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Rates of local cerebral protein synthesis in the rat during normal postnatal development

Y. SUN, G. E. DEIBLER, J. JEHLE, J. MACEDONIA,
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Sun, Y., G. E. Deibler, J. Jehle, J. Macedonia, I. Dumont, T. Dang, and C. Beebe Smith. Rates of local cerebral protein synthesis in the rat during normal postnatal development. *Am. J. Physiol.* 268 (*Regulatory Integrative Comp. Physiol.* 37): R549–R561, 1995.—The degree of recycling of leucine derived from protein breakdown into the precursor pool for protein synthesis was measured in rat brain at different postnatal ages, and age-specific values were used in the calculation of regional (local) rates of cerebral leucine incorporation into protein ($lCPS_{leu}$) in 44 brain regions and the brain as a whole. Early in development, a greater fraction of the precursor leucine pool is derived from protein breakdown, indicating that protein degradation is higher in young rats compared with adults. In whole brain and in most regions, values for $lCPS_{leu}$ were highest at 10 days and gradually decreased with age. By 60 days of age, values in cortex were ~60% of those at 10 days of age. In the paraventricular and supraoptic nuclei of the hypothalamus, however, $lCPS_{leu}$ increased during development, reaching peak values in adults. In white matter of the cerebellum and the cerebrum, peaks of $lCPS_{leu}$ were reached at 14 and 21 days, respectively, approximately at the times of maximum rates of myelination.

leucine; leucine recycling; brain; protein degradation

THE BRAIN OF MANY MAMMALIAN species is largely immature at birth and undergoes profound developmental changes in its composition and structure in the early postnatal period. The maturational process is characterized by a series of events that include the proliferation and migration of nerve cells, the growth of axons and dendrites, the formation of functional synapses, cell death, myelination of axons, and the fine tuning of neuronal specificity (15). Because proteins are fundamental components of all structural elements in tissue, and protein metabolism is directly involved in maturational events, the study of overall cerebral protein synthesis in vivo during postnatal development in various species of animals has been pursued in many different laboratories (see Ref. 17 for review). In all of these previous studies, the measurement of rates of protein synthesis was based on the use of radiolabeled precursors. A critical factor in the determination of protein synthesis rates with a radiolabeled tracer is the accurate evaluation of the specific activity of the precursor amino acid pool. Some of the earlier studies determined only relative incorporation of radioactivity, and many failed to take into account recycling of unlabeled amino acids derived from protein degradation into the precursor pool for protein synthesis. The only studies that did take into consideration the possibility of recycling of amino acids derived from protein breakdown were those in which the "flooding" technique (11) was employed. In the flooding technique, it is assumed that the administration of large doses of the tracer amino acid at low specific activity might so enhance the contribution of the plasma amino acid to the tissue amino acid pools that the relative contribution of the amino acid derived from protein degradation to the precursor pool would become negligible. With this technique, it was shown that, in young rats, rates of protein synthesis in the whole brain and in some dissected brain regions are two to four times higher than those in adults (12). Our studies in adult rats on the effects of flooding on the contribution of amino acids derived from protein degradation to the precursor pool (27) indicate that, under flooding conditions, the precursor pool in brain is not totally overwhelmed by amino acid coming from the plasma and that recycling is still significant and cannot be ignored.

In the interest of determining the developmental time course of cerebral protein synthesis rates in individual brain regions, with no assumptions about the equilibration of the precursor pool specfic activity with the plasma or other tissue pools, we have employed the quantitative autoradiographic L-[1-^{14}C]leucine method for the determination of regional rates of cerebral protein synthesis ($lCPS_{leu}$) in vivo (26). This method takes into account recycling of amino acids, either directly within the cell or via the extracellular space by means of a factor, λ_i, in the operational equation of the method. The factor λ_i is a constant with a value between 0 and 1.0 equal to the fraction of leucine in the precursor pool for protein synthesis in the tissue i derived from plasma; the remainder $(1 - \lambda_i)$ is derived from protein breakdown. The factor λ_i is the only factor in the equation for protein synthesis rates that cannot be measured directly in each experimental animal but can be determined in a separate group of animals as the ratio of the apparent steady-state leucine specific activity in the precursor amino acid pool (tRNA-bound leucine) in the tissue to that of the arterial plasma. In the whole brain of the conscious, adult male rat, this ratio (λ_{WB}) equals 0.58 (26). In the present study, we have examined the effects of postnatal development on the degree of recycling of leucine derived from protein degradation into the precursor pool for protein synthesis in whole brain (λ_{WB}). Local rates of leucine incorporation into protein in 44 brain regions and in the brain as a whole at five postnatal ages were determined with the autoradiographic technique. The age-specific values of λ_{WB} were used in the calculation to correct for dilution of the precursor pool by recycled unlabeled amino acid.

Results of our studies demonstrate that regional rates of protein synthesis are, in general, higher and more heterogeneous at the youngest age studied (10 days of

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age). In most brain regions, rates of protein synthesis gradually decrease during the first 5 wk of life, reaching adult levels by 35 days of age. In the adult rat, rates of protein synthesis are fairly homogeneous in most brain regions, except in certain cell layers and hypothalamic and brain stem nuclei, in which rates of protein synthesis are two to three times those found in the brain as a whole.

MATERIALS AND METHODS

Chemicals. Chemicals and materials were obtained from the following sources: L-[1-^{14}C]leucine (sp act 54 mCi/mmol), Amersham, Arlington Heights, IL; L-[3,4,5-^{3}H]leucine (sp act 155 Ci/mmol), Du Pont-New England Nuclear, Wilmington, DE; *Escherichia coli* tRNA, Sigma Chemical, St. Louis, MO; vanadyl ribonucleoside complex and redistilled nucleic acid-grade phenol, Bethesda Research Laboratories, Gaithersburg, MD; L-norleucine, Cyclochemicals, Travenol Laboratories, Los Angeles, CA; and 5-sulfosalicylic acid, Fluka, Buchs, Switzerland.

Animals. All procedures were carried out in accordance with the National Institutes of Health Guidelines on the Care and Use of Animals and an animal study protocol approved by the National Institute of Mental Health Animal Care and Use Committee. Female Sprague-Dawley rats were obtained between the 11th and 16th day of pregnancy from Taconic Farms (Germantown, NY) and housed one rat per cage. Food and water were provided ad libitum. Litters of six to eight pups remained with their mothers until the day of the experiment or were weaned and separated from their mothers at 21 days of age. Rats were maintained under controlled conditions of normal humidity and temperature with standard alternating 12-h periods of light and darkness. The λ_{WB} was evaluated on postnatal *days 7, 10, 14, 21,* and *35*, and $lCPS_{leu}$ was measured on postnatal *days 7, 10, 14, 21, 35*, and *60*. Studies of 7-, 10-, and 14-day-old rats were carried out on both males and females; the other age groups consisted only of male rats. Rats were prepared for determinations of λ_{WB} or $lCPS_{leu}$ by insertion under light halothane anesthesia of polyethylene catheters (PE-10 for 7-, 10-, 14-, and 21-day-old rats; PE-50 for 35- and 60-day-old rats) into one femoral artery and vein. In the 14-, 21-, 35-, and 60-day-old rats, it was necessary to tunnel the catheters under the skin to exit at the nape of the neck so that the rats could not gain access to the tubing. In the younger animals, this tunneling procedure was unnecessary. At least 1–2 h were allowed for recovery from surgery and anesthesia before initiation of the experimental procedure. To keep the young animals hydrated and normoglycemic, ~0.1–0.2 ml of 10% glucose was administrated by mouth to 7- and 10-day-old rats at 30-min intervals beginning 30 min after surgery to the end of the experiment. The rats were allowed to move freely in their cages throughout the experimental procedure. To maintain normal body temperature, young rats ($\leq$21 days) were placed in a small glass dish kept warm with flexible electric heating tape (Barnstead/Thermolyne, Dubuque, IA) controlled by a thermostat.

Physiological variables. Physiological variables were measured to evaluate each animal's physiological state. Mean arterial blood pressure was measured by means of an air-damped mercury manometer attached to the femoral arterial catheter. Arterial blood pH, CO_2 tension, and O_2 tension were measured with a Corning 158 pH/blood gas analyzer (Corning, Halstead, Essex, UK). Arterial blood hematocrit was determined in blood samples collected in capillary tubes subsequently sealed and centrifuged in a Microspin 24S (Sorvall Instruments, Du Pont, Wilmington, DE). Arterial plasma glucose concentrations were measured with a Beckman Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA). Rectal temperatures were measured with a model BAT-12 thermometer (Sensortek, Clifton, NJ).

Procedure to determine λ_{WB} and Ψ_{WB}. The λ_{WB} and the fraction of leucine in the tissue acid-soluble pool (Ψ_{WB}) were evaluated at 7, 10, 14, 21, and 35 days of age in 4, 6, 6, 5, and 4 rats, respectively, by the method described by Smith et al. (26). The factor λ_i is the ratio of the integrated specific activity of leucine in the tissue precursor pool to that of the arterial plasma 60 min after an intravenous pulse of labeled leucine

$$\lambda_i \approx \frac{\int_0^T [C^*_{pp}(t)/C_{pp}]dt}{\int_0^T [C^*_p(t)/C_p]dt} \quad (1)$$

where C^*_{pp} and C_{pp} are the concentrations of the labeled and unlabeled leucine, respectively, in the precursor pool in the tissue i, and C^*_p and C_p represent the concentrations in arterial plasma of labeled and unlabeled leucine, respectively. If the $C^*_p(t)/C_p$ is held constant for a long enough time (T) for the tissue-free and tRNA-bound leucine pools to reach a steady state with the plasma, then

$$\lambda_i \approx \frac{C^*_{pp}(T)/C_{pp}}{C^*_p(T)/C_p}. \quad (2)$$

The time necessary to achieve this apparent steady state for leucine in the normal, conscious rat is between 30 and 60 min (26). The steady state is designated as apparent, because it pertains to the free and tRNA-bound leucine pools only; a steady state for protein-bound [^{3}H]leucine is not even approached during the 60-min experiments. Analogously, Ψ_i is the steady-state ratio of the specific activity of leucine in the tissue acid-soluble amino acid pools to that of the arterial plasma

$$\Psi_i \approx \frac{[C^*_E + C^*_M](T)/[C_E + C_M]}{C^*_p(T)/C_p} \quad (3)$$

where C^*_E and C_E are the concentrations in the extracellular space of labeled and unlabeled leucine, respectively, and C^*_M and C_M are the concentrations in the intracellular metabolic pool of labeled and unlabeled leucine, respectively.

To determine λ_{WB} and Ψ_{WB}, a constant arterial plasma specific activity for [^{3}H]leucine was maintained for 60 min by means of a scheduled infusion of [^{3}H]leucine designed to achieve and maintain a constant arterial plasma [^{3}H]leucine concentration. The infusion schedule consisted of an initial intravenous pulse followed by a progressively decreasing rate of infusion adjusted every 15 s and administered by a programmed computer-driven infusion pump (Pump 22, Harvard Apparatus, South Natick, MA). Each rat was infused with 3.4–8.3 mCi of [^{3}H]leucine in a total volume of ~0.25 ml for young rats ($\leq$21 days) and 1.5 ml for 35-day-old rats over the 60-min period of the infusion. [^{3}H]leucine was used to determine λ_{WB}, because its high specific activity was needed to measure the specific activity of the leucine in the very small pool of tRNA-bound leucine in brain. The specific activities of [^{3}H]leucine in arterial plasma and in the acid-soluble and tRNA-bound pools in brain were determined as described below. Timed arterial blood samples, 15–25 μl in volume, were collected every 5 or 10 min during the infusion and centrifuged immediately to separate the plasma, which was then deproteinized by addition of one-third of a volume of a solution of 16% (wt/vol) 5-sulfosalicylic acid containing L-norleucine (0.04 mM) as an internal standard for amino acid analyses. The deproteinized plasma samples were stored at −70°C until

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assayed for leucine and [^{3}H]leucine concentrations. At the end of the infusion, the rats were decapitated, and the brains were quickly removed and chilled to 0°C in ice-cold 0.25 M sucrose.

Extraction and purification of aminoacyl-tRNA. The brain from each rat was homogenized by a motor-driven, loose-fitting, all-glass homogenizer in 10 ml of 0.25 M sucrose (0°C) containing 10 mM vanadyl ribonucleoside complex to inhibit ribonuclease, 6 mg of uncharged *E. coli* tRNA as carrier, and L-norleucine (0.02 mM) added as an internal standard. The homogenates were centrifuged at 100,000 *g* for 1 h to remove intact cells, cellular debris, and subcellular organelles, and the pellets were discarded. The cytosolic protein and RNA in the remainder of the supernatant fraction were precipitated by the addition of one-tenth of a volume of 50% (wt/vol) trichloroacetic acid and separated by centrifugation (12,000 *g*, 30 min). The precipitates containing the aminoacyl-tRNA were washed six times in 3% (wt/vol) perchloric acid to remove free amino acids, suspended in 0.3 M sodium acetate (pH 5), and extracted with an equal volume of fresh, water-saturated phenol containing 0.1% (wt/vol) 8-hydroxyquinoline as an antioxidant. The aqueous phase, containing the aminoacyl-tRNA, was separated, and residual phenol was removed by six extractions with anhydrous diethylether. The aminoacyl-tRNA was precipitated overnight at −20°C by addition of 2.5 vol of ethanol containing 0.12 M potassium acetate, pH 5.5. The RNA precipitate was recovered by centrifugation (12,000 *g*, 30 min) at 0°C; washed two times in an ice-cold 1:2.5 (vol/vol) mixture of 0.3 M sodium acetate, pH 5, and 0.12 M potassium acetate in ethanol, pH 5.5; dissolved in 50 mM sodium carbonate (pH 10); and incubated at 37°C for 90 min to deacylate the aminoacyl-tRNA. Deacylated tRNA was precipitated overnight at −20°C by addition of 2.5 vol of ethanol and removed by centrifugation (12,000 *g*, 20 min). The supernatant solutions, which contained the previously tRNA-bound but now free amino acids, were dried in a stream of N_2 and redissolved in 40 μl of 0.2 M sodium citrate (pH 2.2) (26).

Extraction of acid-soluble fraction in brain tissue. A 100-μl volume of the cytosol fraction, i.e., the supernatant solution derived from the 100,000 *g* centrifugation of the whole brain homogenates, was deproteinized by the addition of an equal volume of a solution of 8% (wt/vol) 5-sulfosalicylic acid and stored at −70°C until assayed for leucine and [^{3}H]leucine concentrations. At the time of these assays, the samples were thawed, mixed, and centrifuged for 30 min at 5,000 *g* at 4°C to remove the precipitated protein.

Assay of specific activity of [^{3}H]leucine. Specific activities of [^{3}H]leucine in deproteinized plasma, tissue acid-soluble fractions, and in the fractions derived from the deacylation of the aminoacyl-tRNA were assayed by postcolumn derivatization with *o*-phthaldehyde and fluorometric assay with a Beckman model 7300 amino acid analyzer (Beckman Instruments). This system can measure 10–100 pmol of leucine with a 3% coefficient of variation. Fractions, after passage through the detector, were collected every minute and assayed for ^{3}H with a TRI-CARB liquid scintillation analyzer, model 2250CA (Packard Instrument, Downers Grove, IL). Specific activity was calculated from total ^{3}H in all fractions in the leucine peak and the total measured leucine content in the peak. The leucine concentration and the specific activity of [^{3}H]leucine in the acid-soluble pool in the tissue were corrected for contamination by the leucine in the blood contained in the tissue. The equilibrium distribution of free leucine between red blood cells and plasma was measured and found to be 0.67, and the hematocrit in brain was determined to be 30% (7).

Calculation of values of λ_{WB} and Ψ_{WB}. Values of λ_{WB} were calculated from the ratio of the measured steady-state specific activity of the tRNA-bound leucine in the tissue to that of the acid-soluble leucine in arterial plasma (*Eq. 2*), and values of Ψ_{WB} were calculated from the ratio of the measured steady-state leucine specific activity of the brain acid-soluble pool to that of the arterial plasma (*Eq. 3*). The time course of the specific activity in arterial plasma and the specific activities of acid-soluble and tRNA-bound leucine in whole brain at the end of the experimental interval were determined as described above. The apparent steady-state free-leucine specific activity in the arterial plasma was calculated as the mean of the specific activities determined from 40 min to the end of the interval. Whereas, in some of the experiments, the specific activity of leucine in the arterial plasma is not constant during the first 30 min, from 40 min on, values were within ±12% of the mean and showed no overall trend to increase or decrease over the entire interval. Leucine from the tRNA-bound amino acid fractions was uncontaminated by leucine derived from any blood in the brain tissue because of the procedure used to separate this fraction from the free amino acids in the tissue. The specific activity of [^{3}H]leucine in the acid-soluble pools in the tissue was corrected for leucine in the residual blood contained in the tissue as described above.

Determination of local rates of protein synthesis. Local rates of protein synthesis were determined at 10, 14, 21, 35, and 60 days of age in 7, 6, 6, 6, and 6 rats, respectively. We attempted to measure $lCPS_{leu}$ in 7-day-old rats but were unable to because of technical difficulties. Rats were surgically prepared and catheterized, and their physiological states were monitored as described above. The experimental period was initiated by an intravenous pulse of 100 μCi/kg of L-[1-^{14}C]leucine contained in ~15, 20, 35, 80, and 180 μl of physiological saline at 10, 14, 21, 35, and 60 days of age, respectively. Timed arterial samples were collected during the following 60 min for determination of the time courses of plasma concentrations of leucine and [^{14}C]leucine. Blood sampling was more frequent during the portions of the plasma curve in which the concentration of [^{14}C]leucine changed most rapidly. In the young animals, fewer blood samples (~11–12) were taken to minimize blood loss. The blood samples were immediately centrifuged to remove the red blood cells, and a 5-μl volume of plasma from each sample was diluted in distilled water and deproteinized at 4°C by the addition of a solution of 16% (wt/vol) 5-sulfosalicylic acid containing [^{3}H]leucine (1 μCi/ml) as an internal standard for liquid scintillation counting and L-norleucine (0.04 mM) as an internal standard for amino acid analysis. Labeled and unlabeled leucine concentrations in the acid-soluble fractions were assayed by liquid scintillation counting and by amino acid analysis, respectively. Concentrations of [^{14}C]leucine and total leucine were calculated with correction for recovery based on the internal standard concentrations measured in each sample. In these deteminations of $lCPS_{leu}$, it was necessary to pool equal volumes of plasma samples taken from 10–60 min for a single determination of plasma concentration of unlabeled leucine because of the small blood sample volumes withdrawn from the young rats. [^{14}C]leucine concentrations were determined in each individual plasma sample, and concentrations of unlabeled leucine in plasma were also measured in samples taken at *time 0* and 5 min. The procedure for sampling and pooling samples for analysis was devised to conserve blood in the very young rats but was also used for rats at all ages studied. At the end of the 60-min experimental period, rats were killed by an intravenous injection of pentobarbital sodium, and the brains were rapidly removed and frozen in isopentane cooled to −40°C with dry ice. Sections 20-μm thick were cut in a cryostat at −20°C, thaw mounted on gelatin-coated slides, air dried, and then fixed and washed with five changes of phosphate-buffered 10% Formalin for 30 min each, and washed in running deionized water for 30

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Table 1. *Anatomic delineation of brain regions analyzed*

Structure	Boundary Definition	Level on Coronal Sections (mm rostral to interaural line) for Postnatal Days			
		10, 14	21	35	60
Auditory system					
Auditory cortex (Te1 and Te3), layers 1–6	Rectangle	0	1.2	2	3.2
Medial geniculate body	Outline	0	1.2	2	3.2
Inferior colliculus	Outline	−3.8	−1.9	−1.2	0.2
Lateral lemniscus, dorsal	Outline	−1.4	−0.5	0	1
Superior olivary nucleus*	Outline	−2.2	−1.9	−1.4	−0.3
Ventral cochlear nucleus, anterior	Outline	−3	−2.3	−2.2	−1.2
Visual system					
Visual cortex (Oc1M and Oc1B), layers 1–6	Rectangle	−1.8	−1.2	−0.8	0.4
Dorsal lateral geniculate nucleus	Outline	1.2	2.3	3.2	4.8
Superior colliculus, superficial gray	Outline	−0.9	−0.4	1.2	1.7
Sensorimotor systems & frontal cortex					
Frontal cortex (Fr1 and Fr3), layers 1–6	Rectangle	6.5	8	9	11
Sensorimotor cortex (forelimb area), layers 1–6	Rectangle	5.6	6.5	7.5	9.2
Piriform cortex	Outlined	6.2	7.5	9	10.7
Thalamus, anterior dorsal nucleus	Outline	3.5	4.4	5	7.2
Thalamus, anteroventral nucleus†	Outline	3.5	4.4	5	7.2
Thalamus, reticular nucleus	Outline	3.8	5	5.6	7.6
Subthalamic nucleus	Outline	2	2.9	4.1	5.2
Caudate putamen	Outline	5.6	6.5	7.5	9.2
Globus pallidus	Circle	4.1	5.3	6.2	8.1
Substantia nigra (pars compacta)	Outline	0	0.8	2.6	3.4
Substantia nigra (pars reticulata)	Outline	0	0.8	2.6	3.4
Red nucleus	Outline	0	0.8	1.6	3.2
Inferior olivary nucleus	Outline	−5.4	−5.2	−5.1	−4.7
Pontine nucleus	Outline	−0.9	0.4	1.2	2.2
Medial vestibular nucleus	Circle	−4.6	−4.2	−4	−3.4
Hypoglossal nucleus	Circle	−5.5	−5.4	−5.4	−4.9
Limbic system					
Dorsal hippocampus, CA1-pyramidal cell layer	Outline	2.3	2.6	3.2	5.4
Dorsal hippocampus, CA2-pyramidal cell layer	Outline	2.3	2.6	3.2	5.4
Dorsal hippocampus, CA3-pyramidal cell layer	Outline	2.3	2.6	3.2	5.4
Dorsal hippocampus, dentate gyrus (stratum granulosum)	Outline	2.3	2.6	3.2	5.4
Entorhinal cortex (medial part, layers 2 and 3)	Outline	0	1.2	2	3.4
Anterior cingulate cortex (Cg1 and Cg2)	Outline	5.6	6.5	7.5	9.2
Nucleus accumbens	Circle	6.2	7.5	9	10.7
Basolateral amygdala, anterior	Circle	3.2	3.5	4.7	7.2
Hypothalamus					
Ventromedial nucleus	Outline	3.2	4.1	4.7	6.2
Dorsomedial nucleus	Circle	3.2	4.1	4.7	6.2
Paraventricular nucleus	Outline	3.5	4.7	5.3	7.2
Suprachiasmatic nucleus	Circle	4.1	5.3	6.2	7.7
Supraoptic nucleus	Outline	3.8	5	5.9	7.6
Pineal gland	Outline	−2.6	−1.9	−1.4	0.5
Cerebellum					
External germinal layer	Outline	−5			
Internal granular layer	Outline	−5	−4.2	−3.7	−3.4
White matter					
Cerebellar white matter	Outline	−5	−4.1	−3.6	−3.1
Anterior commissure	Outline	6.2	7.5	8	10.2
Genu of the corpus callosum	Outline	5.6	7	8	9.7

Boundary definition is shape of sampling tool. Level on coronal sections according to Paxinos and Watson (23) for adults and Sherwood and Timiras (25) for other age groups. * Lateral, medial, lateral ventral, and medial ventral; † dorsomedial and ventrolateral parts.

min. The sections were then autoradiographed along with calibrated [^{14}C]methylmethacrylate standards, as previously described (28). The rates of leucine incorporation into protein in individual brain regions and the average for the brain as a whole, weighted for the relative masses of its component parts, were determined by analysis of the autoradiograms with a computerized image processing system (MCID Imaging Research, St. Catharines, Ontario, Canada) with a pixel size of 28 μm. The concentration of ^{14}C in each region of interest in the autoradiograms was determined from the optical density vs. ^{14}C concentration curve for the calibrated plastic standards. Local rates of protein synthesis were calculated by means of the operational equation of the method (26) with the age-specific values of λ_{WB}

$$R_i = \frac{P_i^*(T)}{\lambda_i \left[\int_0^T \frac{C_p^*(t)}{C_p} dt \right]} \quad (4)$$

where R_i is the rate of leucine incorporation into protein in tissue i; $P_i^*(T)$ is the concentration of ^{14}C fixed in the tissue i at any time T after introduction of the tracer into the circulation; λ_i is equal to the fraction of leucine in the precursor pool for protein synthesis in the tissue i derived from plasma; $C_p^*(t)$ and C_p are the concentrations of labeled and unlabeled leucine in the arterial plasma, respectively; and t is variable time. Rates of protein synthesis measured with the [^{14}C]leucine method are rates of incorporation of leucine into all tissue protein, which is comprised of a mixture of many individual proteins. The rate of leucine incorporation into each individual protein is weighted according to not only its individual rate of turnover but also the fraction of total tissue protein which it comprises.

Regions were selected for analysis for the purpose of surveying the brain where it was feasible to do so. Regions were located according to the rat brain atlas of Paxinos and Watson (23) for adult rats and the atlas of the developing brain of Sherwood and Timiras (25) for all other age groups (Table 1).

Statistics. Values of λ_{WB}, Ψ_{WB}, ratios of λ_{WB} to Ψ_{WB}, and weighted average rates of protein synthesis and $lCPS_{leu}$ in 44

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Table 2. *Effects of developmental age on physiological variables*

	Postnatal Age, days											
	7	n	10	n	14	n	21	n	35	n	60	n
Body weight, g	19 ± 1	4	27 ± 1	13	38 ± 2	11	62 ± 3	11	159 ± 4	12	331 ± 8	6
Brain weight, g	0.73 ± 0.03	4	0.96 ± 0.01	6	1.22 ± 0.01	6	1.44 ± 0.03	5	1.69 ± 0.01	4	1.90 ± 0.02	9
Body temperature, °C	37.0 ± 0.1	4	37.0 ± 0.1	13	37.5 ± 0.1	11	37.0 ± 0.1	11	37.0 ± 0.1	12	37.4 ± 0.1	6
Hematocrit, %	31 ± 2	4	31 ± 1	13	30 ± 1	11	32 ± 1	11	42 ± 1	12	51 ± 1	6
Arterial blood												
Mean pressure, mmHg	42 ± 6	4	49 ± 2	13	53 ± 2	11	85 ± 2	11	101 ± 2	12	118 ± 1	6
pH	ND		ND		7.40 ± 0.01	6	7.40 ± 0.01	6	7.43 ± 0.01	6	7.46 ± 0.01	6
Pa_{O_2}, mmHg	ND		ND		81.3 ± 3.4	6	78.2 ± 3.1	6	84.0 ± 1.2	6	85.8 ± 2.4	6
Pa_{CO_2}, mmHg	ND		ND		35.7 ± 0.8	6	39.2 ± 1.0	6	41.4 ± 0.7	6	38.9 ± 0.6	6
Arterial plasma												
Glucose concentration, mM	7.4 ± 0.4	3	7.3 ± 0.2	13	7.7 ± 0.2	10	9.4 ± 0.3	6	9.4 ± 0.2	6	8.4 ± 0.5*	6
Leucine concentration, μM	86 ± 20	4	183 ± 8	13	147 ± 7	11	151 ± 7	11	164 ± 8	12	173 ± 7	6

Values are means ± SE obtained from no. of animals (*n*); ND, not determined. Measurements for body temperature, hematocrit, mean arterial pressure, and 60-day glucose concentration were made 5–10 min before administration of radiolabeled leucine. Measurements for pH, arterial O_2 tension (Pa_{O_2}), arterial CO_2 tension (Pa_{CO_2}), and glucose concentration were made at end of 60-min experiments. Measurements for leucine concentration were made 1–10 min after administration of radiolabeled leucine. *Values from Sun et al. (28).

brain structures, determined in developing rats, were compared with those of adults by means of Dunnett's *t*-tests.

RESULTS

Physiological status. Physiological variables were measured to assess the physiological status of developing animals and adults (Table 2). Body weight and mean arterial blood pressure steadily increased with age and were highest in 60-day-old rats. Brain weight increased steeply until 21 days of age, after which the rate of growth was slow. Hematocrit did not change between 7 and 21 days of age but gradually increased to adult values thereafter. It was crucial to maintain body temperature in the normal range during the experimental period, especially in very young rats, because their body temperatures changed rapidly when they were exposed to room air. Body temperature was, therefore, monitored throughout the experimental period, and only animals with body temperatures within the normal range (36.3–38.5°C) were included in the study. Arterial blood gas tensions and pH were determined only in rats from age 14 days to adult because total blood volume in the younger animals is small; values were found to be relatively stable in the age groups examined. Arterial plasma glucose levels were somewhat lower in 7-, 10-, and 14-day-old rats than in adults, but from 21 days on plasma glucose levels were similar to those of adults. Arterial plasma leucine concentrations were similar at all ages examined except at 7 days, when values were more variable and considerably lower.

Leucine concentrations in plasma and brain during development. In the experiments for determining the values of Ψ_{WB} and λ_{WB}, the steady-state concentrations of unlabeled leucine in the arterial plasma and in brain amino acid pools were measured (Table 3). In the plasma, leucine concentrations were lower in 7-day-old rats compared with those of adults. In brain, acid-soluble and tRNA-bound leucine concentrations were statistically significantly higher at 10 and 14 days of age than in adults. In contrast, leucine levels in both the brain acid-soluble and tRNA-bound pools in 7-day-old rats were similar to those found in adults. The total amount of tRNA-bound leucine per brain was lowest at 7 days and increased with age until 14 days, after which it remained at the same level as that of adult rats. The tissue-to-plasma distribution ratio for the acid-soluble leucine pool was highest at 7 days of age and lowest in adults.

Table 3. *Effects of developmental age on leucine concentrations in plasma and brain amino acid pools in experiments to determine* λ_{WB}

Postnatal Age, days	Acid-Soluble Leucine Pools						tRNA-Bound Leucine Pool			
	Plasma, nmol/ml	n	Brain, nmol/g	n	Tissue-to-Plasma Distribution Ratio	n	Brain Concentration, nmol/g	n	Total Content/ Brain, nmol	n
7	107 ± 24*	4	59 ± 8	4	0.59 ± 0.06†	4	0.133 ± 0.011	4	0.096 ± 0.005†	4
10	155 ± 11	6	80 ± 3†	6	0.52 ± 0.04†	6	0.140 ± 0.017†	6	0.134 ± 0.017*	6
14	169 ± 7	6	85 ± 4†	6	0.51 ± 0.02*	6	0.155 ± 0.008†	5	0.189 ± 0.011	5
21	141 ± 12	5	58 ± 2	5	0.42 ± 0.03	5	0.127 ± 0.007	5	0.183 ± 0.011	5
35	149 ± 14	4	61 ± 1	4	0.42 ± 0.04	4	0.110 ± 0.005	4	0.185 ± 0.007	4
Adults	154 ± 9	9	55 ± 4	9	0.36 ± 0.03	9	0.096 ± 0.006	9	0.182 ± 0.011	9

Values are means ± SE obtained from no. of animals (*n*). Measurements for plasma were made at end of 60-min experiments. Values for tissue-to-plasma distribution ratio are means of individual ratios of brain steady-state acid-soluble leucine concentration to plasma acid-soluble leucine concentration. Values for adults from Sun et al. (28). *$P < 0.05$, statistically significantly different from adult value (Dunnett's *t*-test); †$P < 0.01$, statistically significantly different from adult value (Dunnett's *t*-test).

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Values of λ_{WB} and Ψ_{WB} during development. To evaluate the degree of equilibration of tissue leucine pools with arterial plasma, rats were maintained for 60 min with a relatively constant specific activity of [^{3}H]leucine in arterial plasma, and the steady-state ratios of the specific activity of leucine in the brain acid-soluble pools to that of arterial plasma, i.e., Ψ_{WB}, were measured (Table 4). Values of Ψ_{WB} were considerably <1.0 at all ages, indicating that the acid-soluble tissue pools did not come close to equilibration with the plasma. Values of Ψ_{WB} were relatively constant across the developmental ages studied. In the same animals the steady-state ratios of the specific activity of leucine in the brain tRNA-bound pool to that of the arterial plasma, i.e., λ_{WB}, were also measured (Table 4). At all ages studied values of λ_{WB} were, like values of Ψ_{WB}, <1.0. Values of λ_{WB}, however, were not constant across all ages; they were lowest in 7-day-old rats and increased with developmental age until 35 days, at which time the value of λ_{WB} was the same as that of adult rats.

The differential effects of developmental age on the level of equilibration of the acid-soluble and tRNA-bound leucine pools with the arterial plasma are evident when the steady-state ratios of the specific activity of leucine in the brain acid-soluble pool to that of the brain tRNA-bound pool (Ψ_{WB}/λ_{WB}) are compared across ages (Table 4). In 7-day-old rats this ratio was highest, and it decreased progressively with increasing age. At 7, 10, 14, and 21 days of age the ratio Ψ_{WB}/λ_{WB} was statistically significantly higher than that found in adult rats. These results indicate that, in mature brain, the intracellular source of leucine for the tRNA-bound pool is relatively more diluted with recycled leucine derived from tissue protein breakdown than the total tissue acid-soluble pool. In immature brain the opposite appears to be the case, i.e., the intracellular source of leucine for the total tissue acid-soluble pool is relatively more diluted with recycled leucine derived from tissue protein breakdown than the tRNA-bound pool.

$lCPS_{leu}$ during development. Average rates of protein synthesis in the brain as a whole in 10-, 14-, 21-, and 35-day-old animals were 75, 53, 37, and 14% higher than that of 60-day-old rats, respectively (Fig. 1).

Table 4. *Effects of developmental age on relative steady-state [^{3}H]leucine specific activities in tissue pools*

Postnatal Age, days	Steady-State Ratio of [^{3}H]Leucine Specific Activities		
	Ψ_{WB} Acid-soluble pool/plasma	λ_{WB} tRNA-bound pool/plasma	Ψ_{WB}/λ_{WB} Acid-soluble pool/tRNA-bound pool
7 (4)	0.46 ± 0.03	0.43 ± 0.03*	1.09 ± 0.02*
10 (6)	0.47 ± 0.03	0.45 ± 0.02*	1.05 ± 0.03*
14 (6)	0.53 ± 0.02	0.52 ± 0.01†	1.01 ± 0.03*
21 (5)	0.49 ± 0.03	0.53 ± 0.02	0.94 ± 0.02†
35 (4)	0.53 ± 0.01	0.58 ± 0.01	0.91 ± 0.01
Adults (9)	0.49 ± 0.02	0.58 ± 0.01	0.84 ± 0.02

Values are means ± SE of individual ratios determined in no. of animals indicated in parentheses. Ψ_{WB}, fraction of leucine in acid-soluble pool in whole brain derived from arterial plasma; λ_{WB}, fraction of leucine in precursor pool for protein synthesis in whole brain derived from arterial plasma. Values for adults from Sun et al. (28). *$P < 0.01$, statistically significantly different from adult rats (Dunnett's t-test); †$P < 0.05$, statistically significantly different from adult rats (Dunnett's t-test).

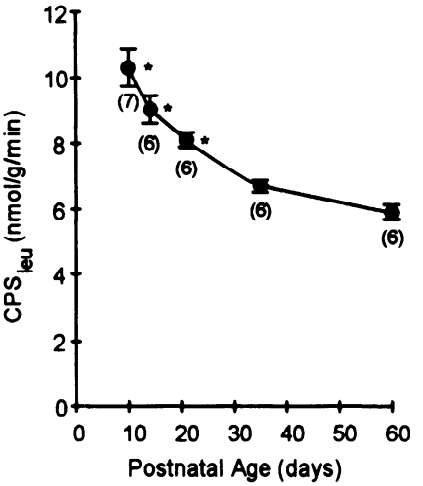

Fig. 1. Average rates of protein synthesis in brain as a whole, weighted for relative masses of its component parts at 5 stages of postnatal development. Each point represents mean ± SE determined in no. of animals indicated in parentheses. Rates of protein synthesis were calculated with age-appropriate values of ratio of the apparent steady-state leucine specific activity in precursor amino acid pool (tRNA-bound leucine) in tissue to that of arterial plasma (λ_{WB}). *$P < 0.01$, statistically significantly different from adult value (Dunnett's t-test).

In most of the structures of the auditory and visual systems examined, rates of protein synthesis were highest at 10 days and gradually decreased with age (Table 5 and Fig. 2). In both the auditory and visual cortices, rates of protein synthesis in 10-day-old rats were 77 and 87% higher than those in 60-day-old adult rats, respectively. In both cortical areas adult rates were reached by 35 days of age. In the subcortical regions of the visual system, the magnitude of the decreases in $lCPS_{leu}$ was generally less than in the auditory system. Furthermore, in subcortical regions of the auditory system, decreases in $lCPS_{leu}$ generally continued over a longer postnatal interval compared with the visual system. Changes in $lCPS_{leu}$ in the lateral geniculate nucleus were small between 10 and 21 days of age, and adult levels were reached by 35 days of age. In the superior colliculus, changes in $lCPS_{leu}$ were slight throughout the developmental time course examined. In contrast, rates of protein synthesis at 10 days of age were more than double those of adults in auditory subcortical structures (except in the inferior colliculus). Rates gradually decreased with age, and in the lateral lemniscus, superior olive, and cochlear nucleus, they continued to decrease until 60 days of age. In the inferior colliculus $lCPS_{leu}$ remained constant through 21 days of age and declined thereafter.

The pattern of changes in $lCPS_{leu}$ in most of the structures of sensorimotor systems and frontal cortex (Table 6 and Fig. 2) was similar to that in whole brain and in most of the other regions examined. In the substantia nigra, however, $lCPS_{leu}$ remained fairly constant until 21 days of age and then decreased with age. The pontine nucleus showed a different pattern in which there was a continuous rise in $lCPS_{leu}$ between 10 and 21 days, followed by subsequent reductions with age. In the

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Table 5. *Auditory and visual systems: effects of developmental age on rates of protein synthesis*

Structure	Postnatal Age, days				
	10	14	21	35	60
Auditory system					
Auditory cortex	12.6 ± 0.8*	10.3 ± 0.4*	9.2 ± 0.2*	7.4 ± 0.3	7.1 ± 0.3
Medial geniculate body	13.8 ± 0.9*	12.0 ± 0.6*	9.6 ± 0.2*	7.8 ± 0.3	6.8 ± 0.2
Inferior colliculus	10.8 ± 0.5*	10.4 ± 0.6*	10.4 ± 0.4*	8.8 ± 0.4	7.6 ± 0.3
Lateral lemniscus, dorsal	17.0 ± 0.8*	14.7 ± 0.9*	12.3 ± 0.3*	9.5 ± 0.3†	8.2 ± 0.3
Superior olivary nucleus	17.6 ± 0.9*	16.3 ± 0.5*	12.1 ± 0.4*	8.8 ± 0.4*	6.9 ± 0.3
Anterior ventral cochlear nucleus	21.7 ± 1.5*	18.4 ± 0.9*	14.1 ± 0.4*	11.4 ± 0.4†	9.7 ± 0.4
Visual system					
Visual cortex	12.5 ± 0.7*	10.5 ± 0.5*	9.1 ± 0.2*	7.5 ± 0.3	6.7 ± 0.3
Lateral geniculate nucleus	10.8 ± 0.7*	10.4 ± 0.5*	9.3 ± 0.3*	7.2 ± 0.2	6.3 ± 0.3
Superior colliculus	8.4 ± 0.5*	7.6 ± 0.4	7.9 ± 0.4	7.0 ± 0.2	6.5 ± 0.3

Values are means ± SE; rates are nmol·g^{-1}·min^{-1}. No. of animals: 7 for 10 days, and 6 for 14, 21, 35, and 60 days of age. *$P < 0.01$, statistically significantly different from 60-day-old rats (Dunnett's t-test); †$P < 0.05$, statistically significantly different from 60-day-old rats (Dunnett's t-test).

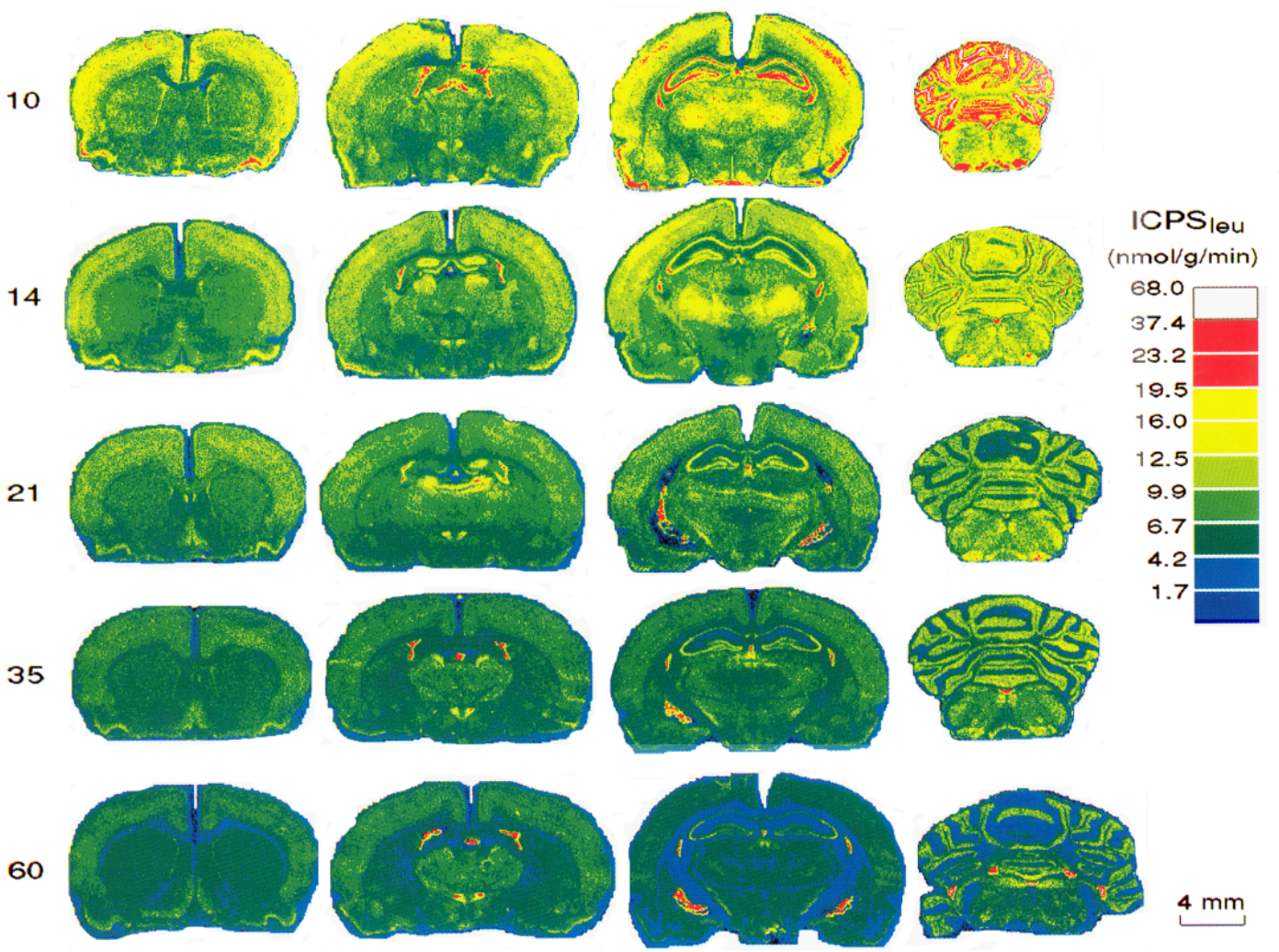

Fig. 2. Digitized autoradiograms of coronal sections color-coded for cerebral leucine incorporation into protein ($lCPS_{leu}$) at 4 different levels in brain from rats representative of 5 age groups studied. Sections *left* to *right* were taken at level of head of caudate-putamen, hypothalamus, thalamus and hippocampus, and cerebellum. Dorsal side is *top*; right side is on *right*. Color bar (*right*) provides calibration scale for range of values of leucine incorporation into protein in nmol·g^{-1}·min^{-1} for each color. Bar (*bottom right*) = 4 mm.

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Table 6. *Sensorimotor systems and frontal cortex: effects of developmental age on rates of protein synthesis*

Structure	Postnatal Age, days				
	10	14	21	35	60
n	7	6	6	6	6
Frontal cortex	11.7 ± 0.5*	10.7 ± 0.6*	9.4 ± 0.3*	7.7 ± 0.1	7.0 ± 0.4
Sensorimotor cortex	12.5 ± 0.6*	11.2 ± 0.5*	9.6 ± 0.3*	7.8 ± 0.2†	6.8 ± 0.2
Piriform cortex	19.2 ± 1.0*	14.8 ± 0.7*	12.0 ± 0.3†	10.7 ± 0.3	10.4 ± 0.4
Thalamus					
Anterior dorsal nucleus	18.0 ± 1.1*	13.6 ± 0.7†	12.0 ± 0.4†	10.7 ± 0.2	10.4 ± 0.4
Anterior ventral nucleus	12.6 ± 0.8*	11.3 ± 0.6*	10.5 ± 0.4*	8.7 ± 0.2	7.6 ± 0.4
Reticular nucleus	9.5 ± 0.5*	9.4 ± 0.3*	9.7 ± 0.4*	8.4 ± 0.2†	6.9 ± 0.2
Subthalamic nucleus	12.7 ± 0.9	11.4 ± 0.7	11.4 ± 0.5	10.4 ± 0.3	9.9 ± 0.3
Caudate-putamen	8.7 ± 0.4*	8.2 ± 0.5*	7.8 ± 0.3*	6.4 ± 0.1	5.3 ± 0.2
Globus pallidus	7.0 ± 0.4*	6.7 ± 0.5*	6.0 ± 0.2*	5.4 ± 0.1*	4.3 ± 0.2
Substantia nigra (pars compacta)	10.0 ± 0.6*	9.7 ± 0.4*	9.7 ± 0.3*	8.4 ± 0.3	7.4 ± 0.2
Substantia nigra (pars reticulata)	5.5 ± 0.3*	5.1 ± 0.3*	5.2 ± 0.2*	4.4 ± 0.2	3.6 ± 0.2
Red nucleus	14.8 ± 0.7*	14.3 ± 1.0*	13.3 ± 0.5*	10.5 ± 0.3	8.9 ± 0.3
Inferior olivary nucleus	16.3 ± 0.9*	14.9 ± 0.8*	11.9 ± 0.2*	10.1 ± 0.4†	8.6 ± 0.3
Pontine nucleus	11.2 ± 0.5	12.1 ± 0.5†	13.0 ± 0.5*	11.2 ± 0.4	10.3 ± 0.4
Medial vestibular nucleus	15.8 ± 0.9*	14.7 ± 0.9*	12.5 ± 0.5*	10.7 ± 0.4	9.7 ± 0.3
Hypoglossal nucleus	14.9 ± 0.7*	13.7 ± 0.7*	12.8 ± 0.6†	11.0 ± 0.5	10.3 ± 0.4

Values are means ± SE, determined in no. of rats, *n*; rates, nmol · g^{-1} · min^{-1}. In 21-day-old group, brain sections were inadequate at the level of inferior olivary nucleus in one rat. Measurements in inferior olivary nucleus were made in only 5 rats at 21 days of age. *$P < 0.01$, statistically significantly different from 60-day-old rats (Dunnett's *t*-test); †$P < 0.05$, statistically significantly different from 60-day-old rats (Dunnett's *t*-test).

subthalamic nucleus $lCPS_{leu}$ remained relatively constant at all ages examined.

At early postnatal periods, the rat cerebellar granule cells are generated in a unique zone, i.e., external germinal layer, and migrate through the molecular layer and Purkinje cell layer to form the internal granular layer. In the present study we found that in 10-day-old rats $lCPS_{leu}$ was very high in the external germinal layer (Figs. 2–4) (31.6 ± 2.4, means ± SE); $lCPS_{leu}$ decreased precipitously during the following 4 days, and by 21 days of age the layer can no longer be seen on the autoradiograms. Developmental changes of $lCPS_{leu}$ in the internal granule layer (Figs. 2–4) followed the typical pattern seen in other cerebral structures.

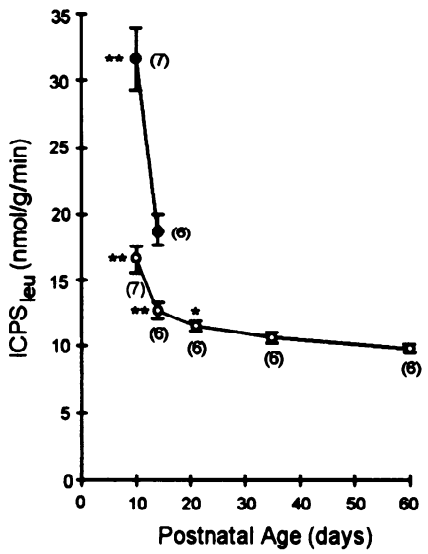

Fig. 3. Rates of protein synthesis in cerebellum at 5 stages of postnatal development. Each circle represents mean ± SE rate of protein synthesis determined in number of animals indicated in parentheses. Filled and open circles pertain to external germinal and internal granular layers, respectively. *$P < 0.05$, statistically significantly different from adult value (Dunnett's *t*-test; **$P < 0.01$, statistically significantly different from adult value (Dunnett's *t*-test).

Hypothalamic and limbic structures showed similar patterns of decreasing rates of protein synthesis during postnatal development, with several dramatic exceptions (Table 7 and Fig. 2). In the pyramidal cell layer of CA1, CA2, and CA3 and the stratum granulosum of the dentate gyrus of the hippocampus, rates of protein synthesis in 10-day-old rats were 70–100% higher than those in 60-day-old adult rats. In both the suprachiasmatic and ventromedial nuclei of the hypothalamus $lCPS_{leu}$ was high in 10-day-old rats and decreased steeply during the following 4 days; thereafter changes were small. In contrast, in the paraventricular and supraoptic nuclei of the hypothalamus rates increased during the course of development, reaching peak values in adults. In the dorsomedial hypothalamic nucleus and pineal gland $lCPS_{leu}$ showed no statistically significant change over the entire range examined.

In the three regions of white matter examined, we found that the peaks of $lCPS_{leu}$ were reached in the cerebellum and cerebrum at 14 and 21 days of age, respectively (Figs. 2 and 5).

DISCUSSION

The results of the present studies demonstrate that the course of normal postnatal development in the rat is accompanied by widespread, progressive decreases in local rates of cerebral protein synthesis. The present study is, to our knowledge, the first study of brain development in rats to apply the quantitative autoradiographic method for measurement of regional rates of cerebral protein synthesis with correction for recycling of amino acids derived from protein degradation into the

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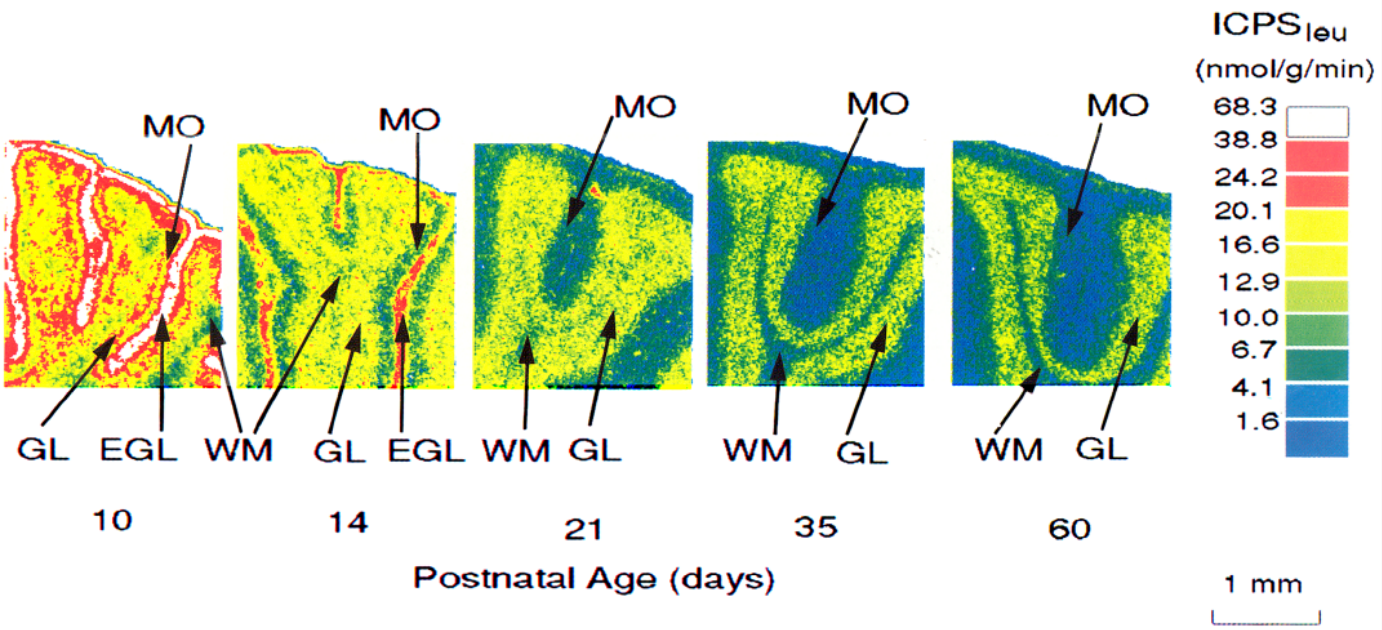

Fig. 4. Digitized autoradiograms of coronal sections at level of cerebellum color-coded for $lCPS_{leu}$ from rats representative of 5 age groups studied. Dorsal side is *top*; right side is on *right*. Color bar (*right*) provides calibration scale for range of values of leucine incorporation into protein in nmol·g^{-1}·min^{-1} for each color. Purkinje cells can be visualized in autoradiogram from 10-day-old rat as a layer of large cells with relatively high rates of protein synthesis (~20–30 nmol·g^{-1}·min^{-1}) just superficial to internal granular layer. EGL, external germinal layer; GL, internal granular layer; MO, molecular layer; WM, white matter. Bar (*bottom right*) = 1 mm.

precursor pool for protein synthesis. Without correction for recycling, rates of protein synthesis determined with a radiolabeled tracer amino acid may be underestimated. Furthermore, it cannot be assumed that the correction for recycling is constant at all developmental ages.

Rates of leucine incorporation into protein in brain were found to vary regionally in adults as well as in the developing animals. Values for $lCPS_{leu}$ in the various cerebral structures ranged from 5.5 to 31.6 nmol·g^{-1}·min^{-1} at 10 days of age and from 3.6 to 20.8 nmol·g^{-1}·min^{-1} at 60 days of age. The highest rates at 10 days of age occurred in regions in which cell proliferation continues postnatally, e.g., external germinal layer of the cerebellum and the ventral cochlear nucleus. In the adult the highest rates of protein synthesis were found in the pineal and in the peptide-producing nuclei of the hypothalamus. At both ages lowest rates of protein synthesis were found in cerebral white matter and

Table 7. *Limbic system, hypothalamus, and pineal: effects of developmental age on rates of protein synthesis*

Structure	Postnatal Age, days				
	10	14	21	35	60
n	7	6	6	6	6
Hippocampal region					
CA1-pyramidal cell layer	17.7 ± 1.0*	14.2 ± 0.6*	11.8 ± 0.2*	9.7 ± 0.3	8.6 ± 0.4
CA2-pyramidal cell layer	24.4 ± 1.4*	18.4 ± 1.0*	15.7 ± 0.4†	13.6 ± 0.3	13.3 ± 0.5
CA3-pyramidal cell layer	22.2 ± 1.3*	16.9 ± 0.9*	14.7 ± 0.3*	12.7 ± 0.2	12.1 ± 0.5
Dentate gyrus-stratum granulosum	17.3 ± 1.0*	14.2 ± 0.7*	13.2 ± 0.4†	10.6 ± 0.3	10.2 ± 0.5
Entorhinal cortex	13.4 ± 0.8*	10.7 ± 0.4*	8.7 ± 0.3*	7.2 ± 0.2	6.7 ± 0.3
Hypothalamus					
Ventromedial nucleus	11.0 ± 0.7*	8.4 ± 0.4	7.8 ± 0.3	8.0 ± 0.2	7.5 ± 0.4
Dorsomedial nucleus	7.5 ± 0.5	6.5 ± 0.3	6.6 ± 0.2	6.3 ± 0.2	6.4 ± 0.2
Paraventricular nucleus	11.6 ± 0.9*	10.8 ± 0.4*	12.5 ± 0.8†	12.9 ± 0.5	15.2 ± 0.5
Suprachiasmatic nucleus	10.6 ± 0.6*	8.0 ± 0.6	7.5 ± 0.4	7.3 ± 0.1	6.8 ± 0.3
Supraoptic nucleus	15.9 ± 1.0	14.1 ± 0.9*	15.0 ± 0.6†	16.8 ± 0.4	18.3 ± 0.7
Anterior cingulate cortex	12.0 ± 0.6*	10.6 ± 0.5*	9.6 ± 0.2*	8.0 ± 0.1	7.2 ± 0.2
Nucleus accumbens	8.7 ± 0.3*	7.8 ± 0.4*	7.4 ± 0.3*	6.5 ± 0.2	5.5 ± 0.2
Basolateral amygdala	12.3 ± 0.7*	10.2 ± 0.4*	9.2 ± 0.3*	8.1 ± 0.2	7.1 ± 0.3
Pineal	21.5 ± 1.3	17.3 ± 0.9	17.2 ± 0.6	18.9 ± 0.8	20.8 ± 0.9

Values are means ± SE determined in no. of rats, *n*; rates, nmol·g^{-1}·min^{-1}. Brain sections were inadequate at level of suprachiasmatic nucleus in 1 rat at 10, 14, and 21 days of age and in 2 rats at 35 days of age. Measurements in suprachiasmatic nucleus were made in 6 rats at 10 days of age, 5 rats at 14 and 21 days of age, and 4 rats at 35 days of age. Brain sections were inadequate at level of pineal in 1 rat at 14 and 60 days of age. Measurements in pineal were made in 5 rats at 14 and 60 days of age. *$P < 0.01$, statistically significantly different from 60-day-old rats (Dunnett's *t*-test); †$P < 0.05$, statistically significantly different from 60-day-old rats (Dunnett's *t*-test).

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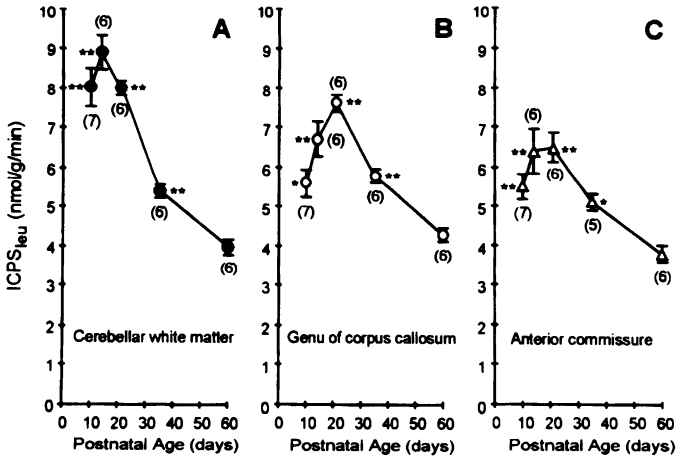

Fig. 5. Rates of protein synthesis in cerebellar white matter (*A*), genu of corpus callosum (*B*), and anterior commissure (*C*) during postnatal development of rat. Each point represents mean ± SE of rate of protein synthesis determined in number of animals indicated in parentheses. *$P < 0.05$, statistically significantly different from adult value (Dunnett's *t*-test); **$P < 0.01$, statistically significantly different from adult value (Dunnett's *t*-test).

substantia nigra pars reticulata. Moreover, in most regions these rates changed with developmental age. In brain as a whole and in most brain regions, values for lCPS$_{leu}$ were highest at 10 days of age and gradually decreased with age. The higher overall rates of cerebral protein synthesis in immature brains found in this study are consistent with those reported by others (12). Although all published results agree qualitatively, there are differences in the magnitude of changes observed. The only studies in which the possible confounding effects of recycling were considered are those which used the [1-^{14}C]valine flooding technique (11), which helps but does not completely correct for recycling (27).

Use of the L-[1-^{14}C]leucine method to study development of specific brain regions. The autoradiographic L-[1-^{14}C]leucine method allowed us to examine the developmental time course of changes in protein synthesis in specific brain regions. Age-specific values of λ_{WB} were used in *Eq. 4* for the rates of protein synthesis in brain as a whole and in all of the local brain regions examined. The λ is needed to derive the integrated specific activity of leucine in the precursor pool in the tissue from the integrated specific activity measured in arterial plasma (*Eq. 1*). The λ corrects for the contribution of unlabeled leucine derived from protein degradation in the tissue. It was assumed that the correction for recycling of leucine is constant from region to region at all ages examined. This is a reasonable assumption inasmuch as previous studies in normal, conscious adult rats have shown that the value of λ_i in most gray matter regions falls within ±5% of the value of 0.58, the average measured for the brain as a whole (28), and remains so even in conditions in which lCPS$_{leu}$ has been shown to be changed, including the regenerating hypoglossal nucleus of the adult rat, in which lCPS$_{leu}$ is increased by 20–30% (29).

lCPS$_{leu}$ in developing cerebellum. In the present study the temporal pattern of lCPS$_{leu}$ in the cerebellar cortex is of particular interest because most of the nerve cells in the cerebellum are generated postnatally (3). The external germinal layer increases in thickness from a layer 4–5 cells deep at birth to a layer ~10 cells deep at 9–10 days after birth as a result of cell proliferation. From 11 days after birth, there is a sharp decline in the thickness of the layer, and eventually, by 21 days of age, it is no longer discernible. During the second and third week of life, granule cells from the external germinal layer migrate through the molecular and Purkinje cell layers and settle in the internal granular layer. At birth, Purkinje cells, which are generated prenatally, form a multicellular layer that disperses into a monolayer beginning on postnatal *day 4* (3). Concomitantly, Purkinje cell bodies increase in size, and during the second and third weeks of life the dendritic arbors are formed.

The results of our analysis of the cerebellar layers correlate well with the histological history. The lCPS$_{leu}$ of 31.6 nmol·g^{-1}·min^{-1} in the external germinal layer of 10-day-old rats was the highest value measured in this study; lCPS$_{leu}$ decreased dramatically (by 40%) during the following 4 days and was not detectable by 21 days of age (Figs. 3 and 4). The very high rate of protein synthesis at 10 days of age occurred during the time of cell proliferation in this layer, and the decreases in lCPS$_{leu}$ during the ensuing 11 days correspond to the time that these cells migrate out of the external germinal layer to form the molecular layer. Compared with the results observed in the external germinal layer, the changes in lCPS$_{leu}$ in the internal granular layer of the cerebellum were relatively small. In 14-day-old rats lCPS$_{leu}$ was 23% lower compared with that of 10-day-old rats; this change was followed by a slower decline in lCPS$_{leu}$ with age (Figs. 3 and 4). It might have been expected that lCPS$_{leu}$ in the internal granular layer would increase between 14 and 21 days of age because during this time the number of neurons in this layer increases as a result of cell migration from the external germinal layer. The gradual decrease of the lCPS$_{leu}$ may be due to the fact that the internal granular layer increases in volume but not in cell density (15). The arrival of mossy fibers from the pontine nuclei at this time (3) is at least in part responsible for the increase in volume. The autoradiograms of cerebellar cortex show both the increase in thickness of the internal granular layer between 10 and 14 days of age and, at least at 14 days of age, a gradient of high to lower lCPS$_{leu}$ in the internal granular layer from the superficial position to the junction with white matter (Fig. 4) which may correspond to the "inside-out" formation of the layer, i.e., the deeper granule cells arrived first, whereas the more superficial cells close to the Purkinje cells are the younger cells. Limitations of spatial resolution of the autoradiographic technique prevented us from making actual measurements of protein synthesis rates in Purkinje cells. However, in [^{14}C]leucine autoradiograms from 10-day-old rats (Fig. 4), a layer of large cells with relatively higher rates of protein synthesis (~20–30 nmol·g^{-1}·min^{-1}) can be delineated superficial to the internal granule cell layer. At the other ages it was impossible to distinguish this layer of cells from the internal granule cell layer on the autoradiograms.

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$lCPS_{leu}$ in developing auditory system. The first cochlear potentials can be observed by 8–9 days of age (32). Opening of the external auditory meatus occurs 12–13 days after birth, at which time there is an increased sensitivity to sound (8). Responses to sound are considered to be mature by 16–20 days of age. In the ventral cochlear nucleus, the first relay station of the auditory system, neurons continue to be generated postnatally (4). Neuron cell counts in the ventral cochlear nucleus, which have only been carried out in mouse (21), peak at 9 days of age and decline by 20–25% to adult levels by postnatal *day 12*. Rates of protein synthesis in the ventral cochlear nucleus at both 10 and 14 days of age were, as in the external germinal layer of the cerebellum, also undergoing cell proliferation, some of the highest rates measured at these ages. It is of interest that in 10-day-old rats subcortical regions of the auditory system have some of the highest rates of protein synthesis relative to adults, i.e., greater than twofold higher (Table 5).

$lCPS_{leu}$ in developing visual system. The eyelids open at ~15 days of age following the development of the electroretinogram (33). In the superficial layer of the superior colliculus, the major target of retinal ganglion cells, rates of protein synthesis changed very little over the developmental time course examined (Table 5). Postnatal development in this region is characterized by cell death during the first postnatal week (14) followed by synaptogenesis, which proceeds in stages until 30–40 days of age (20). In the lateral geniculate nucleus the number of vesicle-filled processes per unit area increases dramatically during the first 2 wk of life, before the eyelids open (18). In both of these regions myelination of fibers begins at about the time of eyelid opening. The subcortical regions of the visual system appear to be more mature at birth than those of the auditory system, where neurogenesis continues postnatally.

$lCPS_{leu}$ in peptide-producing hypothalamic nuclei. The primary function of the neurons in the supraoptic and paraventricular nuclei of the hypothalamus is to synthesize peptides from which two hormones, vasopressin and oxytocin, are derived. Studies of the ontogeny of the hypothalamoneurohypophysial system have shown that vasopressin and the neurons that synthesize this peptide appear around embryonic *day 16–17*, whereas oxytocin peptide is not present until about the time of birth (6, 19). Results of immunohistochemical studies (6), Northern blot analyses (30), and radioimmunoassay (2) have shown that levels of both neurohormones and vasopressin mRNA in both nuclei and in the posterior pituitary increase markedly after birth and peak in the adult. The developmental pattern of change in $lCPS_{leu}$ in these hypothalamic nuclei was unique in that rates increased over the entire time course to peak values in the adult (Table 7). It is in these nuclei that the highest rates of protein synthesis in the brain are found in the adult rat.

$lCPS_{leu}$ and time course of myelination. In the white matter regions examined in this study $lCPS_{leu}$ increased during the first 2–3 wk of life and then steadily decreased to adult values (Fig. 5). Peaks of $lCPS_{leu}$ were reached in cerebellar and cerebral white matter at 14 and 21 days, respectively. These peaks coincide with the rapid phase of myelination in the rat brain (9) which in general follows a caudal to rostral time course (16). The deposition of myelin requires extensive synthesis of lipids and proteins by the oligodendroglia, which undergo morphological transformations during the period of myelination into an "active" state, in which cells become hypertrophic, organelles such as the rough endoplasmic reticulum and Golgi apparatus increase markedly, and chromatin becomes evenly dispersed in the nucleus (31). After the period of myelination, oligodendroglia change to the mature form, in which processes are thinner and the nucleus assumes an eccentric position and is characterized by aggregated chromatin.

Protein degradation during development. In the operational equation (*Eq. 4*) of the autoradiographic L-[1-^{14}C]-leucine method, the factor λ corrects for the effects of recycling of unlabeled leucine derived from protein degradation into the precursor pool for protein syntheis. The factor λ is equal to the fraction of leucine in the precursor pool derived from the arterial plasma. Because leucine cannot be synthesized by the brain, $(1 - \lambda)$ is equal to the fraction of leucine in the precursor pool derived from protein breakdown. In general, $(1 - \lambda)$ can be thought of as an indicator of the rate of protein degradation. If the value of λ decreases under certain conditions, it means that the effect of recycling has increased, which may indicate that the rate of protein degradation has increased. We previously reported that, in the brain as a whole, the value of λ for leucine is 0.58 in normal, conscious adult rats (26), indicating that 42% of leucine entering the precursor pool is derived from protein degradation; the remainder comes from the plasma.

Values of λ_{WB}, measured at 7, 10, 14, 21, and 35 days of age (Table 4), show that early in development a greater fraction of the precursor leucine pool is derived from protein degradation. In 7- and 10-day-old rats, the effects of recycling are as high as 57 and 55%, respectively. In 35-day-old rats the contribution from recycled leucine is 42%, the same as that of adult rats. From the measured rates of cerebral protein synthesis (Fig. 1) and the measured values of λ_{WB} (Table 4), we can calculate that the contribution to the precursor pool for protein synthesis from protein degradation is 2.4 nmol leucine$\cdot g^{-1}\cdot min^{-1}$ in adult rats, whereas, in 10-, 14-, 21-, and 35-day-old rats, it is 5.7, 4.3, 3.8, and 2.7 nmol leucine$\cdot g^{-1}\cdot min^{-1}$, respectively. These data indicate that the rate of protein degradation may be considerably higher in 10-day-old rat pups compared with adult rats. By 35 days of age values have attained adult levels. Results of studies in which rates of protein degradation were estimated by subtracting the rate of accumulation of protein from the rate of protein synthesis determined with the flooding technique (13) showed the same trend, i.e., higher rates of protein degradation in neonatal rats compared with adults.

L-Leucine pools in brain during development. Our measurements of brain acid-soluble leucine concentrations indicate that leucine levels in young rats are

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significantly higher than in adults (Table 3). Similar developmental patterns have been reported previously (1, 5), and it was suggested that higher levels in neonates might merely reflect elevated plasma leucine levels that were not measured. Plasma leucine concentrations were measured in our experiments, and they were, in fact, lower in 7-day-old rats than in adults. Leucine steady-state distribution ratios between brain and plasma were statistically significantly higher in the 7-, 10-, and 14-day-old rats compared with adults by 48, 30, and 28%, respectively (Table 3). These differences cannot be explained solely on the basis of changes in brain water content, which decreases from 88 to 79% of total brain weight between postnatal *day 7* to adulthood (10). The distribution ratio, the ratio of the sum of the rate constants for influx into the pool to the sum of the rate constants for efflux out of the pool, could be higher in the young rats due to either decreased efflux or increased influx. It is unlikely that efflux from the free amino acid pool is lower in the young rats since it has been shown that, in the neonate compared with the adult, rates of leucine decarboxylation measured in vitro are higher (24) and rates of protein synthesis measured in vivo are higher (Fig. 1). Influx to the pool, however, is probably higher in the young rats because it has been shown that the maximal velocity (V_{max}) for blood-brain barrier L-leucine transport in 7-day-old rats is 80% higher than that found in adults (22). Furthermore, protein degradation is higher in the younger animals [i.e., values of $(1 - \lambda)$ are higher in the young rats; Table 4].

Concentrations of leucine in the tRNA-bound pool in brain were higher in the younger rats compared with adults (Table 3), as were the rates of protein synthesis (Fig. 1). From the determinations of rates of leucine incorporation into protein in the brain as a whole and the measured concentrations of leucine in the tRNA-bound pool, we can estimate the rate constant for the turnover of the leucyl-tRNA pool at the different ages studied. From 14 to 60 days of age the rate constant for the turnover of this pool appears to be constant at 1.0/s; at 10 days of age, the calculated value is 1.2/s, indicating that from 14 days of age on the tRNA-bound leucine pool in brain turns over 1/s and at younger ages it may be even faster, e.g., 1/0.8 s.

Perspectives

The results of these investigations show that normal development in the rat is accompanied by widespread decreases in rates of cerebral protein synthesis. The developmental decrease in rates of protein synthesis probably reflects the generalized growth of neuropil and myelin, which decreases the density of cells in which protein synthesis is taking place. The pattern and time course of changes in rates of protein synthesis vary in individual brain regions as do the time courses of cell proliferation, cell death, dendritic arborization, synaptogenesis, and myelination. The regions investigated in these studies are on the macro scale and, therefore, encompass volumes of neural tissue that would include different cell types and cell processes. It would be of interest to expand this autoradiographic method to quantify rates of protein synthesis in individual neurons and to follow the course of cellular protein synthesis, beginning with neurogenesis, through cell migration, maturation, and, in some cases, cell death.

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