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REGIONAL DISTRIBUTION OF SOME ENZYMES INVOLVED WITH PUTATIVE NEUROTRANSMITTERS IN THE HUMAN VISUAL SYSTEM

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SUMMARY

Levels of protein and activity of several enzymes were determined in different portions of the visual system of the human brain. The enzymes studied were total cholinesterase (TChE), acetylcholinesterase (AChE), pseudocholinesterase (ChE), choline acetyltransferase (ChAc), monoamine oxidase (MAO), and N-methyltransferase (NMT). Brains of 17 patients were obtained at autopsy. The specimens were divided into 3 groups. One group consisted of the brains of subjects who were mentally normal prior to death. The second group of subjects had been diagnosed as chronic schizophrenics and the third had an organic brain syndrome. Each group included males and females. Subjects with an organic brain syndrome were significantly older than subjects in either of the other two groups. There were no significant differences in protein among the 3 groups for most of the visual areas. All enzyme activity was expressed per g of protein. TChE, AChE, and ChE activity varied markedly within the visual system, being usually lowest in the optic nerve and highest in the superior colliculus. There were no significant differences in TChE or AChE among the diagnostic groups. Unexpectedly, ChE was elevated in some brain areas in the chronic schizophrenic group. This finding needs further confirmation in a drug free group. ChAc did not differ significantly among the 3 diagnostic groups and, in general, was low throughout the visual system but highest in the superior colliculus and lateral geniculate. MAO activity was not significantly different among the 3 patient groups. Its activity in the gray matter was only slightly higher than in the white matter of the visual system. NMT activity was extremely low (about 0.1 % the activity of the other enzymes) throughout all areas studied and did not differ significantly between areas or diagnostic groups.

INTRODUCTION

There is evidence that lateral geniculate neurons of various animals respond to acetylcholine, norepinephrine, and serotonin applied iontophoretically[21]. The regional distribution of the enzymes associated with each of these putative neurotransmitters in the visual system has been studied previously, especially in animals. Many investigators have included portions of the visual system in their analyses of the regional brain distribution of some of these enzymes. For example, choline acetyltransferase (ChAc, EC 2.3.1.6.) is present in the lateral geniculate nucleus of man and several other animals[8,9,12]. Both acetylcholinesterase (AChE, EC 3.1.1.7) and pseudocholinesterase (ChE, EC 3.1.1.8) are also present in portions of the visual system, especially the lateral geniculate nucleus[1,5,10,11,18–20]. Monoamine oxidase (MAO, EC 1.4.3.4) activity has also been reported[3,4,24]. The distribution of N-methyltransferase (NMT) activity in the visual system has not been previously studied.

We have had a unique opportunity to study the gross distribution of several of these enzymes in the visual system of patients who prior to death were mentally normal, or had chronic schizophrenia or an organic brain syndrome. The data obtained indicate that the cholinergic enzymes are significantly elevated in the lateral geniculate nucleus and superior colliculus of the visual system of man.

MATERIALS AND METHODS

Tissue sources

Brains were obtained from autopsies performed on patients who died at Northville State Hospital, Detroit General or University Hospital (Ann Arbor). A total of 17 brains was examined. Eleven were from Northville, 5 from Detroit General and one from University Hospital. After death, the body was kept at 4 °C. Autopsies were performed within 36 h after death. Most were within 24 h. At autopsy the brain was removed and frozen. One to 4 days after death the brain was transferred to a dry ice freezer (—50 °C) at the Lafayette Clinic and kept there until 24 h before dissection, when it was gradually thawed in a cold room. After dissection, the tissues were either homogenized the same day and the homogenates frozen for assay at a later date or the tissues were packed in plastic bags and frozen for later homogenization and assay.

Patient diagnosis

Three patients (numbers 3, 9, 15) were diagnosed as having an organic brain syndrome (OBS). Seven patients were chronic schizophrenics who had been given psychotropic drugs recently (1, 2, 4, 10, 11, 12, 13) and one was a chronic schizophrenic who was drug free (14). The rest were mentally normal prior to death. The numbers in parentheses refer to the order in which the brain specimens were accepted into the study. All diagnoses were made by the attending psychiatrist and verified by review of the records by one of us.

Drug history

Four patients (3, 9, 14, 17) were drug free for several months or longer prior to

death. Five patients (1, 2, 4, 11, 12) received psychotropic drugs (usually antipsychotics) within 1 week prior to death. These 5 and all mentally normal patients except 17 also received various non-psychoactive drugs (*e.g.*, digitoxin, antibiotics) prior to death. Most of the drugs were discontinued from 1 week to hours before death.

Cause of death

Ten patients (1, 2, 3, 9, 10, 12, 15, 16, 20, 21) died of bronchial pneumonia and/or pulmonary embolism. One (11) died of acute peritonitis and perforated gastric ulcer. One (13) died of a myocardial infarction and terminal pneumonia. One (4) suffered congestive heart failure and one (14) died of an acute myocardial infarction. Another (17) suffocated, one (18) died of renal failure, and one (19) died of lung cancer.

Physical characteristics

Seven patients (3, 4, 14, 15, 17, 19, 21) were males and 10 (1, 2, 9, 10, 11, 12, 13, 16, 18, 20) were females. Their ages ranged from 14 to 90 years. Four (13, 16, 19, 20) were Negro, the rest Caucasians.

There were unavoidable delays in obtaining the brains or performing the enzymatic studies. Delays of from 4 days up to 1 year occurred between date of death and date of enzyme assay. All tissue was frozen during the interim.

Chemical assays

Several assays were performed and standards run. Protein standards were bovine serum albumin and Dade protein standard. Rat brain was the standard enzyme source for total cholinesterase (TChE), AChE, ChE, and ChAc activity. Cow caudate nucleus and rabbit lung were standards for NMT and cow caudate for MAO activity.

Protein determination

Proteins were determined according to the method of Lowry *et al.*[15] using the Folin Cu–EDTA method. Brain homogenates of 10% and 1% were prepared in glass distilled water and 5 ml of Cu–EDTA reagent were added to 1 mg of tissue diluted in water (1 ml total). Ten min later 0.5 ml of a 1:1 diluted phenol reagent was added, mixed, and after 30 min samples were read at 650 nm in a Beckman spectrophotometer.

Acetylcholinesterase and cholinesterase

TChE was measured using the method of Siakotos *et al.*[23]. This is a radiochemical method in which acetyl-[1-^{14}C]choline is used as the substrate. The principle of the assay is that acetylcholine (ACh) is hydrolyzed and after 10 min the reaction stopped by the addition of a strongly acidic cationic exchange resin which binds the unhydrolyzed substrate. TChE activity was measured without any inhibitor. ChE activity was measured using the specific AChE inhibitor, BW 284c51 (1:5,-bis-(4-allyldimethylammoniumphenyl) pentan-3-one dibromide) at 1×10^{-6} *M* final concentration, dissolved in 0.1 *M* phosphate buffer. AChE activity was obtained by subtracting the residual ChE activity from the TChE. Amberlite CG-120 (Mallinckrodt) mixed with dioxane

was used to stop the reaction and bind unhydrolyzed ACh. The reaction mixture consisted of 0.1 ml of 0.1 *M* phosphate buffer, pH 7.4 containing 0.3 *M* sodium chloride and 1% Lubrol WX as a detergent, the brain enzyme source (0.1 ml) and substrate (0.1 ml). Total volume was 0.3 ml. Final substrate concentration was 1×10^{-3} *M* [^{14}C]ACh. The buffer, inhibitor and enzyme source were added to centrifuge tubes and the racks placed in a 37.5 °C water bath. The substrate was then added, mixed and the tubes incubated for 10 min. Nonenzymatic hydrolysis of labeled substrate was measured using 0.1 ml distilled water or boiled enzyme source when possible. Rat brain TChE was run as a standard with each assay. The reaction was stopped by the addition of the resin–dioxane mixture. Additional dioxane was then added, the tubes capped, mixed, and centrifuged at 3000 rev./min for 5 min. One-half (5 ml) of the supernatant was transferred to a scintillation counting vial to which was added 10 ml of a dioxane-based scintillation cocktail (Aquafluor). Samples were counted in a Beckman LS-150 scintillation counter. All enzyme values were expressed in μmole ACh hydrolyzed/g of brain protein/min.

Choline acetyltransferase

ChAc activity was assayed using a combination of the methods of McCaman and Hunt[17] and Schrier and Shuster[22]. A 10% homogenate of the various brain areas was used, 1 mg of tissue being assayed. The 0.1 ml incubation mix consisted of NaCl, 3×10^{-1} *M*; choline chloride, 1×10^{-2} *M*; physostigmine, 2×10^{-4} *M*; $MgSO_4$, 2×10^{-2} *M*; 0.05% serum albumin; phosphate buffer, 0.08 *M*, pH 7.4; and acetyl CoA, 6.2×10^{-4} *M*. The acetyl CoA was a mixture of labeled acetyl-[1-^{14}C]CoA (New England Nuclear) and cold acetyl CoA (Sigma) in such proportion that 0.1 ml of the incubation mix contained approximately 20,000 counts/min. After 30 min incubation at 37.5 °C, the tubes were plunged into boiling water for 5 min to stop the reaction. Incubation mixtures were then applied to the top of prewashed Dowex 1×8 Cl^- (BioRad) columns, 8 cm high, prepared from diSPo pipets plugged with glass wool. Three consecutive 0.5 ml water washes were applied to the column and the effluent collected directly into scintillation vials. Fifteen ml of a dioxane mixture (8 g PPO, 0.1 g POPOP, 110 g naphthalene, 1 liter dioxane) were added to each vial and the samples counted in a Beckman LS-200 liquid scintillation counter. Enzyme activity was expressed as μmole ACh/g protein/min.

Monoamine oxidase

MAO activity was measured by the methods of Ho *et al.*[13] and Wurtman and Axelrod[25]. The method is based on the oxidation of [^{14}C]tryptamine by MAO to form the corresponding aldehyde and further oxidation by aldehyde dehydrogenase to indoleacetic acid (IAA). A 0.1 ml aliquot of a 10% brain homogenate (10 mg), 0.1 ml of [^{14}C]tryptamine, 2.4×10^{-4} *M*, and 1 ml of a 0.5 *M* Na_2HPO_4 buffer, pH 7.4, for a total 1.2 ml final assay volume was used. Final substrate concentration was 2×10^{-5} *M*. Tissue blanks were prepared by substituting boiled enzyme source. Standards included cow caudate and occasionally rat brain homogenate. The enzyme mixture was incubated for 30 min at 37.5 °C and reaction stopped with 0.5 ml 2 *N* HCl and [^{14}C]-

IAA extracted with 5 ml ether. After centrifuging at 2500 rev./min for 5 min the tubes were frozen and the ether layer removed. Three ml of the ether layer were added to scintillation vials with 10 ml toluene scintillation solution and heated at 45 °C to volatilize the ether. The entire procedure was carried out in a well ventilated hood. All enzyme activity was expressed in μmole of tryptamine oxidized/g of protein/min.

N-methyltransferase

NMT was assayed via the method of Mandell *et al.*[16]. This is a radiochemical method based on the methylation of tryptamine to labeled N-methyl tryptamine. The method is similar to that described by Axelrod[2] except that toluene rather than iso-amyl alcohol is used to extract the methylated tryptamine. Blanks were prepared by boiling the enzyme source of the same concentration as that used in the assay. Ten percent cow caudate or rabbit lung homogenized in cold distilled water was used as a standard. Enzyme activity was expressed in nmole tryptamine methylated/g of protein/min.

RESULTS

Age differences

The age (mean $\pm$ S.E.) of the mentally normal group (N = 6) was 53.3 $\pm$ 9.9, that of the chronic schizophrenic patients (N = 8) was 62.2 $\pm$ 4.9, and that of the organic brain syndrome group (N = 3) 83.3 $\pm$ 5.2 years. Using a group comparison '*t*' test the age differences between the normals and the chronic schizophrenics was not statistically significant, but the organic brain syndrome group differed significantly

TABLE I

PROTEIN CONCENTRATION OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

The numbers in parentheses after mean $\pm$ S.E. represent the number of subjects in each group.

Brain area	*Mean $\pm$ S.E. (g prot/g wet weight)*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	0.104 $\pm$ 0.007 (5)	0.099 $\pm$ 0.016 (5)	0.068 $\pm$ 0.006 (3)**
Chiasm	0.105 $\pm$ 0.003 (6)	0.100 $\pm$ 0.011 (2)	0.081 $\pm$ 0 (1)
Optic tract	0.102 $\pm$ 0.004 (6)	0.107 $\pm$ 0.011 (5)	0.104 $\pm$ 0.015 (3)
Lateral geniculate	0.106 $\pm$ 0.003 (6)	0.131 $\pm$ 0.025 (5)	0.098 $\pm$ 0.008 (3)
Optic radiation	0.105 $\pm$ 0.004 (6)	0.116 $\pm$ 0.005 (5)	0.108 $\pm$ 0 (1)
Occipital cortex	0.090 $\pm$ 0.006 (6)	0.102 $\pm$ 0.011 (5)	0.093 $\pm$ 0.007 (2)
Superior colliculus	0.099 $\pm$ 0.006 (5)	0.114 $\pm$ 0.018 (6)	0.101 $\pm$ 0.003 (3)

In this and all subsequent tables the symbols are:
* Significant at the 0.05 level (group comparison of MN *vs.* CS or MN *vs.* OBS).
** Significant at the 0.01 level (group comparison of MN *vs.* CS or MN *vs.* OBS).

TABLE II

TOTAL CHOLINESTERASE ACTIVITY OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

Brain area	*Mean ± S.E. (μmole/g prot/min)*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	2.9 ± 0.2 (5)	3.8 ± 1.4 (5)	5.1 ± 2.1 (3)
Chiasm	8.8 ± 2.0 (6)	4.4 ± 2.2 (2)	8.0 ± 0 (1)
Optic tract	13.9 ± 5.7 (6)	5.5 ± 1.6 (5)	8.1 ± 3.5 (3)
Lateral geniculate	38.7 ± 4.0 (6)	39.1 ± 10.3 (5)	57.1 ± 29.0 (3)
Optic radiation	4.7 ± 1.0 (6)	3.3 ± 0.8 (5)	4.1 ± 0 (1)
Occipital cortex	8.1 ± 1.9 (6)	9.5 ± 1.4 (5)	4.9 ± 1.9 (2)
Superior colliculus	76.3 ± 10.1 (5)	76.8 ± 9.1 (6)	62.2 ± 15.3 (3)

from both the mentally normal ($P < 0.03$) and the chronic schizophrenic patients ($P < 0.02$).

Protein

The concentration of g protein/g of wet weight of brain tissue among the various areas of the visual system was approximately 10%. There were no great differences between the optic nerve, chiasm, optic tract, lateral geniculate and optic radiation. On the other hand, the occipital cortex and superior colliculus had slightly lower protein values in the mentally normal group. There were no significant differences between the 3 diagnostic categories of mentally normal, chronic schizophrenic or organic brain syndrome patients in any of the areas studied except that there was a significantly lower protein concentration ($P < 0.01$) in the optic nerve of the 3 patients with an organic brain syndrome. Two of these patients during life had reasonable

TABLE III

ACETYLCHOLINESTERASE ACTIVITY OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

Brain area	*Mean ± S.E. (μmole/g prot/min)*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	1.7 ± 0.1 (5)	2.4 ± 0.8 (5)	5.3 ± 3.5 (2)
Chiasm	7.2 ± 1.9 (6)	3.0 ± 1.6 (2)	6.9 ± 0 (1)
Optic tract	12.7 ± 5.4 (6)	3.8 ± 1.1 (5)	7.7 ± 5.8 (2)
Lateral geniculate	36.2 ± 3.9 (6)	27.8 ± 8.0 (5)	65.3 ± 20.8 (2)
Optic radiation	3.2 ± 0.7 (6)	1.9 ± 0.5 (5)	4.1 ± 0 (1)
Occipital cortex	6.8 ± 1.6 (6)	6.9 ± 1.1 (5)	4.4 ± 2.4 (2)
Superior colliculus	71.6 ± 9.5 (5)	60.4 ± 7.9 (6)	51.2 ± 31.1 (2)

TABLE IV

PSEUDOCHOLINESTERASE ACTIVITY OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

Brain area	*Mean ± S.E. (μmole/g prot/min)*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	1.1 ± 0.2 (5)	1.7 ± 0.7 (5)	0.2 ± 0.2 (2)
Chiasm	1.6 ± 0.2 (6)	1.5 ± 0.7 (2)	1.1 ± 0 (1)
Optic tract	1.2 ± 0.4 (6)	1.9 ± 0.6 (5)	0.2 ± 0.2 (2)
Lateral geniculate	2.5 ± 0.4 (6)	7.8 ± 2.1* (5)	13.6 ± 12.5 (2)
Optic radiation	1.4 ± 0.3 (6)	1.4 ± 0.4 (5)	0.1 ± 0 (1)
Occipital cortex	1.3 ± 0.3 (6)	2.5 ± 0.5 (5)	0.5 ± 0.5 (2)
Superior colliculus	4.6 ± 0.5 (5)	16.4 ± 1.7** (6)	7.8 ± 5.2 (2)

vision for their age; the third had poor vision but was not blind. The data are listed in Table I.

Total cholinesterase activity

Using [^{14}C]ACh as substrate, marked regional differences in TChE activity were found in the visual system (Table II). The lowest activity (mean ± S.E.) was present in the optic nerve (2.9 ± 0.2 μmole/g protein/min) and the highest activity in the superior colliculus (76.3 ± 10.1 μmole/g protein/min) for the mentally normal. The optic chiasm also had more TChE activity than the optic nerve. Both the optic radiation and occipital cortex had relatively low activity. On the other hand, the superior colliculus and lateral geniculate had an appreciable amount of TChE activity. There were no significant differences between the mentally normal, chronic schizophrenic, and organic brain syndrome patients.

TABLE V

CHOLINE ACETYLTRANSFERASE ACTIVITY OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

Brain area	*Mean ± S.E. (μmole/g prot/min)*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	0.027 ± 0.008 (5)	0.022 ± 0.008 (4)	0.023 ± 0.006 (3)
Chiasm	0.058 ± 0.014 (6)	—	0.044 ± 0 (1)
Optic tract	0.055 ± 0.022 (6)	0.023 ± 0.008 (5)	0.041 ± 0.014 (3)
Lateral geniculate	0.133 ± 0.047 (6)	0.071 ± 0.021 (5)	0.495 ± 0.438 (3)
Optic radiation	0.030 ± 0.009 (6)	0.019 ± 0.005 (5)	0.029 ± 0 (1)
Occipital cortex	0.051 ± 0.011 (6)	0.037 ± 0.012 (5)	0.004 ± 0.002 (2)
Superior colliculus	0.101 ± 0.047 (5)	0.120 ± 0.028 (5)	0.103 ± 0.060 (3)

Acetylcholinesterase activity

Using the specific AChE inhibitor, BW 284c51, and subtracting the residual ChE activity, an estimate of AChE activity was obtained. It can be seen, as has been described previously for brain tissue, that most of the cholinesterase activity was due to AChE[14]. The qualitative distribution of AChE activity in the visual system was similar to that of TChE with no significant differences among the patient groups (compare Tables II and III).

Pseudocholinesterase activity

ChE activity did not show as striking regional differences as were seen with AChE (Table IV). The lateral geniculate and superior colliculus tended to have more activity than the other visual areas. There were no significant differences between mentally normal and organic brain syndrome patients. Surprisingly, ChE activity was enhanced compared to the mentally normal group in the lateral geniculate and superior colliculus of the chronic schizophrenic patients. These differences were significant ($P < 0.05$). When the data were normalized, that is, expressed as a fraction of the ChE standard for each assay, the differences were still statistically significant for both the lateral geniculate and the superior colliculus ($P < 0.01$). It should be remembered that most of the schizophrenic patients prior to death were on neuroleptic medication (see Discussion).

Choline acetyltransferase activity

The same difference between optic nerve, chiasm and optic tract that was observed with AChE activity was also present with the distribution of ChAc. In general, optic radiation and occipital cortex also showed low levels of activity. A similar pattern of distribution of ChAc, being highest in the lateral geniculate and superior colliculus, was seen among the mentally normal, chronic schizophrenic and organic brain syn-

TABLE VI

MONOAMINE OXIDASE ACTIVITY OF MENTALLY NORMAL, CHRONIC SCHIZOPHRENIC AND ORGANIC BRAIN SYNDROME PATIENTS

Expressed as a fraction of cow caudate standard = 1.000. Mean ± S.E. cow caudate MAO activity was 0.092 ± 0.018 μmole/g of tissue/min.

Brain area	*Mean ± S.E.*		
	Mentally normal (MN)	*Chronic schizophrenic (CS)*	*Organic brain syndrome (OBS)*
Optic nerve	0.348 ± 0.018 (4)	0.507 ± 0.118 (4)	0.535 ± 0.220 (3)
Chiasm	0.282 ± 0.036 (3)	0.608 ± 0.237 (2)	0.878 ± 0 (1)
Optic tract	0.363 ± 0.086 (4)	0.367 ± 0.028 (5)	0.459 ± 0.212 (3)
Lateral geniculate	0.471 ± 0.072 (4)	0.480 ± 0.029 (5)	0.424 ± 0.275 (3)
Optic radiation	0.192 ± 0.015 (4)	0.236 ± 0.062 (4)	0.400 ± 0 (1)
Occipital cortex	0.454 ± 0.044 (4)	0.439 ± 0.088 (5)	0.297 ± 0.297 (2)
Superior colliculus	0.639 ± 0.080 (3)	0.604 ± 0.146 (6)	0.676 ± 0.235 (3)

drome patients. There were no significant differences between these subgroups where an N of 3 or more was compared (see Table V).

Monoamine oxidase activity

In view of the fact that there was considerable variation in our standards for MAO activity, all data were expressed as a fraction of 1.000 for cow caudate nucleus standard run simultaneously. It can be seen in the mentally normal group that the differences in distribution of MAO between various visual areas were not as striking as with TChE. The superior colliculus tended to have the greatest activity with the lateral geniculate next. Likewise, the occipital cortex showed somewhat similar activity. White matter areas showed less activity but still appreciable amounts, as shown in Table VI. There were no significant differences between the mentally normal, chronic schizophrenic, and organic brain syndrome patients. In the latter two groups the activity in the optic chiasm was especially high but, in view of the small N, was not tested for statistical significance.

N-methyltransferase activity

NMT activity was determined using tryptamine as the substrate. There were no significant gray–white differences in any of the patient groups. Enzyme activity varied from a low of 0.252 to a high of 0.810 nmole/g protein/min. Of special importance is the fact that NMT activity was extremely low throughout the visual system, approximately 0.1 % the activity of the other enzymes.

DISCUSSION

There are many objections to this study. One is faced with the practical consideration of patients dying from different causes at different times. The time of death to the time of autopsy is a variable which could not be controlled under most circumstances because of the need to obtain family permission, autopsy scheduling, etc. Furthermore, once an autopsy was performed all brains were frozen until subsequent thawing for dissection and regional assay. Thus, the question of enzyme stability with freezing and thawing becomes paramount. Another variable was the fact that the chronic schizophrenic patients in general had various neuroleptic agents given until shortly before their terminal illness, and all patient groups were given various medications including antibiotics, cardiovascular drugs, etc. until just before death. In view of these many variables that could not be controlled, the data obtained are surprising because of their consistency with known literature on the distribution of these enzymes in the visual system of animals where such variables can be controlled[6].

No significant differences in TChE were seen across patient categories. An especially interesting finding was that optic nerve TChE activity was much lower than that in the optic chiasm and optic tract. The present findings in man have previously been observed in animals[11]. A somewhat surprising finding was that the chronic schizophrenic patients may have more ChE activity in the lateral geniculate and superior colliculus. This may be related to the fact that these patients were on neuro-

leptic medication prior to death. It is well known that neuroleptic agents in low concentrations tend to activate ChE activity[7]. One chronic schizophrenic patient (14) was drug free. ChE in his lateral geniculate was 1.1 μmole/g protein/min and 10.7 μmole/g protein/min in his superior colliculus. His lateral geniculate value is less than the mean for all patient groups. His superior colliculus value is higher than the mean of the normal and organic brain syndrome groups but less than that of the other chronic schizophrenics. Thus, the data of this case both support and deny the hypothesis that the increase in ChE activity is due to neuroleptic medication.

The qualitative distribution of ChAc activity in the visual system was similar to that of TChE and AChE activity. These findings again emphasize an important role of the cholinergic system in the human lateral geniculate and superior colliculus. These data are in complete harmony with data in the literature on the distribution of these enzymes in the visual system of animals[6].

There was no significant difference in MAO activity among the 3 patient groups. While there was a difference in the distribution of MAO in white *versus* gray matter, all areas of the visual system showed considerably less activity than the cow caudate nucleus standard. The highest values obtained were in the superior colliculus and lowest in the optic radiation of the normal group. In general, nuclear masses such as the superior colliculus and lateral geniculate tended to have somewhat elevated activity compared to the optic nerve, optic tract and radiation. Of all the enzymes studied, NMT activity was by far the lowest. In general, it was slightly above boiled blanks. It may be that the extremely low levels of NMT activity can be in part related to the fact that this enzyme, when obtained from rabbit lung, is more likely to be destroyed by freezing (unpublished observation, 1972) than the other enzymes such as cholinesterase[10].

It is concluded that the cholinergic enzymes are present in relatively large amounts in the lateral geniculate and superior colliculus of man, irrespective of psychiatric status. If enzyme activity is any indication of functional importance, ACh probably plays an important role in these areas of the human visual system.

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