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Local Brain Functional Activity Following Early Deprivation: A Study of Postinstitutionalized Romanian Orphans

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Early global deprivation of institutionalized children may result in persistent specific cognitive and behavioral deficits. In order to examine brain dysfunction underlying these deficits, we have applied positron emission tomography using 2-deoxy-2-[^{18}F]fluoro-D-glucose in 10 children (6 males, 4 females, mean age 8.8 years) adopted from Romanian orphanages. Using statistical parametric mapping (SPM), the pattern of brain glucose metabolism in the orphans was compared to the patterns obtained from two control groups: (i) a group of 17 normal adults (9 males, 8 females, mean age 27.6 years) and (ii) a group of 7 children (5 males and 2 females, mean age 10.7 years) with medically refractory focal epilepsy, but normal glucose metabolism pattern in the contralateral hemisphere. Consistent with previous studies of children adopted from Romanian orphanages, neuropsychological assessment of Romanian orphans in the present study showed mild neurocognitive impairment, impulsivity, and attention and social deficits. Comparing the normalized glucose metabolic rates to those of normal adults, the Romanian orphans showed significantly decreased metabolism bilaterally in the orbital frontal gyrus, the infralimbic prefrontal cortex, the medial temporal structures (amygdala and head of hippocampus), the lateral temporal cortex, and the brain stem. These findings were confirmed using a region-of-interest approach. SPM analysis showed significantly decreased glucose metabolism in the same brain regions comparing the orphans to the nonepileptic hemisphere of the childhood epilepsy controls. Dysfunction of these brain regions may result from the stress of early global deprivation and may be involved in the long-term cognitive and behavioral deficits displayed by some Romanian orphans. © 2001 Academic Press

Key Words: **positron emission tomography; 2-deoxy-2-[^{18}F]fluoro-D-glucose; hypometabolism; children; maternal deprivation; behavior; limbic system; hippocampus; amygdala; prefrontal cortex; stress; statistical parametric mapping.**

INTRODUCTION

Child neglect and child abuse are chronic societal problems. However, the impact of childhood social deprivation on brain function in humans has been largely unexamined. In contrast, the deleterious effects of early deprivation have been studied extensively in animals, and there is an emerging understanding that there are both short-term and long-term changes in brain function associated with early neglect and deprivation in animals (for reviews see Suomi, 1997; Kaufman *et al.*, 2000). Social policies and severe economic problems in Romania in the 1980s resulted in early global deprivation for a large number of children. Over 65,000 children were placed in orphanages during this period, 85% of whom were placed within the first month of life (Ames and Carter, 1992; O'Connor and Rutter, 2000). Child–caregiver ratios were 10:1 for infants and 20:1 for children over 3 years of age (McMullan and Fisher, 1992), and infants spent up to 20 h per day in their cribs unattended (Ames and Carter, 1992). Studies of children following removal from the orphanages and adoption by families in the United Kingdom and North America revealed the presence of cognitive, social, and physical deficits (Ames, 1997; Rutter, 1998). At the time of adoption, the majority of the children showed cognitive performance in the mental retardation range based upon parent report on the Denver scale (Rutter, 1998). Longitudinal studies have demonstrated that these children showed considerable recovery by age 4 years (Ames, 1997; Rutter, 1998), but that deficits remaining at 4 years were also present at 6 years of age (O'Connor and Rutter, 2000). Interestingly, behavioral abnormalities reported in these children are qualitatively similar to some seen in socially deprived nonhuman primates (Suomi, 1997). Motor stereotypies and self-stimulatory behaviors were observed, and indiscriminately friendly behavior and insecure attachment were measured (Chisholm *et al.*, 1995). Physical deficits could be attributed largely to malnutrition as 59% of the children were below the 5th

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TABLE 1

Characteristics of the Romanian Orphan Sample

Patient	Age (years)	Sex	Hand preference	Duration in orphanage (months)	Duration in adoptive home	Head circumference *Z* score	Height *Z* score	Weight *Z* score	Family relationship index[a]	Family personal growth index[a]	Family system maintenance index[a]
1	9.6	M	R	90	24	−2.2	1.2	0.87	61.33	50	56.5
2	11.3	M	R	22	113	−2.6	0.17	−1.7	65.33	55.8	53.5
3	8.1	F	L	32	65	−2.1	0.6	−1	51.67	55	59
4	7.3	M	R	72	15	−2	−0.2	0.48	53.33	50.6	61.5
5	7.1	M	R	16	78	−2.1	−1.7	−0.44	64.33	34.8	50.5
6	7.8	M	L	20	76	−0.44	1.5	0.02	63.33	46.6	48
7	9.5	F	R	32	81	−1.6	0.22	0.14	57.67	51	56.5
8	8	M	L	33	63	−2.6	−2.6	−2.16	64	63.2	61.5
9	8.3	F	R	27	58	−1.4	0.33	0.22	39.67	65.2	73
10	11.3	F	R	36	99	−1.2	−1	−0.7	57.33	53.8	59
Mean	8.83			38	67.2	−1.824	−0.148	−0.427	57.799	52.6	57.9

[a] Family Environment Scale summary scores (mean = 50; SD = 10).

percentile for weight at the time of adoption (Ames, 1997).

In order to examine brain dysfunction underlying persistent cognitive and behavioral deficits, we have applied functional neuroimaging with positron emission tomography (PET) using 2-deoxy-2-[^{18}F]fluoro-D-glucose (FDG) in a group of children older than 6 years adopted from Romanian orphanages. The study was designed to identify objectively focal abnormalities of brain glucose metabolism in order to assess brain regions involved in the long-term deficits resulting from early global deprivation.

METHODS

Subjects

Ten children (6 males, 4 females, mean age 8.83 years; age range 7.1–11.3 years) adopted by U.S. families from orphanages in Romania were included in the study (Table 1). All subjects had been placed into the orphanages at approximately 4–6 weeks of age after being released from the hospital and had resided in the orphanage for a mean of 38 months (range 16–90 months) before being adopted. Mean duration spent in the adoptive home was 67.2 months (range 15–113 months). Exclusion criteria for orphans included having an ongoing relationship with the biological parents during the period of institutionalization; known pre- or perinatal complications, including substance abuse by the biological mother; previous or current medical conditions; use of psychotropic medication; and evidence of underlying neurological condition based on physical examination. However, given the sparse historical data on the children and institutions, it was not possible to control for a number of potentially important variables (e.g., favoritism in the orphanage, undetected medical conditions, and variability among the orphanages).

Two control groups were employed in this study. The first control group consisted of 17 normal adults (9 males, 8 females, mean age 27.6 years; age range 21–36 years). All of these subjects were screened for history of medical and/or psychiatric conditions via physical examinations, blood workup, and structured clinical psychiatric interview. The application of statistical parametric mapping (SPM) in children older than 6 years of age has been validated in our previous study using the same adult control group (Muzik *et al.,* 2000). The second control group consisted of 7 children (mean age 10.74 years, age range 7.9 to 13.5 years, 5 males and 2 females; Table 2) with medically intractable partial epilepsy selected according to the following criteria. All children were diagnosed with a unilateral seizure focus based on seizure semiology and scalp ictal EEG as well as FDG PET, which was performed as part of the presurgical evaluation. Medications taken

TABLE 2

Clinical Data of Seven Epileptic Children Whose PET Data were Used for Comparison

Age/sex	Seizure focus	FDG PET	Drug
1. 7.9/M	R T	R T-F	CBZ, GBP
2. 8.8/M	L T-O	L > R O	LAM
3. 10.3/M	L T	L T-P	LAM
4. 10.5/M	R hemisphere	R F-T-P	CBZ
5. 11.5/M	R T	R T	CBZ
6. 12.7/F	R T	R T	CBZ, LAM
7. 13.5/F	R T(post.)	R T-P(post.)	VPA, TPX

Note. Location of seizure focus (as defined by scalp ictal EEG), corresponding FDG PET abnormalities (areas of hypometabolism) by visual analysis, and drugs taken at the time of the PET scan are summarized. Abbreviations: R, right; L, left; F, frontal; T, temporal; P, parietal; O, occipital; CBZ, carbamazepine; GPB, gabapentin; LAM, lamotrigine; TPX, topamax; VPA, valproate.

TABLE 3a

Neurocognitive Profile for the Romanian Orphans

Domain	Mean standard score[a]	SD
Intellectual (WISC-III)		
Full scale IQ	81.4	19.6
Verbal Comprehensional Index score	81.5	18.3
Perceptual–Organizational Index score	84.3	17.71
Processing Speed Index score	89.8	18.47
Freedom from Distractibility Index score	75.8	16.51
Nonverbal IQ (Ravens CPM)	90.6	20.41
Language		
Receptive language (CELF-III)	75.1	16.42
Receptive vocabulary (PPVT-3)	82.6	23.92
Expressive language (CELF-III)	75.1	16.86
Expressive vocabulary (EOWPVT-R)	96.8	25.99
Memory (WRAML)		
Verbal Memory Index	75.1	13.09
Visual Memory Index	79.9	11.23
Executive functioning (Trails A&B, GDS)		
Sustained attention	77.2	20.29
Impulse control	51.85	49.0
Cognitive efficienc	72.1	17.58
Manual dexterity (grooved pegboard)		
Dominant hand	82.15	17.57
Nondominant hand	78.7	24.65

[a] Standard scores (normal mean = 100; SD = 15).

by these subjects included carbamazepine ($n = 4$), gabapentin ($n = 1$), lamotrigine ($n = 3$), valproate ($n = 1$), and topiramate ($n = 1$). The region of epileptic focus as verified by EEG showed hypometabolism in all cases. No cortical or subcortical lesions on magnetic resonance imaging (MRI) were observed; however, patients with unilateral hippocampal atrophy were not excluded. We have shown previously (Muzik *et al.*, 2000) that the pattern of glucose metabolism in the hemisphere contralateral to the epileptic focus in this same control group is not different from the pattern in the adult control group.

This study was performed in accordance with the policies of the Wayne State University Human Investigations Committee and written informed consent was obtained for all studied subjects.

Neuropsychological Evaluation

All children enrolled in the study received a comprehensive neuropsychological assessment. Tests for the orphan group included evaluation of global, verbal, and nonverbal intellectual functioning; expressive and receptive vocabulary and language processing; visual and verbal memory; executive functioning (attention, impulsivity, cognitive efficiency, and flexibility); manual dexterity; and behavioral functioning. The battery included the following measures: Weschler Intelligence Scales for Children—Third Edition (WISC-III). Ravens Progressive Matrices, Peabody Picture Vocabulary Test—Third Edition (PPVT-3), Expressive One-Word Picture Vocabulary Test—Revised (EOWPVT-R), Comprehensive Evaluation of Language Functions—Third Edition (CELF-III), Wide Range Assessment of Learning and Memory (WRAML), Gordon Diagnostic System (GDS), Trails A&B and, Grooved Pegboard (Table 3a, values reported as standard scores). In addition, the Child Behavior Checklist (CBCL) was administered to evaluate behavior problems (Table 3b, values reported as *T* scores, higher values represent higher dysfunction). The psychometric properties of the above measures have been well established and the above measures are widely used with both clinical and research populations (Sattler, 1990). The evaluation also included a social–historical interview with the child's adoptive parent(s). Data collected in the interview included the physical, developmental, and behavioral status of the children at the time of adoption and at 1 year postadoption and the status at the time of the evaluation. We also included a measure of the adoptive family environment. The summary indices Family Relational, Family Personal Growth, and Family Systems Maintenance are provided in Table 1.

PET Imaging

PET imaging of brain glucose metabolism using FDG in the awake resting state was performed in both the study group and the two control groups. Subjects were fasted for 4 h prior to the PET studies. Initially, a venous line was established for injection of FDG (5.3 MBq/kg) produced using a Siemens RDS-11 cyclotron (Knoxville, TN). External stimuli were minimized during the FDG uptake period (0–40 min postinjection) by dimming the lights and discouraging interaction so that studies reflected the resting awake state. None of the subjects required sedation. The epileptic children also had EEG monitoring during the tracer uptake period in order to verify that the study was performed

TABLE 3b

Behavioral Profile for the Romanian Orphans

Behavior (CBCL)	Mean *T* score	SD
Total behavior problems	70.1	7.43
Withdrawn	61.2	8.21
Somatic complaints	58	8.75
Anxious/depressed	67.8	14.14
Social problems	69.3	8.88
Thought problems	71.3	9.09
Attention problems	75.7	7.93
Delinquent behavior	63.6	10.09
Aggressive behavior	64.9	12.07

Note. *T* scores: normal mean = 50; SD = 10. *T* score of 67–69, borderline range; *T* score ≥70, clinically significant range.

in the interictal state, which was indeed the case in all subjects.

FDG images were acquired using a CTI/Siemens EXACT HR whole-body PET scanner. This scanner has a 15-cm field of view and generates 47 image planes with a slice thickness of 3.125 mm. The reconstructed image in-plane resolution obtained is 6.5 ± 0.35 mm at full width at half-maximum (FWHM) and 6.0 ± 0.49 mm in the axial direction (reconstruction parameters: Shepp–Logan filter with 1.1 cycles/cm cutoff frequency and Hanning filter with 0.20 cycles/pixel cutoff frequency). Forty minutes after FDG injection, patients were positioned into the scanner. Using a low-laser beam system the position of the patient's head was adjusted so that the imaging planes were parallel to the canthomeatal line. Subsequently, a static 20-min emission scan in 2D mode was initiated collecting approximately 1 million net true counts per plane. Calculated attenuation correction was performed on all images using the CTI/Siemens reconstruction software. The outline of the head was derived directly from the raw data by threshold fits to the sinograms according to the method of Bergstroem *et al.* (1982).

Statistical Parametric Mapping

The pattern of glucose metabolism derived from the orphans was compared to those obtained from the two control groups using SPM. The benefits of employing SPM in analyzing the data include that no *a priori* assumptions about anatomical regions have to be made and that SPM provides overall information about changes in the pattern of glucose metabolism throughout the brain. However, this technique relies heavily upon an accurate spatial normalization of image volumes to a standard image template. We have shown previously that spatial normalization of PET image volumes using an adult template is feasible for children older than six years of age and that such a comparison does not produce artifacts as a result of SPM analyses (Muzik *et al.*, 2000; also see Discussion). All FDG images were analyzed using the SPM96 software package (Friston *et al.*, 1995).

Prior to spatial normalization, the FDG images of children with left-sided epileptic foci were reversed (patients 2 and 3, see Table 2), so that hypometabolic regions related to epilepsy were lateralized on the right side in all patients. PET image volumes were spatially normalized to the PET image template using default normalization parameters (12 linear and 4 × 5 × 4 nonlinear basic functions, eight iterations). Following spatial normalization, all images were smoothed using a 3D Gaussian kernel of 12-mm FWHM. The confounding effect of global activity was removed using proportional scaling. An unpaired *t* test based on two contrasts was applied (Romanian orphan sample–controls; controls–Romanian orphan sample). The resulting SPM(t) statistic was transformed to a normal distribution, SPM(Z), and thresholded at $P = 0.0001$ (uncorrected). In addition, only regions of more than 50 voxels attaining a corrected P value of less than 0.05 were considered. For comparisons of the Romanian orphans to the pediatric epilepsy control group, differences between groups *only* in the hemisphere contralateral to the seizure focus were considered relevant, due to the confounding effect of the seizure focus on glucose metabolism in the epileptic hemisphere. The resulting foci were then characterized in terms of spatial extent (k) and peak height (μ). The significance of each region was estimated using distributional approximations from the theory of Gaussian fields (Friston *et al.*, 1995). The SPM(Z) maps were analyzed using two corrected levels of statistical inference: a voxel level (Z) and a cluster level (k, Z). The voxel level represents the probability (corrected for multiple comparisons based on the number of resolution elements in the image volume) of observing a Z score of Z or higher, whereas the cluster level represents the probability of observing a cluster size k or larger with a maximal Z score of Z or higher. The corrected significance level was chosen as $P < 0.05$.

The resulting significant differences in 3D image space were displayed collapsed into three orthogonal planes ("glass brain," Fig. 1). Regions of significant difference were overlaid on normalized T1-weighted images to facilitate correlation with anatomy (Fig. 2). The Talairach coordinates (Talairach and Tournoux, 1988) are given in millimeters describing the location of significant voxels: x defining the lateral displacement of this voxel from the midline (left = negative), y defining the anteroposterior position relative to the anterior commissure (posterior = negative), and z defining the vertical position relative to the line connecting the anterior and posterior commissure (down = negative) (Table 4).

Region of Interest (ROI) Analysis

In order to determine if regions identified as having decreased or increased glucose metabolism in the Romanian orphans compared to the normal *adult* control group by the SPM analysis represent real differences between groups or if they represent artifacts caused by inaccuracies during the realignment and warping procedures, we reanalyzed the PET data by drawing ROIs in the original unwarped PET image volumes. Significant regions determined during the SPM analysis were overlaid onto an MRI template showing the extension of significant decreases or increases in relation to anatomical structures (Talairach and Tournoux, 1988). Based on this information, we defined ROIs in corresponding image planes of the FDG study (Fig. 3). The smallest ROI was defined at the location of Brodmann area 25 with a volume of about 0.5 cm^3, whereas the

largest ROI was at the location of the lateral temporal cortex with a volume of about 8 cm^3.

Statistical Assessment

In order to determine whether normalized ROI values differed between the Romanian orphans and the normal control group, a mixed-design repeated-measures ANOVA was performed, with the group as the between-subject factor and the ROIs as the within-subject factor. Initially, significance for the two main effects (group, region) and the group by region interaction was determined. If significance was shown for the overall test, simple effect tests for each individual region were conducted. Each of these tests was performed using a composite of the between-subjects and within-subjects error terms as suggested by Winer (1971). Correction for multiple comparisons was performed using the modified Bonferroni correction (Keppel, 1991). Using this correction, the initially chosen uncorrected *P* value of 0.05 was transformed to a critical *P* value of 0.04.

RESULTS

The neuropsychological profile determined for the orphans at the time of PET scanning suggested low average global intellectual functioning (WISC-III) with relatively evenly developed verbal and nonverbal skills (Table 3a). A relative weakness on intellectual testing was found for sustained concentration and attention. This finding was consistent with results of attentional testing (GDS), which also indicated mildly impaired performance. Performance on expressive and receptive language processing scores (CELF-III), which were both measured in the borderline range, was also below that which would be expected given the IQ scores, as were verbal and visual memory (WRAML) tasks. Cognitive efficiency was relatively mildly impaired, and a measure of impulsivity (GDS) indicated performance in the severely impaired range. Parent report of behavioral problems indicated significant behavioral difficulties with Total Problems *T* score falling in the clinical range, and clinically significant elevations on the Attention and Thought Problems subscales, as well as borderline elevations on the Anxiety/Depressed and Social Problems subscales (Table 3b). All 10 children had at least one subscale that fell in the clinically significant range. Total Problems scores were in the clinically significant range in 6 children and were in the borderline range in 3 children.

The social/historical interview revealed that at the time of adoption, all of the children were described as physically small for their age, and malnutrition was suspected in 9 of the 10 children. Five of the children had physical scarring from suspected lacerations, burns, or broken bones. Eight of the children were not yet walking, and 9 were not yet speaking at the time of adoption. Developmental assessments completed shortly after adoption revealed severe gross and fine motor, language, and social delays in all 10 children. Furthermore, all the children were reported to be manifesting substantial "postinstitutionalized" socioemotional sequelae and/or behavioral anomalies (e.g., absence of crying or expression of pain, failure to seek out nurturance from caregivers, rocking, head-banging), as well as sensory oddness (e.g., preference to be wrapped tightly for sleeping, persistently mouthing objects, bothered by noises).

At 1 year in the adoptive home, substantial "catch-up" in motor and language skills was reported in all 10 children. All were walking within months of placement, and phrase speech had emerged by 1 year post-adoption in all of the children. However, development delays in language, fine and gross motor skills, and social domains remained in 8 of the 10 children. Parents continued to observe absence of crying, expression of pain, or fear in a majority of the children, as well as a continuing absence of utilizing the parents as a secure base. A number of the parents reported that such behavior alternated with indiscriminate friendliness with adults. Absence of toy play and/or immature and/or unusual toy play was also reported in the majority of the children. Motoric overactivity, difficulty attending to task, disinterest in or unusual play with peers, trouble falling and/or staying asleep, and nightmares were also typical across the group. All the children now manifested sensory difficulties including difficulty with loud noises and crowds and tactile defensiveness.

At the time of the PET scan, while 9 of the 10 children were short in stature and appeared younger than their chronological age, parents described their children as having largely caught up to their peers in gross motor functioning, with substantial developmental progress across domains. However, they reported continued concerns with regard to mild and more specific fine motor, language (e.g., need to give instructions over and over), attentional (e.g., difficulties staying on task), and behavioral difficulties (e.g., acts without thinking, hoards food); difficulties with academic achievement; and problems getting along with peers. Most parents also complained that the children now, rather than exhibit an absence of crying, seemed to alternate between excessive expressions of such emotions and the lack of any expression. With regard to measures of the family environment, the families in the study could be characterized (as a group) as having increased conflict and control (variables commonly increased in distressed families), but also increased independence, cohesion, expressiveness, moral/religiosity, active/recreational, and intellectual/cultural orientations (all variables associated with well-being). The data are presented (Table 1) using three summary

TABLE 4

Listing of the Probabilities and Statistics of Significant Clusters Obtained using SPM

P value cluster level (*k*, *Z*)	*P* value voxel level (*Z*)	Talairach coordinate *x*, *y*, *z* (mm)	Region (Brodmann's area)
Orphans vs normal adults			
Lower in the orphans			
$P < 0.001$ (740, 6.36)	$P < 0.001$ (6.36)	−34, 44, −14	L orbital frontal cortex (BA 11)
$P < 0.001$ (658, 5.68)	$P < 0.001$ (5.68)	48, 32, −14	R orbital frontal cortex (BA 11)
$P = 0.03$ (5832, 6.33)	$P < 0.001$ (6.33)	38, −20, −16	R medial temporal structures
	$P < 0.001$ (6.18)	60, −42, −20	R lateral temporal cortex (BA 20)
$P = 0.44$ (3599, 6.13)	$P < 0.001$ (6.13)	−58, −46, −22	L lateral temporal cortex (BA 20)
	$P < 0.001$ (6.06)	−34, −16, −20	L medial temporal structures
$P = 0.09$ (1226, 5.77)	$P < 0.001$ (5.77)	−2, 12, −12	Infralimbic cortex (BA 25)
$P = 0.05$ (1029, 5.61)	$P < 0.001$ (5.61)	2, −36, −26	Brain stem
Higher in the orphans			
$P < 0.001$ (11,761, 6.20)	$P < 0.001$ (6.20)	−38, −52, 34	L parietal cortex (BA 7)
Orphans vs children with unilateral (right) epileptic focus; lower in the orphans:			
$P = 0.006$ (4397, 5.43)	$P < 0.0001$ (4.93)	−44, −20, −8	L medial temporal structures
$P = 0.022$ (391, 4.88)	$P = 0.005$ (4.88)	−16, 8, −34	L lateral temporal cortex (BA 38)
	$P = 0.053$ (4.33)	−28, 48, −16	L orbital frontal cortex (BA 11)

Note. All SPM statistical results were thresholded on the basis of the significance of both the spatial extent and the peak intensity of regions which survived the initial height threshold ($P = 0001$ uncorrected). Significance of activations was evaluated at $P = 0.05$. The resulting cluster level represents the probability of observing a cluster of size *k* or larger with a maximal *Z* score of *Z*, and the voxel level represents the probability of observing a *Z* score of *Z* or higher. Finally the Talairach coordinate reports the location of voxel with maximal *Z* values within the observed cluster. The next highest *Z* score in the same cluster, that is at least 8 mm apart, is also reported. Abbreviations: L, left; R, right; BA, Brodmann's area.

indices: Family Relational, Family Personal Growth, and Family Systems Maintenance. All three summary indices were suggestive of healthy family functioning (and none of these predicted any of the PET, neurocognitive, or behavioral data).

In the first SPM analysis, adult controls were compared to the Romanian orphans. This SPM analysis showed significant regional decreases of normalized glucose metabolism bilaterally in prefrontal cortex, the medial temporal structures (including amygdala and hippocampus), the lateral temporal cortex, and the brain stem in orphans compared to the control group (Table 4). Specifically, significant decreases in prefrontal cortex were determined in the left (*Z* score 6.36, $P < 0.001$) and right (*Z* score 5.68, $P < 0.001$) orbital frontal cortices (Brodmann's area 11) (Figs. 1 and 2, Table 4), which were also significant at the cluster level ($P < 0.001$ with 740 voxels above threshold on the left and $P < 0.001$ with 658 voxels above threshold on the right side). Significant bilateral decreases of glucose metabolism were also detected in the prefrontal infralimbic cortex (Brodmann's area 25) (*Z* score = 5.77, $P < 0.001$). This decrease showed a trend for significance at the cluster level ($P = 0.09$, 1226 voxels above threshold). In the temporal lobe, significant decreases at the voxel level were found in the right (*Z* score = 6.33, $P < 0.001$) and left (*Z* score = 6.06, $P < 0.001$) medial temporal structures (Fig. 2), but only the decrease on the right side was significant at the cluster level ($P = 0.03$, 5832 voxels above threshold). This cluster was quite large and included also a significant decrease in the *right* lateral temporal cortex (Brodmann's area 20) (*Z* score = 6.18, $P < 0.001$). Decrease of glucose me-

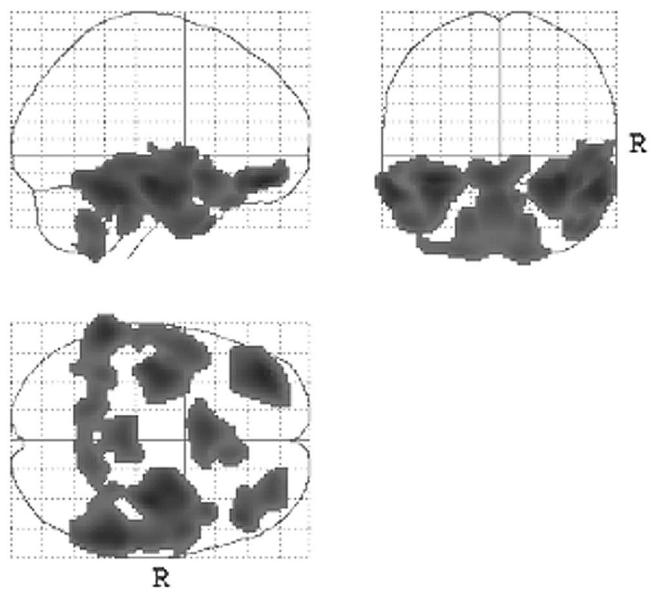

FIG. 1. Composite showing regions of decreased glucose metabolism in the group of Romanian orphans compared to the adult control group as identified by the SPM analysis at the cluster level. The results are displayed (at an uncorrected $P = 0.0001$) as though viewed in a "glass brain," with transverse, coronal, and sagittal views shown. R, right side.

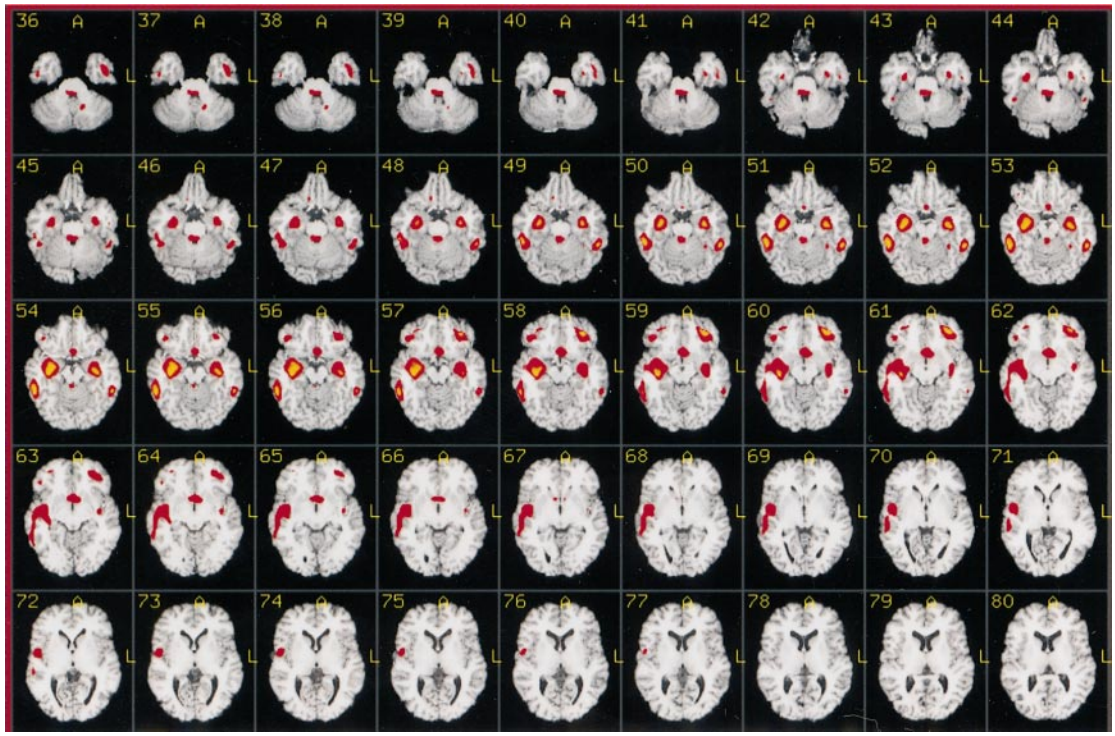

FIG. 2. SPM maps showing regions with lower glucose metabolism in the Romanian orphan group in comparison to the adult control group superimposed onto a representative MRI scan in standardized space. The left side of the image represents the right side of the brain. Regions in red and yellow represent significant ($P < 0.05$) decreases of activity in the Romanian orphan group in comparison to the adult controls.

tabolism in the left lateral temporal cortex (Brodmann's area 20) was significant only at the voxel level (Z score = 6.13, $P < 0.001$). Finally, SPM analysis detected a significant decrease of glucose metabolism in the medial rostral brain stem (Z score = 5.61, $P < 0.001$) and showed a trend for significance at the cluster level ($P = 0.05$, 1029 voxels above threshold). A single peak of *increased* metabolism was detected in the left parietal cortex (Z score = 6.20, $P < 0.001$), which was significant at the cluster level ($P < 0.001$).

ROI analysis performed in native space on original PET scans showed significantly decreased normalized values for glucose metabolism in all regions which showed significant decreases by the SPM analysis (Fig. 3, Table 5). Results of the repeated-measures ANOVA showed that the group effect ($P < 0.001$), the region effect ($P < 0.001$), and the region-by-group interaction ($P < 0.001$) were highly significant. Furthermore, simple-effect tests showed highly significant differences for all SPM-determined regions between the orphan and the control group, except left parietal cortex. The single peak of apparently *increased* glucose metabolism in the left parietal lobe was not confirmed by the ROI analysis ($P = 0.42$), indicating that this result is likely to be an artifact (Table 5).

The second SPM analysis involved comparison of the Romanian orphan group to the age-matched group of children with focal epilepsy. This SPM analysis showed a significant regional decrease of glucose metabolism in the left orbital frontal cortex (Brodmann's area 11), left medial temporal structures, and left lateral temporal cortex (maximum in Brodmann's area 38) in the Romanian orphans (Fig. 4, Table 4). Thus, the regions with decreased glucose metabolism in the Romanian orphans compared to the pediatric control group were similar to those seen in the comparison of the orphans to the adult control group, although the location of the *peak* difference within the lateral temporal cortex differed (Brodmann's area 38 vs 20; see Table 4).

DISCUSSION

The major finding in the present study is that early global deprivation in the Romanian orphans is associated with dysfunction in a number of brain regions, including orbital frontal cortex, prefrontal infralimbic cortex, lateral temporal cortex, medial temporal structures, and brain stem. We selected a rigorous analysis approach using SPM to compare the pattern of glucose metabolism in Romanian orphans to that of normal adults, followed by an independent ROI approach and, finally, a second SPM analysis in which the pattern of glucose metabolism of the Romanian orphans was compared to that of the "normal" hemisphere of age-matched children with focal epilepsy. The same brain regions emerged as significantly different between Ro-

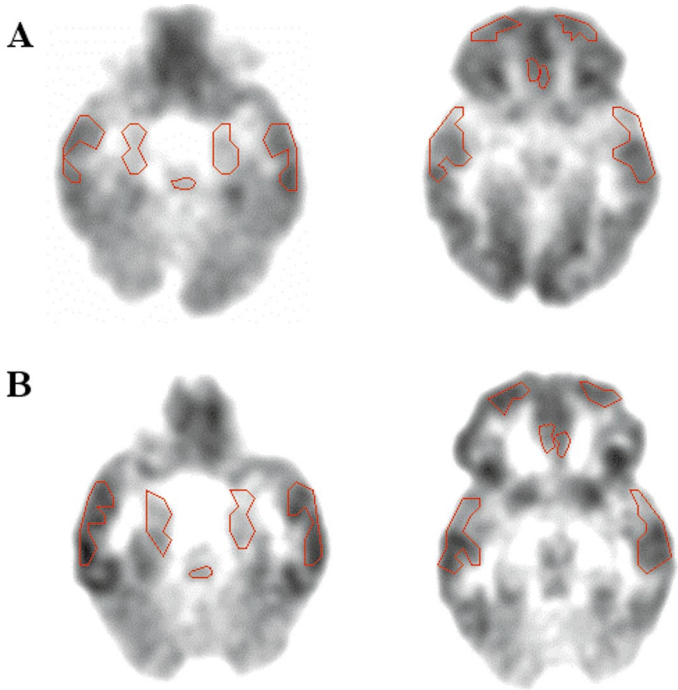

FIG. 3. Transaxial FDG PET images in (A) a child adopted from a Romanian orphanage and (B) a normal adult control. Outlined regions show representative ROIs defined bilaterally for regions found abnormal by the SPM analysis: orbital cortex, infralimbic cortex, inferior temporal cortex, medial temporal structures, and brain stem. These regions were defined in all PET image planes in which the particular structure could be identified.

manian orphans and controls across all three independent analyses.

The neurocognitive findings for the orphan group in this study are largely consistent with those reported by others (e.g., O'Connor and Rutter, 2000). Specifically, the "late-placed" group in the O'Connor study had a mean global cognitive index that fell in the low average range. Global intellectual functioning for the orphan sample in this study was also measured in the low average range. Of note, however, is that, where others have reported group means in the normal range and therefore interpret these children as having recovered in the cognitive domain, more extensive assessment across cognitive, language, memory, executive functioning, and motor domains, as carried out in this study, yields findings indicative of more specific and relative deficit. Specifically, mild neurocognitive relative deficits in language processing, memory, and executive functioning were found. Clinically significant behavioral problems were also found, including attentional, thought, and social deficits. Thus, the children in the present study appear to represent adequately that segment of the population exposed to early deprivation with persistent cognitive and behavioral problems, but who appear to be functioning within normal limits (O'Connor and Rutter, 2000).

TABLE 5

Region-of-Interest Analysis of Normalized Regional FDG PET Values Determined in the Romanian Orphans and the Adult Controls

Region	Romanian orphans	Adult controls	P value[a]
R orbital frontal cortex	1.09 (0.11)	1.24 (0.04)	$P < 0.001$
L orbital frontal cortex	1.15 (0.12)	1.25 (0.06)	$P = 0.008$
R lateral temporal cortex	0.99 (0.07)	1.14 (0.04)	$P < 0.001$
L lateral temporal cortex	1.03 (0.09)	1.13 (0.04)	$P < 0.001$
R medial temporal structures	0.62 (0.08)	0.81 (0.04)	$P < 0.001$
L medial temporal structures	0.62 (0.07)	0.81 (0.05)	$P < 0.001$
R infralimbic cortex	1.04 (0.17)	1.25 (0.09)	$P < 0.001$
L infralimbic cortex	1.08 (0.18)	1.31 (0.08)	$P < 0.001$
Brain stem	0.59 (0.14)	0.86 (0.06)	$P < 0.001$
Parietal cortex	1.23 (0.08)	1.21 (0.04)	$P = 0.42$

Note. The regional mean and SD in both groups as well as the corresponding P value are shown.

[a] Cut-off threshold for significance is $P = 0.04$ according to the modified Bonferroni correction for multiple comparisons.

Suitability of an Adult Control Group

The rationale for employing an adult control group is based upon our previous studies showing that although there are large changes in the quantitative values of regional brain glucose metabolism between 1 year of age and adulthood, the overall *pattern* of brain glucose metabolism at 1 year of age is similar to that seen in adults (Chugani *et al.*, 1986, 1987). We reasoned that if the regional *pattern* of glucose utilization is indeed "fixed" by 1 year of age, comparison of glucose PET scans using SPM (Friston *et al.*, 1995), a technique

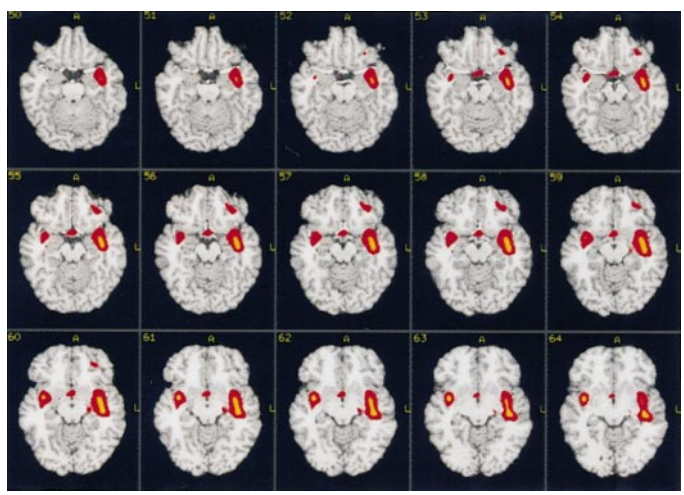

FIG. 4. SPM maps showing regions with lower glucose metabolism in Romanian orphan group compared to the age-matched pediatric epilepsy controls, superimposed onto a representative MRI scan in standardized space. The left side of the image represents the right side of the brain. Regions in red and yellow represent significant ($P < 0.05$) decreases of activity in the Romanian orphan group in comparison to the age-matched pediatric epilepsy controls.

which compares data which are normalized to the global mean, might be a useful approach for the study of pediatric disorders. Normal adult PET data might then be used as a control group, since obtaining absolutely normal pediatric PET data is not feasible due to ethical constraints.

However, SPM relies upon the accurate normalization and spatial registration of images to a standard template. In order to determine whether spatial normalization of PET image volumes to a PET image template could be successfully used in the pediatric population, we applied PET-derived transformation parameters to coregistered MRI volumes (Muzik *et al.*, 2000). We then compared coronal, sagittal, and transaxial contours of spatially normalized MRI volumes obtained from epileptic children ages 2–14 years with those derived from adult controls. The children selected for this comparison all had unilateral (and concordant) epileptic foci based on their electroencephalograms and visual assessment of their PET scans. Our results indicated that the spatial normalization of pediatric brains to an adult template causes a higher level of artifacts in statistical parametric maps compared to SPM analyses which involve only adult subjects. Indeed, the error associated with this procedure in children younger than 6 years precludes the application of SPM in this age group. However, although the error in the spatial normalization procedure for children ages 6 to 14 years was higher than in adults, the error did not result in artifacts in the SPM analysis. In addition, children over 6 years of age appeared to display the same pattern of glucose utilization as adults, with the exception of the expected focal decreases due to their epilepsy. Therefore, it appears that normal adult subjects are suitable controls for studies of glucose utilization in pediatric study groups but only if the children are over the age of 6 years (Muzik *et al.*, 2000). Importantly, the group of epileptic children studied (also a control group in the present study) did not show the pattern of decreased glucose metabolism observed in the group of Romanian orphans, indicating that the present findings are not due merely to age effect when using the SPM analysis. When interpreting results of an SPM analysis comparing children with adults, however, it must be kept firmly in mind that it is only the *pattern* that is being compared and that there are large global changes of absolute glucose utilization rates with age in children (Chugani *et al.*, 1987).

Regions with Decreased Glucose Metabolism Are Functionally Connected

It is interesting to note that the brain areas with significantly decreased glucose metabolism in the Romanian orphans are strongly interconnected and are known to be damaged as a result of prolonged stress (see below). For example the infralimbic (Brodmann's area 25) and orbital (Brodmann's area 11) cortices are reciprocally connected with amygdala and hippocampus (Barbas and Pandya, 1989; Barbas and De Olmos, 1990; Morecraft *et al.*, 1992; Barbas, 1993). Furthermore, layer V neurons of infralimbic and posterior orbitofrontal cortex project directly to subcortical autonomic centers (Neafsy, 1990; Hurley *et al.*, 1991). Infralimbic cortex has been called the autonomic motor cortex (Hurley *et al.*, 1991) as its ventral efferent pathway projects to autonomic cell groups in the brain stem and spinal cord, including the periaqueductal gray matter, the parabrachial nucleus, the nucleus of the solitary tract, the dorsal motor vagal nucleus, the nucleus ambiguous, and the ventrolateral medulla. Thus, the infralimbic cortex plays a role in regulating visceral responses to emotional stimuli (Freedman *et al.*, 2000), and dysfunction of this circuit has been implicated in impaired autonomic response to emotionally significant stimuli (Chu *et al.*, 1997). Furthermore, Rinaman *et al.* (2000) have demonstrated significant postnatal maturation of limbic forebrain projections to central autonomic neurons in rats, and they hypothesized, therefore, a critical period during early postnatal development during which experience might affect synapse formation of limbic–autonomic circuits.

The interconnected brain regions showing decreased glucose metabolism in the Romanian orphans also are integrally involved in the brain response to stress (reviewed by Lopez *et al.*, 1999). The presence of stress activates a series of pathways involving the brain and the endocrine system. Within the brain, a group of regions is activated in response to different types of stressors. For example, restraint, swim, or audiogenic stress in rats resulted in c-fos induction in neocortex, allocortex, hippocampus, nucleus accumbens, lateral septum, hypothalamus, amygdala, dorsal raphe, locus coeruleus, and brain-stem nuclei (Campeau and Watson, 1997; Cullinan *et al.*, 1995; 1996). The integration of the brain and endocrine stress response is mediated via the limbic–hypothalamic–pituitary–adrenal axis (Selye, 1936; McEwen *et al.*, 1974), the activation of which results in a series of events leading to glucocorticoid release. There is growing evidence that chronic elevations of glucocorticoids may cause damage to limbic brain regions (Kaufman *et al.*, 2000; McEwen, 2000; Sapolsky, 2000). Stress and glucocorticoids have been implicated in dendritic remodeling (McEwen and Sapolsky, 1995) and inhibition of neurogenesis in the hippocampus (Gould *et al.*, 2000). For example, chronic psychosocial stress in the tree shrew inhibited neurogenesis in the dentate gyrus resulting in a 30% reduction in its size (Gould *et al.*, 2000). Furthermore, stress and adrenal steroids cause reversible impairments in episodic and spatial memory in animals and humans (de Quervain *et al.*, 2000; Lupien and McEwen, 1997), and repeated stress can result in cognitive dysfunction (McEwen and Sapolsky, 1995).

Effects of Stress During Development

There is considerable evidence from animal studies that stressors applied early in development can affect behavior in adulthood (for review, see Lopez *et al.*, 1999). Similarly, early adverse experiences in humans are associated with elevated rates of major depression and other psychiatric disorders in adulthood (for review, see Kaufman *et al.*, 2000). Maternal separation is a potent stressor as evidenced by behavior (Harlow *et al.*, 1965; Harlow, 1969; Suomi, 1997; Newman and Bachevalier, 1997) and neuroendocrine response (Kuhn *et al.*, 1990; Higley, 1992; Pihoker *et al.*, 1993). Early primate models of social deprivation utilized paradigms involving total social deprivation throughout infancy and resulted in severe social and emotional disturbances, including aggression, motor stereotypies, and reproductive problems (Harlow *et al.*, 1965). Maternal deprivation with access to peers results in less severe behavioral abnormalities, but these monkeys have a characteristic phenotype in which they are highly reactive and impulsive. Adult monkeys which had been maternally deprived typically rank at the bottom of the dominance hierarchy (Harlow, 1969). Physiologically, maternally deprived monkeys show higher adrenocortical and noradrenergic responses to social separation, as well as consistently lower concentrations of the serotonin metabolite 5-hydroxyindole acetic acid in cerebrospinal fluid beginning before 6 months of age and continuing through adolescence (Higley *et al.*, 1992). Structural abnormalities in socially deprived monkeys include reduction in Purkinje cell size (Floeter and Greenough, 1979) and decreases in cortical dendritic branching (Struble and Riesen, 1978). Alterations in neurofilament protein immunoreactivity in the dentate gyrus granule cell layer of the hippocampus was observed in socially deprived monkeys compared with socially reared animals (Siegel *et al.*, 1993). Prolonged maternal separation of rat pups resulted in increased limbic–hypothalamic–pituitary–adrenal axis activity (Kuhn *et al.*, 1990; Pihoker *et al.*, 1993) and increased reactivity to stressors during adulthood (Plotsky and Meaney, 1993). However, different strains of rats show different sensitivity to early stress, suggesting that long-term effects of stress are mediated by both exposure and genetic variables (reviewed by Anisman *et al.*, 1998).

Similarly, human infants show heterogeneity in their behavior and neuroendocrine response to stressful situations. Toddlers who were classified as having disorganized/disoriented attachment had higher salivary cortisol concentrations in response to a strange situation, compared to the securely attached or the avoidant/resistant groups (Hertsgaard *et al.*, 1995). Carlson and Earls (1997) measured the diurnal variation of salivary cortisol in home-reared Romanian children compared to that of orphanage-raised children at 2 years of age. Morning cortisol levels were significantly lower, while noon and evening levels were higher in the orphan group. However, only mean values were presented, and thus variability among the children cannot be assessed. Genetic differences in the response to stress may partly explain why some children subjected to early deprivation are more severely impacted by the experience and show incomplete recovery following adoption.

Effect of Age at Which "Damage" Occurs

Kennedy and colleagues (1982), in studying the relationship between infant rhesus monkey behavior and regional cerebral glucose utilization, concluded that the time during development when a particular brain region became metabolically active marks the time when that structure contributed to the behavioral repertoire of the individual. In this regard, the early physiological maturation in humans of the amygdala and hippocampus (already active in newborns) and the orbital frontal cortex (about 6 months) as shown on glucose metabolism PET scans highlights the likely importance of this network in social interactions even in the very young infant (Chugani, 1998, 1999).

The notion that damage to temporal lobe regions occurring early in life can have different consequences compared to the same lesions suffered during adulthood has been studied in nonhuman primates. Bachevalier *et al.* (1999) studied memory and socioemotional behavior in monkeys after bilateral hippocampal damage incurred in infancy or in adulthood. Both neonatal and adult hippocampal lesions resulted in impairment of specific memory processes such as automatic recognition memory of visual, tactile, and spatial information and relational learning, as well as severe global anterograde amnesia. However, neonatal, but not adult, lesions resulted in a progressive loss of social behavior, the development of locomotor stereotypies, decreased eye contact, and blank facial expressions. Bachevalier *et al.* (1999) suggested that neonatal lesions of the hippocampus cause dysfunction in neural systems remote from the damage and cited evidence that behavioral and cognitive processes of the prefrontal cortex and caudate were disrupted by neonatal, but not adult, hippocampal lesions. Newman and Bachevalier (1997) studied the effect of bilateral lesions of amygdala or inferior temporal cortex (area TE) during the first week of life on vocal response to social separation in rhesus monkeys. Animals with bilateral ablations to amygdala produced calls with a lower slope in the rate of frequency change over time. Animals with bilateral inferior temporal cortex (area TE) lesions produced higher frequency calls, and males also produced noisy calls at a higher rate. These authors concluded that the amygdala and inferior temporal

neocortex are important for regulating the vocal response to social separation during development.

The Romanian orphans in the present study showed evidence of bilateral dysfunction (as indicated by decreased glucose metabolism) of medial temporal structures including the amygdala and hippocampus, as well as bilateral dysfunction of the inferior temporal cortex. Characteristics of behavioral abnormalities at the time of adoption, including absence of crying, stereotyped behaviors such as rocking and head-banging, and social difficulties, may be related to bilateral dysfunction in these brain regions during early brain development. However, the time course of the emergence of abnormal behaviors differed somewhat between the lesioned monkeys and the deprived children. In the primates, the social difficulties and stereotyped behavior emerged over time, whereas in the orphans these symptoms tend to dissipate after removal from the deprived environment.

In summary, children exposed to early social deprivation show long-term cognitive and behavioral deficits, associated with dysfunction (indicated by decreased glucose utilization) in a group of limbic brain regions known to be activated by stress and damaged by prolonged stress. We suggest that chronic stress endured in the Romanian orphanages during infancy in these children resulted in altered development of these limbic structures and that altered functional connections in these circuits may represent the mechanism underlying persistent behavioral disturbances in the Romanian orphans.

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