked cabinets in the BEIP Research Laboratory. Some data (e.g., psychopathology raw data, EEG/ERP raw data, CANTAB raw data) will be stored on a secure HP ProLiant ML 350 70GB server based in the BEIP Research Laboratory and transferred to the United States for additional processing, scoring or coding.

Data Transfer to the US

Data that require additional processing, scoring, or coding will be transmitted via Virtual Private Network to a dedicated, secure 500GB server maintained by the Information Services Department (ISD) at Boston Children's Hospital. Data may also be transferred using Business Dropbox and Syncplicity. Both are secure methods of file transfer.

The BEIP server uses FlexProtect to protect against hardware failure and all data are backed up daily, weekly and monthly. The BEIP server contains 4 folders which vary in degree of access:
Archives – This folder contains raw and final data files from all BEIP assessments (baseline to 12 year assessments). Only the PI, project manager and data manager have access to this folder.

Groups – This folder is divided into 4 folders, one for each US performance site location (BCH, UMD and Tulane) and one for collaborators not affiliated with BCH, UMD or Tulane. This folder stores copies of all incoming project data that require additional processing, scoring or summarization (EEG, ERP, ANS data, videos, etc). Additionally, these folders serve as internal workspace for BEIP users at each site. Access is granted to approved BEIP users by performance site.

Collaborative – This folder includes approved IRB materials, administrative updates, and BEIP publications. This folder can be accessed by all approved BEIP users.

Incoming - This folder serves as the temporary storage site for all data transmitted from Romania. Data are encrypted according to Research Computing policies and transmitted to this folder daily. Once data are posted to this folder, they are moved to the designated assessment folder within the Archives folder and copied to the appropriate Groups folder for additional processing, scoring or summarization. Only the PI, co-PIs, project manager and Romanian research staff have access to this folder.

BCH-ISD has published security and privacy policies that govern the use of systems and data and maintains a high standard of system security in clinical and administrative systems that support clinical, research operations and clinical trials. These policies are designed to address compliance with the privacy and security requirements of the Health Information Portability and Accountablility Act (HIPAA) and other regulations such as Section 21 CFR Part 11.

Access to the BEIP server will be approved by the PIs and granted only to those users who are listed on the BEIP IRB protocol. Each user will apply for Associated Personnel status, receive a BCH employee number, secure password and Virtual Private Network (VPN) access to the BEIP server for one year. Continued

access to the BEIP server will be granted at the discretion of the PIs to users with updated human subjects training. Data may be transferred to approved Associated Personnel or to established subawardees (UMD, Tulane, UNC- at Chapel Hill, and the University of Washington) through secure Business Dropbox or Synchplicity. Additionally, we have established a Materials Transfer Agreement with Northwestern University (under the direction Thomas McDade, BCH Agreement# 18748, date of execution 12/13/2016) to perform the dried blood spot analysis and a Data Use Agreement with Newham College (under the direction of Adela Apertroia, date of execution 03/01/2017), and the Bill and Melinda Gates Foundation (under the direction of Sasha Jumbe, date of execution 10/14/2016).

Questionnaire Data

All questionnaire data proposed in this application will be collected and managed using Research Electronic Data Capture (REDCap) tools hosted at Boston Children's Hospital. REDCap is a secure, web-based application that provides automated export procedures for seamless data downloads to Excel and common statistical packages (SPSS, SAS, Stata, R), as well as a built-in project calendar, a scheduling module, ad hoc reporting tools, and advanced features, such as branching logic, file uploading, and calculated fields.

REDCap (Research Electronic Data Capture) data collection projects rely on a thorough study-specific data dictionary defined in an iterative self-documenting process by all members of the research team with planning assistance from the Clinical Research Program. The iterative development and testing process results in a well-planned data collection strategy for individual studies. REDCap servers are housed in a local data center at Children's and all web-based information transmission is encrypted. REDCap has been disseminated for use locally at other institutions and currently supports > 200 academic/non-profit consortium partners on six continents and 13,000 research end-users (www.project-redcap.org).

Upon IRB approval, the project coordinator and data manager will update the existing BEIP REDCap database to include the measures proposed above. As questionnaire data are collected, they will be double-entered by BEIP research staff into the REDCap database to be matched with the data previously obtained on this sample in the original BEIP project. The project coordinator will monitor data entry and complete comparisons of the double data entries to identify any discrepancies. Any identified discrepancies will be corrected by the BEIP research staff. Once data collection is complete, the data manager will be responsible for reviewing all data for obvious outliers, summarizing output variables and exporting final data sets that can be merged with existing data files and shared with BEIP project collaborators.

All BEIP collaborators will be required to submit a BEIP data request detailing their planned analyses. The data request will be reviewed and approved by the PI and co-PIs. Once approved, the data request will be sent to the data manager for processing. These steps help assure that all collaborators use the same data values. The data manager has authority to make documented changes in data values if they are found necessary by users. Final data sets (in SAS and SPSS format) will be stored in the Archives folder on the BEIP server.

7. Quality Control Method

All research assistants responsible for collecting the proposed data have worked extensively with human participants in this age range. The research staff will receive additional training by a licensed psychologist on the administration of the proposed measures that are new to our repertoire (SBST/SSA, WASI, WISC, PPVT, Vineland). They will also receive training on risk-taking measures (Stoplight Task, Probabilistic Gambling Task) and questionnaires (Future Orientation Scale and Barratt Impulsivity Scale).

All assessments involving the use of standardized, clinical measures will be administered by trained Romanian psychologists on our staff. The Romanian research staff will be responsible for sending weekly 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.

The data collected in Bucharest will be analyzed by project staff on an ongoing basis to ensure quality. All questionnaire data will be entered into a secure REDCap database maintained by Boston Children's Hospital Information Services Department. This database will only be accessible by the BEIP Research Team, project coordinator, data manager, and PI.

8. Data Analysis Plan

Since we are working with a modest sample and there are a large number of measured outcomes, statistical methods will be limited to simple and direct approaches. Smaller samples preclude application of complex longitudinal analyses, but most of the hypotheses appearing with the Specific Aims can be addressed directly. It will, however, be impossible to develop large scale statistical models involving comparisons across measurement domains. Most of our hypotheses may be evaluated using t and chi-squared tests for two-sample comparisons, analyses of variance/covariance and logistic regression for higher order analyses, and simple and multiple correlations for measures of association. In cases where distributional assumptions are questionable, we will use either transformation or rank-based distribution-free approaches. The most frequent comparisons will be between subjects in the CAUG and FCG. Inference from these analyses will be supported by the randomization that took place at the outset of our study and thus these comparisons will involve an intent-to-treat rationale and any significant findings may be subjected to causal interpretation. We will conduct similar analyses involving the NIG for comparison purposes. These would be outside the randomized trial context.

It is not feasible within the limited space to project detailed statistical methods for every one of our hypotheses. However for each specific aim we provide a detailed analysis for one of the primary outcome measures as an example. Statistical analysis for the other outcome measures described above will follow the same broad outline. Specific statistical methodology will vary depending upon the nature of the measure (categorical or continuous, symmetrically or skewed distribution). Power analyses are provided below using the example measure.

The proposed research will examine the differences between adolescents with a history of institutionalization and adolescents who have never been institutionalized (Aim 1) on the Brain, Cognitive Functioning, Psychopathology, Executive Functioning, Stress/Emotion Reactivity, Reward Seeking/Sensitivity & Risk Taking, and Social Skills & Social Relationships measures detailed in the sections above. Within the group of adolescents with a history of institutionalization, those who were randomized to a family/foster care intervention and those who were randomized to care as usual also will be compared on these outcome measures (Aim 2a). In addition, within the family/foster care intervention group, age of placement into foster care will be used to examine the presence of sensitive periods on the outcome measures listed above (Aim 2b). Furthermore, the longitudinal data collected on this sample will be examined for mediating mechanisms linking the amount of early social deprivation (i.e., percentage of life spent in an institution) to psychiatric outcomes at the proposed 16-year assessment. Specifically, measures previously collected as part of the larger longitudinal study will be examined as mediators of the effect of institutionalization on internalizing and externalizing disorders and symptoms (Aim 3).

Analyses for Aims 1 and 2 will be conducted in SPSS 18.0 with an expected 16-year-old sample of 120 adolescents with a history of institutionalization and 60 adolescents with no history of institutionalization. Analyses for Aim 3 will be conducted using structural equation modeling (SEM) software (Mplus 6.12)[75], which offers all of the features necessary to analyze these mediation effects and allows for missing data by using maximum likelihood estimation. Thus, the sample size for the Aim 3 models will include the entire sample of children with a history of institutionalization (N=136). GPower 3.1.2[76] was used to analyze power estimates for Aims 1 and 2, while Monte Carlo simulation analyses were run in Mplus[77] to test power estimates for Aim 3 mediation models.

Long-term effects of institutionalization and intervention on outcomes (Aims 1 and 2a): Analyses of the group differences on outcomes proposed in Aims 1 and 2a will use 2 (group) x 2 (gender) ANCOVAs. Using a moderate effect size of Cohen's f = .30, and power to detect a significant between-subjects effect of .80, would require at least 90 subjects. Thus, given our preliminary data showing robust group differences across multiple assessments within this sample and our expected sample size of 180 for Aim 1 (institutionalized group vs. community group) and 120 for Aim 2a (family/foster care group vs. care as usual group), we have confidence in our ability to examine the proposed effects of institutionalization (Aim 1) and intervention (Aim 2a) on outcomes at 16 years of age.

Sensitive periods for intervention effects on outcomes (Aim 2b): Within the foster care group, regression analysis will be used to explore the effect of the age of placement in foster care on the outcome measures at 16 years of age. Using a modest effect size of f^2= .15, and power to detect a significant linear regression effect of .80, would require at

least 55 subjects for a linear regression. Thus, given our preliminary data showing multiple effects of foster care intervention timing on outcomes within this sample and our expected sample size of 60 for Aim 2b, we have confidence in our ability to examine the proposed effects of age of placement on the outcome measures at 16 years of age.

Mediating mechanisms for links between early institutionalization and psychopathological outcomes (Aim 3): Within a SEM framework, the effects of early institutionalization will be modeled as a predictor of psychopathology at 15-16 years of age. In addition, the role of multiple mediators in an indirect effect between early institutionalization and later psychopathology will be tested. A measure of *percent time spent in the institution* across the first 12 years of life will be used as a measure of early institutionalization. Two types of outcomes will be tested in separate models: 1) internalizing symptoms and 2) externalizing symptoms. For the internalizing model (Aim 3a), previously collected measures of attachment security (42 months), reward sensitivity (12 years) & emotion regulation (12 years) will be examined as mediating mechanisms. For the externalizing model (Aim 3b), previously collected measures of EEG power (12 years), white matter volume (8 years), reward sensitivity and reward processing/risk taking (12 years), executive functioning (12 years) & emotion regulation (12 years) will be examined as mediating mechanisms.

9. Statistical Power and Sample Considerations

A set of Monte Carlo simulation models was run to test the stability and power of the proposed effects, using the overall sample size of 136. The first set of simulations was run to test the stability and power to detect a moderate direct effect (.40, standardized beta) between *percent time in institution* and psychopathological symptoms. Then, the second set of simulations was run to test the stability and power to detect moderate indirect effects between *percent time in institution* and the psychopathology measure. Results indicated good estimation of the associations in the models predicting indirect effects of *percent time in institution* and psychopathology symptoms in terms of average parameter bias (-.01 -.00), standard error bias (-.09, -.02), coverage (.91, .94) and power (1.00, 1.00) for the internalizing and externalizing models, respectively. Given our preliminary data showing mediation effects and these Monte Carlo analyses, we are confident in our ability to detect these associations with our overall sample size of 136.

10. Study Organization

Charles A. Nelson, Ph.D. (Boston Children's Hospital), Nathan A. Fox, Ph.D. (University of Maryland), and Charles H. Zeanah, M.D. (Tulane University) have collaborated on the Bucharest Early Intervention Project (BEIP) for the past 13 years. In this time, they have established a cooperative partnership that allows for joint oversight of the research project. They each play an integral role in the continued success of this longitudinal study and will oversee data collection and analysis related to their areas of expertise. Drs. Nelson, Fox and Zeanah will also work closely and all BEIP collaborators to analyze and integrate all data collected from this project with data previously collected on this sample.

The Project Manager will be responsible for coordinating all administrative aspects of the project, including completion of human subjects approval (local and abroad), completion of all required reports related to the project, budget management, and organization of meetings of all BEIP collaborators in the United States. She will maintain weekly contact with the Romanian research team via video-conference calls and will forward notes to the PI and co-PIs following their discussion. Agenda items will include updates on participant enrollment, data entry, and concerns regarding any participants. She will also maintain weekly contact with the Data Manager to review any data management issues and discuss new data requests.

The PI and co-PIs will hold monthly conference calls with the Project Manager to review data collection and analyses, and to discuss any necessary scientific decisions or conflicts. Calls will be scheduled more frequently if needed. Conference calls will also be used to review data analysis proposals and publications related to the proposed assessment, as well as review and revise policies to ensure data integrity among collaborators.

In addition to monthly conference calls, the PIs and Project Manager will meet in person twice a year to discuss administrative and scientific issues related to the project. During these meetings, they will review the project budget, discuss enrollment progress and review any concerns related to data collection. These meetings will also be used to review data analysis proposals and publications related to the proposed assessment, as well as develop, review and revise policies to ensure data integrity among collaborators. These meetings will provide dedicated time to address all administrative issues related to the project.

The BEIP Research Team consists of five research assistants and two social workers. The research assistants will be responsible for translation and submission of all IRB materials submitted to the local Ethics Committee in Romania. They will also assist with participant enrollment, data collection, data coding, scoring, data entry and translation. The team social workers will be responsible for establishing convention agreements with all participating sectors of Child Protective Services in Bucharest so we may obtain updated placement status and contact information for legal guardians of the ever-institutionalized children in the BEIP sample. They will also be responsible for participant enrollment and will assist in data collection activities, data entry and translation, as needed. They will also be responsible for updating the extensive life histories we have maintained on all ever-institutionalized children in the BEIP sample since the inception of the study.

Kate McLaughlin, Ph.D. (Univ. of Washington) and Margaret Sheridan, Ph.D., (BCH) will be responsible for overseeing all aspects of data collection related to physiological reactivity, including processing and analysis of these data. They will provide direct supervision to the research staff via Skype calls and will work closely with the PI and co-PIs to integrate these data with existing data collected previously collected on the sample.

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