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Maturational Changes in Cerebral Function in Infants Determined by ^{18}FDG Positron Emission Tomography

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2-Deoxy-2[^{18}F]fluoro-D-glucose positron emission tomography performed in human infants during development revealed progressive changes in local cerebral glucose utilization. In infants 5 weeks of age and younger, glucose utilization was highest in the sensorimotor cortex, thalamus, midbrain-brainstem, and cerebellar vermis. By 3 months, glucose metabolic activity had increased in the parietal, temporal, and occipital cortices and the basal ganglia, with subsequent increases in frontal and various association regions occurring by 8 months. These functional changes measured with positron emission tomography are in agreement with behavioral, neurophysiological, and anatomical alterations known to occur during infant development.

SINCE GLUCOSE AND OXYGEN ARE the principal substrates for meeting the energy demands of the brain, measurements of the rates at which these substrates are utilized provide an assessment of the level of neuronal function in the brain. Measurement of the rates of regional substrate utilization in the brain during maturation provide a means whereby local functional activity can be related to various stages of behavioral development. Previous investigations of changing metabolic patterns in the maturing brain of animals (*1*) and humans (*2*–*4*) have involved the Kety-Schmidt method (*5*), which estimates average rates of blood flow and substrate utilization for the brain as a whole. Subsequently, measurements of local cerebral blood flow (LCBF) by quantitative autoradiography (*6*) permitted indirect assessment of local cerebral metabolic activity during development (*7*) because of the close relation between LCBF and local cerebral metabolic activity (*8*). With the introduction of the 2-deoxyglucose method for determining the local cerebral metabolic rate for glucose (LCMRglc) in animals (*9*), a number of investigators directly measured local changes in glucose utilization in the developing brain of the dog (*10*), monkey (*11, 12*), and sheep (*13*). However, the lack of a suitable noninvasive method precluded the measurement of LCBF and LCMRglc in the developing human brain.

Positron emission tomography (PET) (*14*), which involves tracer kinetic measurements of compounds labeled with positron-emitting isotopes, provides a noninvasive approach with which the principles of the 2-deoxyglucose method can be directly applied in humans to visualize and quantify LCMRglc (*15*) during postnatal development. In using PET with 2-deoxy-2[^{18}F]-fluoro-D-glucose (FDG) to measure LCMRglc in infants with certain neurological disorders, we obtained valuable data on LCMRglc during development. Although our studies were conducted in infants who were not completely normal, we believe that the data are reasonably representative of the normal state because all these infants had episodic neurological events but remained neurodevelopmentally normal (follow-up period, 8 to 14 months).

All studies were performed in accordance with the policies of the UCLA Human Subject Protection Committee. From over 60 infants and children who had been measured for LCMRglc, we selected nine infants whose status at the time of the procedure was judged to be nearly normal (Table 1).

In infants 5 weeks of age and younger (n = 4), LCMRglc was highest in the sensorimotor cortex, thalamus, midbrain-brainstem (*16*), and cerebellum (particularly the vermis, the centrally located, phylogenetically older portion of the cerebellum) (Fig. 1). The rate of glucose utilization was very low in the basal ganglia and the remaining cortex. By about 3 months of age (n = 2), a relative increase in LCMRglc was noted in much of the cerebral cortex, and LCMRglc in the striatum approached that of the thalamus. Glucose utilization in the cerebellum, previously highest in the vermis, now also extended laterally into the hemispheres. The frontal cortex and several association cortical regions, however, remained less metabolically active than the rest of the brain. A pattern of glucose utilization resembling that seen in adults (*17*), with prominent activity in frontal and association cortices, was established in infants 7.5 months, 1 year, and 1.5 years old.

In Fig. 2 the ratios of local cerebral to thalamic glucose utilization for several selected regions are plotted as a function of age, revealing the marked heterogeneity in the rates of functional maturation for different brain regions. Compared to the thalamus, the caudate and lenticular nuclei were relatively hypometabolic at birth, but LCMRglc rapidly approached that of the thalamus by the third month. The sensori-

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motor cortex (consisting of precentral and postcentral gyri) and the transverse temporal gyrus (which includes both primary and association sensory areas) were relatively active at birth, and activity there continued to increase during the first 3 to 4 months compared to that in the thalamus. The primary and association visual cortices, anterior cingulate gyrus, and middle frontal gyrus were inactive at birth relative to the thalamus, but all showed gradual increases during the first year. In contrast, LCMRglc for the cerebellum (taken as a whole) at birth appeared to exceed its rate at maturity, gradually decreasing during the first year.

An additional four infants and children (2 to 6 years of age) who had suffered from severe anoxia at birth were also studied with FDG-PET. All four had marked psychomotor retardation, with full-scale intelligence quotients less than 30 and computerized tomographic evidence of diffuse brain atrophy. Glucose utilization was, for the most part, restricted to the sensorimotor cortex, thalamus, midbrain-brainstem, and cerebellum, a pattern similar to that of neonates (Fig. 1). Furthermore, there was little glucose utilization in the visual areas of three of these subjects, and indeed all three were cortically blind.

Our results agree with previous observations in animals relating LCMRglc to level of function. Using 2-deoxyglucose autoradiography (*9*), Kennedy *et al.* (*12*) found that structures above the midbrain generally had lower metabolic rates in newborn monkeys than in adult monkeys. In addition, LCMRglc in neonatal auditory and somatosensory cortical areas resembled mature rates, whereas rates in the striate cortex and the large frontal and parietal association areas were well below the rates at maturity. Neonatal thalamic metabolic rates were relatively high compared to those for other structures. This pattern of glucose metabolic activity in the neonatal monkey is consistent with its behavior at that age, when auditory and somatosensory systems are relatively mature but visual function is less advanced. These observations led Kennedy *et al.* to suggest that "at any given developmental age, structures having metabolic rates equal to or exceeding their mature levels are those that dominate the behavior at that age" (*12*, p. 339). This hypothesis is supported by studies measuring regional brain tissue uptake of oxygen (*18*), LCBF (*7*), and LCMRglc (*10, 11, 13*) in various animal species characterized by different degrees of maturity at birth. Our findings support Kennedy *et al.*'s hypothesis not only in the normal human infant, but also in brain-damaged children.

In human neonates the prominent LCMRglc of sensorimotor cortical and subcortical regions, midbrain-brainstem, and paleocerebellum is consistent with the predominantly subcortical functional level at this age. The low LCMRglc seen in the striatum contrasts with the high LCMRglc of the thalamus, a pattern resembling that of adults with Huntington's chorea (*19*). Since neonates manifest nonpurposeful limb movements not unlike those seen in Huntington's chorea, a relatively active thalamus and less active striatum may be related to the choreiform movements seen in both Huntington's chorea and the "physiological chorea" of normal neonates. The former results from loss of function in the striatum while the latter appears to occur before development of function in the striatum.

The increasing LCMRglc in the basal ganglia and many cortical regions during the second and third months of life is consistent with several organizational changes that occur in the infant brain during this period (*20*). For example, the electroencephalogram (EEG) (which reflects predominantly cortical activity) matures rapidly during the second and third months, with the disappearance of neonatal patterns (*21*) and appearance of alpha-rhythm precursors (*22*). Behaviorally, several intrinsic subcortical reflexes, such as the tonic neck reflex, grasping, and the startle reflex begin to be suppressed, presumably as a result of increasing cortical input (*23*). By 3 to 4 months, the purposeless limb movements begin to be replaced by more coordinated motions as the infant reaches out for objects. The increasing LCMRglc in the cerebellar hemispheres and parietal cortex (containing centers vital to visuo-sensorimotor integration) may be important in this context.

By 8 to 9 months, cognitive or hypothesis-forming development is thought to begin in the human infant (*24*), and indeed, the PET images now show increased LCMRglc in frontal and association corti-

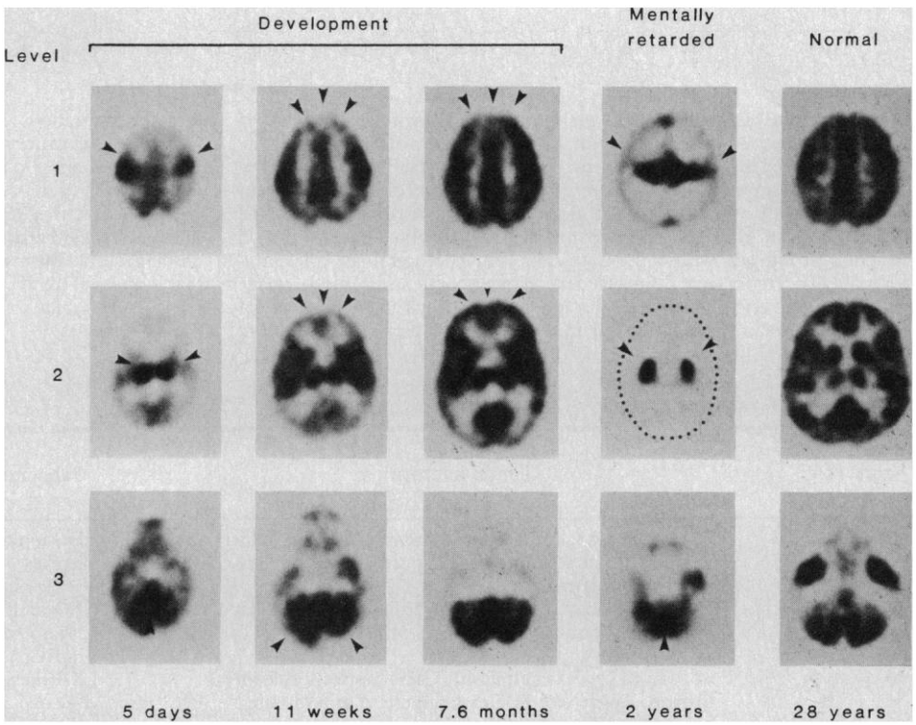

Fig. 1. FDG-PET images illustrating some of the developmental changes in LCMRglc in the normal human infant with increasing age (adult brain images not on same scale with respect to size). These maturational changes appear to have been halted at a neonatal stage in the 2-year-old infant with severe psychomotor retardation. All infants were studied awake, with eyes open. Continuous EEG recordings were made in all subjects during the study; in no case was there EEG or behavioral evidence of seizure activity. Although anticonvulsants were being administered to some of the infants, the doses were nonsedating. The dose of FDG was 0.143 mCi/kg, intravenously. In seven infants, timed blood samples were collected after injection for plasma glucose and FDG determinations. The samples were collected through an arterial catheter (if one was in place) or a venous catheter, placed in an extremity, which was inserted into a 44°C water bath to allow "arterialization" of the venous blood (*15*). Approximately 40 minutes after FDG administration, imaging of the brain was initiated with a NeuroECAT positron tomograph (CTI), which provides a spatial resolution of 8.4 mm in the plane of section and a 12.4-mm slice thickness (*31*). A series of 12 tomographic images was obtained parallel to the canthomeatal line. LCMRglc was determined with the operational equation of Sokoloff *et al.* (*9*) as modified for use in PET (*15*). In two cases absolute quantitation of LCMRglc was not possible due to difficulty in access to blood sampling.

ces, areas intimately involved with higher cortical function. This progressive increase in glucose utilization is consistent with anatomical studies showing both an expansion of dendritic fields (*25*) and an increase in the capillary density (*26*) of the human frontal cortex during this period. Behaviorally, the infant now exhibits anxiety in the presence of strangers and interacts more meaningfully with its surroundings (*27*).

Although cerebral blood flow and cerebral oxygen consumption have been studied in normal and neurologically abnormal children (*3*) with the Kety-Schmidt method (*5*), this technique does not permit regional measurements. Moreover, in the study by Kennedy and Sokoloff (*2*) the youngest child was 3 years old, and most of the patients studied by Garfunkel *et al.* (*3*) were neurologically markedly abnormal. Nevertheless, cerebral blood flow and cerebral oxygen consumption seemed to correlate directly with the developmental quotient. In our studies of children with psychomotor

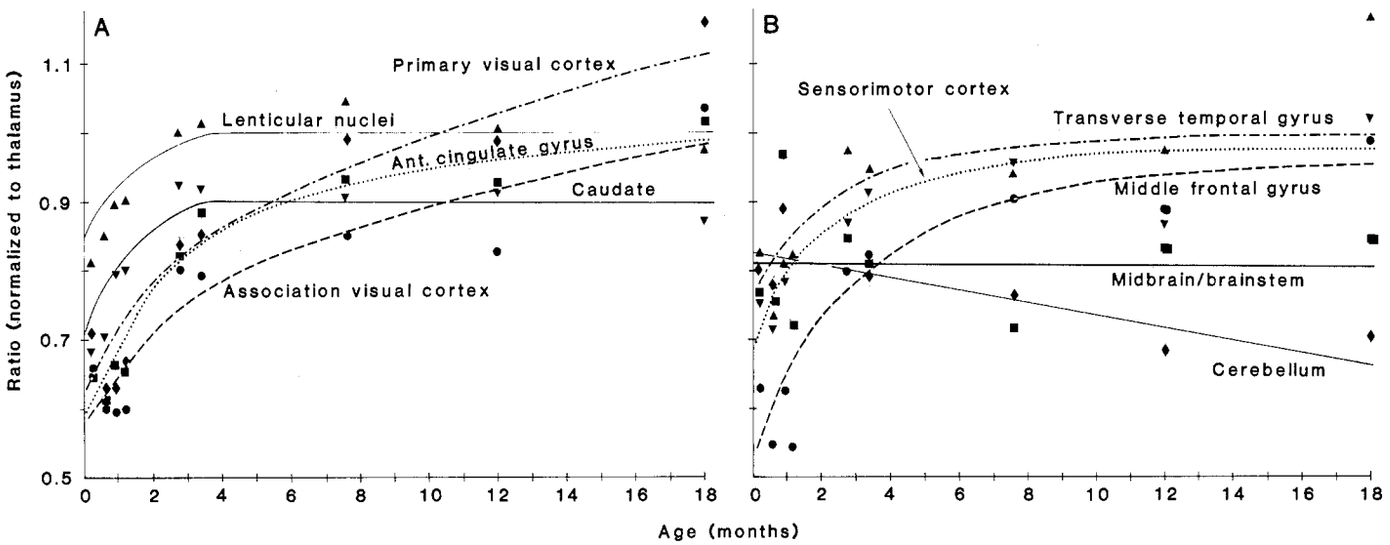

Fig. 2. (A and B) LCMRglc for several brain regions, expressed as a ratio to thalamic glucose utilization and plotted as a function of age for all nine infants described in Table 1. Since absolute glucose metabolic rates were not obtained in all subjects, the data were analyzed by several different approaches. The absolute values of LCMRglc were normalized to supratentorial global values of glucose utilization for all subjects with successful blood sampling. In subjects whose blood was not sampled, local tissue concentrations of radioactivity were normalized to average supratentorial global image counts to avoid errors intrinsic in absolute measurements and variable use of alternate substrates known to occur in early postnatal periods. In addition, local concentrations of radioactivity were normalized to values for the thalamus, a structure serving as a clear reference landmark in all our studies. Glucose utilization in the thalamus was well developed even in the youngest infant (5 days); furthermore, preliminary data indicate that the absolute LCMRglc for this structure (26.97 ± 2.61 μmol per 100 g per minute, mean ± standard error) does not undergo any significant change during the ensuing years. Both the global and thalamus reference yielded the same developmental trends, although the thalamus reference demonstrated somewhat less variability.

Table 1. Patient summary.

Age at time of study	Clinical information	Laboratory data	Medication on day of study	Period of follow-up after study
5 days	Post-term (42 weeks gestation). Caesarian section for fetal distress. Apgar scores: 2 at 1 minute, 8 at 5 minutes. Seizures on days 1 and 2.	EEG multifocal spikes	Phenobarbital	13 months
19 days	Normal pregnancy and delivery. Cardiac surgery on day 9 for congenital heart disease. Seizures on days 10 and 11.	EEG multifocal sharp wave activity	Phenobarbital	9 months
26 days	Post-term (43 weeks gestation). Caesarian section for fetal distress. Apgar scores: 3 at 1 minute, 6 at 5 minutes. Seizures on days 1 and 2.	EEG normal	Phenobarbital	8 months
5 weeks	Normal pregnancy, labor, and delivery. Onset of tonic seizures 4 days before study.	EEG normal, CT scan normal	Phenobarbital	6 months
11 weeks	Normal pregnancy, labor, and delivery. Onset of myoclonic jerks at 3 weeks of age.	EEG normal, CT scan normal	Valproic acid, phenobarbital	14 months
13.5 weeks	Normal pregnancy, labor, and delivery. Left facial port-wine nevus at birth. (?Sturge-Weber syndrome)	EEG normal, CT scan normal	None	9 months
7.6 months	Normal pregnancy, labor, and delivery. Opsoclonus and myoclonus developed at 6.5 months. Responded well to adrenocorticotropic hormone.	CT scan normal, EEG paroxysmal generalized spike and wave activity	Phenobarbital, clonazepam	10 months
1 year	Normal pregnancy, labor, and delivery. Left facial port-wine nevus at birth. (?Sturge-Weber syndrome)	EEG normal, CT scan normal	None	9 months
1.5 years	Normal pregnancy, labor, and delivery. Bilateral facial port-wine nevi (left > right) at birth. (?Sturge-Weber syndrome)	EEG normal	None	8 months

retardation, not only are global values of cerebral glucose utilization lower than in relatively normal children, there is a striking regional heterogeneity of functional brain anatomy. The pattern, in fact, resembles that of relatively normal neonates (Fig. 1). Congruent with this metabolic pattern is the persistence of intrinsic subcortical reflexes (such as grasping and tonic neck reflexes) and the overall neonate-like behavior of many such children. Diminished glucose utilization in psychomotor retardation is also consistent with reported dendritic spine loss and dysgenesis in children with mental retardation (*28*), since nerve endings and dendrites utilize more glucose than other neuronal constituents (*29*).

Since the extent of neurological compromise in brain-damaged children can vary, it will be important to determine whether the pattern of glucose metabolism in development and during various stimulation paradigms can predict the type and extent of neurological affliction. Furthermore, similar studies in children with specific learning disabilities may provide important insight into the basis of their deficits by identifying sites of disturbed cerebral function.

PET provides a novel approach to studying the developing human brain, both in normal children and in the brain-impaired child. The use of FDG to measure LCMRglc is but one example of many neurodevelopmental processes that can be investigated with PET. By using appropriately labeled compounds, it will be possible to quantify developmental changes in regional brain protein synthesis, blood flow, oxygen consumption, myelination, and neuroreceptors (*30*).

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L-Isoleucine and L-Leucine: Tumor Promoters of Bladder Cancer in Rats

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A 4-week assay for screening tumor promoters of bladder cancer has been developed in which increased agglutinability of isolated rat bladder cells with concanavalin A is used as an indicator. On the basis of this assay system, L-isoleucine and L-leucine were suspected of being possible tumor promoters. Results of 40- to 60-week carcinogenesis experiments in which *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine was used as an initiator to demonstrate that L-isoleucine and L-leucine promote bladder cancer in rats. This finding may be relevant to the high incidence of human bladder cancer in Western countries, where the diet is rich in protein.

A TWO-STAGE CARCINOGENESIS PROcess, consisting of initiation and promotion, has been established in mouse skin (*1*). The same two-stage process has also been demonstrated to apply in experimental bladder carcinogenesis, where substances such as sodium saccharin, DL-tryptophan, and sodium ascorbate act as tumor promoters (*2–4*). About 70 percent of human bladder cancers are of the superficial papillary type, which is characterized by frequent intravesical ectopic recurrence after transurethral resection of the tumors. This recurrence may be due to new tumor growth from epithelial lesions unrecognized on cystoscopy (*5*). Tumor promoters may play a key role in promoting the growth into recognizable tumors of epithelial lesions too small to be visible by cystoscopy. Thus, identification of promoters of human bladder carcinogenesis may be crucial for the management of superficial papillary bladder cancer.

We have developed a 4-week assay to detect promoters of bladder carcinogenesis in rats (*6–8*). Isolated bladder cells from rats that have been treated with various types of bladder carcinogens, such as *N*-butyl-*N*-(4-hydroxybutyl)nitrosamine (BHBN) or *N*-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide (FANFT) show increased agglutinability with concanavalin A (Con A) (*7*). This increased agglutinability with Con A disap-

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