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EFFECTS OF AGEING ON LOCAL RATES OF CEREBRAL PROTEIN SYNTHESIS IN SPRAGUE-DAWLEY RATS

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SUMMARY

The effects of ageing on local rates of protein synthesis in 39 brain structures in resting conscious rats have been examined. Young adult rats (aged 6 months) have been compared with a group of middle-aged/aged rats (aged 15–23 months). The results show that ageing is associated with significant decreases in rates of protein synthesis in the brain as a whole as well as in several specific brain regions. Brain regions involved in visual and auditory function were selectively affected, perhaps due to a chronic lack of sensory input. Several regions involved in motor function and two areas in the limbic system had significantly decreased rates of protein synthesis in the old rats. Notably, there was a significant age-related decrease in protein synthesis in the locus coeruleus which contains the cell bodies of origin of the major ascending noradrenergic innervation of the cortex.

INTRODUCTION

Senescent changes in brain structure and function are well known, but the underlying causes of these changes are poorly understood. In man, psychological testing has shown impairments in cognitive functions (Birren *et al.*, 1963; Granick and Patterson, 1971). These changes are accompanied by a reduction in average glucose consumption of the brain as a whole even while cerebral blood flow and oxygen consumption may remain within the normal range (Dastur *et al.*, 1963). In addition, degenerative changes such as neuronal loss and dendritic regression can be found postmortem in some brain regions in normal aged subjects (Brody, 1955; Scheibel *et al.*, 1975). In rats, ageing is also accompanied by behavioural as well as histopathological changes in the brain (Feldman, 1976; Vaughan, 1977; Ordy *et al.*, 1978). Studies with the deoxyglucose method have demonstrated that age-related, region-selective reductions in glucose utilization occur in both Sprague-Dawley (Smith *et al.*, 1980*b*) and Fisher-344 rats (London *et al.*, 1981). Most brain regions

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that are associated with visual and auditory functions show significant reductions in glucose utilization that may be secondary to degenerative changes in the primary sense organs. With only a few exceptions the rates of glucose utilization in the structures of the limbic and motor systems of the rat remain unchanged with age.

Measurements of energy metabolism do not differentiate between the immediate functional demands of cerebral structures and the longer term maintenance processes within the nervous system. Long-term effects that are related to changes in morphology, structural maintenance, and remodelling in the nervous system are more likely to be reflected in biosynthetic biochemical processes, such as protein synthesis. In this study the effects of ageing on local rates of protein synthesis in brain have been examined by means of a quantitative autoradiographic method (Smith *et al.*, 1980*a*). The results of these studies show that in middle-aged and aged rats, as compared with young adult animals, the overall rate of cerebral protein synthesis is decreased. On a local level some specific structures are affected, notably the locus coeruleus, substantia nigra, and structures associated with visual and auditory functions.

MATERIALS AND METHODS

Animals

Normal male Sprague-Dawley albino rats between the ages of 6 and 23 months were obtained from Zivic Miller Laboratories, PA. Studies were completed in 6 young adult rats (6 months of age), 8 middle-aged rats (15 months of age), and 4 aged rats (22–23 months of age). The animals were group-housed (2–3 per cage) under a laminar flow hood designed to reduce the exposure to infectious agents. The animals were maintained on Purina Laboratory Chow and water *ad libitum* until 14 h prior to the measurement of protein synthesis at which time they were deprived of food. The physiological state of the animals at the time of the measurements of protein synthesis was assessed by monitoring rectal temperature, mean arterial blood pressure, haematocrit, and arterial blood pH, pCO_2 and pO_2. Only animals with values for these physiological variables within the normal range (Sokoloff *et al.*, 1977) were included in these studies.

Determination of Local Rates of Protein Synthesis

Local rates of protein synthesis were determined by means of a recently developed autoradiographic method (Smith *et al.*, 1980*a*). A more detailed description of this method has been published elsewhere (Smith *et al.*, 1984). The method is based on the use of L-[1-^{14}C]leucine as a tracer to measure leucine incorporation into protein. Carboxyl-labelled leucine has been chosen as the tracer because the only pathway for its metabolic degradation entails a transamination followed by decarboxylation. Therefore, in the metabolism of L-[1-^{14}C]leucine the label is transiently transferred to α-ketoisocaproic acid and ultimately to $^{14}CO_2$ which is negligibly reincorporated because of dilution by the large amount of unlabelled CO_2 produced by cerebral carbohydrate metabolism (Banker and Cotman, 1971). There are, therefore, no residual radioactive products of [^{14}C]carboxyl-labelled leucine other than the labelled protein. By mathematical analysis of the kinetics of exchange of leucine between plasma and tissue, its metabolic degradation and its incorporation into protein, an equation (fig. 1) has been derived that defines the rate of L-leucine incorporation into protein in terms of the time course of the plasma L-leucine specific activity, the final tissue concentration of ^{14}C, and experimentally determined rate constants.

$$v_i = \frac{C_i^*(T) - C_e^*(T)}{\int_0^T \left(\frac{C_p^*}{C_p}\right) dt - e^{-(k_2+k_3+k_4)T} \int_0^T \left(\frac{C_p^*}{C_p}\right) e^{(k_2+k_3+k_4)t} dt}$$

FIG. 1. Operational equation for calculation of local rates of protein synthesis (v_i). C_p and C_p^* represent the concentrations of free leucine and [1-^{14}C]leucine, respectively, in the arterial plasma. $C_i^*(T)$ represents the total tissue concentration of ^{14}C determined by the quantitative autoradiographic technique. The constants k_1, k_2, k_3 and k_4, represent the rate constants for carrier-mediated transport of leucine from plasma to tissue, for carrier-mediated transport back from tissue to plasma, for metabolic degradation of leucine, and for incorporation of leucine into protein, respectively. The numerator of the equation is the total amount of label incorporated into protein in the tissue at the end of the experiment. It is calculated from the total amount of label in the tissue, $C_i^*(T)$, minus the free [^{14}C]leucine in the tissue, $C_e^*(T)$. $C_e^*(T)$ is estimated as follows:

$$k_1 e^{-(k_2+k_3+k_4)T} \int_0^T C_p^* e^{(k_2+k_3+k_4)t}\, dt.$$

In a separate series of animals, $C_e^*(T)$ has been measured and determined to constitute less than 5 per cent of $C_i^*(T)$ 60 min after an i.v. pulse injection of [1-^{14}C]leucine. $C_e^*(T)$ can be reduced to zero by washing tissue sections repeatedly in 10 per cent phosphate buffered (pH 7) formalin. At the time of these ageing experiments, fixation in formalin vapour followed by washing in tap water was carried out rather than the immersion fixation and washing procedure. It was later found that the vapour fixation and washing procedure used in these experiments washed out only half of the unincorporated label. $C_e^*(T)$ in this study was therefore divided by 2. This was considered reasonable in view of the very small effect the second term in the numerator has on the final result and the fact that the study had been carried out on valuable, aged animals. In subsequent studies the procedure of immersion fixation followed by washing will be used routinely.

The animals were prepared for the determination of local rates of protein synthesis by the insertion under light halothane anaesthesia of polyethylene catheters into one femoral vein and artery. The rat's hindquarters were then restrained by the application of a loose-fitting plaster cast which was taped to a lead brick. After a period of 4 h for recovery from the effects of surgery and anaesthesia, the procedure was initiated by the administration of an intravenous pulse of L-[1-^{14}C]leucine (specific activity 59 mCi/mmol, Amersham Corporation, Arlington Heights, IL); the dose was 100 μCi/kg body weight contained in 0.1 to 0.4 ml of physiological saline. Timed arterial blood samples were then collected during the following 60 min for the determination of plasma concentrations of leucine and [^{14}C]leucine. The blood was immediately centrifuged to remove the red cells, and the plasma was stored on ice and assayed later. At the end of the 60 min experimental period the rats were killed by an intravenous injection of sodium pentobarbital, and the brains were quickly removed and frozen in isopentane cooled to $-40°$C with dry ice.

The brains were cut into sections 20 μm in thickness in a cryostat maintained at $-18°$C, and the sections were mounted on gelatin-coated glass slides and air dried. They were then fixed in 37 per cent formalin vapour overnight, and washed in running water for 1 h. This fixation and washing procedure was designed to remove unincorporated [^{14}C]leucine and any ^{14}C-labelled α-ketoisocaproic acid from the tissue sections without loss of labelled protein. The sections were then dried and autoradiographed along with calibrated [^{14}C]methylmethacrylate standards as previously described (Sokoloff *et al.*, 1977). Integrated optical densities of regions of the autoradiographs corresponding to selected brain structures were measured with either a manual densitometer (Photovolt Model 520-A densitometer, Photovolt Corporation, NY) equipped with a 0.2 mm aperture or with a Photoscan System P-1000 densitometer (Optronics International, Chelmsford, MA) as previously described (Goochee *et al.*, 1980). The structures on the autoradiographs were localized and identified by comparison with the

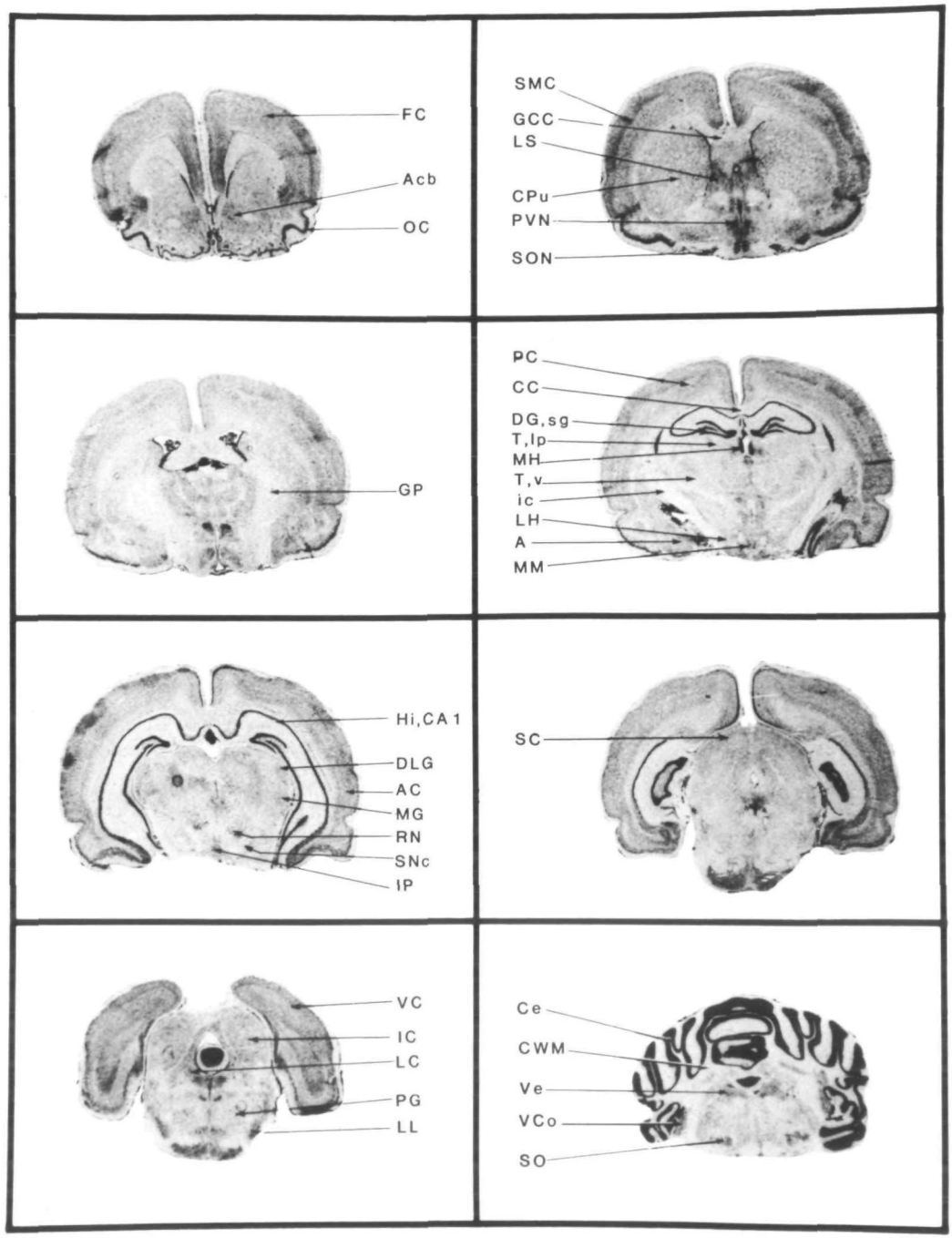

FIG. 2. Cresyl violet-stained sections of rat brain illustrating the location of the structures (Tables 3–5) in which rates of protein synthesis were determined. FC = frontal cortex; Acb = nucleus accumbens; OC = olfactory cortex; SMC = sensorimotor cortex; GCC = genu of the corpus callosum; LS = lateral septal nucleus; CPu = caudate putamen; PVN = paraventricular nucleus; SON = supraoptic nucleus; GP = globus pallidus; PC = parietal cortex; CC = corpus callosum; DG, sg = dentate gyrus, supragranular zone; T,lp = thalamus, lateral posterior; MH = medial habenula; T,v = ventral thalamus; ic = internal capsule; LH = lateral hypothalamus; A = amygdala; MM = mamillary body; Hi, CA1 = CA1 of hippocampus; DLG = dorsal lateral geniculate; AC = auditory cortex; MG = medial geniculate; RN = red nucleus; SNc = substantia nigra compacta; IP = interpenduncular nucleus; SC = superior colliculus; VC = visual cortex; IC = inferior colliculus; LC = locus coeruleus; PG = pontine grey; LL = lateral lemniscus; Ce = cerebellar cortex; CWM = cerebellar white matter; Ve = vestibular nucleus; VCo = ventral cochlear nucleus; SO = superior olivary nucleus.

atlas of König and Klippel (1963) and the cortical maps of Krieg (1946) (fig. 2). These optical densities were used to determine the local tissue concentrations of ^{14}C by comparison with the optical densities produced by the calibrated standards.

The plasma samples were deproteinized with 4 per cent sulphosalicylic acid, and [^{14}C]leucine and leucine concentrations were assayed by liquid scintillation counting and by amino acid analysis (Beckman Amino Acid Analyzer, Model 121 MB, Beckman Instruments, Fullerton, CA), respectively. It is necessary to deproteinize the plasma samples because a significant amount of [^{14}C]leucine is incorporated into plasma protein. From the time courses of the concentrations of [^{14}C]leucine and leucine in the deproteinized plasma and the local tissue concentration of ^{14}C, local rates of protein synthesis were calculated by means of the operational equation (fig. 1).

All calculations were carried out with a Hewlett-Packard System 9845B computer (Hewlett Packard, Loveland, CO).

RESULTS

Survival

The middle-aged and aged groups of rats used in the studies were derived from a population of retired breeders segregated specifically for ageing research at the age of 10 months. At the ages of 6, 15, and 22 to 23 months the survival rates of the populations were about 100, 91, and 26 per cent, respectively (Bruce Rose, Manager, Aged Rat Colony, Zivic Miller Laboratories, personal communication). Of the 31 rats obtained from Zivic Miller, 8 were young adults, 9 were middle-aged, and 14 were aged. None of the rats had any evidence of cataracts. One of the young animals was hypothermic, and one of the aged animals was hypotensive during the experiment. One each of the young adult and the middle-aged groups had brain tumours. In the aged group there was one case of each of the following: bowel tumour, lung tumour with metastases, lung tumour without metastases, pituitary tumour, fibroma of the abdominal wall, and an invasive tumour of the right hip. In addition, 3 of the aged rats died from bronchopneumonia, and 1 of these 3 animals had a pituitary tumour. All these animals were excluded from the study. This reduced the aged group to 4 and the percentage of 'survivors' in this group to 7 per cent. In the middle-aged group the number was reduced to 8 which represented 81 per cent of the original population. Because the aged group was too small for statistical evaluation, the aged and middle-aged animals were combined to form a group of old rats with an age span of 15 to 23 months. These survivors represent about 60 per cent of the original population. There were no statistically significant differences in rates of protein synthesis in any brain structures between the 4 aged and 8 middle-aged animals, and the values found in the two groups overlapped completely.

Physiological Status

There were no significant differences in the physiological status of the 2 groups of animals (Table 1). Only body weight exhibited an age-related change; body weight was higher in the older group of animals as compared with the young adults.

TABLE 1. PHYSIOLOGICAL VARIABLES IN YOUNG AND OLD RATS DURING MEASUREMENT OF LOCAL RATES OF CEREBRAL PROTEIN SYNTHESIS†

Parameter	*Young adult* (*6*)	*Middle-aged/aged* (*12*)
Body weight (g)	771 ± 30	879 ± 21*
Haematocrit (%)	50 ± 3	46 ± 1
Mean arterial blood pressure (mmHg)	117 ± 3	118 ± 2
Arterial blood pH	7.46 ± 0.01	7.44 ± 0.01
Arterial blood pO_2 (mmHg)	82.4 ± 2.1	81.6 ± 1.1
Arterial blood pCO_2 (mmHg)	35.3 ± 1.0	34.6 ± 0.4
Arterial plasma leucine concentration (nmol/ml)	193 ± 14	188 ± 7

† The values are the means ± standard errors obtained in the number of animals indicated in parentheses. * Significantly different from young adult rats, as determined by Student's t test for group comparisons ($P < 0.01$).

Average Rates of Protein Synthesis in the Brain as a Whole

The mean rates of protein synthesis in the brain as a whole were calculated from the local values of all of the brain structures weighted for their volumes by the image-processing technique of Goochee *et al.* (1980). In comparison with the young adult rats, the weighted average rate of protein synthesis in the older animals was significantly reduced by 17 per cent below that of the young animals (Table 2).

Local Rates of Protein Synthesis

Local rates of protein synthesis were determined in 39 brain structures. For the purpose of presentation these structures have been grouped according to the functional systems with which they are presumed to be associated.

Rates of protein synthesis were determined in the brain structures of three sensory systems. In the auditory system (Table 3) 6 structures were examined. Of these structures rates of protein synthesis were significantly reduced in the older animals

TABLE 2. EFFECTS OF AGEING ON AVERAGE RATE OF PROTEIN SYNTHESIS IN BRAIN AS A WHOLE†

Age group	*Age* (*months*)	*Cerebral protein synthesis* (*nmol leucine incorporated/g tissue/min*)
Young adult (6)	6	4.2 ± 0.1
Middle-aged/aged (12)	15–23	3.5 ± 0.2**

† Values are the means ± standard errors obtained in the number of animals given in parentheses. ** Significantly different from the young adult rats, as determined by Student's t test for group comparisons ($P < 0.005$).

TABLE 3. AUDITORY, VISUAL, AND EXTRAPYRAMIDAL MOTOR SYSTEMS: EFFECTS OF AGEING ON RATES OF PROTEIN SYNTHESIS

	Local cerebral protein synthesis† *(nmol leucine incorporated/g tissue/min)*	
Structure	*Young adult (6 months)*	*Middle-aged/aged (15–23 months)*
Auditory system		
Auditory cortex (cortical area 41)	5.2 ± 0.2 (6)	4.7 ± 0.2 (12)
Medial geniculate body	5.4 ± 0.2 (6)	4.7 ± 0.3 (12)
Inferior colliculus	6.2 ± 0.3 (6)	4.9 ± 0.2*** (12)
Lateral lemniscus, ventral	5.8 ± 0.3 (6)	4.6 ± 0.2** (11)
Superior olivary nucleus	5.2 ± 0.6 (6)	4.4 ± 0.3 (11)
Cochlear nucleus, ventral	6.4 ± 0.4 (6)	5.3 ± 0.3* (10)
Visual system		
Visual cortex (cortical area 17)	5.7 ± 0.2 (6)	4.8 ± 0.2* (12)
Lateral geniculate body	4.8 ± 0.2 (6)	4.1 ± 0.2* (12)
Superior colliculus	4.9 ± 0.2 (6)	4.1 ± 0.2* (12)
Thalamus: posterior lateral nucleus	3.9 ± 0.2 (5)	3.6 ± 0.2 (8)
Extrapyramidal motor system		
Caudate-putamen	3.7 ± 0.2 (6)	3.2 ± 0.2 (12)
Globus pallidus	2.4 ± 0.1 (6)	2.2 ± 0.1 (12)
Substantia nigra (zona compacta)	2.0 ± 0.1 (6)	1.7 ± 0.1* (12)
Inferior olivary nucleus	5.9 ± 0.3 (6)	4.4 ± 0.2*** (10)
Pontine grey matter	3.9 ± 0.2 (6)	3.1 ± 0.2** (12)
Thalamus: ventral nucleus	5.3 ± 0.2 (6)	4.9 ± 0.2 (12)
Vestibular nucleus, superior	7.4 ± 0.3 (6)	6.2 ± 0.3* (12)
Red nucleus	5.2 ± 0.2 (6)	3.9 ± 0.2*** (11)

† Values are the means ± standard errors obtained in the number of animals given in parentheses. Asterisks indicate difference from the young adult rats, as determined by Student's t test for group comparisons: * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$.

by between 17 and 21 per cent in the inferior colliculus, lateral lemniscus, and cochlear nucleus. In the visual system (Table 3), rates of protein synthesis in visual cortex (area 17), lateral geniculate nucleus, and superior colliculus were all significantly decreased in the older animals by about 15 per cent. The rate of protein synthesis in the olfactory cortex (cortical area 51) was significantly lower in the older animals by 20 per cent as compared with the young controls (Table 4). In 4 other cortical areas (Table 4), frontal (area 10) and parietal (area 7), sensorimotor (area 2), and cerebellar cortex, rates of protein synthesis were unaltered in the older animals.

Of the 8 structures examined in the extrapyramidal motor system (Table 3), 5 had reduced rates of protein synthesis in the older animals. Some of the largest changes were found in the inferior olivary nucleus and the red nucleus, both of which were decreased by 25 per cent. The rates of protein synthesis in the substantia nigra,

TABLE 4 CORTICAL STRUCTURES AND REGIONS OF WHITE MATTER: EFFECTS OF AGEING ON RATES OF PROTEIN SYNTHESIS

	Local cerebral protein synthesis† *(nmol leucine incorporated/g tissue/min)*	
Structure	*Young adult (6 months)*	*Middle-aged/aged (15–23 months)*
Cortical structures		
Parietal cortex (cortical area 7)	5.2 ± 0.2 (6)	4.6 ± 0.2 (12)
Olfactory cortex (cortical area 51)	7.7 ± 0.3 (6)	6.2 ± 0.4* (10)
Frontal cortex (cortical area 10)	5.1 ± 0.2 (6)	4.9 ± 0.2 (12)
Sensorimotor cortex (cortical area 2)	5.2 ± 0.2 (6)	4.7 ± 0.2 (12)
Cerebellar cortex	5.9 ± 0.2 (6)	5.3 ± 0.3 (12)
White matter regions		
Corpus callosum, splenium	2.0 ± 0.1 (6)	1.7 ± 0.1 (12)
Genu of corpus callosum	2.2 ± 0.1 (6)	2.0 ± 0.1 (12)
Internal capsule	2.1 ± 0.1 (6)	1.7 ± 0.1** (12)
Cerebellar white matter	1.7 ± 0.1 (6)	1.4 ± 0.1* (12)

† Values are the means ± standard errors obtained in the number of animals given in parentheses. Asterisks indicate significant difference from the young adult rats, as determined by Student's t test for group comparisons: * $P < 0.05$; ** $P < 0.01$.

TABLE 5. LIMBIC SYSTEM AND OTHER AREAS: EFFECTS OF AGEING ON RATES OF PROTEIN SYNTHESIS

	Local cerebral protein synthesis† *(nmol leucine incorporated/g tissue/min)*	
Structure	*Young adult (6 months)*	*Middle-aged/aged (15–23 months)*
Hippocampus; CA1	6.5 ± 0.4 (6)	5.5 ± 0.4 (12)
Dentate gyrus, supragranular zone	8.4 ± 0.6 (6)	7.0 ± 0.3* (12)
Amygdala, lateral	5.9 ± 0.3 (6)	5.0 ± 0.3 (12)
Septal nucleus, lateral	3.9 ± 0.3 (6)	3.6 ± 0.2 (12)
Nucleus accumbens, medial	3.9 ± 0.3 (6)	3.1 ± 0.2* (9)
Habenula	11.0 ± 0.8 (4)	9.2 ± 0.9 (9)
Interpeduncular nucleus	5.5 ± 0.3 (6)	5.0 ± 0.2 (12)
Locus coeruleus	7.6 ± 0.4 (6)	5.9 ± 0.4** (10)
Hypothalamus:		
Ventral medial	5.4 ± 0.4 (6)	4.9 ± 0.4 (7)
Paraventricular	11.5 ± 0.6 (6)	10.0 ± 0.7 (8)
Supraoptic	12.0 ± 1.0 (4)	11.8 ± 1.2 (7)
Mamillary body	4.9 ± 0.2 (6)	4.8 ± 0.3 (12)

† Values are the means ± standard errors obtained in the number of animals given in parentheses. Asterisks indicate significant difference from the young adult rats, as determined by Student's t test for group comparisons: * $P < 0.05$; ** $P < 0.01$.

vestibular nucleus, and pontine grey matter were also significantly reduced in the aged animals.

In the limbic system (Table 5), only the dentate gyrus and the nucleus accumbens were affected in the older animals. The rate of protein synthesis in the locus coeruleus (Table 5) was very significantly decreased by 22 per cent in the older group. In the 4 white matter structures examined, the internal capsule and cerebellar white matter were significantly affected in the older animals (Table 4).

DISCUSSION

The results of these studies demonstrate that in the normal Sprague-Dawley rat, ageing is associated with decreases in the rates of protein synthesis in the brain as a whole and in some specific brain regions. The regional changes are clearly selective and show a predilection for certain neural systems. Specifically, sensory and extrapyramidal motor systems are particularly affected whereas most areas of cortex and regions involved in higher functions are spared. When the results of the present studies are compared with those of a previous study (Smith *et al.*, 1980*b*) on the effects of ageing in Sprague-Dawley rats on local rates of cerebral glucose utilization (Table 6), it is apparent that structures of the visual and auditory systems are affected with respect to both biochemical processes throughout the entire extent of their respective pathways. On the other hand, the only significant age-related effects on glucose utilization outside the auditory and visual systems are in the caudate-putamen and white matter structures, whereas senescent decreases in protein synthesis are found in several structures of the limbic system, four regions involved in motor function, several white matter structures, the locus coeruleus, and pontine grey matter.

The decreases in glucose utilization in the visual and auditory systems may reflect a partial deafferentation due to retinal degeneration (Schardein *et al.*, 1975; Lai *et al.*, 1978) and degenerative changes in the organ of Corti. An acute decrease in visual or auditory input results in marked reductions in glucose consumption in components of the visual or auditory systems, respectively (Kennedy *et al.*, 1975; Sokoloff, 1977). The consequences of chronic decreases in sensory input are, however, still unknown. Glucose consumption, which is so closely linked to functional activity, would presumably remain reduced but, in addition, involutional changes in the brain structures involved in sensory function might result. Whether the senescent decreases found in glucose utilization in structures of the visual and auditory systems are related to such involutional changes or merely to a lack of input could not be distinguished on the basis of the studies on regional glucose utilization. The results of the present studies, in which decreased rates of protein synthesis were found in visual and auditory structures in the aged animals, suggest that there has been some involution in both of these systems. Because protein synthesis is a process necessary for long-term maintenance and remodelling in the nervous system and is less reflective of immediate functional activity, it may more nearly reflect the

TABLE 6. COMPARISON OF EFFECTS OF AGEING ON LOCAL RATES OF CEREBRAL GLUCOSE UTILIZATION AND PROTEIN SYNTHESIS

	Glucose utilization†		*Protein synthesis*	
Structure	*Significance*	*Percentage change*	*Significance*	*Percentage change*
Visual system				
Visual cortex (area 17)	***	−22	*	−16
Lateral geniculate	*	−18	*	−15
Superior colliculus	***	−17	*	−16
Thalamus, posterior lateral nucleus	*	−14	n.s.	
Auditory system				
Auditory cortex (area 41)	n.s.		n.s.	
Medial geniculate	***	−18	n.s.	
Inferior colliculus	**	−16	***	−21
Lateral lemniscus, ventral	**	−16	**	−21
Superior olivary nucleus	**	−16	n.s.	
Cochlear nucleus	n.s.		*	−17
Other cortical regions				
Olfactory cortex (area 51)	n.s.		*	−19
Parietal cortex (area 7)	n.s.		n.s.	
Frontal cortex (area 10)	n.s.		n.s.	
Sensorimotor cortex (area 2)	n.s.		n.s.	
Cerebellar cortex	n.s.		n.s.	
Limbic system and related structures				
Hippocampus: CA1	n.d.		n.s.	
CA2	n.s.		n.d.	
CA3	n.s.		n.d.	
Dentate gyrus, supragranular zone	n.s.		*	−17
Lateral amygdala	n.d.		n.s.	
Medial amygdala	n.s.		n.d.	
Lateral septal nucleus	n.s.		n.s.	
Nucleus accumbens	n.s.		*	−21
Habenula	n.d.		n.s.	
Interpenduncular nucleus	n.s.		n.s.	
Locus coeruleus	n.d.		**	−22
Hypothalamus: Ventral medial	n.s.		n.s.	
Paraventricular	n.d.		n.s.	
Supraoptic	n.d.		n.s.	
Mamillary body	n.s.		n.s.	

† Derived from data of Smith *et al.* (1980*b*). Values for the middle-aged and aged groups were combined to form a middle-aged/aged group equivalent to that used in the present study of protein synthesis. Asterisks indicate significant difference between the young adult and the middle-aged/aged rats as determined by Student's t test for group comparisons: n.s. = not significant; * $P < 0.05$; ** $P < 0.01$; *** $P < 0.005$; n.d. = not determined.

	Glucose utilization†		*Protein synthesis*	
Structure	*Significance*	*Percentage change*	*Significance*	*Percentage change*
Extrapyramidal motor system				
Caudate putamen	***	−16	n.s.	
Globus pallidus	n.s.		n.s.	
Substantia nigra (zc)	n.s.		*	−15
Inferior olivary nucleus	n.s.		***	−25
Pontine grey matter	n.s.		**	−21
Thalamus, ventral nucleus	n.s.		n.s.	
Red nucleus	n.d.		***	−25
Superior vestibular nucleus	n.s.		*	−16
White matter structures				
Splenium corpus callosum	*	−26	n.s.	
Genu of corpus callosum	n.s.		n.s.	
Internal capsule	*	−24	**	−19
Cerebellar white matter	***	−27	*	−18

structural and functional capacity of the nervous system. In the visual and auditory systems, at least, the changes in protein synthesis and glucose utilization may both indicate a retrogressive response in these structures to a chronic lack of input. The loss of pyramidal cell dendritic spines and branches found in both the visual (Feldman, 1976) and auditory (Vaughan, 1977) cortex of aged rats supports this idea.

Cortical areas, other than primary sensory areas, and structures of the limbic system, were generally unaffected by ageing (Table 6) with respect to either glucose utilization or protein synthesis. The significantly decreased rate of protein synthesis found in the supragranular zone of the dentate gyrus may be related to the reported senescent losses of synaptic terminals and dendrites in this area (Geinisman *et al.*, 1977, 1978). The decreased protein synthesis in the nucleus accumbens is of particular interest because of the mounting evidence that this nucleus has a significant role in mediating locomotor behaviour *via* a dopaminergic pathway (Pijenenburg and van Rossum, 1973; Jackson *et al.*, 1975; Kelly *et al.*, 1975, 1982) and that mesotelencephalic dopaminergic motor pathways are affected by the ageing process (reviewed by Finch *et al.*, 1981).

The nigrostriatal dopaminergic system is particularly affected by the ageing process. In the present study a decreased rate of protein synthesis was found in the zona compacta of the substantia nigra in the old rats. The evidence that this pathway is affected with age is considerable. Clinically, human aged show an increased incidence of parkinsonian signs (Critchley, 1956) and of extrapyramidal side effects to neuroleptic drugs (Crane, 1974). In aged rats, it has been observed (Marshall and Berrios, 1979) that there are deficits in swimming behaviour which are diminished by

the administration of apomorphine. Other studies (Gage *et al.*, 1983) of aged rats have shown that intrastriatal dopaminergic grafts are associated with significant improvement in the scores on some tests of motor coordination. In a number of species decreases with age in dopamine turnover (Finch, 1973), number of dopamine receptors (Makman *et al.*, 1979; Severson and Finch, 1980; Severson *et al.*, 1982), and dopamine-sensitive adenylcyclase (Govoni *et al.*, 1977; Puri and Volicer, 1977) in the striatum are well documented. In the study of the effects of ageing on local rates of glucose utilization (Smith *et al.*, 1980*b*) significant decreases were found in the caudate-putamen (Table 6). Of the many senescent changes that have been found in the striatum, most involve the dopaminergic terminals so rich in this area. Protein synthesis is unchanged in this region (Table 4), but it is significantly decreased in the substantia nigra where the cell bodies of origin of the striatal dopaminergic terminals are located.

Apart from a significant decrease in glucose utilization in the caudate putamen, there are no effects of ageing on glucose utilization in motor areas (Table 6). In contrast, protein synthesis rates are decreased in the older animals in many regions involved in motor function (Tables 4 and 6), particularly in the red nucleus and the inferior olivary nucleus. It is possible that these effects are related to more general effects on the extrapyramidal motor system inasmuch as well-defined neuronal circuits exist between the striatum and cerebellum *via* the inferior olivary nucleus (Fox and Williams, 1970) and between the red nucleus and inferior olivary nucleus (Walberg, 1956).

There may also be effects of ageing on noradrenergic pathways. The rate of protein synthesis was significantly reduced in the locus coeruleus in the old animals (Table 5). The locus coeruleus contains the cell bodies of origin of the major ascending noradrenergic innervation of the basal telencephalon and the entire isocortex. The effect of age on glucose utilization in the locus coeruleus was not determined in the previous study of senescent changes in energy metabolism (Smith *et al.*, 1980*b*); a further examination of both glucose utilization and protein synthesis in this region and other catecholaminergic nuclei and their projection sites is warranted.

The present studies utilize a quantitative autoradiographic method to measure local rates of cerebral protein synthesis that is still being refined (Smith *et al.*, 1980*a*, 1984). The details of its development and validation will be published separately. Briefly, the kinetic model for the behaviour of leucine in brain used in formulating the operational equation (fig. 1) assumes a single free leucine precursor pool for protein synthesis that exchanges with plasma but with no admixture of leucine derived from protein degradation. Although this model is probably oversimplified, it is likely to be adequate for the present studies for the following reasons. There are two major requirements for a method of this type: (1) a means of determining the amount of labelled product formed (the numerator of the operational equation (fig. 1)), and (2) a means of determining the integrated specific activity of the precursor pool for the reaction (the denominator of the operational equation (fig. 1)). The

total amount of ^{14}C in the tissue at the end of the experiment, Ci*, is determined by quantitative autoradiography; it represents the combined concentrations of [^{14}C]leucine incorporated into protein (i.e. product) and residual free [^{14}C]leucine remaining in the tissue represented by the second term in the numerator. As calculated with the equation this second term equals 2 to 5 per cent of the Ci* at 60 min after the pulse. Also, acid-soluble ^{14}C, which includes free [^{14}C]leucine and α-[^{14}C]ketoisocaproic acid, if any, has been measured experimentally and found to be approximately 5 per cent of the Ci* 60 min after the pulse of [1-^{14}C]leucine. The correction of Ci* for free [^{14}C]leucine is, therefore, an almost minimal source of error in the rat. The integrated specific activity of the precursor pool is calculated from the history of the plasma specific activity (first term in denominator) and the lag between the plasma and the precursor pool in the tissue (corrected for in the second term in denominator). The half-life of the precursor pool has been estimated in a separate series of experiments to be less than 3.5 min. With a pool turning over at such a rapid rate the difference between its integrated specific activity and that of the plasma will be relatively small 30 to 60 min after a pulse of [^{14}C]leucine, and correction for the lag is almost negligible.

The assumption that there is no admixture of leucine derived from protein degradation with the precursor pool is one for which there is evidence derived from *in vitro* experiments (Gainer *et al.*, 1975; Robertson and Wheatley, 1979). Such admixture, if present, would dilute and, therefore, reduce the precursor pool specific activity because it would provide a source of constant dilution of the radioactive amino acid entering the precursor pool from the plasma. Experiments are currently in progress to assess the degree of admixture but, in the meantime the rates of protein synthesis in the present studies were calculated with the assumption that the admixture is negligible. If there is, indeed, significant admixture, then the data presented here represent the minimal rather than the actual rates of leucine incorporation into protein.

The design of the leucine method which utilizes a pulse injection followed by a 60 min interval for clearance of free [^{14}C]leucine from the brain minimizes possible artefacts due to differences in blood flow and amino acid transport. It does not rule out the possibility, however, that in extreme conditions blood flow or transport could be limiting the rate of protein synthesis. The results of studies with the leucine method show changes in the rate of protein synthesis, but they do not demonstrate the cause of the changes.

The finding of decreases in brain protein synthesis with age agrees well with ideas of the involutional nature of the ageing process. Of particular interest in the present study is the finding that changes in protein synthesis are regionally selective. A decreased rate of protein synthesis in a brain region may reflect either a decrease in the number of cells or an atrophy of existing cells in the region. Either neurons or glial cells could be affected. In the case of neurons, an atrophy of the terminal regions might be reflected in decreased rates of protein synthesis in the cell bodies of origin. The findings of senescent decreases in protein synthesis in substantia nigra,

inferior olivary nucleus, and locus coeruleus are of interest in light of studies of the effects of ageing on neuron number in these regions (Monagle and Brody, 1974; McGeer *et al.*, 1977; Vijayashankar and Brody, 1979; Goldman and Coleman, 1981). In regions in which neuron number does not appear to change significantly with age decreases in protein synthesis may indicate that the extent of the terminal fields of these neurons has decreased or, alternatively, changes may have occurred in glial cells.

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