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Amino acids and N-acetyl-aspartyl-glutamate as neurotransmitter candidates in the monkey retinogeniculate pathways

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AMINO ACIDS AND N-ACETYL-ASPARTYL-GLUTAMATE AS NEUROTRANSMITTER CANDIDATES IN THE MONKEY RETINOGENICULATE PATHWAYS

by

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ABSTRACT

AMINO ACIDS AND N-ACETYL-ASPARTYL-GLUTAMATE
AS NEUROTRANSMITTER CANDIDATES IN THE
MONKEY RETINOGENICULATE PATHWAYS

by

Ricardo A. Molinar-Rode

Advisor: Professor Pedro Pasik

The lateral geniculate nucleus (LGNd) receives chemically identified inputs from brain stem structures, the thalamus and visual cortex. The identity of the neurotransmitter(s) in the retinal input, however, is unknown.

To investigate the possibility that some amino acids and certain dipeptides, such as N-acetyl-aspartyl-glutamate (NAAG), fulfill this function, changes in their concentration were measured in the optic tract, parvocellular and magnocellular segments of the LGNd, superior colliculus and visual cortex of six monkeys (Macaca fascicularis), seven days after right optic tractotomy. The LGNd was studied also in two additional macaques, three months after occipital lobectomy. Tissue was frozen within five minutes of death, regions were dissected with the micropunch technique, and substances were analyzed by HPLC.

Of the ten compounds measured in the normal side, glutamate, glutamine, glycine, and alanine had homogeneous distributions. GABA was highest in the superior colliculus, cysta-

thionine and NAAG decreased in the rostrocaudal direction, and N-acetyl-aspartate showed an opposite gradient of concentration. The heterogeneity in taurine and aspartate was less systematic.

Optic tract section induced significant, large reductions in NAAG, glutamate and aspartate in the optic tract distal to the lesion. Significant decreases in NAAG, and to a lesser extent in glutamate, were observed in the LGNd. Changes in the dipeptide were apparent in both the parvocellular and magnocellular segments. Reductions in glutamate reached significance in the parvocellular laminae, and those of aspartate approached significance in the magnocellular division. No significant differences were detected in the superior colliculus and striate cortex.

Occipital lobectomy produced large declines in aspartate and glutamate in the LGNd, as well as moderate reductions in alanine and GABA, and minor changes in glutamine and glycine.

The results of optic tractotomy support the role of NAAG as a neurotransmitter candidate in the monkey retinogeniculate pathways; its significant decrease in both geniculate segments suggests that X- and Y- retinal axons utilize this substance. Although at times the reductions in glutamate or aspartate failed to reach significance, their role cannot be excluded. The findings after occipital lobectomy strongly favor these latter substances as corticogeniculate transmitters.

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CHAPTER 1

INTRODUCTION

One of the main goals in neuroscience is to understand the processing of sensory information in the brain. The visual system has been employed fruitfully as a model to understand this question. The rapid advancement of protein biochemistry, combined with the introduction of sensitive histochemical methods has made it possible to study the chemical neuroanatomy of the visual system with greater detail. These type of studies have been more successful in the retina and the visual cortex, two of the components of the primary visual system, but they have provided also important information in the lateral geniculate nucleus (LGNd), the intermediate center located in the thalamus. In the past, this nucleus was considered as a mere relay station along the visual pathways, based on the similarity in receptive field properties between geniculate neurons and those of retinal ganglion cells [Hubel and Wiesel 1961; Shapley and Lennie 1985]. More recently, this viewpoint has been changed, particularly on the basis of the complexity of both the inputs it receives, as well as on its intrinsic synaptic organization. For example, the LGNd receives a cholinergic input from the mesencephalic reticular formation, a serotonergic input from the dorsal raphe, and a

noradrenergic input from the locus coeruleus; it also receives a reciprocal input from the primary visual cortex that is probably glutamergic [for review see Jones 1985c]. The direct input from the retina is, in terms of the number of terminal synapses, a minor element of the LGNd organization; however, it is the most important input in terms of the content of visual information. The neurotransmitters that retinal ganglion cells utilize to relay information into the LGNd are unknown.

Investigations on the identity of the retinogeniculate transmitter(s) began approximately in the 1950's, when Evarts [1955] and Bishop [1958] studied the effects of serotonin and LSD on the responses evoked on LGNd neurons by light or optic nerve stimulation. In the 1960's, acetylcholine and several analogs of this molecule were studied using the same approach by Curtis [1962], McCance [1966], Phillis [1967] and other investigators. In the 1970's these type of studies continued, while at the same time the possibility that glutamate (GLU) and/or aspartate (ASP) could be the excitatory neurotransmitter(s) released from optic nerve terminals began to be investigated. Tebecis and colleagues [1972, 1973, 1974] concluded in their studies that GLU was probably not the optic nerve transmitter, although the possibility was left open. At about the same time, several investigators obtained evidence in the avian and amphibian brain indicating that excitatory amino acids could be the neurotransmitters in the

retinotectal pathways [Beart 1976; Roberts 1976; Bondy 1977; Wang 1978; Canzek 1981; Fonnum 1982]. In 1982, with work in the cat, Kemp and Sillito reintroduced the possibility that GLU, ASP or a similar substance could be the optic nerve transmitter in the mammalian visual system.

The monkey visual system offers several advantages as a neurobiological model to study this question. First, it is a highly developed laminated structure, with a parvocellular and a magnocellular segments. Second, it receives the input from the retina in a segregated, parallel fashion, with the X- ganglion cells projecting to the parvocellular laminae and the Y- ganglion cells projecting to the magnocellular laminae. This segregation of inputs into clearly discernible laminae allows the investigator to obtain information from each channel independently.

The purpose of this project is to examine the role of amino acids and related substances as neurotransmitters in the monkey retinogeniculate pathways, observing some of the criteria proposed by several investigators [Watkins and Evans 1981; Werman 1966].

CHAPTER 2

BACKGROUND

1. MORPHOLOGICAL FEATURES OF THE MONKEY DORSAL LATERAL GENICULATE NUCLEUS

1.1 NORMAL STRUCTURE

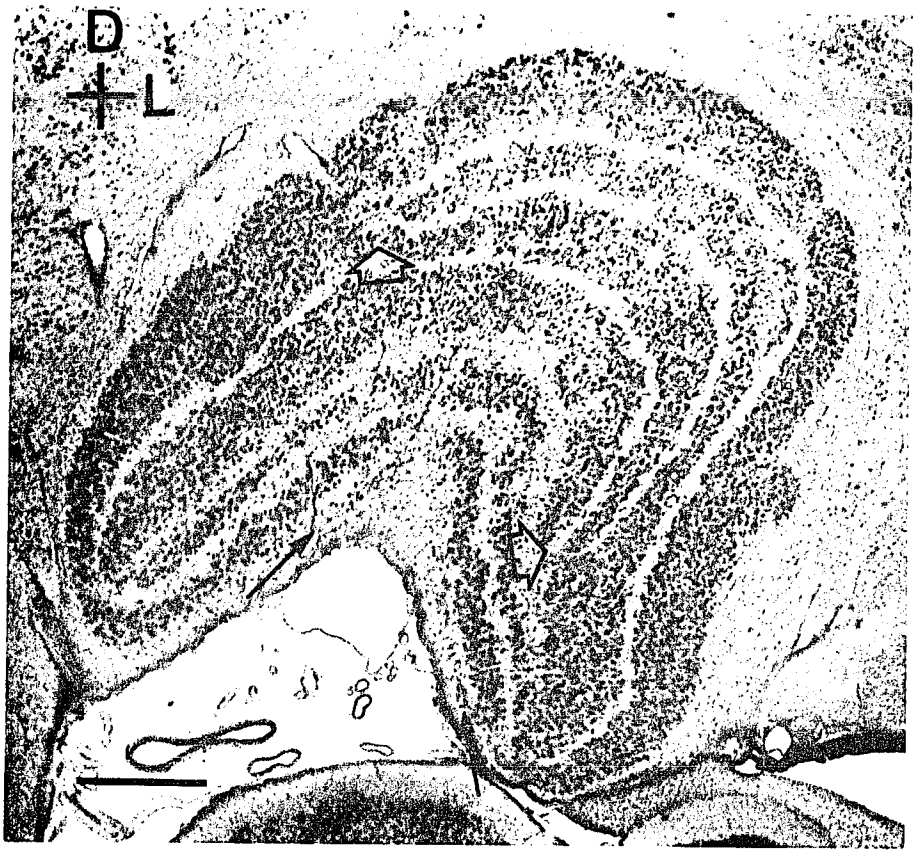
The dorsal lateral geniculate nucleus (LGNd) is an essential component of the mammalian visual system that receives the main output from the retina and projects directly to the striate cortex. In the monkey it exhibits some characteristic features:

a) Lamination: the LGNd is a clearly laminated structure (Fig.1), with six cellular layers present at least in its 2/3 caudal portion. They are conventionally labeled 1 to 6 from ventral to dorsal. The two ventral or magnocellular layers are composed of cells whose maximal diameter varies from 28-36 μm; the four dorsal or parvocellular layers contains smaller cells, with a maximal diameter that does not usually exceeds 20 μm [Le Gros Clark 1941; Szentágothai 1973; Jones 1985b; Kaas 1986]. This lamination can be noticed even with the naked eye by the trained observer, which allows the dissection of individual layers under low magnification (10X) for biochemical analysis.

FIGURE 1

Nissl section of the monkey LGNd

Coronal section through the middle of monkey LGNd showing the organization of cell laminae (dark bands). The two ventral magnocellular laminae (1 and 2) and the four dorsal parvocellular laminae (3-6) are self evident. Note also the areas of continuity between laminae 3 and 5, and 4 and 6 indicated by open arrows, and the thin laminae S (arrows). D, dorsal; L lateral. Cresyl violet stain. Scale 1 mm (corrected for shrinkage) [From Pasik and Pasik 1987].


D
L

b) Retinal projections: parvocellular layers 6 and 4, together with magnocellular layer 1 receive inputs from the nasal retina of the contralateral eye. Inputs from the temporal retina of the ipsilateral eye terminate in layers 2, 3 and 5. Each laminae, therefore, carries a representation of the contralateral visual field. This segregation of ocular projections into ipsilateral temporal retina and contralateral nasal retina occurs at the level of the chiasm. The optic tract and subsequent caudal structures will carry fibers according to this segregation [Le Gros Clark 1941; Szentágothai 1973; Jones 1985b; Kaas 1986].

c) Projection columns: groups of cells in each lamina that receive input from the same region of the visual field and project to the same cortical areas stand in register, that is, in continuous columns along all the six laminae [Le Gros Clark 1941; Szentágothai 1973; Jones 1985b; Kaas 1986]. This columns correspond to the "lines of projection" defined electrophysiologically in the cat [Bishop 1962].

d) Segregation of X- and Y-retinal ganglion cell inputs into different laminar types: there are two basic classes of retinal ganglion cells, originally classified according to their electrophysiological responses to grating stimuli [Enroth-Cugell and Robson 1966; for reviews see Stone et al. 1979, and Stone and Dreher 1982; see also Lennie 1980, and Wiesel and Hubel 1966]. The X-cells comprise a system with properties which include: (1) sustained response to a standing

contrast, (2) linear summation to stimulus intensities, (3) highly tuned response to selective bands of spatial frequencies, (4) color sensitivity, (5) slow axonal conduction velocity. These electrophysiologic properties correlated well with anatomic features, such as: (1) medium size somata, (2) small dendritic fields, (3) predominance in central retina, and (4) axonal termination in the parvocellular laminae of the LGNd. The segregation of the X-system remains in the geniculocortical projection, terminating in layers 4A and 4C-beta.

The Y-cells have opposite properties to the X-cells, namely: (1) transient response to a standing contrast, (2) non-linear summation, (3) preferential response to low frequencies, (4) insensitivity to color, (5) fast axonal conduction velocity, (6) large somata, (7) large dendritic fields, (8) predominance in the peripheral retina, (9) axonal termination in the magnocellular laminae of the LGNd and in the superior colliculus. The LGNd magnocellular laminae project to area 17 (layer 4C-alpha) and area 18 of the visual cortex.

There is the possibility that the X- and Y- inputs to the LGNd differ not only in their anatomical and physiological properties, but also in their biochemical characteristics. Since these two systems remain segregated into the parvocellular and magnocellular layers of the LGNd, it is possible to analyze them separately and determine if they

carry different neurotransmitters. This type of analysis will constitute one of the main objectives of the present study.

In addition to the X- and Y- systems, a W- system of cells has been described. Apparently, it projects only to the superior colliculus, and its properties will not be reviewed here [see Stone et al. 1979, Stone and Dreher 1982].

e) Cell types: on the basis of Golgi impregnated material in monkey, the LGNd cells can be classified into Golgi type I neurons or principal cells (P-cells), with a long axon essentially projecting outside the nucleus, and Golgi type II neurons, also called interneurons (I-cells), with short axons arborizing in the immediate vicinity of the cell body [Pasik et al. 1973; Saini 1981; for review see Jones 1985a].

These two different neuronal cell types can be recognized also at the ultrastructural level [Le Vay 1971; Wong-Riley 1972; Pasik et al. 1973; Hâmori et al. 1983]. The P-cell has a globular or occasionally ovoid soma and is characterized by its variable size (14-30 μm generally), a central nucleus without indentations, a cytoplasm rich in organelles, numerous and large mitochondria of medium density, and abundant polyribosomes that are compactly arranged, giving the perikaryon a dense appearance. This type represents the majority of the neuronal population, and it shows consistent acute chromatolytic alterations after complete excision of the visual cortex, strengthening the evidence that these are projection neurons [Hâmori et al. 1983]. The I-cell possesses

an ovoid shape and small soma. The nucleus is large, eccentric and with indentations facing the major portion of the perikaryon. This type of cell has a pale matrix, and mitochondria are dense and much smaller than in the P-cell. Characteristically present in the perikaryon of this type of cell are clusters of small pleomorphic synaptic vesicles. Furthermore, the cell membrane can become presynaptic at any location (cell body, dendrites, axon initial segment and axon terminals) [Hámori et al. 1978].

f) Neuropil: several boutons may be recognized in the LGNd neuropil [Guillery and Colonnier 1970; Guillery 1971; for review see Jones 1985b]:

1) RLP terminals: these are large (L) terminals containing round (R) vesicles and pale (P) mitochondria. They are identified as optic terminals since many of their axons degenerate after removal of one eye [Colonnier and Guillery 1964; Pecci Saavedra et al. 1969]. These terminals are presynaptic to dendrites, dendritic spines, F terminals and occasionally to cell bodies. They have never been seen postsynaptic to other axon terminals.

2) RSD terminals: these are small (S) terminals with round (R) vesicles and dense (D) mitochondria that originate mostly from corticogeniculate fibers.

3) F terminals: they contain flattened (F) vesicles; these vesicles may be found tightly or loosely bound, and are classified accordingly as F1 and F2 terminals, respectively.

In general, F2 terminals are postsynaptic to RLP or to F1 or F2 terminals, in the form of serial synapses. F boutons may belong to Golgi type II neurons.

The three types of terminals described above can be found associated in several combinations [Guillery and Colonnier 1970; Hamori et al. 1974; Hamori et al. 1976; Pasik et al. 1976; for review see Jones 1985a]:

1) Triadic synaptic arrangement: this is by far the most characteristic arrangement found in the LGNd, and in the thalamus in general. It is defined by the presence of three contacts: 1) a retinal axon terminal (RLP) presynaptic to a P-cell dendrite, 2) the same retinal axon terminal presynaptic to an interneuron dendrite, and 3) this interneuron dendrite presynaptic to the same P-cell element (Fig.2).

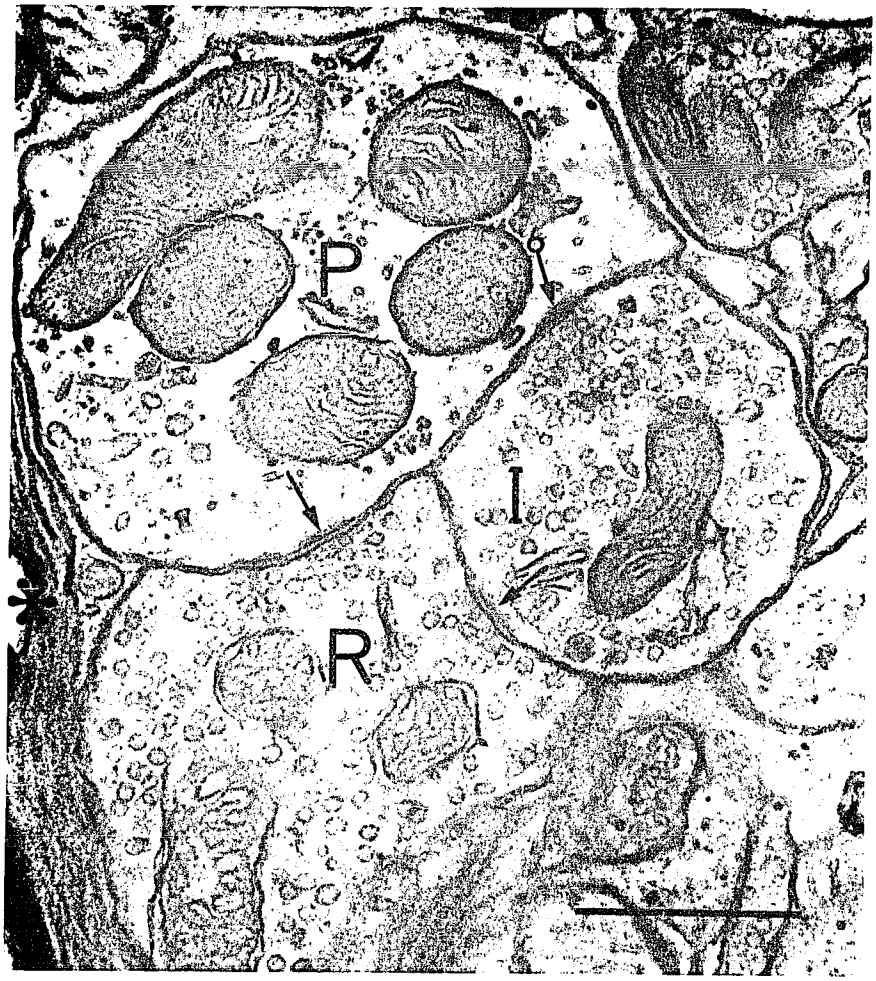
2) Serial synapse: this is another synaptic arrangement commonly found in the LGNd, where the dendrite (or soma) of the interneuron is postsynaptic to an axon (e.g., retinal axon) or to another I-cell dendrite, and becomes itself presynaptic to a third element, that can be a dendrite or a soma (e.g., of a P-cell).

3) Reciprocal synapse: this is an arrangement only rarely found, where a profile is presynaptic to an I-cell dendrite, which is in turn presynaptic to the same original profile [Pasik et al. 1986b].

FIGURE 2

Triadic synaptic arrangement in the monkey LGNd

A retinal terminal (R) is presynaptic at arrows to a P cell dendrite (P) and an I cell dendrite (I). The I cell dendrite, in turn, is presynaptic at ringed arrow to the same P cell dendrite. Partial glial wrappings indicated by asterisk. This arrangement is characteristic of the LGNd synaptic organization. Scale: 0.5 μm. [From Pasik and Pasik 1987].


P
6
I
R

1.2 DEGENERATIVE CHANGES

Since the present study is concerned with biochemical changes in the LGNd following optic tract section, it is pertinent to review here the possible morphological counterparts.

Following axotomy, either by eye enucleation or by optic nerve or optic tract section, several structures are affected, including the retinal ganglion cells (retrograde degeneration), optic axons and terminals (anterograde degeneration), as well as the neurons (transneuronal degeneration), and glial cells of the LGNd. In fact, this nucleus has been a preferred model to study the changes that occur as a consequence of axotomy [for reviews see Glees 1968; and Cowan 1970].

Several factors influence the severity and temporal course of the degenerative changes. These include the animal species, the developmental stage, the location of the lesion, and the survival time.

a) Species: the LGNd is perhaps one of the best examples to illustrate differences in the reactivity of neurons to deafferentation in adult animals of different species. Eye enucleation in primates leads within a few days to transneuronal changes in the cells that are in contact with degenerating axon terminals, whereas in cats and rabbits the alterations begin to be noticed not until about two and six months, respectively, and in rats no detectable changes appear to occur in neuronal somata ten months after enucleation. These results may reflect subtle differences in the

connectivity patterns which are present in the LGNd of different species [for review see Cowan 1970].

2) Developmental stage: lesions in immature animals produce earlier onset and greater severity of transneuronal modifications than in fully developed subjects. When deafferentation is carried out before or during a critical period, when normal synaptic connections are being established, the geniculate neurons may die. This may occur because the development of a full set of synaptic contacts provide the cells with factors that help in their survival when one of the inputs is eliminated [Cowan 1970].

c) Location of the lesion: optic tract lesions produce in general an earlier onset of retinal terminal degeneration as compared to enucleation. The variation in the temporal course of the alterations has been explained in terms of the differences in the amount of axoplasmic materials that are available in the distal portion of the axon for the maintenance of normal structure and function. In the cat it has been estimated that synaptic transmission can be maintained about 12 hours for each centimeter of remaining optic fibers attached to the terminals [Pecci Saavedra 1971]. The degree of transneuronal degeneration can be less dramatic in cats with a "balanced" lesion resulting from optic tract section or from bilateral enucleation, than in animals with section of one optic nerve or unilateral enucleation. Although the mechanism is not clear, interlaminar interactions

in the monocular and binocular segments of the LGNd may be a part of the reason [Garey et al. 1976; Ghetti et al. 1972].
d) Survival time: this factor plays a clear role in the magnitude of the alterations observed, so that the longer the animal is allowed to survive, the further progress in the sequential stages of degeneration will be noted.

All of the above factors must be kept in mind when interpreting the results from different sets of experiments.

In the next section, the time course of the changes that develop during anterograde and retrograde degeneration will be described in some detail because of their relevance in the experimental design of this study.

a) Anterograde degeneration:

Most experimental studies of LGNd degeneration have been carried out in an effort to identify the morphologic features of the retinal terminals and the type of synaptic contacts they establish, as well as to illustrate the morphological changes leading to the final phagocytosis of the terminals by glial cells.

A classical investigation of the anterograde alterations that take place in the LGNd has been performed in the cat [Szentágothai et al. 1966]. In primates, several studies of this nature exist [Glees and Clark 1941; Glees and Hasan 1968; Pecci Saavedra et al. 1969; Cowan 1970; Le Vay 1971; Pecci

Saavedra et al. 1971; Ghetti et al. 1972; Wong-Riley 1972; Wisniewski et al. 1972; Novotny 1979, 1980 a,b].

The changes occurring in retinal terminals following optic deafferentation have been grouped into three stages: (1) neurofilamentous hyperplasia; (2) electron-dense degeneration; (3) phagocytosis of terminals by glial cells.

The stage of neurofilamentous hyperplasia is observed as early as two days after enucleation in the squirrel monkey [Wong-Riley 1972], and it is characterized by the appearance of neurofilaments within the terminal. The synaptic vesicles are pushed to the periphery of the bouton, and their numbers decrease. The mitochondria, synaptic junctions and myelinated axons appear normal. By the fourth to sixth day the neurofilaments increase largely in number and are in a clear stage of hyperplasia. The number of vesicles continue to decrease and the mitochondria begin to disintegrate. Some synaptic junctions start to show signs of detachment. Hyperfilamentosis can still be seen in some boutons until the tenth to fifteenth day.

The stage of electron-dense degeneration, also called dark degeneration, begins at about the sixth day postoperatively, and becomes more prominent by the tenth to fifteenth day. The terminals turn highly electron dense, and gradually diminish in size. All organelles disintegrate into dense fragments which are scattered within the dark matrix. The synaptic junctions are sometimes detached. In this stage,

degeneration of the axon in its preterminal myelinated portion can be observed.

Finally, in the stage of glial reaction, processes of astrocytes and phagocytic cells invade the synaptic complexes, gradually displacing the postsynaptic elements and ultimately engulfing the whole electron dense retinal terminal. This stage is already present at the peak of the dark degeneration (tenth to fifteenth day) and is active for as long as degenerating elements remain present.

b) Transneuronal degeneration:

It is well known that geniculate neurons suffer a process of transneuronal degeneration after optic deafferentation [Matthews et al. 1960; Glees et al. 1966; Glees et al. 1967; Pecci Saavedra et al. 1969; Le Vay 1971; Ghetti et al. 1972; Wong-Riley 1972; Wisniewski et al. 1972; Garey et al. 1976; Novotny 1980 a,b]. Morphologic changes were noted in the cebus monkey as early as two days after enucleation, but they were represented only by a slight increase in the number, size and variety of lysosomes [Pecci Saavedra et al. 1969]. In the macaque monkey, the earliest changes were apparent four days after enucleation [Wisniewski et al. 1972]. Alterations included dilation of the Golgi apparatus, distentions of the cisterns of the rough endoplasmic reticulum, and some dispersion of ribosomes. By the eleventh to twelfth day there was cytoplasmic vacuolization and infoldings in the nuclear

membrane [Le Vay 1971; Wisniewsky et al. 1972]. Two weeks after denervation there was a reduction in the size of the neurons [Mathews et al. 1960]. Actual cell loss could not be demonstrated in this study, even after four months, but other investigators have reported such a loss [Wisniewsky et al. 1972].

Some metabolic changes have been described in the LGNd of the monkey after enucleation. Apparently, there was an increase in the RNA level, together with an increase in glucose oxidation and protein synthesis during the first 1-2 days after optic tract section in the rhesus monkey [Kupfer and Downer 1967]. However, the RNA level was already 85% of control by the third day after denervation, and decreased to 50% of control by the fifth day. Subsequently. the decreases were more gradual, reaching steady values (30% of control) at 23 days after surgery. This lowering of RNA level correlated well with the decrease in the Nissl staining intensity observed at the light microscopic level. Accompanying this change there was a concomitant decrease in glucose utilization, which reflected a decreased metabolic capacity, and a reduction in protein synthesis.

The mechanism of transynaptic degeneration is not well understood. It is possible that damage to the cellular membrane of the terminal produces an increase in its permeability, allowing the efflux of substances from degenerating terminals that can prove toxic to the postsynaptic element.

It is possible also that during phagocytosis some elements of the postsynaptic membrane are disrupted, allowing the leakage of vital cellular components [Wisniewsky et al. 1972].

2. NEUROACTIVE SUBSTANCES IN THE LATERAL GENICULATE NUCLEUS

The identity of the optic nerve transmitter has been sought without success for many years. All the substances known to play a neurotransmitter role in the central nervous system have been examined in the retinogeniculate pathways according to the criteria postulated by several investigators [Werman 1966; Wilkins and Watkins 1981]. Presently, the amino acids glutamate and aspartate have received the strongest support. Glutamate is also a major candidate as corticogeniculate transmitter. Other substances, such as acetylcholine, serotonin and norepinephrine, seem to influence LGNd activity mainly through ascending pathways originating in the brain stem.

The purpose of the present section is to review the neurotransmitter systems operating in the monkey LGNd, with special emphasis on the retinogeniculate pathways.

2.1 AMINO ACIDS

A) GLUTAMATE AND ASPARTATE

The most important excitatory amino acids in brain are L-glutamate (GLU) and L-aspartate (ASP) [Curtis and Johnston 1974]. Other naturally occurring substances with similar properties include L-cysteate, L-homocysteate, L-cystein-sulphinate and L-homocystein-sulphinate; the latter are

sulphur-containing amino acids present in brain in concentrations 100-1000 times lower than GLU, and their properties as neuroactive compounds are being investigated [Curtis and Johnston 1974; Cuénod et al. 1986].

Glutamate is the most abundant amino acid in the adult CNS, with an average concentration of 5-10 μmol/g wt (approximately 10 mM) [Krnjevic 1974]. ASP has a concentration 3-4 times lower than GLU, but it is still one of the amino acids in highest concentrations in the brain, together with taurine, glutamine and GABA. The concentration of these amino acids in the visual system of several species is presented in Table 1. GLU shows a rather homogeneous distribution, except in the optic nerve, where it may be significantly lower. For example, in the cat it has been observed that the concentration of GLU in the optic nerve is significantly low in relation to 12 gray matter structures [Johnson and Aprison 1971]. One of the findings of the present study will offer a comparison on the levels of several amino acids along the primary visual pathways in the monkey (see Results). Due to the "low" levels of GLU in the optic nerve, it has been argued that it may not be the optic nerve transmitter [Tebecis and DiMaria 1972; Tebecis 1974]. However, it may be pointed out that the concentration of GLU in the optic nerve (3-5 μmol/g wt, 3-5 mM) is high compared to other neurotransmitters in brain (0.3 mM for free ACh, [MacIntosh 1981]); this concentration may even rise a thousand fold in the nerve terminal if

TABLE 1
AMINO ACID CONCENTRATION IN THE VISUAL SYSTEM (REVIEW)

SPECIES	TAU	ASP	GLU	GLN	GLY	ALA	GABA	REF
I. OPTIC NERVE OR OPTIC TRACT								
Pigeon	3.0	1.1	4.6	2.5	0.5	0.5	0.1	5
Rabbit	3.7							16
Cat			3.5					7
II. LATERAL GENICULATE NUCLEUS								
Rat	3.4	2.0	12.0	4.0	1.2	1.0	2.6	8
Rabbit							2.3	9
Rabbit		5.3	5.9	3.5		1.0	3.2	2
Cat	4.3							6
III. SUPERIOR COLLICULUS (OPTIC TECTUM)								
Frog		0.6	3.0	1.8	0.6	0.3	1.3	14
Pigeon	1.9	3.2	8.0	5.0	0.7	0.6	3.3	1
Rat	2.8							10
Rat							7.7	9
Rat	3.1	2.5	11.2	4.1	1.8	1.2	6.1	8
Rabbit		3.8	3.5	3.7		0.6	4.3	2
Rabbit	1.5							16
Rabbit							4.9	9
Cat			8.0					7
Monkey							4.2	9
Monkey							4.2	4
IV. OCCIPITAL CORTEX								
Rat		1.6	7.9		1.1	0.6	1.7	11
Rat	7.4							10
Rabbit		3.2	7.7	4.0		0.8	3.9	2
Cat	1.6	1.7	5.9	4.0	0.6	0.6	1.0	13
Cat	2.1							6
Monkey	2.4							17
Human		2.2	5.7				1.3	15
Human	1.1	2.6	8.6	3.7	1.8	1.3		12
Human	0.7	2.4	6.9	1.3			0.9	3

TABLE 1

Amino acid concentration in the visual system (review)

Values are expressed in μmol/g wt. When the original values were expressed in μmol/g protein, it was assumed that 1 g wet wt. = 0.1 g protein [Sheridan et al. 1967]. References: (1) Beart [1976]; (2) Davis et al. [1969]; (3) Ellison et al. [1987]; (4) Fahn and Côté [1968]; (5) Fonnum and Henke [1982]; (6) Guidotti et al. [1972]; (7) Johnson and Aprison [1971]; (8) Lund Karlsen and Fonnum [1978]; (9) Okada et al. [1971]; (10) Palkovits [1986]; (11) Peinado and Mora [1986]; (12) Perry et al. [1971]; (13) Perry et al. [1972]; (14) Roberts and Yates [1976]; (15) Sasaki et al. [1986]; (16) Sturman [1979] (value above is the average of two samples); (17) Volpe and Laster [1970].

the substance is stored inside synaptic vesicles (200 mM for ACh).

It has been shown that when a particular substance is associated with a specific pathway in the brain, its concentration decreases in the terminal region after the pathway is severed. For example, a significant decrease in GLU content has been demonstrated in the termination field of the corticostriate, entorhinal-hippocampal and cerebellar granule cells after these elements were lesioned [McGeer and McGeer 1981]. The interpretation of lesion-induced decreases in levels, however, must be done with caution. Some of these changes may occur as a result of disturbance of metabolic activity and/or the increase in the proportion of glia relative to neurons. Nonetheless, when this criterion is used in combination with others, especially physiological or pharmacological in kind, it has proven very useful to suggest neurotransmitter candidates.

In the frog optic tectum (the main receiving structure of optic fiber terminals), unilateral enucleation results in a significant reduction in GLU concentration as early as eight days after surgery, with a maximum of 40% decrease five weeks later; ASP concentration, however, does not change significantly [Roberts and Yates 1976]. In the pigeon optic tectum, an even higher decrease in GLU (75%) is observed after retinal ablation, and ASP levels are also significantly reduced. These amino acids decrease in the optic nerve as

well, two weeks after ablation [Fonnum and Henke 1982], but not after one week [Beart 1976].

The criterion of decrease in concentration after lesion has not been documented for the retinogeniculate pathways in mammals. The only study close to this objective reported that ablation of the visual cortex in rat produced a moderate reduction (32%) in GLU levels in the LGNd, and a lesser one in the superior colliculus; other amino acids, including ASP, remaining unchanged [Karlsen and Fonnum 1978]. It is possible that the portion of GLU still present belonged to other geniculate components, including retinal terminals. The present study provides results bearing on this issue in the visual system of the monkey.

Immunocytochemical studies have provided some of the strongest evidence to support the role of amino acids as neurotransmitters. The first studies on the direct visualization of GLU/ASP in the brain were carried out by Storm-Mathisen et al. [1983]. Initially, it was expected that GLU and/or ASP would occur in all neurons regardless of the transmitter they use, since the bulk of these amino acids participate in the intermediary metabolism, or in protein synthesis and degradation. What has been observed however, is a considerable variation in the levels of immunoreactivity among neurons, which are very low in cell groups thought to use GABA for neurotransmission, and intermediate to high in populations of putative GLU/ASP-ergic, cholinergic and

dopaminergic cells [Ottersen and Storm-Mathisen 1986]. In the LGNd of the mouse and rat, weak GLU-like immunoreactivity has been observed at the light microscopic level [Ottersen and Storm-Mathisen 1984]. However, it is considered that the GLU antisera is not specific for glutamergic elements, since it apparently does not discriminate between the "transmitter pool" and the "metabolic pool" of GLU under the experimental conditions employed.

In the search for specific biosynthetic markers for GLU/ASP-ergic neurons, the interest has focused on glutamate dehydrogenase (GDH, formation of GLU from alpha-ketoglutarate), aspartate aminotransferase (AAT, interconversion of ASP and GLU) and Glutaminase (formation of GLU from glutamine). GDH is rich in optic and auditory nerves compared to other cranial nerves, such as the trigeminal and facial nerves. [Wenthold 1980]. The first two carry mostly sensory fibers, while the other two contain mixed sensory and motor fibers. The level of AAT has been more correlated with metabolic activity than with transmitter identity. Moreover, it has been suggested that this enzyme and cystein-sulphinic acid transaminase may be the same protein [Ottersen and Storm-Mathisen 1986]. Glutaminase is particularly high in the auditory nerve [Wenthold 1980], but the significance of this enzyme as a marker is not clear, since it is abundant in glia [Fonnum et al. 1986]. Glutaminase has also a rather uniform distribution in brain; in particular, it does not show a high

concentration in hippocampal mossy fibers, which is one of the systems where GLU is a strong neurotransmitter candidate [Ottersen and Storm-Mathisen 1986]. However, in conjunction with other methods, it may serve as an indicator of transmitter identity. Nevertheless, none of these markers have been studied in the LGNd.

The vesicular storage of excitatory amino acids has been a long sought process [Watkins and Evans 1981]. Preliminary electron microscopic visualization of direct GLU-like immunoreactivity in the hippocampus suggests that this amino acid is significantly concentrated in synaptic vesicles [Storm-Mathisen et al. 1983]. Unfortunately, ultrastructural studies of this nature in the LGNd are lacking at this time.

For a substance to be established as a neurotransmitter, it is important to demonstrate its release upon nerve stimulation or by K^+-induced depolarization in a calcium-dependent manner. It has been shown that GLU and ASP are released in the optic tectum of the pigeon following optic nerve stimulation [Canzek et al. 1981]. Moreover, increased amounts of these amino acids have been collected in dialysates of the cat LGNd after local electrical stimulation [Sandberg and Lindstrom 1983]. However, in these latter experiments, increased amounts of GABA were collected also. Since this amino acid is not known to be present in optic fiber terminals [Pasik et al. 1986], it must have originated from stimulation of terminals from other sources, such as the thalamic

reticular nucleus and geniculate interneurons, which are known to be gabaergic (see section on GABA below). Therefore, the specificity of the release of GLU and ASP after LGNd stimulation remains unclear.

It is generally accepted that reuptake from the extracellular space is an important process by which transmitter amino acids are removed from the vicinity of their receptors. It is thought that the sodium-dependent high affinity uptake system for GLU/ASP occurs selectively in neurons using these substances as transmitters [Curtis and Johnston 1974]. If presynaptic terminals are caused to degenerate, reuptake from the synaptosomal fraction is expected to be depleted. Although uptake measurement is a very sensitive marker, decreasing almost twice as compared to the respective changes in amino acid content [Ottersen and Storm-Mathisen 1986], it presents some disadvantages. The transport system does not distinguish between L-GLU, L-ASP and D-ASP, and uptake also occurs in glial cells [Ottersen and Storm-Mathisen 1986]. The screening capability of the uptake techniques is limited, having to test each amino acid individually. Despite all these restrictions, this type of analysis has provided very useful insights into the transmitter role of GLU/ASP in the brain.

The visual system has not been very fortunate in this respect. The effects of enucleation on GLU uptake have been studied in the optic tectum of the frog and the pigeon

[Roberts and Yates 1976; Beart 1976], as well as in the LGNd of the rat [Karlsen and Fonnum 1978], where no significant decreases were observed. However, in the chick optic lobe a 32% decrease in total GLU uptake was observed, 23 days after enucleation [Bondy and Purdy 1977]. In addition, cortical ablation in adult rats, as opposed to newborns, does produce a large reduction in GLU/ASP uptake in the LGNd [Karlsen and Fonnum 1978; Kvale and Fonnum 1983]. The remaining portion of the uptake may represent its presence in other geniculate components, including retinal terminals.

Another possible mechanism by which GLU may be inactivated is via decarboxylation. Consistent with this idea is the finding that thiosemicarbazide, a glutamate decarboxylase inhibitor, enhances the action of iontophoretically administered GLU onto thalamic cells in the rat [Steiner and Ruf, 1966; Phillis 1971].

The excitatory properties of GLU and ASP on geniculate neurons have been known for many years [Curtis and Davis 1962, 1963; Phillis 1971; Curtis and Johnston 1974]. The principal characteristics of GLU action are its quick onset and an almost instantaneous cessation. With these characteristics, it is ideally suited to be a transmitter at geniculate synapses [Phillis 1971; Krnjevic 1974]. Under optimal conditions, cells can be excited with as little as 10^{-15} molar. The depolarizing action of GLU is caused by an increase in membrane permeability that enhances sodium conductance. The

effects produced by ASP are similar, and some cells respond even more vigorously to ASP than to GLU [Morgan et al. 1972].

Electrophysiological studies indicate that the excitatory action of GLU is mediated by multiple receptors. Four receptors have been characterized by their selective sensitivity to N-methyl-D-aspartate (NMDA), kainic acid (KA), quisqualic acid (QA) and 2-amino-4-phosphonobutyric acid (APB). Another classification is based on their ionic dependence, by which GLU receptors are grouped into (1) sodium-dependent, (2) sodium-independent, chloride/calcium-dependent, and (3) sodium-independent, chloride/calcium-independent [for reviews see Cotman et al. 1987; Foster and Fagg 1984; Monaghan et al. 1983]. The sodium-dependent GLU binding sites may represent the recognition sites for an acidic amino acid membrane transport system. Pharmacological analyses show that chloride/calcium-dependent binding sites cannot be classified as NMDA, QA or KA receptors, but may be equated with the APB-sensitive class of glutamate receptors. Less information is available by which to assign a role to chloride/calcium-independent glutamate binding sites; one possibility is that at least some of these sites may represent NMDA receptors.

Several ligands and experimental conditions have been employed in an effort to differentiate the various subtypes of GLU-related receptors. ^{3}H-GLU labels the major subclasses of NMDA, quisqualate, kainate and APB sites. If binding of

^{3}H-GLU is displaced by NMDA, an estimate of the distribution of the latter receptor type can be obtained. ^{3}H-AMPA (amino-3-hydroxy-5-methyl-4-isoxazolepropionate) has higher selectivity for quisqualate sites, and ^{3}H-KA labels preferentially its own class of receptors. When binding of any of these ligands is performed in sodium, calcium or chloride free medium, their ionic dependence can be determined.

The distribution of GLU-related receptors in the visual system of several species is summarized in Table 2. In the monkey, the LGNd has a low density of ^{3}H-GLU binding sites, with no preferential distribution over the magnocellular or parvocellular segments [Shaw and Cynader 1986]. In the rat, the LGNd has moderate to low levels of GLU-related receptors, either NMDA displaceable [Monahan and Cotman 1985], AMPA [Rainbow et al. 1984; Monahan et al. 1984], or sodium-independent ^{3}H-GLU sites [Halpain et al. 1984]. In comparison to the superior colliculus and the striate cortex, the rat LGNd shows the highest levels of sodium-independent ^{3}H-GLU binding sites [Schliebs et al. 1986]. The dorsolateral thalamus of the pigeon, which is the equivalent to the mammalian LGNd, has high levels of ^{3}H-KA binding, in reference to the paleostriatum [Vischer et al. 1982].

The superior colliculus has a slightly lower proportion of GLU-related receptors with respect to the LGNd. In the rat, the levels of NMDA displaceable sites are about one-half of those found in the LGNd [Monahan and Cotman 1985], and the

TABLE 2
GLUTAMATE-RELATED BINDING IN THE VISUAL SYSTEM (REVIEW)

BINDING	SPECIES	LGN	SC	VCTX	REF
^{3}H-Glu	monkey	low		high	6
NMDA displaceable	rat	35%	18%		3
^{3}H-AMPA	rat	15%		30%	4
^{3}H-AMPA	rat	11%			2
^{3}H-KA	pigeon	65%	59%	57%	7
Na^+-independent	rat	30%	29%	41%	1
Na^+-independent	rat	100%	39%	15%	5

Abbreviations: (LGN) lateral geniculate nucleus; (SC) superior colliculus (monkey, rat) or optic tectum (pigeon); (VCTX) visual cortex. Percentages are relative to the richest area reported in the study; this area is indicated in parenthesis (below), together with the corresponding level of binding, expressed in fmol/mg protein. References: (1) Halpain et al. [1984] (neocortex, 434); (2) Monahan et al. [1984] (hippocampus, 213); (3) Monahan and Cotman [1985] (hippocampus, 1285); (4) Rainbow et al. [1984] (hippocampus, 1257); (5) Schliebs et al. [1986] (LGN, 2155); (6) Shaw and Cynader [1986] (non-quantitative); (7) Vischer et al. [1982] (paleostriatum, 332).

levels of sodium-independent sites are almost equal [Halpain et al. 1984] or less than half [Schliebs et al. 1986]. In the pigeon, the levels of KA sites are approximately the same in both regions [Vischer et al. 1982].

The visual cortex, conversely, tends to have a larger proportion of GLU-related receptors than the LGNd. In the monkey, high density of ^{3}H-GLU is observed in layers 1-4A, and moderate label in layers 4C-6, with a gap in layer 4B [Shaw and Cynader 1986]. In the rat, twice as many AMPA sites are found in the striate cortex [Rainbow et al. 1984], and the levels of sodium-independent sites range from 136% [Halpain et al. 1984] to 15% [Schliebs et al. 1986] relative to the geniculate nucleus. In the pigeon, the density of KA sites is 88% of that found in the dorsolateral thalamus [Vischer et al. 1982].

The most conclusive evidence in support of excitatory amino acids as optic nerve transmitters has derived from the use of competitive antagonists which block the excitatory postsynaptic potential (EPSP) generated by visual stimulation. D-alpha-aminoadipate (DaAA), which is thought to interact preferentially with the NMDA receptors [Evans et al. 1978], effectively depresses the response elicited by photic or GLU stimulation when applied iontophoretically to cells of the cat LGNd [Kemp and Sillito 1982]. Gamma-D-glutamylglycine (DGG), another excitatory amino acid antagonist, reversibly blocks the optic nerve evoked EPSP and the depolarization elicited

by GLU or quisqualic acid in rat LGNd slices [Crunelli et al. 1987]. Because D-2-amino-5-phosphonovalerate (APV), a potent and selective NMDA antagonist, has no effect on the EPSP, and because of the competitive nature of the antagonism evoked by DGG, it is suggested that the type of receptors activated are of the QA/KA type. On the basis of the results with DaAA, it appears that a real difference in species exists, with the transmitter of the cat optic nerve acting on NMDA receptors and that of the rat on quisqualate/kainate receptors [Crunelli et al. 1987]. Additional support for the possibility that GLU acts as an excitatory transmitter in the optic nerve has derived from studies in the pigeon, where the alkaloid nuciferine (L-5,6-dimethoxyaporphine, a GLU/ASP antagonist [Ben-Ari and Kelly 1975]) and L-glutamate diethylester (GDEE) depressed the synaptic response specifically [Wang et al. 1978]. In the cat, however, GDEE proved to be of little selectivity [Kemp and Sillito 1982].

It has been observed that tryptamine derivatives, including lysergic acid diethylamide (LSD) and serotonin, decrease the response of geniculate cells to visual stimulation, even at doses that have little effect on the excitatory action of amino acids [Bishop et al. 1959; Curtis and Davis 1962; Freund 1973; Tebecis and DiMaria 1972; Tebecis 1973, 1974]. Some of these authors have argued that the inhibitory action of serotonin and LSD can be at the postsynaptic level, or at the level of synthesis and/or release of the optic nerve

transmitter [Tebecis and DiMaria 1972; Tebecis 1973, 1974]. They have added that if the action is at the postsynaptic level, the optic nerve transmitter may not be an excitatory amino acid (but rather a substance related to serotonin); however, if the inhibitory action is at the level of synthesis or release of the transmitter, this may still be an excitatory amino acid. To this reasoning it may be added that the depressant effect of serotonin and LSD can be at the postsynaptic level, and still an excitatory amino acid can be the retinogeniculate transmitter. This can be possible if the postsynaptic element contains receptors for both serotonin and GLU. The effect of serotonin, acting on its own receptor, may be to depress the general excitability of the postsynaptic element, so that it becomes refractory to the excitatory effect of the optic nerve transmitter (see section on Serotonin below).

In summary, the presence of GLU/ASP has been demonstrated in the optic nerve and LGNd (or optic tectum) of several species. A reduction in their concentration after lesion of the retinogeniculate (retinotectal) pathways has been documented only in the frog and the pigeon. Studies of this sort are not available in mammals.

The visualization of presumed glutamergic terminals by direct GLU/ASP antibodies has not been possible; conditions for the specific localization of the "transmitter pool" of these amino acids are being developed. Ultrastructural

studies under these conditions would help to document the vesicular storage of these amino acids.

There are no specific biosynthetic markers for GLU/ASP neurons. Glutamate dehydrogenase and glutaminase, in combination with other methods, may serve as indicators of transmitter identity.

The release of GLU/ASP upon nerve stimulation or chemically induced depolarization has been proven in the pigeon optic tectum. In the cat LGNd the specificity of the release remains uncertain.

A decrease in the activity of the uptake system after enucleation or optic nerve lesion has been described in the chick optic tectum. Negative results were obtained in the frog, pigeon and rat. Cortical ablation in the rat does not eliminate completely GLU uptake in the LGNd. It remains to be determined if the residual portion is present in retinal terminals.

GLU/ASP are excitatory on LGNd neurons, with quick onset of action and rapid cessation. GLU action is blocked competitively by specific antagonists, such as DaAA in the cat, DGG in the rat, and nuciferine and GDEE in the pigeon. Inconsistent results with these antagonists may point out to real species differences.

GLU-related receptors (NMDA, QA, KA and Na^+-independent sites) are present in the LGNd of the monkey and the rat at moderate to low levels. A portion of the Na^+-independent

sites measured in rat may represent NMDA or APB-sensitive receptors.

The depressant action of serotonin and other tryptamine derivatives on the response of geniculate neurons to visual or GLU stimulation may be at the presynaptic and/or postsynaptic levels. The elucidation of the exact mechanism of action may be important in discovering the identity of the optic nerve transmitter.

In conclusion, GLU is one of the strongest candidates as neurotransmitter in the retinogeniculate pathways. It fulfills several of the necessary criteria, specially in the pigeon. Since there is concern about species differences in the identity of the optic nerve transmitter, it is important to document all the criteria in the same species.

B) GABA

Gamma-aminobutyric acid (GABA) is perhaps one of the best documented neurotransmitters in the LGNd. It participates in inhibitory mechanisms that are important to maintain the center-surround organization of the receptive fields, in binocular interactions and in directional selectivity bias [Sillito and Kemp 1983; Jones 1985c; Pape and Eysel 1986; Shotwell et al. 1986].

The levels of GABA are low in the optic nerve, moderate in the LGNd and visual cortex, and high in the superior colliculus (see Table 1). However, the significance of this

comparison needs to be demonstrated statistically. The present study will provide some results bearing on this issue, in the visual system of the monkey.

Retinal ablation or eye enucleation have produced significant losses of GABA in the optic tectum of the frog [Roberts and Yates 1976] and the pigeon [Fonnum and Henke 1982], 2-4 weeks after operation. However, in the pigeon no such changes have been observed after one week [Beart 1976]. In mammals, studies of this sort apparently have not been reported.

Of all the enzymes that are relevant in the metabolism of GABA, glutamic acid decarboxylase (GAD), which is responsible for the conversion of glutamate to GABA, has proved to be a specific marker to visualize the distribution of GABA in brain [Mugnaini and Oertel 1985]. In the LGNd, GAD-immunoreactivity is associated with small neurons that have the anatomical characteristics of interneurons [Ohara et al 1983, rat; Fitzpatrick et al. 1984, cat; Hendrickson et al. 1983, monkey]. More recently, immunocytochemical studies of the monkey LGNd have been carried out with direct GABA antibodies. These studies have confirmed previous findings about the gabaergic nature of geniculate interneurons. For example, it was shown that when horseradish peroxidase was injected in the visual cortex, none of the GABA (+) neurons appeared to be labelled [Montero 1986]. Moreover, at the ultrastructural level, stained profiles were observed forming

presynaptic contacts with triadic elements within glomeruli, and they had the morphologic characteristics of presynaptic dendrites of interneurons. Outside the glomeruli, additional labeled axon terminals probably derive from neurons of the thalamic reticular nucleus, which labels intensely with GABA antibodies [Pasik et al. 1986].

Immunocytochemical studies with direct antibodies have also been realized to estimate the proportion of gabaergic interneurons in the LGNd. It was found that they correspond to 22% of the total neuronal population of the LGNd, in the rat [Gabbott et al. 1986] and 27% in the cat [Madarasz et al. 1985]. In the monkey, different ratios of gabaergic cells have been found between the magno- and parvocellular layers. Figures, however, of 35% and 25%, respectively, reported in one study [Montero and Zempel 1986], appear too high in relation to other criteria [Hâmori et al. 1983]. The reason for this discrepancy is not clear, but part of it may be fact that only one animal was used in the first study.

Developmental investigations have been carried out at the biochemical and immunocytochemical levels in order to study the time course of appearance of gabaergic interneurons and their possible role in the formation of connections. McDonald, Speciale and Parnavelas [1981] found that the enzymatic activity of GAD in the rat LGNd reached a peak at postnatal day 24, and subsequently declined to reach steady levels from day 150 on. This correlated well with the

morphological appearance of interneurons, which reach maturity by the third postnatal week. In the cat, specific staining was seen as early as 2 weeks before birth, confined mainly to perigeniculate neurons [Shotwell et al. 1986]; staining of cells and terminals was not seen in the LGNd until birth; five weeks postnatally the first glomerular clusters of terminals could be seen; the pattern of immunostaining was almost adult-like by two months after birth, and the final pattern appeared one month later. Therefore, the first source of gabaergic inhibition seems to be provided by the PGN during fetal life, with the major portion of the inhibition supplied by intrinsic geniculate neurons after birth.

There appears to be no enzymatic mechanism for the rapid destruction of GABA extracellularly. However, it seems to be efficiently taken up into presynaptic endings by a sodium-dependent mechanism [see Cooper et al 1982]. The high affinity uptake of GABA has been measured in the optic nerve of the newborn chick, where it is 20 times more active than GLU-uptake [Bondy and Purdy 1977]. In the optic tectum of the adult frog this relationship is also observed, but to a lesser extent [Roberts and Yates 1976]. On the contrary, the activity of the GABA-uptake system is markedly lower than the uptake of GLU in the adult rat LGNd [Karlsen and Fonnum 1978; Kvale et al. 1983]. This probably reflects the higher contribution of corticogeniculate synapses in the LGNd, which are probably glutamergic. The presence of a high affinity

uptake system of GABA in the optic nerve is interesting, in view of the fact that this structure contains only small amounts of GABA (see Table 1) and that its presence has not been detected immunocytochemically in retinal terminals of the LGNd [Pasik et al. 1986; Montero 1986].

The uptake capacity for GABA is not affected in the LGNd of several species after eye enucleation [Bondy and Purdy 1977; Karlsen and Fonnum 1978]. However, in the optic tectum of the frog a marked reduction in the high affinity uptake of GABA was measured 41 days after surgery. This decrease was attributed to transynaptic effects following degeneration of the afferent nerve terminals. It is worth mentioning that removal of the visual cortex of adult rats does not affect the high affinity uptake of GABA in the LGNd and the superior colliculus [Karlsen and Fonnum 1978], supporting the idea that the reduction in GLU uptake seen after cortex ablation is not due to a general decrease in neuronal activity, and that GABA is localized in non-principal cells (interneurons).

The specific release of GABA after electrical stimulation has been difficult to demonstrate in the CNS [Cooper et al 1982], due to the fact that GABA is mostly associated with interneurons having short axons that terminate in the immediate vicinity of the region. Studies of this nature are specially lacking in the LGNd. In one report, increased levels of GABA were collected in dialyzates of the cat LGNd after local electrical stimulation, with a concomitant release

of GLU, ASP and TAU. The authors interpret these changes as representing a non-specific response caused by the massive activation of the geniculate area [Sandberg and Lindstrom 1983].

GABA has a widespread function in the brain as an inhibitory neurotransmitter. Electrophysiological and pharmacological studies indicate that this action is mediated by at least two types of receptors: $GABA_A$ and $GABA_B$ receptors [for review see Bowery et al. 1984]. $GABA_A$ receptors represent the "classical" bicuculline-sensitive sites that have high affinity for muscimol and isoguvacine; they exhibit allosteric sites for benzodiazepine and barbiturates, and increase chloride ion conductance upon activation. $GABA_B$ sites have been defined as bicuculline-, isoguvacine-insensitive, that are activated stereospecifically by (-)baclofen and inhibited allosterically by GTP; these receptors are calcium-, magnesium-dependent and decrease calcium ion conductance upon agonist binding; benzodiazepines and barbiturates have no effect on their properties.

Gabaergic synapses operating via the $GABA_A$ receptor induce an inhibitory postsynaptic potential (IPSP) that does not markedly hyperpolarizes the cell. That is, activation of the $GABA_A$ receptor produces a "silent" or "shunting" inhibition that short-circuits any excitatory input arriving during increased chloride conductance. On the other hand, $GABA_B$ receptors mediate a strong, long lasting hyperpolariza-

tion (IPSP), inhibiting the electrical activity of the cell by opposing the rise of the EPSP [for review see Sherman and Koch 1986].

Until more evidence is collected, it is considered that the majority of the gabaergic inhibition in the LGNd occurs via the $GABA_A$ receptor [see Sherman and Koch 1986]. In support of this idea are pharmacological studies showing that local injection of chloride into the relay cells reverses the IPSP generated by geniculate interneurons [Lindstrom 1982], and that application of bicuculline leads to loss of inhibitory mechanisms [Sillito and Kemp 1983; Berardi and Morrone 1984; see also Sherman and Koch 1986]. Autoradiographic techniques indicate that the monkey LGNd has a marked concentration of [^{3}H]muscimol and [^{3}H]flunitrazepam binding sites, specially in the magnocellular layers [Shaw and Cynader 1986]. The LGNd of the rat is one of the areas with highest levels of $GABA_A$ binding sites, which are labeled with either [^{3}H]muscimol [Palacios 1981], [^{3}H]GABA in the presence of baclofen (to inhibit interaction with $GABA_B$ sites) [Bowery et al. 1987], or with monoclonal antibodies to purified $GABA_A$/benzodiazepine receptor complex [Richards et al. 1987]. In the dorsolateral thalamus of the pigeon, high levels of muscimol binding have also been measured [Vischer et al. 1982].

As for the $GABA_B$ receptors, they seem to be fairly common in the mammalian brain, including the thalamus [Bowery et al.

1984, 1985]. In the LGNd of the rat, a high density of $GABA_B$ receptors was found, and the ratio of these receptors to $GABA_A$ receptors is close to unity. Since the majority of the gabaergic inhibition in the LGNd seems to occur via the $GABA_A$ receptor, as pointed out above, the role of $GABA_B$ receptors in this nucleus remains uncertain at this time. Studies performed in rat geniculate slices showed a long lasting, long latency hyperpolarization after stimulation of the optic tract, which was enhanced by baclofen (a $GABA_B$ agonist), and not blocked by bicuculline [Hirsch and Burnod 1987; Crunelli et al. 1988]. Geniculate interneurons are capable of generating this late IPSP, since it occurred in slices devoid of the influence of the thalamic reticular nucleus. Studies with phaclofen, a new selective $GABA_B$ antagonist, support these results, since this substance inhibits the K^+-dependent, long IPSP, leaving intact the early, brief, Cl^--dependent response to GABA [Soltesz et al. 1988]. It would be of interest to study the role of $GABA_B$ receptors in the superior colliculus, a structure where there is a greater proportion of $GABA_B$ to $GABA_A$ binding sites [Bowery et al. 1987].

In summary, GABA is present in the LGNd of several species at moderate levels. The superior colliculus may have a significantly higher concentration of GABA relative to other structures of the visual system, but this has to be confirmed statistically.

Retinal ablation or eye enucleation does produce a significant decrease in GABA concentration in the optic tectum of the frog and the pigeon. However, similar studies apparently are not available in mammals.

Glutamic acid decarboxylase (GAD) has proved to be a specific marker for gabaergic elements in brain. More recently, direct GABA antibodies have been used successfully to visualize GABA. In the LGNd, it is present mostly in interneurons, as well as in terminals from the thalamic reticular nucleus. Ultrastructurally, GABA has been observed participating in triadic synaptic arrangements within glomeruli.

A specific high affinity uptake system for GABA has been demonstrated in the optic nerve of the chick, in the optic tectum of the frog and in the LGNd of the rat. However, it seems not to be affected by enucleation, confirming the predominant role of GABA in intrinsic elements of the LGNd. Even more, the uptake of GABA is not affected after striate cortex ablation in rats.

The specific release of GABA in the LGNd has been difficult to demonstrate, mostly due to its major presence in short axon terminals of interneurons.

The inhibitory action of GABA is mediated primarily by $GABA_A$ and $GABA_B$ receptors. In the rat LGNd, these two receptors are found at high concentrations, and in a ratio close to unity. The majority of gabaergic inhibition in the

LGNd seems to be mediated by $GABA_A$ receptors, and the role of $GABA_B$ receptors in this structure is beginning to be comprehended.

C) GLYCINE

Glycine appears to play a role as inhibitory neurotransmitter in the CNS, although with a more circumscribed participation than GABA. Glycine is one of the amino acids that are in low concentration in brain, relative to glutamate. In the visual system, it seems to have a rather homogeneous distribution, with levels ranging between 0.5-1.3 μmol/g wt. (see Table 1).

The levels of glycine do not decrease significantly after lesion of the retinogeniculate pathways [Roberts and Yates 1976, frog; Beart 1976, pigeon; Fonnum and Henke 1982, pigeon; Karlsen and Fonnum 1978, rat]. In fact, this amino acid and alanine are often taken as reference to demonstrate that the reduction in glutamate or aspartate levels after the lesion has been specific.

Few other criteria for neurotransmitter identification have been documented for glycine in the geniculate nucleus. However, the presence of glycine receptors can be considered as a suitable indicator. Two types of receptors have been described for glycine: strychnine-sensitive and strychnine-insensitive [see Cotman et al. 1987]. The density of strychnine-sensitive glycine receptors is greatest in the grey

matter of the spinal cord, and it decreases progressively toward more rostral regions, so that the LGN has only traces of binding [Zarbin et al. 1981, rat]. In the pigeon, it was found that the density of binding was minimal in the dorsolateral thalamus [Vischer et al. 1982]. The distribution of strychnine-insensitive receptors is also not very striking in the thalamus and striate cortex of the rat [Bristow et al. 1986]. It remains to be established more specifically the amount of strychnine-insensitive binding sites in the lateral geniculate nucleus, their differential distribution among layers, and the proportion of them that are physiologically relevant before drawing any conclusions about the status of glycine as neurotransmitter in this nucleus.

2.2 PEPTIDES

Several neuropeptides have been described in the vertebrate visual system; they include: substance P, enkephalin, beta-endorphin, cholecystokinin, somatostatin, neurotensin, vasoactive intestinal polypeptide, neuropeptide Y, bombesin, glucagon, thyrotropin releasing hormone, avian pancreatic polypeptide and luteinizing releasing hormone. The following section reviews much of the evidence supporting the presence of these peptides in the visual system, with special attention to the retinal ganglion cell layer and the lateral geniculate nucleus.

A) SUBSTANCE P

Several lines of evidence indicate that substance P (SP) is associated with the retinogeniculate pathways in several species, possibly as a neurotransmitter or neuromodulator. First, the presence of SP has been demonstrated by several procedures (bioassay, radioimmunoassay, immunocytochemistry, high-pressure liquid chromatography) in the retina, optic nerve, LGNd and optic tecta of various vertebrate species [for review see Aronin et al. 1983; Brecha 1983; Brecha and Karten 1983]. A marked variability in the concentration of immunoreactive SP among species can be noted, specially in the optic nerve, where the values range from 0.6 pmol/g wt. in the human, to 64.5 pmol/g wt. in the rat. In the other regions of the visual system, the variability is less pronounced (see

Table 3). Second, immunocytochemical studies indicate that SP is localized in the ganglion cell layer (GCL) of several vertebrates, including the goldfish, frog, toad, lizard, rabbit, cat and monkey retinae [Brecha and Karten 1983], although not in the GCL [Eriksen and Larsson 1981; Brecha and Karten 1983] or in the LGNd [Mantyh and Kemp 1983] of the rat. A portion of the substance P-immunoreactive neurons in the GCL may belong to retinal ganglion cells. In Macaca nemestrina, two distinct SP-containing neuronal populations were located in the GCL [Brecha et al. 1982]. One of these cell populations gave rise to one or more primary processes that ramified in lamina 3 of the inner plexiform layer (IPL); this type of cell was considered to be a displaced amacrine. A second cell type gave rise to several processes, which ramified within lamina 5 of the IPL. Some of the secondary processes that entered the ganglion cell nerve fiber layer resembled axons as they might appear in Golgi preparations. However, the unequivocal identification of this type of cells has yet to be established. The strongest evidence supporting the presence of SP within retinal ganglion cells is provided by the study of Brecha et al. [1987]. They found that, in the rabbit, 25-35% of the retinal ganglion cells contain SP-like immunoreactivity; these cells were identified by retrograde labeling with fluorescent tracers injected into the superior colliculus, or by retrograde degeneration after optic nerve section. In the LGNd, ultrastructurally identified retinal

TABLE 3
SUBSTANCE P CONCENTRATION IN THE VISUAL SYSTEM (REVIEW)

REGION	SPECIE	CONCENTRATION[a]	REF
Optic nerve	pigeon	35.0	4
	rat	64.5	3
	rabbit	4.5	5
	human	0.6	2
Lateral geniculate	rat	90.0	1
	rat	81.6	3
	human	35.7	2
Superior colliculus	pigeon	42.0	4
	rat	177.0	1
	human	135.3	2
Striate cortex	rat	23.0	3
	human	4.6	2

[a]The concentration of SP was determined by radioimmunoassay. Values are expressed in pmol/g wt. When the original data was expressed in pmol/mg protein, it was assumed that 1 mg protein = 10 mg tissue [Sheridan et al. 1967]. References: (1) Brownstein et al. [1976], (2) Cooper et al. [1981], (3) Kanazawa and Jessell [1976], (4) Reubi and Jessell [1978], (5) Unger et al. [1981].

terminals were immunostained for SP, and participating in triadic synapses within complex glomeruli; the labeled terminals disappeared after optic nerve section or eye enucleation.

B) ENKEPHALIN

In his review on retinal neurotransmitters, Brecha [1983] reported the presence of enkephalin in the retina of several species. However, it was found only in different classes of amacrine cells, but not in ganglion cells. In the same year, Mantyh and Kemp [1983] reported that only very light immunoreactive enkephalin fibers were present in the rat LGNd. Other fibers were present also in the intrageniculate leaflet (IGL), a small region lying between the dorsal and the ventral divisions of the LGN. Heavy enkephalin staining was contained within both cell bodies and nerve fibers in the LGNv. It is possible that some of the enkephalin fibers in the LGNd originate not only from cell bodies of the LGNv, but also from the dorsal raphe nuclei, which is known to contain some enkephalin neurons and to project to the LGN [Finley et al. 1981].

Kuljis and Karten [1985] reported the presence of a distinct pattern of enkephalin immunoreactivity in the optic tectum of the frog that decreases rapidly after deafferentation. They suggest that the changes may be due to

the existence of a previously unrecognized population of retinal ganglion cells and/or to rapid transynaptic effects.

Beta-endorphin has been localized in several layers of the vertebrate retina, but not in the ganglion cell layer [Brecha 1983; Brecha and Karten 1983; Kuljis and Karten 1983]. Harlan et al. [1987] studied the localization of individual neurons containing the messenger RNA for the opioid peptide precursor preproenkephalin in the rat by in situ hybridization. The mRNA marker was found in multiple regions of the brain and the spinal cord, including several neocortical areas and the LGN. These results indicate that many more neurons may contain enkephalin than previously estimated on the basis of immunocytochemistry.

The binding of etorphine, an opiate analog, to different regions of the pigeon visual system was described by Vischer et al. [1982]. No marked regional differences were detected for etorphine binding, except for a low value in the nucleus rotundus. In comparisons within animal, the tectum always ranked first, followed by the paleostriatum, Wulst (the equivalent to the visual cortex in mammals), dorsolateral thalamus and ectostriatum.

C) CHOLECYSTOKININ

Initial studies indicated that cholecystokinin (CCK)-like immunoreactivity was absent from the retinal ganglion cell layer and LGN of several species [Eriksen and Larsson 1981;

Mantyh and Kemp 1983; Brecha 1983; Brecha and Karten 1983; Dockray 1983]. However, in the anuran optic tectum, CCK-like immunoreactivity disappeared within ten days after deafferentation, suggesting a population of retinal ganglion cells innervating the optic tectum [Kuljis and Karten 1983]. The authors discussed that these effects could be also due to rapid transneuronal degenerative changes. Later on, a moderately dense fiber plexus was shown at the border of the optic tract and LGNd in the rat [Fallon and Seroogy 1984]. When these animals were treated with colchicine, nearly one-fourth of geniculate cells appeared labelled. Furthermore, the same number of cells were shown to project to the visual cortex, in a combined immunofluorescence and retrograde tracing procedure, demonstrating the presence of a geniculo-cortical CCK-containing pathway. When tracers were injected in the LGNd, no double-labelled cells were visualized in the striate cortex, suggesting the absence of the reciprocal corticogeniculate pathway. The authors argued that although the immunohistochemical staining was specific, there was the possibility that the CCK immunoreactivity observed in the LGN was an acute response to colchicine toxicity (dose was 1 ul of 1% colchicine intraventricularly, plus 0.1 ul into the LGN). More recently, CCK-like immunoreactive fibers have been visualized in the LGNd, superficial layers of the optic tectum and other regions of the brain in non-colchicine treated hamsters [Miceli et al 1987]. However, even in colchicine-

treated hamsters and one rat, no perikarya were observed within the LGNd; in this case, the same dose of colchicine was injected intraventricularly, but no colchicine was injected into the LGN.

The laminar distribution of CCK receptors was studied in the geniculostriate system of the monkey, by *in vitro* autoradiography using ^{3}H-pentagastrin as a selective ligand [Shaw and Cynader 1986]. CCK receptors were found in negligible amounts in the LGNd. In the visual cortex, layers 4C, upper 5 and 6 were preferentially labelled, with moderate levels in layers 1-4A. In the same report, the distribution of adenosine receptors was also described. Adenosine-A1 receptors, labelled with ^{3}H-cyclohexyladenosine, were not found in appreciable concentrations in the LGNd. In the visual cortex, they were more concentrated in layers 2, 3, 4A and 4Cb, with lighter density in the other layers. It has been suggested that adenosine may subserve a role as inhibitory neuromodulator at presynaptic terminals [Goodman *et al*. 1983].

D) SOMATOSTATIN

The presence of somatostatin (SS) in retinal ganglion cells is uncertain. An increase in SS levels was observed in retinal extracts one year after transection of the optic nerve in the rat, suggesting that SS is entirely intrinsic to the retina [Rorstad *et al*. 1979]. However, SS-containing cell

bodies were found throughout the inner nuclear layer and ganglion cell layer, and their processes found in both the outer plexiform layer and inner plexiform layer, implying the existence of SS-containing bipolar, horizontal, interplexiform amacrine and possibly ganglion cell populations in the rat retina [Krish and Leonhardt 1979]. This study is in contrast with most others, where SS is confined to a more limited number of cells. In addition, SS has been detected in the optic nerve of the frog by radioimmunoassay, at the level of 11.6 $\pm$2.3 pmol/mg protein [see also Reichlin 1983].

Initially, immunocytochemical studies failed to detect SS in the rat retina or LGN [Eriksen and Larsson 1981; Mantyh and Kemp 1983]. More recently, however, SS-like immunoreactive neuronal elements were identified in the optic tract, the ventral portion of the LGN, superior colliculus, pretectum and visual cortex of the rat [Laemle and Feldman 1985]. In the optic tract, dispersed fibers formed a thin band within the lateral border of the LGNv. Only scattered immunoreactive fibers were present in the ventrolateral portion of the LGNd. Occasionally, these fibers were found innervating non-immunoreactive neurons within the nucleus. In the superior colliculus, labelled perikarya were distributed predominantly in the superficial grey layer, and the remaining were scattered throughout the optic fiber layer and the other collicular layers. Immunoreactive fibers were likewise distributed preferentially to the superficial layers,

but were also present with different densities in all the collicular layers. In the visual cortex, SS was present in layers 2-6, and in a variety of morphologically defined cell populations within these layers. Positive neurons were identified also in the nucleus of the posterior commissure, the nucleus of Edinger-Westphal, nucleus of Darkschewitsch, nucleus of the optic tract and pretectal nuclei, suggesting a role for somatostatin in visual and visuomotor reflex pathways.

E) OTHERS

Neurotensin: the distribution of neurotensin in the central nervous system of the monkey has been reported by Kataoka et al. [1979] using a radioimmunoassay procedure. The highest concentrations were found in the hypothalamus (30-50 pmol/g wet wt). In areas of the visual system, the highest content was measured in the LGN (4.6 $\pm$1.1 pmol/g wet wt), followed by the superior colliculus (3-6 pmol/g wet wt) and the visual cortex (2.3 $\pm$0.9 pmol/g wet wt) [for review see Aronin et al. 1983].

Neurotensin has been observed in the retina, but confined only to some populations of amacrine cells [for reviews see Brecha 1983; and Brecha and Karten 1983]. The immunocytochemical study of Mantyh and Kemp [1983] failed to detect any neurotensin-like immunoreactivity in the divisions of the rat LGN.

Vasoactive intestinal polypeptide (VIP): the immunoreactivity of this peptide has been localized in the retina of several vertebrates, but it is confined to some populations of amacrine cells [Brecha 1983; Brecha and Karten 1983]. In the rat, Eriksen and Larsson [1981] found that the number of VIP immunoreactive somata and fibers in the retina increases with the amount of light entering the eye. In the lateral geniculate nucleus of the rat, VIP is found only in the ventral division [Mantyh and Kemp 1983].

The autoradiographic distribution of VIP binding sites was studied in the rat central nervous system by Besson et al. [1986]. The highest density of binding sites were in the pineal gland. High densities were found in other nuclei including the dorsolateral geniculate nucleus and the superior colliculus.

Neuropeptide Y (NPY): initially, neuropeptide Y-like immunoreactivity (NPYLI) was detected only in cell bodies of the rat intrageniculate leaflet, and in fibers of the ventral LGN [Mantyh and Kemp 1983]. More recently, NPYLI has been detected also in somata and fibers of the LGNd in the rat, hamster, chipmunk and cat; in the monkey, however, no immunoreactive elements were observed [Ueda et al. 1986].

Bombesin: Kuljis and Karten [1985] observed that bombesin-like immunoreactivity disappeared within ten days after deafferentation of the frog optic tectum, suggesting the presence of some bombesin-containing retinal ganglion cells. However, this effect could be attributed also to rapid transneuronal degenerative changes.

Mantyh and Kemp [1983] did not detect any bombesin-like immunoreactivity in the divisions of the rat LGN.

Glucagon, thyrotropin releasing hormone (TRH), avian pancreatic polypeptide (APP) and luteinizing hormone releasing hormone (LHRH) have been localized in several layers of the vertebrate retina, but not in the ganglion cell layer [Brecha 1983; Brecha and Karten 1983; Kuljis and Karten 1983].

In summary, substance P is a promising candidate as neurotransmitter and/or neuromodulator in at least some retinogeniculate pathways. It has been localized in the retina, ganglion cell layer, optic nerve and LGNd of several species. Moreover, in the rabbit, identified retinal ganglion cells contain SP-like immunoreactivity, and their terminals have been traced to the LGNd. However, other criteria will have to be fulfilled before considering SP a more definitive candidate as neurotransmitter in the visual system.

Retinal enkephalin seems to be confined to different classes of amacrine cells in most vertebrate species. In the

frog, however, it may be present also in a population of ganglion cells, but further experimentation is needed to confirm this suggestion. The frog optic tectum and the rat LGNd contain some enkephalinergic fibers. Their origin in the rat seems to be the ventral LGN and/or the dorsal raphe nucleus. Neurons of the rat LGNd contain also the messenger RNA for preproenkephalin. Opiate binding has been described in several regions of the pigeon visual system.

The LGNd of some mammalian species contains a moderate plexus of CCK-containing fibers, and most probably also some CCK immunolabeled neurons. The levels of CCK receptors in the monkey LGNd seem to be negligible. The CCK-containing neurons of the rat geniculate nucleus seem to form part of a geniculocortical CCK pathway. Receptors for this peptide have been observed also in most layers of the monkey striate cortex. The origin of CCK fibers present in the LGNd, as well as the presence of CCK immunoreactivity in optic nerve fibers and retinal ganglion cells remains to be determined.

The presence of somatostatin in retinal ganglion cells is controversial. Scattered immunoreactive fibers are found in the optic tract and in the ventrolateral portion of the rat LGNd. Immunoreactive fibers and perikarya are found in all layers of the superior colliculus, but more concentrated in the superficial grey layer. Somatostatin is also distributed in fibers and neurons of layers 2-6 of the striate cortex. The additional presence of somatostatin in the

nucleus of the optic tract, nucleus of Edinger-Westphal, nucleus of Darkschewitsch, pretectal nuclei, and nucleus of the posterior commissure suggests a role for somatostatin in visual and visuomotor reflex pathways.

Neurotensin and neuropeptide Y have been detected in the LGNd, but their role in this nucleus needs to be investigated. The presence of vasoactive intestinal polypeptide and bombesin is suggested by some experiments, but it needs to be confirmed. For the rest of the peptides, the only available studies do not indicate that they may have a role as putative neurotransmitters in the retinogeniculate pathways.

2.3 ACETYLCHOLINE

The mammalian central nervous system contains moderate amounts of acetylcholine (ACh), but there is little of it in nerves containing mainly sensory fibers, such as in the optic nerve, where the concentration ranges from 1.10 nmol/g wt. in the sheep [recalculated from Cobbin et al. 1965; Ach chloride FW=182 g/mol], to 1.65 nmol/g wt. in the cat [recalculated from MacIntosh 1941]. The LGNd has higher concentrations of ACh, varying closely from 18.1 nmol/g wt. in the cat [recalculated from MacIntosh 1941], to 22.0 nmol/g wt. in the rat [recalculated from Hoover et al. 1978]; this latter value represents about 20% of the highest levels found in the caudate nucleus. Acute eye enucleation increases ACh concentration in the cat LGNd [Deffenu et al. 1967]. However, its concentration decreases significantly three weeks after this procedure in the rabbit [Miller et al. 1969]; this decrease was attributed by the authors to possible transneuronal changes following deafferentation. The amount of Ach remaining may belong to geniculate elements other than retinal and cortical terminals. Their probable origin will be described below. Enucleation and/or cortical ablation did not have any effect on the concentration of ACh in the superior colliculus [Miller et al. 1969].

The activity of the cholinergic markers choline acetyltransferase (ChAT) and acetylcholinesterase (AChE) appears to be low in the optic nerve [Deffenu et al. 1967;

Feldberg and Vogt, 1948; MacIntosh, 1941; Graham 1974]. However, De Roeth [1951] found appreciable levels of ChAT in acetone-dried powder of horse and rabbit optic nerve. Moderate levels of both markers are present in the LGNd, superior colliculus and visual cortex [Lund Karlsen 1978].

Histochemical visualization of AChE has revealed a particularly dense staining in the parvocellular layers of the owl monkey LGNd, as well as in two layers of the LGNd in Galago senegalensis. The intensity of the staining was unchanged following transneuronal atrophy induced by long-standing enucleation, or after destruction of neurons by injections of kainic acid. However, ablation of a portion of the visual cortex led to significant loss of AChE staining in the visuotopically related parts of these two layers. These findings indicate an association of the enzyme with corticogeniculate fibers rather than with geniculate cells or optic tract axons [Fitzpatrick and Diamond 1980]. No cholinergic cell bodies in the LGNd were detected by these or other authors [Mesulam et al. 1984; Shute and Lewis 1967; Jacobowitz and Palkovits 1974].

Butyrylcholinesterase (BuChE, a pseudocholinesterase) shows a differential distribution relative to AChE, in the macaque monkey LGNd [Graybiel and Ragsdale 1982]. AChE is concentrated in all laminae, but is particularly high in the magnocellular layers, whereas BuChE is virtually confined to the parvocellular layers. Moreover, only BuChE is sensitive

to the effects of eye removal. Since the histochemical localization of pseudocholinesterase is independent of AChE both in its distribution and its response to eye enucleation, it was concluded that an endogenous substrate of BuChE may be a neuroactive substance in the primate brain.

Choline acetyltransferase, in contrast to AChE, is thought to exist exclusively within cholinergic neurons and fibers, and its immunocytochemical visualization can be considered the most selective method available to assess the distribution of cholinergic elements. The LGNd and the PGN (perigeniculate nucleus, the visual segment of the thalamic reticular nucleus in the cat) are densely innervated by fibers with ChAT-like immunoreactivity. Axons made synaptic contacts with interneurons in the PGN and within synaptic glomeruli of the LGNd. In addition, positive terminals formed intra- and extraglomerular synapses with dendrites of the principal cells. Terminals of retinogeniculate and corticogeniculate fibers (RLP and RSD profiles, respectively) remained unlabelled, suggesting that these pathways may not exert cholinergic influence in the LGNd [De-Lima et al. 1985]. A similar dense innervation with immunoreactive fibers was observed in the cat LGNd using a monoclonal antibody to ChAT [Fitzpatrick and Raczkowski 1987].

The possible origin of the cholinergic innervation of the LGNd has been placed in several nuclei within the midbrain and pontine reticular formation [Lysakowski et al. 1986; Woolf

and Butcher 1986; Hallanger et al. 1987; De-Lima and Singer 1987; De-Lima et al. 1985]. Some specific structures include the nucleus cuneiformis [Shute and Lewis 1967] and the parabigeminal and parabraquial nuclei [Fitzpatrick and Raczkowski 1987; Fitzpatrick, D., personal communication].

The contribution of cholinergic fibers to the LGNd taking origin in the striate cortex is controversial. On one hand, visual cortex ablation produces no alteration in the ACh concentration in the LGNd of the rabbit four weeks after operation [Miller et al. 1969]. Moreover, corticogeniculate terminals (RSD profiles) remain unlabelled after treatment with ChAT antibodies [De-Lima et al. 1985]. On the other hand, ablation of a portion of the visual cortex led to loss of AChE staining in visuotopically related areas of the monkey LGNd [Fitzpatrick and Diamond 1980].

The possibility of a small contribution of cholinergic elements coming from the retina cannot be excluded, since small amounts of ACh are found in the optic nerve [Deffenu et al. 1967; Feldberg and Vogt, 1948; MacIntosh, 1941], and a significant decrease in ACh concentration occurs in the LGNd after enucleation [Miller et al. 1969]. However, all the cholinergic cells present in the ganglion cell layer of the retina seem to be displaced amacrine cells [Brecha 1983]. Moreover, retinogeniculate terminals (RLP profiles) remained unlabelled with ChAT antibodies, and the intensity of AChE

staining was unchanged after enucleation [De-Lima et al. 1985].

Cholinergic receptors of both the muscarinic and nicotinic type are present in the thalamus [for review see Jones 1983c]. The nicotinic receptors are only present in trace amounts in the rat LGNd, as determined with alpha-bungarotoxin [Segal et al. 1978; Hunt and Schmidt 1978]. Cholinergic muscarinic sites are in moderate to high levels in the LGNd, when binding is measured with ^{3}H-quinuclidinyl benzylate (QNB) or with ^{3}H-N-methyl-scopolamine (NMS) [Shaw and Cynader 1986, monkey; Wamsley et al. 1980, rat; Schliebs et al. 1982a, rat; Dohanich et al. 1985, guinea pig; Vischer et al. 1982, pigeon]. However, with ^{3}H-propylbenzilylcholine mustard, very low levels of binding are detected in the rat [Rotter et al. 1979]. The cause of discrepancy might be related to the way tissue was prepared (lightly fixed vs fresh tissue), or to differences in the capacity of the various ligands to recognize these sites. The ligands named above do not discriminate between the M1 and M2 subclasses of muscarinic receptors; a more selective ligand, such as pirenzepine [Hammer et al. 1980], may be used to study the distribution of muscarinic receptor subtypes in the LGNd.

Ach has excitatory actions on most geniculate neurons when it is injected iontophoretically, but antagonists have little effect on the responses evoked by light or by optic nerve stimulation [Curtis and Davis, 1963; Matsuoka and

Domino, 1972; Phillis et al. 1967]. The excitatory action of ACh can influence LGNd activity by (1) direct facilitation of relay cells, (2) enhancement of stimulus-specific inhibition via facilitatory actions on the inhibitory interneurons, and (3) reduction of inhibitory influences from the thalamic reticular nucleus [Sillito et al. 1983; Eysel et al. 1986].

In summary, the evidence in favor of Ach as retinogeniculate transmitter is weak. No retinal ganglion cells appear to be cholinergic, ChAT immunoreactivity is not present within retinogeniculate terminals, AchE staining remains unchanged after long standing enucleation, and ACh antagonists have little effect on the responses evoked by light or by optic nerve stimulation. The significance of small to moderate amounts of ACh and its markers in the optic nerve, as well as the effects of eye enucleation on geniculate ACh concentration, in the presence of full retinal degeneration but minimal transneuronal changes, needs to be evaluated. Butyrylcholinesterase may prove to be a useful marker in the identification of new neuroactive substances in the visual system. The distribution of muscarinic M1 and M2 receptor subtypes using discriminating ligands such as pirenzepine may suggest novel roles of ACh in the lateral geniculate nucleus.

2.4 MONOAMINES

A) SEROTONIN

Serotonin concentration has an uneven distribution in brain [Saavedra et al. 1973]. In the visual system of the rat, the highest concentration is found in the LGNd, relative to the superior colliculus and the visual cortex [Cano and Reinoso-Suárez 1982]. In the optic nerve, very little serotonin exists [Amin et al. 1954; Bodganski et al. 1957; Cobbin et al. 1965]. The actual concentration values differ significantly, sometimes more than a thousand fold, in some of these reports, probably due to the different methods of determination (bioassay, enzymatic-isotopic or fluorometric). Visual deafferentation does not seem to change serotonin levels in the LGNd, suggesting that it may not play a role in the transmission of visual impulses from the retina [Crawford 1958, dog; Deffenu et al. 1967]. Moreover, no serotonergic retinal ganglion cells have been detected so far [Brecha 1983].

The serotonergic pathway constitutes a major representative of the non-specific subcortical afferent systems providing innervation to the dorsal thalamus [for review see Jones 1985c]. The origin of the serotonergic fibers in the LGNd is the dorsal raphe nuclei, as indicated by anatomical evidence [Bobillier et al. 1985; MacKay-Sim et al. 1975; Pasquier and Villar 1982], and by histochemical

[Fuxe 1965], immunocytochemical [Steinbuch 1981; Cropper et al. 1984], biochemical [Palkovits et al. 1984; Geyer et al. 1976], pharmacological [Yoshida et al. 1984] and uptake studies [Chan-Palay 1977] demonstrating the serotonin content of these fibers. Light microscopic observations with direct serotonin antibodies have revealed a fine plexus of immunostained fibers that appear to enter the LGNd through the ventral surface and become finer and fewer toward the parvocellular layers, with no apparent rostrocaudal gradient. Ultrastructurally, these fibers show strictures and dilations, and are filled with dense round particles. Most of the labeled profiles did not form identifiable synapses, even when followed in serial sections. The few synapses observed showed asymmetry in their membrane thickenings, with the postsynaptic profiles identified in most cases as belonging to interneurons. This appearance suggests a possible excitatory role of serotonin on interneurons, which in turn would inhibit principal cells [Pasik et al. 1983, 1988].

Evidence for serotoninergic cell bodies in the lateral geniculate nucleus is weak. This evidence comes from two sources. First, a single immunoreactive cell body was found in the study by Pasik et al. [1983, 1988]. Second, some small neurons of the cat LGNd are destroyed after intraventricular injection of 5,6-dihydroxytryptamine [Pecci Saavedra and Perez Lloret 1979]. If these cells exist in fact, their functional significance must be small.

Biosynthesis of serotonin is effected by the participation of tryptophan hydroxylase and 5-hydroxytryptophan (5HTP) decarboxylase. The most important enzyme in the catabolism of serotonin is monoamino oxidase (MAO). Any of these enzymes could be used as potential markers for the visualization of serotonin, but the availability of histofluorescence techniques [Fuxe 1965] and more recently of direct antibodies [see Pasik et al. 1983, 1988, above] has made this approach unnecessary.

Serotonin can be inactivated enzymatically by MAO or by an uptake system present in serotonergic and other monoaminergic endings [see Brownstein 1981]. Studies of these processes can be performed to demonstrate the neurotransmitter role of serotonin, and/or to study the anatomical distribution of serotonin neurons and their projections in brain [Chan-Palay 1977].

It has been found that a number of tryptamine derivatives, including serotonin and lysergic acid diethylamide (LSD), have a depressant action on most geniculate neurons [Bishop et al. 1958; Bishop et al. 1959; Phillis et al. 1967; Satinsky, 1967; Tebecis and DiMaria, 1972; Yoshida et al. 1984]. Serotonin and LSD can also block the response of orthodromic (retinogeniculate) and antidromic (corticogeniculate) stimulation in a dose dependent manner [Tebecis and DiMaria, 1972]. At low doses, serotonin depresses the firing induced by visual or electrical

stimulation of the optic nerve; antidromic or chemical (glutamate) excitation is not blocked at these small doses. At higher concentrations, serotonin also depresses these excitatory effects. Serotonin actions can occur by presynaptic or postsynaptic mechanisms. Presynaptically, it may block the release of the transmitter from retinal terminals [Curtis and Davis, 1962; Tebecis and DiMaria 1972]. Postsynaptically, it can depress the spontaneous and evoked firing of the principal cell and facilitate the activity of interneurons [Satinsky 1967; Rinaldi et al. 1975; Rogawski and Aghajanian 1980]. It has been suggested that it may also act as a competitive antagonist of the optic nerve transmitter [Curtis and Davis, 1962; Tebecis and DiMaria 1972].

Serotonin receptors of both the 5-HT_1 and 5-HT_2 types have been found in the LGNd. Serotonin-1 receptors are present in moderate amounts, about 30% of the richest area in brain [Pazos and Palacios 1985, rat; Vischer et al. 1982, pigeon], whereas serotonin-2 receptor levels are low, approximately 5% of the highest value in brain [Pazos et al. 1985]. In the superior colliculus, serotonin-1 and serotonin-2 receptors are in similar proportions to those in the LGNd, but in the striate cortex the levels are higher, specially of the 5-HT_2 type [Pazos and Palacios 1985; Pazos et al. 1985; see also Peroutka and Snyder 1981]. The reason for the differential proportion of serotonin receptor types in these structures in unclear. Inhibition induced by serotonin

appears to be mediated by 5-HT_1 receptors, and a subclass of these receptors may function as autoreceptors. Serotonin-2 receptors seem to mediate both central and peripheral excitatory responses that can be blocked by classical 5-HT antagonists such as methysergide [see Cooper et al. 1986a]. These observations seem to support the view of serotonin having both excitatory and inhibitory actions in the LGNd [see Pasik et al. 1988]. It is possible that serotonin, acting on 5-HT_1 receptors located on retinal terminals, modulates their output towards a decrease in excitation of P-cells. On the other hand, serotonin would have an excitatory effect on 5-HT_2 receptors located presumably on interneurons, which in turn would inhibit principal cells through the action of GABA.

B) NORADRENALINE

The presence of noradrenaline (NA) in the optic nerves, optic tracts and LGNd of several species was barely detected using bioassay procedures [Vogt 1954; Cobbin et al. 1965]. With more sensitive techniques, the content of NA in the rat LGNd was estimated to be 20% of that found in the locus coeruleus, which is the major source of noradrenergic innervation in the brain [Versteeg et al. 1976; Kromer and Moore 1980]. No attempts have apparently been made to study the effects of retinogeniculate lesions on the noradrenaline content in the LGNd. However, when lesions of the locus

coeruleus have been made, a significant reduction in content is observed [Kromer and Moore 1980].

As suggested above, the principal, if not the only, source of noradrenergic innervation of the LGNd is the locus coeruleus, located in the caudal pontine central gray. Fibers arising from this nucleus form part of the large dorsal catecholamine bundle running along the midbrain tegmentum; in the diencephalon they ascend in close association with the superior cerebellar peduncle, and enter the LGNd from the dorsal portion of the thalamic reticular nucleus [Jones 1985c; Kromer and Moore 1980]. Evidence for the noradrenaline content of these fibers is given by fluorescence histochemical studies [Fuxe 1965; Lindvall et al. 1974; Kromer and Moore 1980], by immunofluorescence techniques using antibodies to dopamine-beta-hydroxylase [Swanson and Harman 1975] and by autoradiography [Jones and Moore 1977; Kromer and Moore 1980] [for review see Moore and Bloom 1979; Jones 1985c]. It is interesting to observe that the monkey LGNd has been found virtually devoid of noradrenergic fibers, as determined with an antiserum to dopamine-beta-hydroxylase. However, the pulvinar and lateral posterior complex were richly innervated. [Morrison and Foote 1986]. These results were suggested by the authors to indicate that the noradrenergic system has a more specialized role in the monkey, innervating preferentially regions involved in spatial analysis and visuomotor

responses, rather than those involved in feature extraction and pattern analysis.

Electrical stimulation in the region of the brainstem reticular formation has been shown to facilitate the excitability of geniculate neurons [Ogawa 1963]. Some of these actions are of cholinergic nature, as described in section 2.3, above. In addition, electrical stimulation of the locus coeruleus enhances the spontaneous and evoked activity of geniculocortical relay cells [Nakai and Takaori 1974; Rogawsky and Aghajanian 1980a,b,c, 1982]. Iontophoretic application of NA mimics the effects of locus coeruleus stimulation [Rogawsky and Aghajanian 1980a,b,c; Satinsky 1967], and this action is blocked by prazosin, a selective blocker of alpha-1 adrenoceptors [Rogawsky and Aghajanian 1982]. In earlier experiments it was found that iontophoretically applied NA produced depression of the activity of geniculate cells [Curtis and Davis 1962; Phillis and Tebecis 1967; Phillis et al. 1972]. However, in a reevaluation of these studies, Tebecis and DiMaria [1972] found that the depressant effects were produced only with high iontophoretic currents (where loss of responsiveness may occur), but that a delayed activation was produced with low NA doses [see Rogawsky and Aghajanian 1980b,c]. Since NA does not produce enhanced responsiveness in the absence of synaptic (tonic retinogeniculate) or glutamate-induced excitation, its action in the LGNd is viewed as typically neuromodulatory, to

distinguish it from the action of the conventional neurotransmitters which produce direct excitation or inhibition [Rogawsky and Aghajanian 1980a].

Noradrenaline in brain acts through at least four classes of receptors, which have been termed alpha-1, alpha-2, beta-1 and beta-2 receptors [see Kemp and Downes 1986]. The facilitatory action of NA is attributed to its interaction with alpha receptors, whereas beta receptors mediate its depressant actions [see Rogawsky and Aghajanian 1982]. As previously indicated, the stimulatory action of NA in the LGNd is mediated through alpha-1 adrenoceptors [Rogawsky and Aghajanian 1980b, 1982]. It has been shown that activation of these receptors in LGNd slices leads to inositol phospholipid breakdown, to yield diacylglycerol (DG) and inositol 1,4,5-triphosphate ($InsP_3$) [Kemp and Downes 1986]. Both compounds have second messenger functions: DG activates protein kinase C, and $InsP_3$ releases intracellular calcium. Accumulation of inositol phosphates was reduced in kainic acid-lesioned animals, indicating that this response occurred within geniculate neurons and not in afferent terminals [Kemp and Downes 1986]. Information on the distribution of alpha-1 adrenoceptors in the LGNd is apparently lacking. Beta-noradrenergic receptors are present in moderate to low levels in the LGNd and superior colliculus of the rat, and in moderate to high levels in the retina and visual cortex [Schliebs et al. 1982]. In the visual system of the pigeon,

high levels of beta-noradrenergic receptors are found in the dorsolateral thalamus and optic tectum, and moderate levels in the Wulst (the equivalent to the visual cortex in mammals) [Vischer et al. 1982]. The cellular localization of this type of receptors remains to be determined, as a substantial proportion of them are known to occur on glial cells and blood vessels.

C) ADRENALINE

The adrenaline content of the rat LGNd is approximately 3% of that of noradrenaline [Van Der Gugten 1976]. Part of this may reflect innervation of capillary vessels.

By immunocytochemistry, epinephrine-containing neurons are defined as those that are positively stained (in serial sections) with antibodies to tyrosine hydroxylase, dopamine-beta-hydroxylase and phenylethanolamine-N-methyltransferase [see Cooper et al. 1986b]. By these criteria, two large adrenergic cell groups have been defined, which are intermingled with noradrenergic cells of the lateral tegmental system and the dorsal medulla [Cooper et al. 1986b]. However, investigations on the influence of this system in the LGNd have apparently not been pursued [see Jones 1983c].

The activation of most geniculate neurons observed with the application of NA at low iontophoretic currents is mimicked with greater potency by adrenaline, through interaction with alpha-1 adrenoceptors [Rogawsky and

Aghajanian 1980b]. This action may be related to the adrenaline role in blood pressure control [Cooper et al. 1986b].

D) DOPAMINE

The dopamine concentration in the LGNd is very low, about 5-10% of that of noradrenaline [Versteeg et al. 1976; Kromer and Moore 1980]. These dopamine levels are within the precursor range expected for regions which contain only noradrenergic axons or neurons [Brownstein and Axelrod 1974].

The dopaminergic system, arising in the pars compacta of the substantia nigra and the ventral tegmental area, does not appear to play an important role in the thalamus [see Jones 1985c]. It was shown that a small proportion of retinal ganglion cells contained dopamine [Ehinger, 1966; Kramer et al. 1971], raising the possibility that some influence from the retina may be mediated by this substance. More recently, however, this view has been abandoned [Brecha 1983].

Dopamine does not depress significantly the orthodromic excitation of geniculate cells and has in general weaker effects than serotonin [Tebecis and DiMaria 1972]. Very low levels of dopamine binding have been measured in the dorsolateral thalamus of the pigeon [Vischer et al. 1982], reflecting the minor role that dopamine may have in this structure.

In summary, the monoamines serotonin, noradrenaline, adrenaline and dopamine do not seem to play a role as neurotransmitters in the retinogeniculate pathways. Serotonin was considered a strong candidate at one time, but this view has lost impetus. This substance has not been detected in retinal ganglion cells, and its concentration in the LGNd, even if appreciable, does not decrease after visual deafferentation. Immunocytochemical procedures have not revealed serotonergic terminals of retinal origin in the LGNd. It is worth observing that serotonin and other tryptamine derivatives block the response of geniculate cells evoked by photic or optic nerve stimulation at small doses; however, this action seems to be neuromodulatory at the level of retinal terminals, and excitatory on gabaergic interneurons.

The noradrenergic influence on LGNd transmission originates mostly from the locus coeruleus. Its action is viewed as tipically neuromodulatory and, like acetylcholine, it may function to facilitate the transfer of sensory information to the cortex.

The other two monoamines, adrenaline and dopamine, do not seem to play a major role in LGNd neurotransmission.

CHAPTER 3

MATERIALS AND METHODS

1. SUBJECTS

Nine adolescent monkeys (Macaca fascicularis), of 1.7-3.6 Kg in body weight were employed in this study. Seven received an optic tract section, and the other two an occipital lobectomy. For details on individual animals see Table 4.

2. PROCEDURES

2.1 SURGERY

Animals were given water and food without restrictions until one day before surgery, when only water was allowed. All surgical procedures were carried out under aseptic conditions and with the monkeys anesthetized with an intraperitoneal dose of sodium pentobarbital (40 mg/Kg). After the operation, the subjects received 300,000 units of penicillin G (Longicil; Fort Dodge Laboratories).

2.1.1 RIGHT OPTIC TRACT SECTION

An intravenous infusion of 30% (w/v) urea solution in 10% invert sugar in water was administered (3.2 ml/Kg) over 30 minutes before surgery, to induce a temporary shrinkage of the brain thereby facilitating the operation. A dose of 2 mg of

TABLE 4
EXPERIMENTAL SUBJECTS

MONKEY No.	SEX (+)	WEIGHT (Kg)	SURGERY (+)	SURVIVAL TIME (DAYS)
1028	M	2.2	ROT	7
1029	M	2.0	LOL	90
1031	F	2.0	LOL	90
1033	M	1.9	ROT	7
1034	M	1.7	ROT	6*
1035	M	2.3	ROT	7
1036	M	2.0	ROT	7
1037	M	2.2	ROT	7
1040	M	3.6	ROT	7

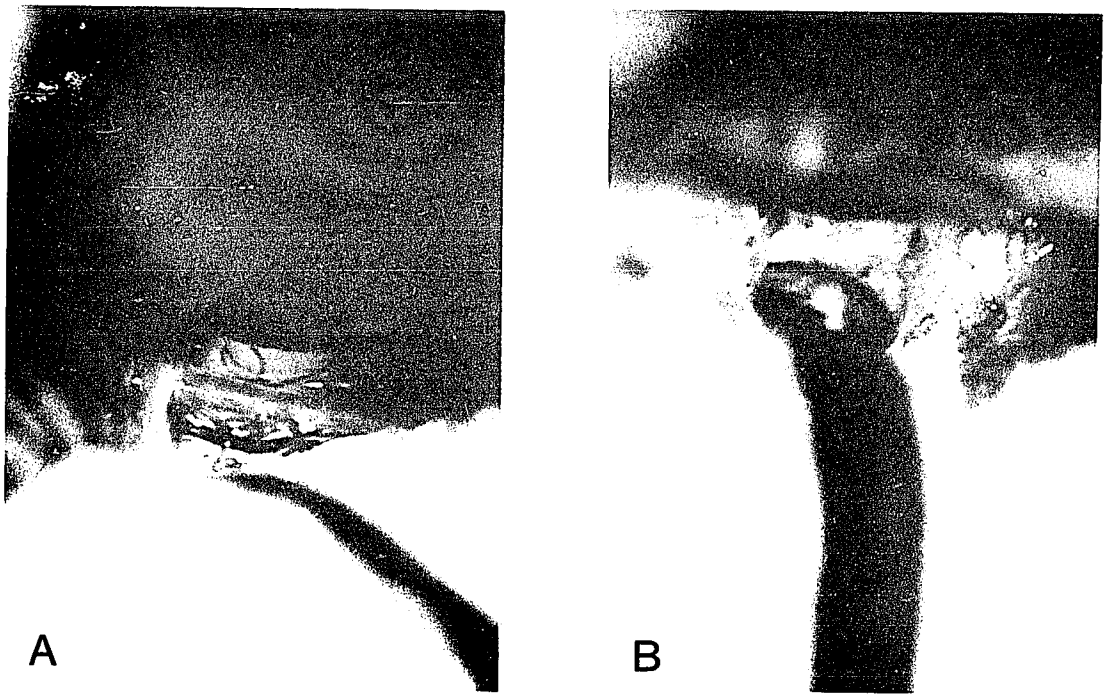
(+) abbreviations: (M) male, (F) female, (ROT) right optic tract section, (LOL) left occipital lobectomy.
(*) subject was found dead 6 days after surgery, and therefore the material was not considered suitable for analysis.

methylprednisolone (Depo-Medrol; Upjohn Co.) was also given to reduce brain edema. The animal was mounted in a stereotaxic frame and the body wrapped to the instrument to be able to tilt it. The right optic tract was approached from under the frontal lobe through a single bone flap which included the frontal area, the supraorbital ridge and part of the orbital roof. The remainder of the thin roof was removed with fine rongeurs. After opening the dura, which was hinged on the supraorbital line, the monkey was positioned so that the occiput pointed downward (about a 120° tilt from the prone position). The chiasmatic cistern was exposed by gentle separation of the frontal lobe from the orbital dura, with the aid of retractors applied on cotonoid. Minor bleeding was controlled with gel foam or electrocoagulation of the blood vessels. The region of the optic tract was visualized through an operating microscope at 10X magnification. In order to document the lesion, the microscope was equipped with a half-frame photographic camera (Robot Recorder 24; Urban Engineering Co., Hollywood, CA) loaded with Ektachrome film (200 ASA, daylight, ED 135-36; shutter speed 1/60 sec.). The chiasmatic cistern was opened by tearing the arachnoid membrane with fine needles. The anterior cerebral artery and the hypothalamus were clearly recognized. Subpial transection of the tract was made approximately one millimeter caudal to the posterior angle of the optic chiasm with the aid of microsurgical instruments (knives, probes, etc.) (Fig.3).

FIGURE 3

Right optic tract during surgery

(A) View through the operating microscope of the right optic tract, running in the rostral (top) to caudal (bottom) direction towards the lateral geniculate nucleus. The optic tract is seen crossed by the anterior cerebral artery from its medial (left) to lateral (right) margins. (B) The optic tract is shown completely transected.


A
B

Dural substitute material was placed to avoid adhesions, and the dural flap closed with 5-0 silk. In monkeys # 1035 to 1040, the bone flap was not replaced, and a drain left through a small skin opening caudal to the incision. The wound was closed in anatomical layers. After surgery, the subjects were placed in a warm room, and water was restricted for 18 hrs. A dose of 2 mg of Depo-Medrol every 12 hours was maintained for the first three postoperative days, and then it was reduced by half each consecutive day, for three days. A soft diet of fruit was given the second postoperative day, together with 30-60 ml of water. On subsequent days small pieces of solid food pellets were added to the diet, and the amount of water was increased progressively, so that by the third day the animals were drinking about 150 ml. When the monkey took less than 100 ml, additional fluid (5% glucose in isotonic saline) was given subcutaneously in the area between the scapular bones. The animals were maintained under these conditions for a total survival period of seven days.

2.1.2 LEFT OCCIPITAL LOBECTOMY

The monkey was fixed on a special "U" frame of a stereotaxic instrument with raised ear bars. After craniectomy, made with a turbine-driven osteotome, and dural opening, the left occipital lobe was amputated at the level of the lunate sulcus in a plane approximately perpendicular to the falx using a 20 gauge suction tip and bipolar electro-

cautery of the major blood vessels. Thereafter, the cross section of the calcarine fissure was visualized, and the cortex at both the superior and inferior banks was aspirated with a 22 gauge suction tip under visual guidance through an operating microscope (Fig.4). The rostral end of the empty calcarine fissure was identified by its pial wall and the entrance of the calcarine blood vessels. The dura was closed over a piece of Dacron cloth impregnated with silicone rubber to prevent adhesions. Bone was replaced and soft tissues closed in anatomical layers. The animal was placed in a warm room overnight. From the following day, food and water were provided without restrictions, for a total survival period of three months.

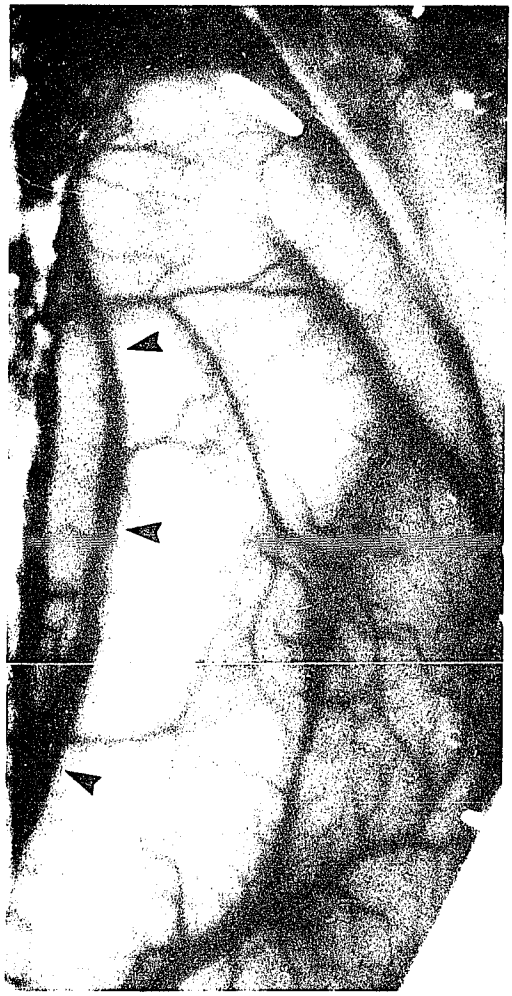
2.2 NEUROLOGICAL EXAMINATION

The pupillary reflexes and the extension of the visual fields were tested in order to evaluate the effect of the lesions on the visual system. The pupillary reflexes were tested with the animal placed in a normal-lit room and each eye stimulated independently with a small spot of light, protecting the opposite eye from the stimulus. The size of the pupil on each eye was estimated. To assess the extension of the visual fields, a tray containing a horizontal row of seven small pieces of apple spaced four inches from each other was presented to the monkey, whose head was manually held oriented toward the tray. A record was kept of the order in

FIGURE 4

Occipital lobectomy during surgery

(A) View taken through the operating microscope of the dorsolateral surface of the occipital lobe. Amputation was made along the lunate sulcus, indicated by large arrow heads. (B) View of the surface of the section after amputation of the occipital lobe. Cortical tissue has been aspirated from both the upper and lower banks of the calcarine fissure (small arrow heads).


A


B

which the animal picked up the morsels, as well as of any which were ignored. Any other abnormal behavior was also noted.

2.3 SPECIMEN COLLECTION

Animals were deeply anesthetized with an intraperitoneal dose of 45 mg/Kg of sodium pentobarbital. A catheter was inserted transcutaneously 15-20 cm deep into a saphenous vein and secured in place. Isotonic saline solution was infused at a slow drip flow rate. A syringe containing 3 ml of euthanasic solution (Sleepaway; Fort Dodge Laboratories; composition: 26% sodium pentobarbital, 10% isopropyl alcohol, and 20% propylene glycol in distilled water) was adapted to a "Y" connector in the infusion set. The monkey was mounted on a stereotaxic frame and transported to a cold room. The calvarium and the dorsal portion of the dura were rapidly removed to expose the entire brain. The frontal lobe was carefully lifted and the olfactory tract and the optic chiasm were severed. Just before sectioning the carotid arteries, a lethal dose of the euthanasic solution was delivered rapidly through the catheter placed in the saphenous vein. Removal of the brain was effected in less than fifteen seconds. The specimen was placed in a polyethylene bag and immersed in iced water for 3-4 minutes to accelerate cooling and increase the consistency in order to facilitate dissection. The right and left sides were separated by a midsagittal section.

In animals with right optic tract surgery, a block was cut containing the right dorsal lateral geniculate nucleus (LGNd) together with about three millimeters of the entering optic tract; the levels of the cuts were estimated with the aid of the stereotaxic atlas of Szabo and Cowan [1984]. The block was frozen immediately in isopentane (2-methylbutane) cooled at -20°C with a mixture of dry ice and acetone. The time span between administration of the euthanasic solution and freezing of the tissue was less than five minutes. After the block was equilibrated at -20°C, it was taken slowly to a final temperature -70°C, in order to avoid fractures. The block was identified properly and stored at -70°C until further processing. The same procedure was applied simultaneously to slabs of tissue containing the superior colliculus and striate cortex. Equivalent structures on the opposite (left) hemisphere were collected by the same method. The tissue rostral to the block containing the LGNd, which included the right optic tract at the level of the transection, was fixed in 10% formalin for histological verification.

In animals with occipital lobectomy, an identical technique was employed to obtain the LGNd and superior colliculus specimens of the operated and unoperated sides. In addition, the caudal portion of the left hemisphere, containing the surface of the section of the occipital lobe and the remainder of the calcarine fissure, was fixed in 10%

formalin to ascertain the complete removal of the striate cortex.

2.4 HISTOLOGY

After a two-week treatment in 10% formalin, the blocks of tissue containing the right optic tract at the level of the lesion and the caudal portion of the left hemisphere were embedded in celloidin, and serial sections of 40 μm in thickness were obtained with a sledge microtome (Model 1400; E. Leitz, Inc.). One every ten sections were stained with the method of Klüver-Barrera [1953]. Photographs of these sections were taken with a camera adapted to a dissecting microscope with transillumination through a blue filter No.12 and Kodachrome film, ASA 64, at magnifications between 0.8X to 3.0X (see Results).

2.5 TISSUE PREPARATION FOR BIOCHEMICAL ANALYSES

The blocks of frozen tissue containing the desired structures were mounted onto the specimen holder of a microtome-cryostat (Model CTF; Damon, International Equipment Division, Needham, Mass.). This instrument was totally frost-free. The block of tissue was surrounded with a bed of iced-water, to provide better support while sectioning. The specimen mounted onto the holder was attached to the microtome-cryostat and it was left there for about 15-30 minutes to allow it to equilibrate to the temperature optimal

for sectioning, which in this case was -8°C. The block was positioned to the correct plane and coronal sections 256 μm in thickness were obtained. The sections were mounted onto glass microscope slides, and they were maintained frozen on a metallic tray placed over dry-ice. They were stored at -20°C for 1-3 days inside an airtight container until dissected.

In order to be able to dissect differentially the magnocellular and parvocellular laminae of the LGNd, as well as selected laminar regions of the superior colliculus and the striate cortex, the micropunch technique of Palkovits was followed [Palkovits 1973, 1975; Palkovits and Brownstein 1983]. Microdissecting punches were adapted from hypodermic gauge 22 needles. In the last experiments (monkeys 1037-1040), a custom-made punching needle with a thin plastic handle was employed, to provide better thermal insulation. The punches were 4 cm in length, and had their tips cut at an angle of 90° from the longitudinal axis. The tips were sharpened to a cutting edge, and their inner diameters calibrated before use. The calibration procedure consisted in making an impression of the edge onto scotch tape attached to a glass microscope slide. The inner diameter of the punch was measured under the microscope with an ocular piece provided with a reticle, in turn calibrated with a stage micrometer (5+ 100/100; Zeiss). The mean of five values was obtained, and the coefficient of variation was less than 6%.

The mean diameter for different punches ranged between 435 μm and 640 μm. Other procedures to measure the inner diameter of the punch were attempted, such as making the impression onto a wax block, or on graph paper, but they were less reproducible. The amount of tissue equivalent to one punched disc was calculated from the thickness of the section (256 μm), and the area of the disc, given by the diameter of the punch. The volume of the disc (in mm^3) is equivalent to the weight of the tissue (in mg), assuming that the density of the tissue is 1 mg/mm^3. This assumption was tested by collecting 4 to 15 mg of tissue from the thalamus, and counting the number of discs necessary to obtain this material. The procedure consisted in punching out the tissue and depositing it on the walls of a tared light-weight plastic test tube containing 1 ml of water to create a moist chamber. This volume of water was decanted carefully and any remaining droplets were absorbed before weighing the test tube for the second time. An electrobalance (Model DTL; Cahn Instruments, Inc.) was used in this procedure. The time span between collecting the first disc of tissue and weighing the test tube containing all the material was less than fifteen minutes, to minimize dehydration. The theoretical volume of the total number of discs (volume of one disc X number of discs) was plotted against the actual weight of tissue obtained experimentally. A straight line was obtained, with slope equal to unity (see Fig.5). The slope of this line (in

mg/mm^3) is equivalent to the density of the tissue. Therefore, with a section of 0.256 mm in thickness and a punch of 0.500 mm in diameter, a disc of 0.05 mg of tissue would be obtained.

The amount of tissue necessary for analysis was calculated on the basis of the concentration of amino acids in brain, on the limit of detection of the amino acid analyzer, and on pilot studies made in the thalamus of the rat to estimate the recovery. For glutamate, which is the richest amino acid in brain (5-10 μmol/g wt), with a level of quantitation two times above the limit of detection (2 X 50 pmol/injection) and with an 80 percent recovery, 0.01 mg to 0.02 mg of tissue would be approximately needed for one analysis. For glycine, which is present in moderate concentrations in brain (0.4 to 3.6 μmol/g wt), following the same reasoning, 0.03 mg to 0.3 mg of tissue would be needed. For amino acids present in low concentration in brain, such as tyrosine, phenylalanine, beta-alanine, etc., two to twenty times more material would be needed.

Once the microdissection needles were calibrated and the amount of material necessary for analysis was estimated, the sampling of the tissue followed. The frozen slide containing the desired section was attached by the edges with two drops of iced-water onto a thermoelectric freezing stage, 7.5 cm X 8.0 cm in size (Model BFS-25TC; Bailey Instruments Co., Inc.; Saddle Brook, NJ), adjusted to -10°C. This stage was placed

FIGURE 5

Verification of tissue density

The accuracy in tissue weight determination using the micropunch technique is influenced by several factors, including the density of the material, which is assumed to be 1 mg/μl. This assumption was verified by collecting samples from the thalamus, following the procedure described in the text. The volume of tissue (thickness of the section X inner diameter of the needle X total number of discs) was plotted against its actual weight obtained experimentally. The regression curve was a straight line with slope equal to unity (in mg/mm^3), which is equivalent to the tissue density.

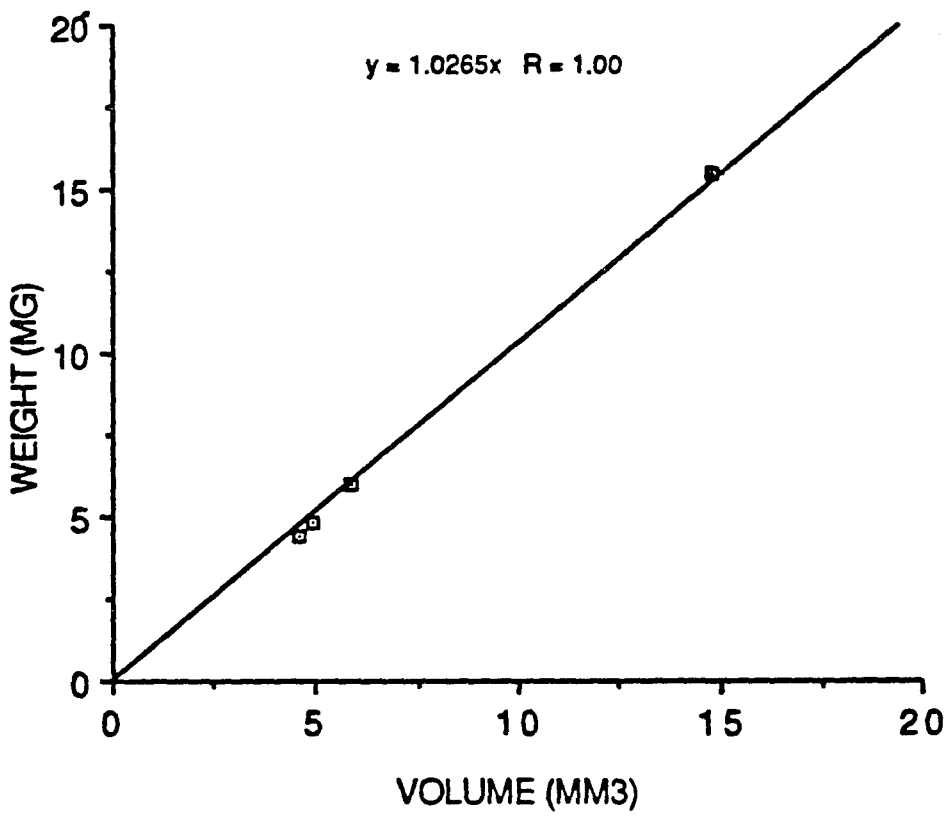


Figure 5

under an operating microscope (Model 25842; Zeiss) that provided a magnification of 6X to 40X. The small amount of frost that accumulated on the surface of the section (specially during the summer) was scraped away very gently with the edge of a beveled needle maintained cold in dry-ice. The operating microscope was tilted slightly away from the vertical position so that the light that came from it did not produce glare. Pictures were taken through the microscope with the Robot camera loaded with Ektachrome film (daylight, 200 ASA, ED 135-36), at a shutter speed of 1/30 or 1/15 sec. Dissection was done with the punches maintained cold in dry-ice. Portions of the optic tract distal to the lesion yielded approximately 12 to 18 discs of tissue per section (see Fig.6A). The ventral portion of the LGNd surrounding the hilum, corresponding to the magnocellular layers, produced 5 to 6 discs per section. The same numbers of discs were collected from the parvocellular layers, on the same section. The remainder of the tissue from the parvocellular layers was also collected and saved for future analysis (see Fig.6B). Samples from the superior colliculus were centered on the stratum opticum, the layer of entrance of retinal fibers; approximately 6 to 8 discs were obtained from each section (Fig.6C). The visual cortex was dissected in the area centered around the stripe of Gennari in the regions corresponding to the central and peripheral visual fields, located on the dorsolateral surface and in the upper and lower

banks of the calcarine fissure, respectively. About 20 discs of tissue were recovered from each of these two regions (Fig.6D). The punch technique was repeated on sequential slides containing the structures under study until a total of 40 to 60 discs were collected, equivalent to an average of approximately 3 mg of tissue (Table 5).

Discs of tissue were air blown with a syringe into a microhomogenizer containing 500 μl of iced-cold perchloric acid 0.4 N. This solution has proved to be the most efficient among six others to extract acidic, neutral and basic amino acids from brain [Saifer 1971]. The tissue was homogenized together with 10 nmoles of norleucine (NLEU) used as internal standard. After 30 minutes under occasional stirring in the cold, the suspension was centrifuged for 10 minutes at 10,000 rpm, in a refrigerated Beckman microfuge. The supernatant fluid was adjusted to pH 2.5-4.5 by the addition of 4N and 0.1N KOH. After being chilled in iced-water for 1 hour, the precipitated potassium perchlorate was removed by centrifugation. The resulting supernatant was frozen at -70°C until analysis.

TABLE 5
AMOUNT OF TISSUE COLLECTED FOR ANALYSES[a]

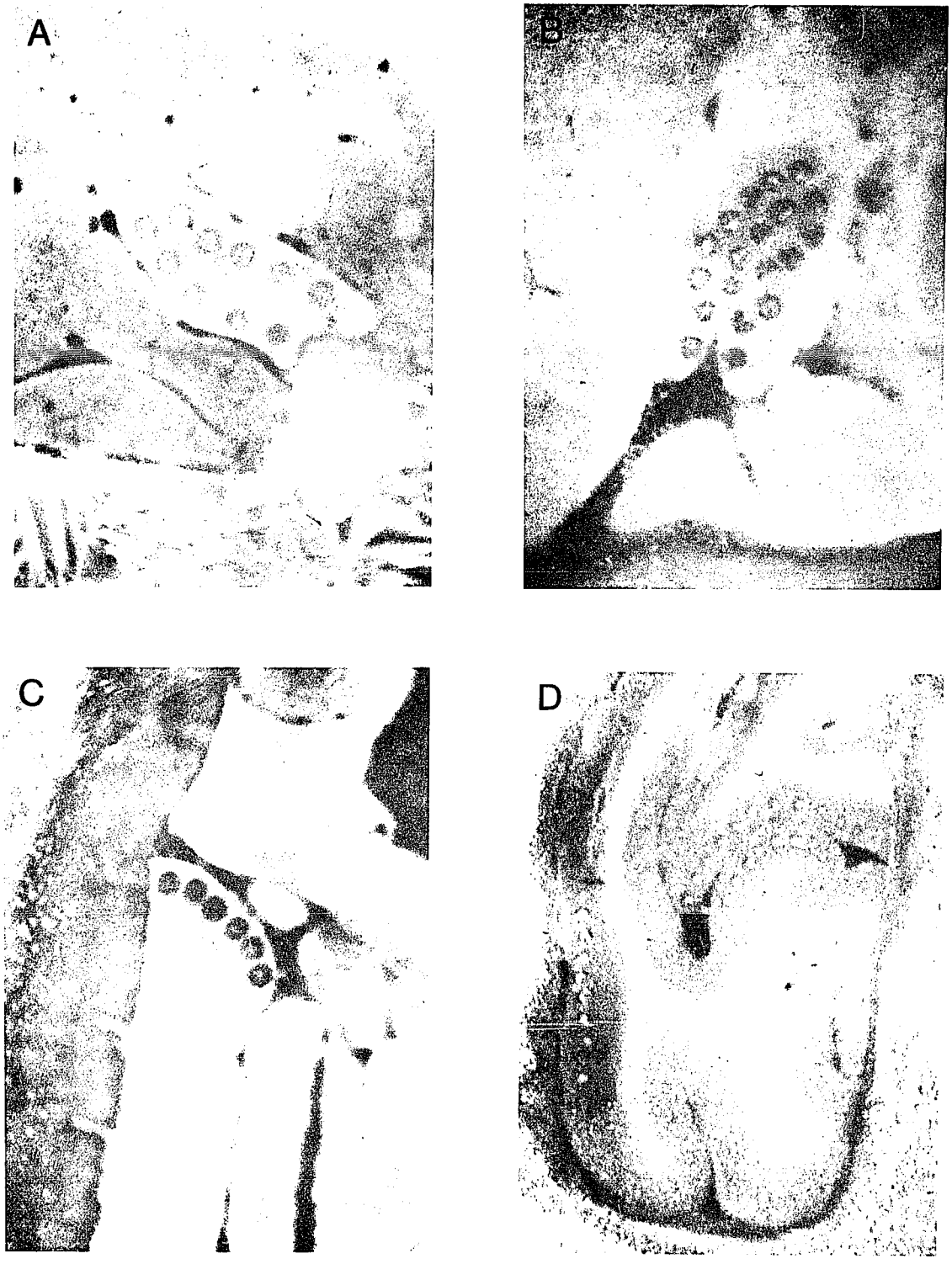
MONKEY	REGION	LEFT	RIGHT
1028	MAGNO	1.74	2.64
	PARVO	8.32	7.28
1029	MAGNO	1.50	2.14
	PARVO	7.28	8.82
1031	MAGNO	2.01	2.62
	PARVO	7.28	8.06
1033	MAGNO	2.96	2.66
	PARVO	4.48	6.28
1035	TRACT	1.35	1.26
	MAGNO	1.35	1.78
	PARVO	8.32b	2.81
	S.COL	1.00	2.05
	CTX.C	1.61	2.49
	CTX.P	1.56	2.49
1036	TRACT	2.27	1.94
	MAGNO	2.88	2.55
	PARVO	2.88	2.30
	S.COL	1.50	2.22
	CTX.C	2.22	2.22
	CTX.P	2.22	2.22
1037	TRACT	3.45	3.45
	MAGNO	2.66	2.66
	PARVO	2.66	2.66
	S.COL	2.66	2.66
	CTX.C	2.66	2.85
	CTX.P	2.66	2.85
1040	TRACT	1.90	2.17
	MAGNO	1.98	1.79
	PARVO	1.98	1.67
	S.COL	2.09	1.56
	CTX.C	1.90	2.28
	CTX.P	1.94	2.28

(a) Tissue was collected by the micropunch technique, as described under Materials and Methods, and the amount is expressed in milligrams. (b) Material was collected from the entire parvocellular segment of monkey #1035. Tissue was collected from a total of 64 structures, with an average of 3.05 mg per structure.

FIGURE 6

Dissection of four regions of the monkey visual system by the micropunch technique

Frozen sections were mounted on a microscope glass slide and microdissected with a 22-gauge calibrated punch. (A) optic tract; (B) lateral geniculate nucleus; (C) superior colliculus; (D) striate cortex. Approximately 15-20 discs were collected from the optic tract in each section. The 5-6 discs removed from the ventral portion of the LGNd correspond to its magnocellular segment, and the remaining discs to the parvocellular segment. Samples from the superior colliculus were centered around the incoming optic fiber layer (stratum opticum), and approximately 6-8 discs were collected from each section. Discs from the striate cortex were centered at the stripe of Gennari, in the dorsolateral surface (corresponding to the representation of the central visual fields), and in the upper and lower banks of the calcarine fissure (corresponding to the representation of the peripheral visual fields); approximately 20 discs were collected from each region in each section.


A
B
C
D

2.6 BIOCHEMICAL ANALYSES

2.6.1 AMINO ACID ANALYSIS

Amino acids and other free-amino compounds were analyzed by high-performance liquid chromatography (HPLC) with fluorometric detection. The instrument used was a Perkin-Elmer series 4 quaternary solvent delivery system, equipped with an injection valve (Rheodyne model 7125), a zero-volume filter (PN 3102-9030), a guard column, an OPA (o-phthalaldehyde) module for post-column delivery of the fluorophoric reagent, and an LCI-100 computing integrator. The separation was carried out on a 250 X 3 mm column, maintained in a heating unit at 42°C. The buffers used were lithium citrate 0.24 N, pH 2.75 (Li275), lithium citrate 0.64 N, pH 7.50 (Li750) and lithium regenerant solution 0.30 N, pH>11 (RG003). Column and buffers were from Pickering Laboratories, Inc., Mountain View, CA. These buffers were run according to the gradient program shown in Table 6. The fluorophoric reagent was an aqueous solution of fluoraldehyde (o-phthalaldehyde crystals (OPA), Pierce cat. 26015), first dissolved in 3 ml of methanol, and then taken to a final concentration of 0.5 g/l in potassium borate buffer 0.5N, pH 10.5, containing 10 ml of Brij-35 (Pierce cat. 20150) and 5 ml of 2-mercapto-ethanol (Pierce cat. 35600). The final solution was filtered through a Millipore membrane of 0.45 μm and degassed by stirring under vacuum for 10 minutes. This solution was stored in the cold for up to ten days because in various

TABLE 6
HPLC GRADIENT PROGRAM FOR AMINO ACID ANALYSES

TIME	E V E N T			NOTE
t (min)	Li275 (%)	Li750 (%)	RG003 (%)	
0	100	----	----	Inject sample
0-20	100	----	----	Isocratic
20-70	100-65	0-35	----	Linear
70-128	65-0	35-100	----	Linear
128-151	----	100	----	Isocratic
151-185	----	100-94	0-6	Linear
185-185.1	100	94-0	6-0	Linear
185.1-210	100	----	----	Re-equil.
210	100	----	----	Ready

attempts to use it beyond this time it was found that the sensitivity of detection started to decrease progressively. The instrument conditions were: buffer flow rate: 0.3 ml/min; fluorophoric reagent flow rate: 0.3 ml/min; column temperature: 42°C; fluorescence development: 1 min at room temperature; detector: excitation wavelength of 360 nm, absorption wavelength of 440 nm; recorder: sensitivity of 128, chart speed of 3mm/min.

A standard solution containing forty amino acids present typically in physiological fluids was employed to prepare standard curves in order to calculate the concentration of the amino acids in the analytical samples. Two stock standard solutions were prepared first. They were made at a concentration of 25 nmol/ml and 5 nmol/ml, respectively, from a commercially available product containing 38 acidic, neutral and basic amino acids and ammonia, at a concentration of 0.5 μmol/ml each (Pierce ANB, cat. 20077); in addition, asparagine and glutamine dissolved in 0.1 N HCl were added fresh, because these two compounds degrade rapidly and are therefore not included in the commercial product. These two stock solutions were divided into 1 ml aliquots and stored at -70°C. For usage, one of these aliquots was freeze-thawed no more than three times, and stored at -20°C for up to one month.

Twenty to two hundred microliters of the working standard solutions, containing 0.05 to 2.0 nmoles of each amino acid, were analyzed in the HPLC. Under the conditions described

above, the sensitivity of the method was at least 50 pmol/injection. The areas under the peaks of the chromatogram corresponding to each amino acid were integrated with an electronic graphic digitizer (Numonics Corp., North Wales, PA). This method was preferred over the LCI-100 computing integrator because on occasions it was found that the values obtained with the latter method were highly overestimated; the reason for this unreliability was the failure of the baseline to return to zero after certain peaks. The mean of three measurements (in cm^2) was plotted against the amount of the amino acid injected (in nmoles). The regression curve obtained was a straight line with zero intercept in the ordinate axis, and of slope (in cm^2/nmol) equal to the area produced by one nmole of the amino acid in question (Fig.7). The quotient (area standard/nmole standard) is called the fluorescence constant (FK), which is characteristic for each amino acid under the conditions of the analysis. Standard curves were also constructed using the height of the peaks as the basis for quantitation, but the curves obtained were not linear in the range tested.

Once standard curves for each amino acid were constructed, analysis of the biological samples followed. The sample was thawed and adjusted to pH 2.8-3.2 with either KOH 0.1 N or HCl 0.1 N before being injected. A volume of 75-150 μl (equivalent to approximately 0.5 mg of tissue) was analyzed in the same fashion as described above. The areas

FIGURE 7

Standard curve of selected amino acids

The amount of amino acid injected to the HPLC (ranging from 0.05 to 2.0 nmoles) was plotted against the area (in cm^2) under the corresponding peak. Each point represents the mean of triplicate determinations, with a coefficient of variation less than 5%. The slope of the regression line (in cm^2/nmole) is equivalent to the area produced by one nmole of the amino acid in question. This value, termed the fluorescence constant, is characteristic for each amino acid under the conditions of the analysis, and it was employed to calculate its final concentration in the sample, according to the formula in Materials and Methods, Section 2.6.1.

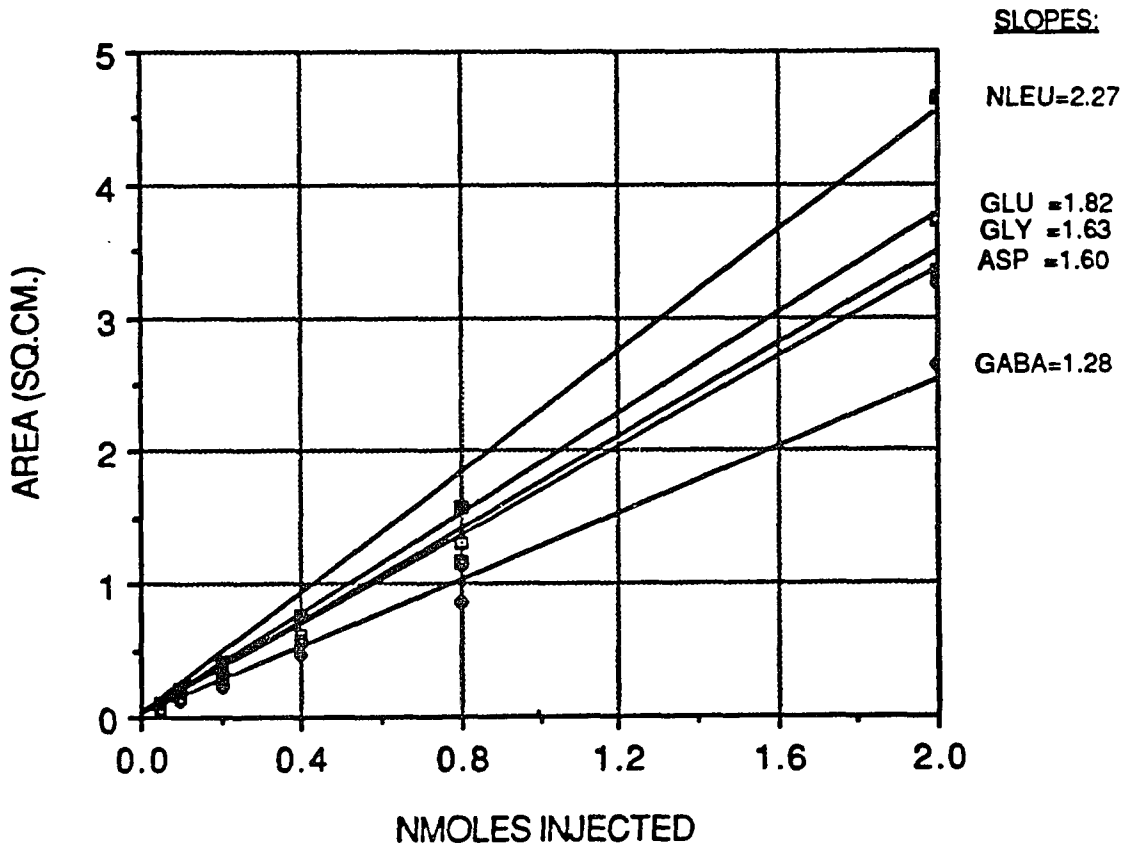

SLOPES:
NLEU=2.27
GLU =1.82
GLY =1.63
ASP =1.60
GABA=1.28
AREA (SQ.CM.)
NMOLES INJECTED
0
1
2
3
4
5
0.0
0.4
0.8
1.2
1.6
2.0

Figure 7

under the peaks recorded on the chromatograms were measured with the digitizer, and the mean value (in cm^2) of three measurements was obtained. This value was divided by the fluorescence constant (in cm^2/nmol) of the amino acid in question, and the result was equal to the number of nanomoles of amino acid in the injected sample. It should be recalled that 10 nmoles of norleucine were added to the tissue before homogenization as an internal standard (see above). The amount of norleucine present in the injected sample was calculated by dividing the area under the peak by its fluorescence constant. The quotient of the total number of nanomoles of norleucine (10 nmoles) and the amount of norleucine injected with the sample (in nanomoles) represents the fraction of the total sample that was injected to the HPLC. Since the amount of tissue obtained for analysis is known, the concentration of each amino acid in the sample, expressed in μmol/g wt, was calculated as follows:

$$\text{concentration} = \frac{A}{FK} \times \frac{10}{NLEU} \times \frac{1}{WT} = \mu\text{mol/g wt}$$

where A is the integrated area (in cm^2) under the amino acid peak; FK is the fluorescence constant obtained from the standard curve (in cm^2/nmol), equivalent to the area produced by one nmole of the amino acid (see Table 7 in Results); "10" is the amount of NLEU (in nmoles) used as internal standard; NLEU is the amount of this amino acid present in the sample (in nmoles), given by the quotient (area NLEU / FK NLEU); and

WT is the total amount of tissue (in mg) that was prepared for biochemical analysis.

2.6.2 NASP AND NAAG ANALYSIS

The levels of N-acetyl-aspartate (NASP) and N-acetyl-aspartyl-glutamate (NAAG) were measured with an isocratic anion HPLC method adapted from Koller et al. [1984; see also Lavollee et al. 1987], with two main differences: (1) the extraction solvent was perchloric acid instead of ice-cold methanol, allowing the use of the same sample for both amino acid and NAAG analysis; (2) glutamate, aspartate and other free-amino compounds were not removed from the sample as reported by Koller et al. [1984]. The two differences were validated in preliminary experiments. Thus, whole rat brain samples were extracted with perchloric acid and analyzed by HPLC; two determinations showed levels of NASP of 6.70 and 6.94 nmol/mg wt. (mean of 6.82 nmol/mg wt), and of NAAG of 0.27 and 0.36 nmol/mg wt. (mean of 0.32 nmol/mg wt.); these values are in almost complete agreement with those reported previously using methanol [NASP = 6.82 nmol/mg wt., NAAG = 0.37 nmol/mg wt.; Koller and Coyle 1984]. Similarly, when the amino acid standard solution (described above), containing 10 nmoles each of 41 amino compounds, was injected into the HPLC equipped for peptide analysis (see next paragraph), a large off-scale deflection appeared at approximately 3 minutes and returned to baseline values at approximately 7 minutes, with

no further peaks appearing for up to one hour. When a mixture containing 1.0 nmole each of GLU, ASP, NASP and NAAG was analyzed by this HPLC method, retention times were of 4.9, 5.5, 10.5 and 27.0 minutes, respectively. Therefore, all the amino acids present in the physiological standard solution eluted at least three minutes earlier than NASP and twenty minutes earlier than NAAG, and consequently their presence was not a confounding factor in the analysis.

The instrument employed was the Perkin-Elmer series 4 solvent delivery system used for amino acid analysis, equipped with a LC-95 spectrophotometric detector and the LCI-100 computing integrator. The separation was carried out in a Partisil 10-SAX column, 250 x 4.6 mm (Alltech Assoc., Deerfield, IL), protected with a guard column that was packed with a pellicular anion-exchange resin (Whatman; Clifton, NJ). The mobile phase consisted of 75 mM monobasic potassium phosphate (pH 4.5) at a flow rate of 1 ml/min. Periodically, the column was regenerated with 200 mM monobasic potassium phosphate at the same flow rate for one hour. Absorbance was monitored at a wavelength of 210 nm. Under these conditions, the limit of detection was 0.25 nmol/injection. Each injection represented approximately 0.5-1.0 mg of tissue. External standards consisted of N-acetyl-Glutamate (Sigma cat. A-9000), NASP (Sigma cat. A-8901), and NAAG (Biochem; Torrance, CA). N-Ac-Glu eluted at 9.4 minutes. The elution times of NASP and NAAG were given above. The purity of the

NAAG standard was tested by amino acid analysis before and after acid hydrolysis: 100 nmoles of NAAG were injected directly into the HPLC, but no free amino acids were detected, including GLU or ASP; after hydrolysis, only two peaks at equimolar concentrations were present, corresponding to the constituent amino acids. Standard curves were plotted for NAAG and NASP at four different amounts varying from 0.5 nmol to 2.0 nmoles. In this case, and differing from the amino acid analysis (see above), the concentration correlated linearly with both the height of, and the area under the peaks; therefore only height measurements were performed on the resulting chromatograms. The slope of the line (in cm/nmol) was equivalent to the height of the peak produced by one nmole of the compound, and it was called the absorbance constant, in analogy to the fluorescence constant for the amino acid analysis. Its value was equivalent to 1.10 cm/nmol for both NASP and NAAG. The recovery of the sample was calculated on the basis of the amino acid analysis of the same sample, and it was therefore expressed in terms of NLEU equivalents. The final concentrations of NASP and NAAG in the analytical samples, expressed in μmol/g wt., were calculated as follows:

$$\text{concentration} = \frac{H}{AK} \times \frac{10}{NLEU} \times \frac{1}{WT} = \mu\text{mol/g. wt}$$

where H is the height of the peak (in cm); AK is the absorbance constant obtained from the standard curve (in

cm/nmol), equivalent to the height of the peak for one nmole of the compound; (10/NLEU) is the NLEU recovery obtained from the amino acid analysis of the same sample, and WT is the total amount of tissue (in mg) that was prepared for biochemical analysis.

2.7 STATISTICAL ANALYSES

2.7.1 CONTROL (NORMAL) STRUCTURES

The differences among control (normal) structures were subjected to an analysis of variance model (ANOVA) for unbalanced situations. Note that for the optic tract, superior colliculus and striate cortex, the number of animals was four, but for the magnocellular and parvocellular layers of the LGNd the number was six. If the calculated F of the unbalanced ANOVA were at least as large as the critical value, then the null hypothesis of "no difference among regions" would be rejected. To determine between which means the inequalities lie, the Bonferroni and Tukey multiple comparison tests were employed [for review see Miller 1981].

Bonferroni $\underline{t}$ tests control the overall alpha at the desired level (i.e., 0.05) by setting the error rate per comparison (e) to a sufficiently small value, defined by e=alpha/c, where (c) is the total number of comparisons. Under the Bonferroni tests, two means are declared to be significantly different if

$$|X2-X1|/S(1/N1+1/N2)^{1/2} \geq t(e;v),$$

where |X2-X1| is the absolute difference of the means, the denominator is the standard error of the mean difference, (N1) and (N2) are the sample sizes for each mean, (t) is the critical value of the t test, (e) is the error rate per comparison, and (v) are the degrees of freedom obtained from the error term in the unbalanced ANOVA procedure.

Two means are considered to be significantly different by the Tukey (Tukey-Kramer) criterion if

$$|X2-X1|/S((1/N1+1/N2)/2)^{1/2} \geq q(alpha;k,v),$$

where q(alpha;k,v) is the alpha-level critical value of the q distribution of (k) means with (v) degrees of freedom. Both the Bonferroni and the Tukey's tests control the probability of committing a Type I error (which is the probability alpha of expressing a "significant difference" by mistake). However, the Bonferroni t tests are more conservative, generally having a higher Type II error than Tukey's (higher probability of declaring a difference "not significant").

2.7.2 CONTROL AGAINST EXPERIMENTAL STRUCTURES

The differences between control and experimental sides for each region of the visual system were analyzed by the Student's paired t test. The t value for the null hypothesis of no differences between means is given by

$$t = md/S_{md}$$

where (md) is the mean difference among pairs of values and (S_{md}) is the standard error of the mean difference. For a

two-tailed hypothesis with paired samples, (n) is the number of differences, (v = n-1) are the degrees of freedom, and t(alpha,2,v) is the critical value of the $\underline{t}$ statistic. A significant result is declared when t > t(alpha,2,v).

All statistical calculations were performed with the computer programs from Statistical Analysis System [SAS Institute Inc. 1982] which were supported under the operating systems of the Graduate School Computer Center of the City University of New York (CUNY).

CHAPTER 4

RESULTS

1. NEUROLOGICAL EXAMINATION

1.1 RIGHT OPTIC TRACTOTOMY

As soon as the animals recovered from anesthesia, they exhibited varying degrees of circling in the direction of the lesion, which disappeared 1-3 days postoperatively. Subjects showed dilated pupils on both eyes (5-7 mm), with a tendency for the pupil contralateral to the lesion to be more dilated. The direct and consensual reflexes were both present, as evidenced by pupillary constriction down to 3-4 mm with flash light stimulation from each eye separately. They tended to constrict more briskly upon stimulation of the right eye.

The horizontal extension of the visual fields was tested as described under Materials and Methods. Monkeys started to select morsels from the extreme right of the tray, and going towards the center. After some delay, they selected also from the left off center, but ignoring the extreme left. This test was repeated three times and results became more evident on restraining one arm at a time. Some difficulties were experienced because the animals tended to explore tactually the surface of the testing board. These results indicated a left hemianopia. Eyes were not tested independently, so that

the homonymous character of the field defect could only be presumed.

1.2 LEFT OCCIPITAL LOBECTOMY

After surgery, some monkeys exhibited circling movements to the side of the lesion, but they disappeared after the first one or two postoperative days. Contralateral hemianopia was observed after evaluation of the extension of the visual fields. Ocular movements in response to tactile or auditory stimuli were normal, as well as the pupillary and blink reactions.

2. HISTOLOGICAL VERIFICATION

2.1 RIGHT OPTIC TRACTOTOMY

Serial sections of the right optic tract at the level of the lesion were stained for myelin sheaths and for Nissl substance (Fig.8). The planes of the microtome section and of the surgical lesion were oblique with respect to each other. The lesion was apparent at all levels of the tract, from rostrolateral to ventromedial portions. Myelin was still well preserved, which is a common finding after a seven day lesion. Sections from animals #1033, 1036 and 1040 showed some involvement of the ventral hypothalamus.

2.2 LEFT OCCIPITAL LOBECTOMY

Serial sections of the left (operated) hemisphere stained for Nissl substance showed absence of striate cortex (area OC) from both the dorsolateral surface and from within the calcarine fissure (Fig.9). In addition, the ablations included a large amount of prestriate cortex, estimated as 80% of area OB and 30% of area OA [Bonin and Bailey 1947].

FIGURE 8

Histological verification of optic tract section

Serial sections of the right optic tract at the level of the lesion, taken 80 μm appart. The completeness of the lesion is demonstrated by its presence at all levels of the tract, from the dorsomedial portion (A) to the ventrolateral portion (D), including intermediate levels (B and C). Myelin staining is still present, which is a common finding after a 7 day lesion. KlÜver-Barrera method. Magnification: 10X.

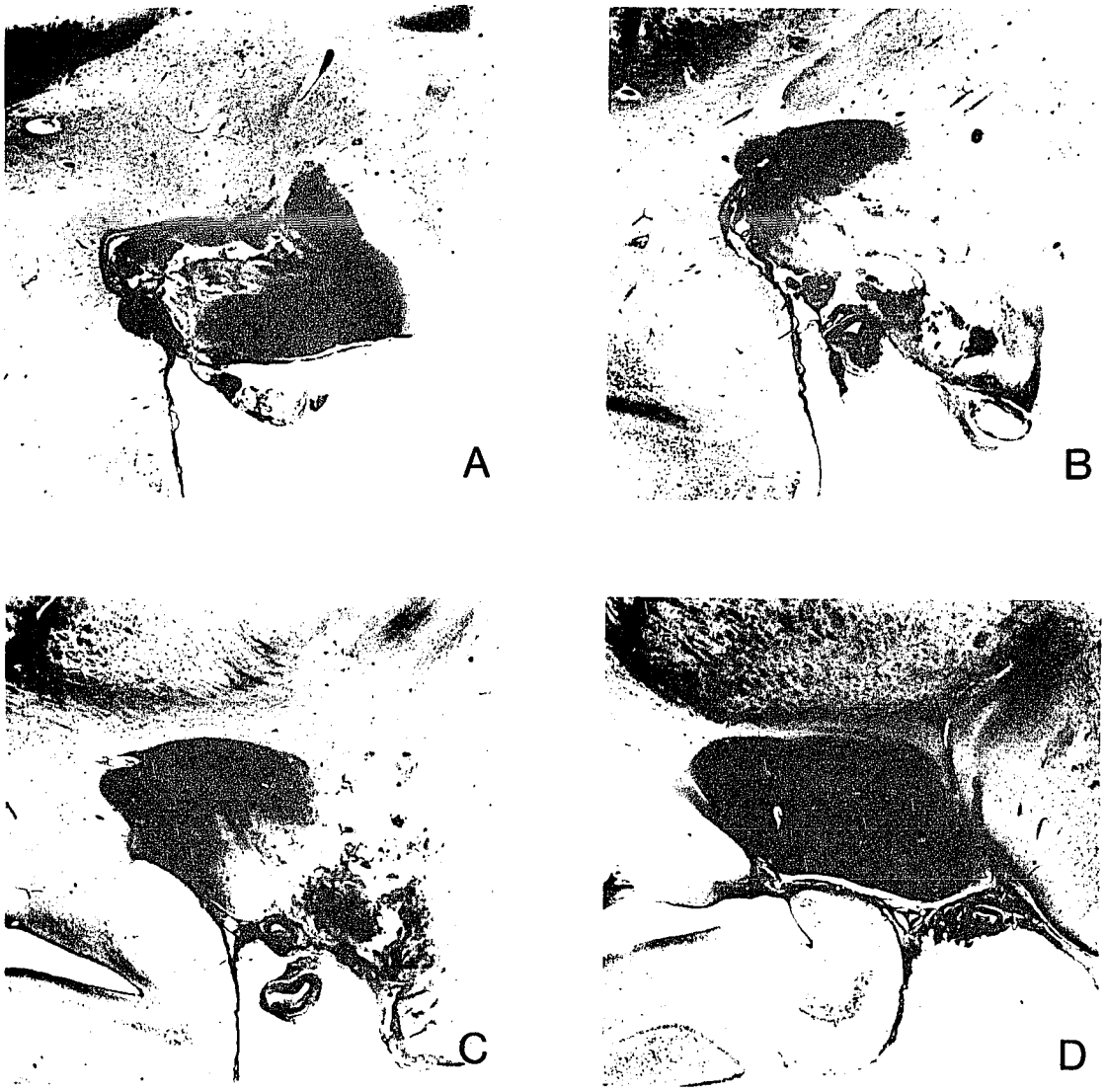
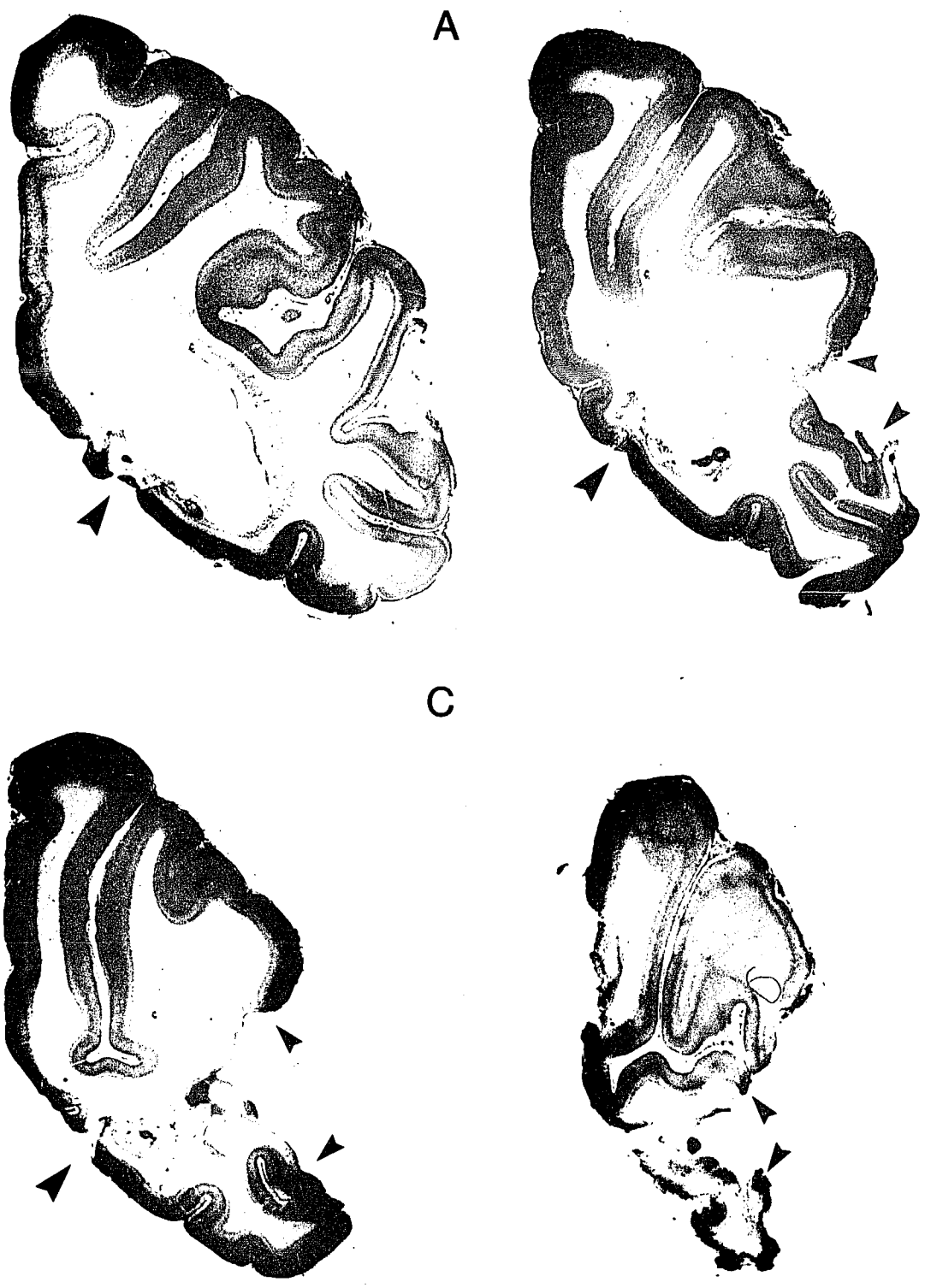

A
B
C
D

FIGURE 9

Histological verification of occipital lobectomy

Serial sections of the caudal portion of the operated (left) hemisphere, taken 240 μm appart. Note that the striate cortex of the calcarine fissure has been completely aspirated (large arrow heads), and that there are not remnants of striate cortex on the dorsolateral surface (small arrow heads). Cresyl violet stain. Magnification: 3X.


A
B
C
D

3. BIOCHEMICAL ANALYSES

3.1 AMINO ACID ANALYSIS

3.1.1 CRITERIA FOR EXCLUSION/INCLUSION

The standard solution contained forty amino acids. After HPLC analysis, eleven of them were excluded on the basis of the following criteria: (1) Absence of the primary amino group necessary to react with the fluorophoric reagent (OPA) to yield a detectable derivative: hydroxyproline, proline, creatinine and sarcosine. (2) Poor reaction with OPA, in spite of the presence of the primary amino group: cystine and homocystine. (3) Elution in pairs not completely resolved: serine/threonine. (4) Frequent co-elution with ammonia, a component of the commercial standard solution (Pierce ANB): hydroxylysine. (5) Artifactual peaks appearing in the region of alpha-amino-beta-guanidino propionate and arginine, the last two amino acids on the chromatogram, making their quantitation problematic.

The remaining twenty nine amino acids present in the standard mixture were efficiently resolved and showed high fluorescence yields (Table 7). However, after analysis of the biological samples, sixteen of them were also excluded on the following bases: (1) Values below the level of quantitation (0.10 nmol/injection): asparagine, citrulline, alpha-amino-butyrate, methionine, isoleucine, beta-alanine, beta-amino-isobutyrate, 3-methylhistidine, 1-methylhistidine and anser-ine. (2) Great variability in the retention times of the amino

acids that eluted last, tentatively identified as ethanolamine, ornithine, lysine, histidine and carnosine (Fig.10). (3) Uncertain identification of alpha-aminoadipate, because of a short and uncharacteristically broad peak present at its elution time.

Of the thirteen amino acids that did not fall into the previous categories, valine, leucine, tyrosine and phenylalanine were present at concentrations below 0.2 μmol/g wt in normal and experimental LGNd samples. They were analyzed in only two monkeys with optic tract section (#1028 and 1033), and in two monkeys with occipital lobectomy (#1029 and 1031). The results, however, were inconsistent, and due to the fact that large amounts of tissue would have been necessary for their reliable analyses, it was decided to exclude them from the rest of the study, to save material for the measurement of the dipeptide NAAG.

The eight amino acids that were present in concentrations higher than 0.2 μmol/g wt were taurine, aspartate, glutamate, glutamine, glycine, alanine, cystathionine and GABA; they were measured in all the animals. In addition, norleucine was used as internal standard.

TABLE 7
FLUORESCENCE CONSTANTS FOR SOME AMINO ACID DERIVATIVES

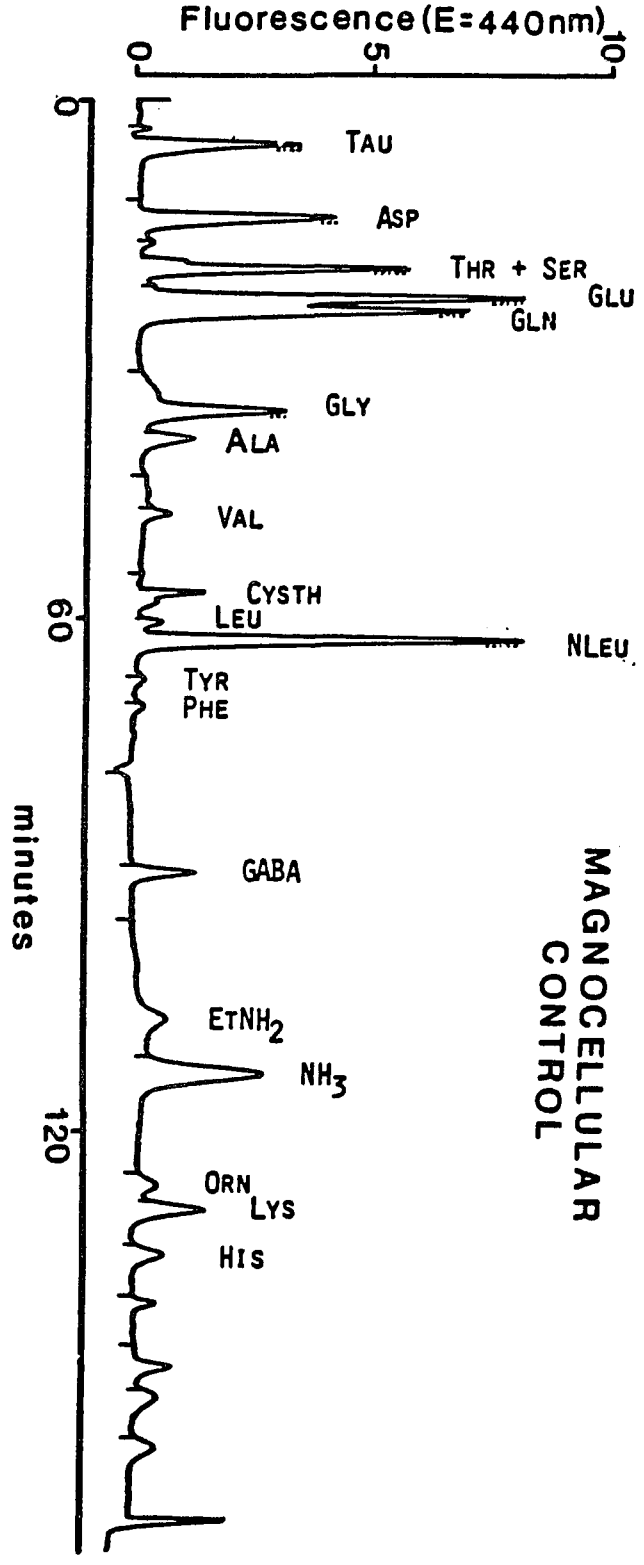
No.	AMINO ACID	ABBRV.	FLUORESCENCE CONSTANT* FK	FK'
01	TAURINE	TAU	1.87	0.82
02	ASPARTATE	ASP	1.60	0.70
03	ASPARAGINE	ASN	1.58	0.70
04	GLUTAMATE	GLU	1.82	0.80
05	GLUTAMINE	GLN	1.68	0.74
06	alpha-AMINOADIPATE	AAAD	1.27	0.56
07	GLYCINE	GLY	1.63	0.72
08	ALANINE	ALA	1.58	0.70
09	CITRULLINE	CIT	1.69	0.74
10	alpha-AMINOBUTYRATE	AABA	1.81	0.80
11	VALINE	VAL	2.18	0.96
12	METHIONINE	MET	1.86	0.82
13	CYSTATHIONINE	CTH	3.02	1.33
14	ISOLEUCINE	ILE	2.50	1.10
15	LEUCINE	LEU	2.09	0.92
16	NORLEUCINE	NLEU	2.27	1.00
17	TYROSINE	TYR	1.91	0.84
18	PHENYLALANINE	PHE	2.15	0.95
19	beta-ALANINE	BALA	1.44	0.63
20	beta-AMINOBUTYRATE	BABA	1.29	0.57
21	gamma-AMINOBUTYRATE	GABA	1.28	0.56
22	ETHANOLAMINE	ETA	1.47	0.65
23	ORNITHINE	ORN	2.58	1.13
24	LYSINE	LYS	3.58	1.58
25	HISTIDINE	HIS	1.30	0.57
26	3-METHYLHISTIDINE	HIS3	1.00	0.44
27	1-METHYLHISTIDINE	HIS1	1.09	0.48
28	CARNOSINE	CAR	1.14	0.50
29	ANSERINE	ANS	1.17	0.51

(*) Fluorescence constants (FK) are equivalent to the area under the peak generated by 1 nmole of the amino acid, and are expressed in (cm^2/nmole) (see Materials and Methods). The values refer only to the conditions of the analysis (chart speed=3 mm/min, attenuation=128). FK' are the relative fluorescence constants, in reference to the internal standard NLEU = 1. Eleven amino acids present in the standard solution, excluded on the bases described in the text, are not shown: hydroxyproline, threonine, serine, sarcosine, proline, cystine, homocystine, hydroxylysine, creatinine, alpha-amino-beta-guanidino propionate and arginine.

FIGURE 10

HPLC chromatogram of amino acid analysis

The sample is a perchloric acid extract of the monkey LGNd (control side of the magnocellular segment), equivalent to approximately 0.5 mg of wet weight of original brain. Amino compounds were detected at a wavelength of 340 nm after post-column derivatization with o-phthalaldehyde. Chart speed was 3 mm/min, and the attenuation was 128. Other chromatographic conditions are described in Materials and Methods. The amino acids included in the final analysis were taurine (TAU), aspartate (ASP), glutamate (GLU), glutamine (GLN), glycine (GLY), alanine (ALA), cystathionine (CTH) and gamma-aminobutyrate (GABA); in addition, norleucine (NLEU) was used as internal standard. The rest of the amino compounds were excluded according to several criteria, as described in Results, Section 3.1.1.


Fluorescence (E=440nm)
0
5
10
0
60
120
minutes
TAU
ASP
THR + SER
GLU
GLN
GLY
ALA
VAL
CYSTH
LEU
NLEU
TYR
PHE
GABA
ETNH2
NH3
ORN
LYS
HIS
MAGNOCELLULAR
CONTROL

3.1.2 NORMAL REGIONAL DISTRIBUTION

The normal regional distribution of amino acids in the visual system was studied on left brain structures, considered unchanged after a seven day lesion of the right optic tract. Values from right side structures in animals with left occipital lobectomy were not included because of the possibility that transneuronal changes occurred following the degeneration of commissural fibers of prestriate cortical origin after a three month survival period. The structures studied were the optic tract (TRACT), the LGNd, the superior colliculus (S.COL), and the striate cortex (CTX). Samples from the LGNd included separate specimens of its magnocellular (MAGNO) and parvocellular (PARVO) segments. The mean concentration value for the entire LGNd was obtained as a weighted mean that compensated for the differences in volume of these two segments. In a young macaque monkey, the volume ratio of the parvocellular and magnocellular segments of the LGNd was reported as PARVO:MAGNO=6.7 [Gottlieb et al. 1985]. Therefore, the weighted mean value of the LGN.T was calculated with the formula:

$$X = \frac{(MG) + (6.7 * PV)}{(7.7)}$$

where (X) is the weighted mean concentration (in μmol/g wt tissue), and (MG) and (PV) are the concentrations of the amino acid in the magnocellular and parvocellular segments, respectively. Specimens of the striate cortex comprised

separate samples of dorsolateral and calcarine cortices, corresponding to the central (CTX.C) and peripheral (CTX.P) portions of the visual fields. The volumes of these two segments of striate cortex are of similar magnitude in the young macaque [Gottlieb et al. 1985], and therefore the mean value for the entire striate cortex (CTX.T) was not weighted. Statistical differences among regions were determined with the Bonferroni multiple procedure, at an overall significance level alpha of 0.05, as described in Materials and Methods.

Only the eight amino acids present in moderate to high concentrations were quantitated for this part of the study, to save material for the analysis of NAAG. The results are shown in Table 8 (for a representative chromatogram see also Fig.10).

Taurine concentrations varied closely among regions of the visual system, from 2.73 μmol/g wt in the optic tract, to 1.82 μmol/g wt in the parvocellular segment of the LGNd. These two extremes were significantly different from each other, but not the intermediate values (Table 8A; Fig.11A).

Aspartate concentrations were significantly lower in the optic tract (2.13 μmol/g wt) than in the LGNd (3.75 μmol/g wt), both in its magnocellular (3.96 μmol/g wt) and parvocellular segments (3.71 μmol/g wt). All other comparisons showed no significant differences(Table 8B; Fig.11B).

Glutamate had a homogeneous distribution along the visual pathways, with concentrations ranging from 4.43 μmol/g wt to 7.04 μmol/g wt. The optic tract (4.43 μmol/g wt) and the superior colliculus (4.69 μmol/g wt) had the lowest values, but were not significantly different from the rest (Table 8C; Fig.11C).

Glutamine, glycine and alanine also had homogeneous distributions in structures of the visual system (Table 8D-8F; Fig. 11D-11F).

Cystathionine concentration decreased progressively in the rostrocaudal direction, being highest in the optic tract (0.69 μmol/g wt) and lowest in the striate cortex (0.20 μmol/g wt). Significant regional differences are indicated in Table 8G. For example, the concentration of cystathionine was significantly higher in the optic tract than in the superior colliculus and both segments of the striate cortex. The two segments of the LGNd had comparable concentrations of this amino acid; the magnocellular segment had a significantly higher concentration of cystathionine than the superior colliculus and the striate cortex, and the parvocellular segment differed significantly only from the peripheral visual field segment of the striate cortex (Fig.11G).

GABA concentration in the superior colliculus (3.36 μmol/g wt) was significantly higher than in the rest of the structures, where the average was 1.10 μmol/g wt. Although the level in the optic tract was only 0.28 μmol/g wt, the

differences with the LGNd (1.12 μmol/g wt) and striate cortex (1.28 μmol/g wt) were not significant (Table 8H; Fig.11H).

TABLE 8
NORMAL REGIONAL DISTRIBUTION OF AMINO ACIDS IN THE MONKEY VISUAL SYSTEM

A:			T A U R I N E	
No	REGION	N	CONCENTRATION	DIFF. VS REGION No
1	TRACT	4	2.73 ±0.18	4
2	LGN.T	6	1.92 ±0.19	
3	MAGNO	6	2.60 ±0.31	
4	PARVO	6	1.82 ±0.19	1
5	S.COL	4	2.25 ±0.39	
6	CTX.T	4	2.44 ±0.35	
7	CTX.C	4	2.26 ±0.40	
8	CTX.P	4	2.62 ±0.30	
B:			A S P A R T A T E	
1	TRACT	4	2.13 ±0.15	2, 3, 4
2	LGN.T	6	3.75 ±0.47	1
3	MAGNO	6	3.96 ±0.61	1
4	PARVO	6	3.71 ±0.48	1
5	S.COL	4	3.39 ±0.71	
6	CTX.T	4	3.40 ±0.69	
7	CTX.C	4	3.37 ±0.78	
8	CTX.P	4	3.44 ±0.62	
C:			G L U T A M A T E	
1	TRACT	4	4.43 ±0.24	
2	LGN.T	6	6.71 ±0.71	
3	MAGNO	6	7.04 ±1.19	
4	PARVO	6	6.66 ±0.70	
5	S.COL	4	4.69 ±0.97	
6	CTX.T	4	6.80 ±1.41	
7	CTX.C	4	7.03 ±1.42	
8	CTX.P	4	6.58 ±1.42	

TABLE 8 (continued)

No	REGION	N	CONCENTRATION	DIFF. VS REGION No
D:			G L U T A M I N E	
1	TRACT	4	4.79 ±0.56	
2	LGN.T	6	5.56 ±0.86	
3	MAGNO	6	6.03 ±0.94	
4	PARVO	6	5.49 ±0.88	
5	S.COL	4	5.48 ±1.44	
6	CTX.T	4	5.89 ±1.55	
7	CTX.C	4	6.04 ±1.66	
8	CTX.P	4	5.73 ±1.62	
E:			G L Y C I N E	
1	TRACT	4	1.45 ±0.08	
2	LGN.T	6	1.30 ±0.46	
3	MAGNO	6	1.70 ±0.48	
4	PARVO	6	1.24 ±0.46	
5	S.COL	4	2.53 ±0.71	
6	CTX.T	4	1.87 ±0.63	
7	CTX.C	4	2.35 ±1.06	
8	CTX.P	4	1.39 ±0.31	
F:			A L A N I N E	
1	TRACT	4	0.47 ±0.05	
2	LGN.T	6	0.65 ±0.17	
3	MAGNO	6	0.72 ±0.20	
4	PARVO	6	0.63 ±0.21	
5	S.COL	4	0.85 ±0.34	
6	CTX.T	4	0.88 ±0.33	
7	CTX.C	4	1.12 ±0.53	
8	CTX.P	4	0.63 ±0.12	

TABLE 8 (continued)

G:	C Y S T A T H I O N I N E			
No	REGION	N	CONCENTRATION	DIFF. VS REGION No
1	TRACT	4	0.69 ±0.04	5, 6, 7, 8
2	LGN.T	6	0.48 ±0.08	6, 8
3	MAGNO	6	0.60 ±0.16	5, 6, 7, 8
4	PARVO	6	0.46 ±0.08	8
5	S.COL	4	0.32 ±0.10	1, 3
6	CTX.T	4	0.23 ±0.04	1, 2, 3
7	CTX.C	4	0.26 ±0.05	1, 3
8	CTX.P	4	0.20 ±0.05	1, 2, 3, 4
H:			G A B A	
1	TRACT	4	0.28 ±0.10	5
2	LGN.T	6	1.12 ±0.12	5
3	MAGNO	6	1.36 ±0.20	5
4	PARVO	6	1.09 ±0.12	5
5	S.COL	4	3.66 ±0.82	ALL
6	CTX.T	4	1.28 ±0.16	5
7	CTX.C	4	1.18 ±0.16	5
8	CTX.P	4	1.39 ±0.17	5

TABLE 8

Normal regional distribution of amino acids in the monkey visual system

Amino acid concentrations are the mean ±SEM in μmol/g wt. As indicated in column (N), six monkeys were used in the analysis of LGNd structures, and four monkeys for the remaining structures. The regions analyzed were the optic tract (TRACT), the magnocellular (MAGNO) and parvocellular (PARVO) segments of the LGNd, the superior colliculus (S.COL), and the central (CTX.C) and peripheral (CTX.P) representations of the visual fields in the striate cortex. The values for the total LGNd (LGN.T) represent the weighted means of the constituent segments, as defined in Results, Section 3.1.2. The numbers in the last column (DIFF VS REGION No) refer to the numbered regions as in the first column, and indicate statistically significant differences in amino acid concentrations; for example, they show that taurine concentration in the optic tract (region No.1) is significantly different from the parvocellular segment (region No.4) of the LGNd. Blanks in the last column indicate the absence of significant differences between regions. Statistical analysis was made with the Bonferroni's multiple comparisons procedure, at the overall level of significance of 0.05, as described in Materials and Methods.

FIGURE 11

Graphic representation of the normal regional distribution of amino acids in the visual system

Bars represent the mean amino acid concentration ±S.E.M., expressed in μmol/g wt. Six monkeys were used in the analysis of LGNd structures, and four monkeys for the remaining structures. Notations as in Table 8.

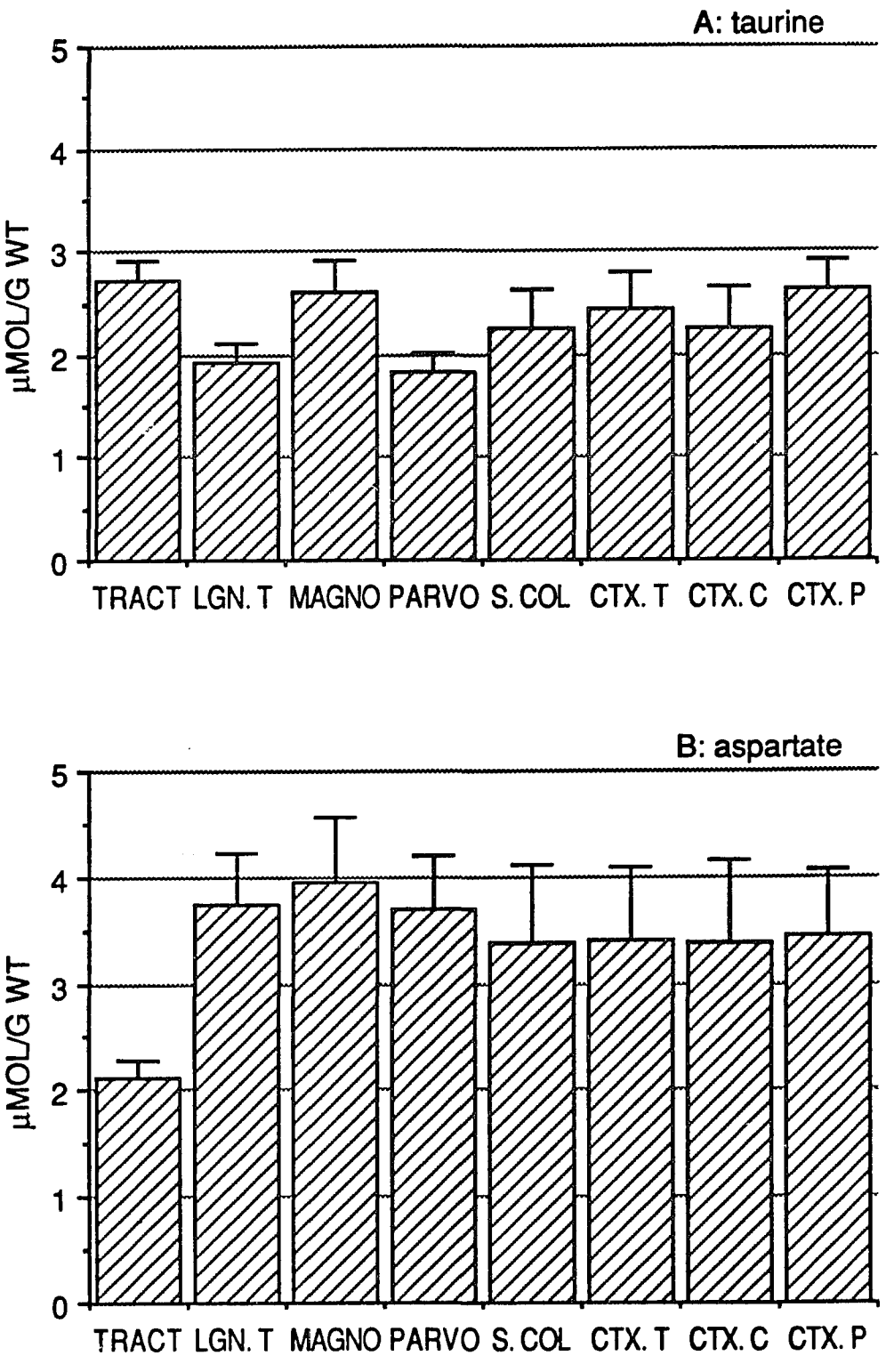
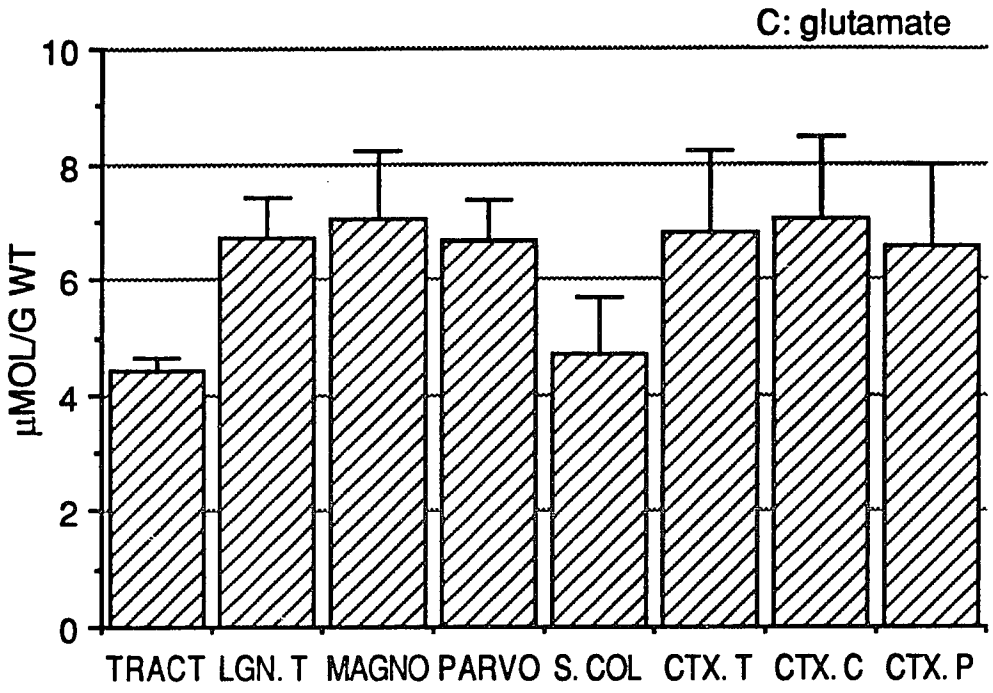

A: taurine
µMOL/G WT
5
4
3
2
1
0
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P
B: aspartate
µMOL/G WT
5
4
3
2
1
0
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P

Figure 11



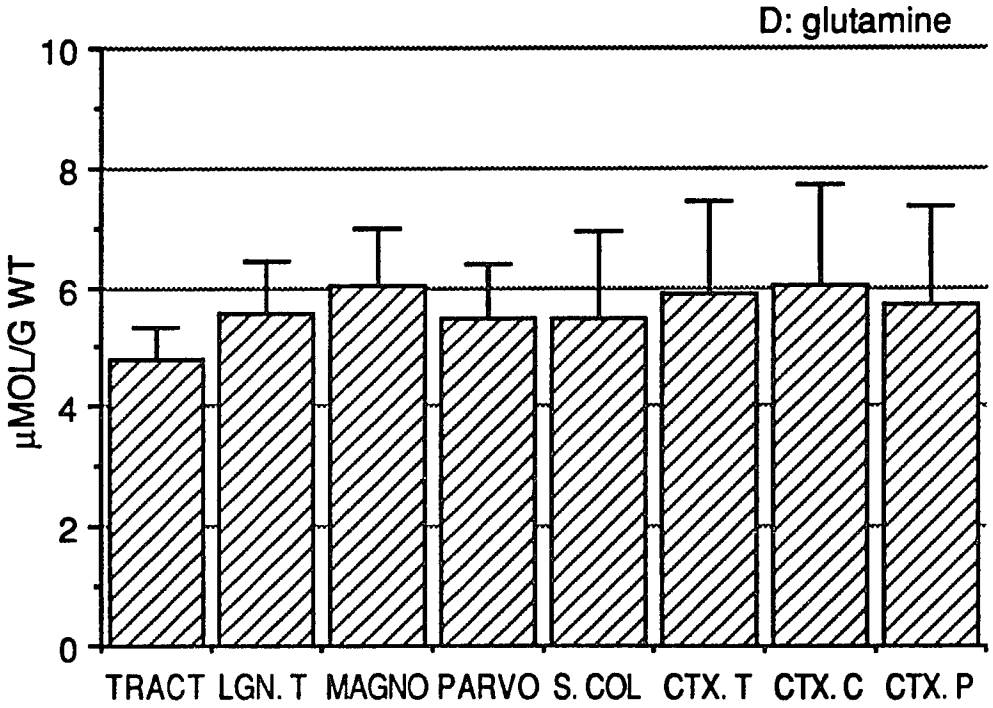
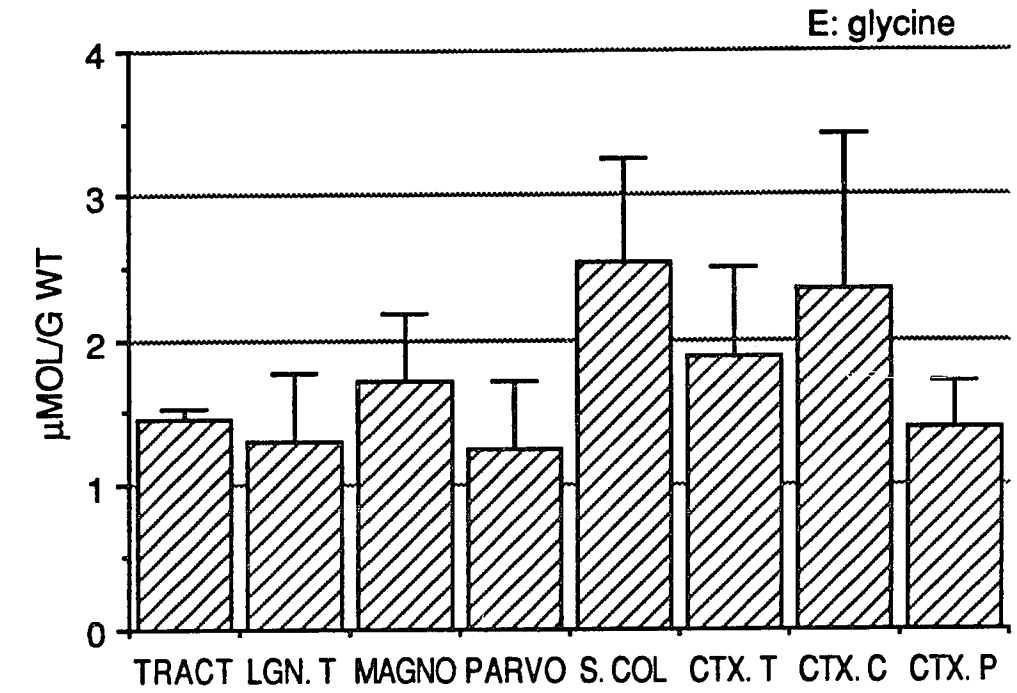


Figure 11 (continued)



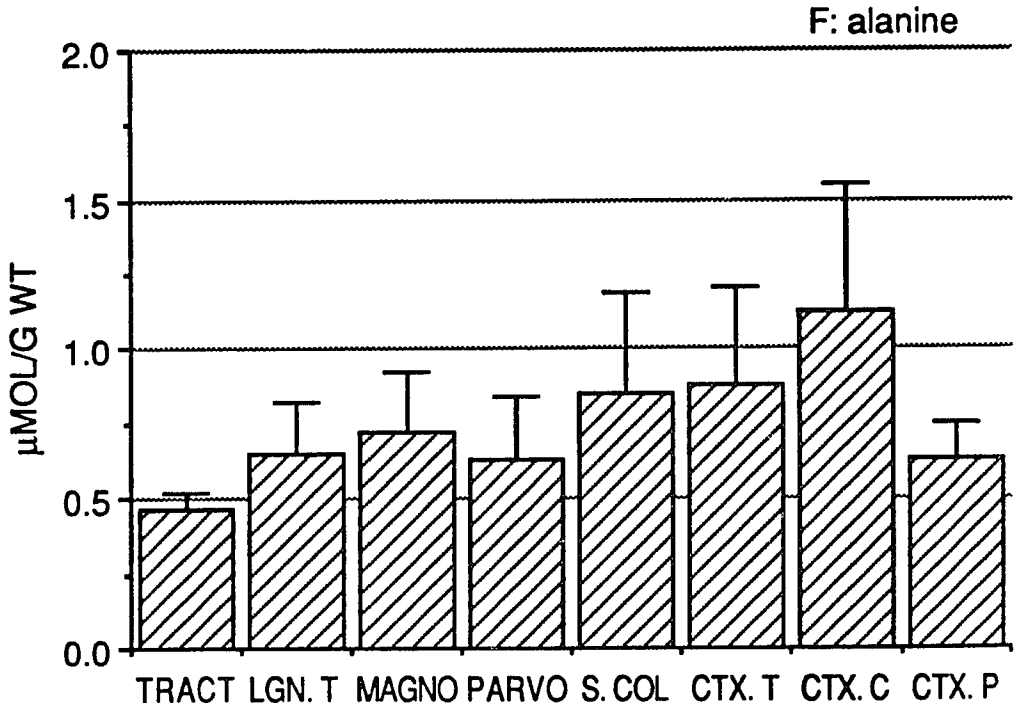
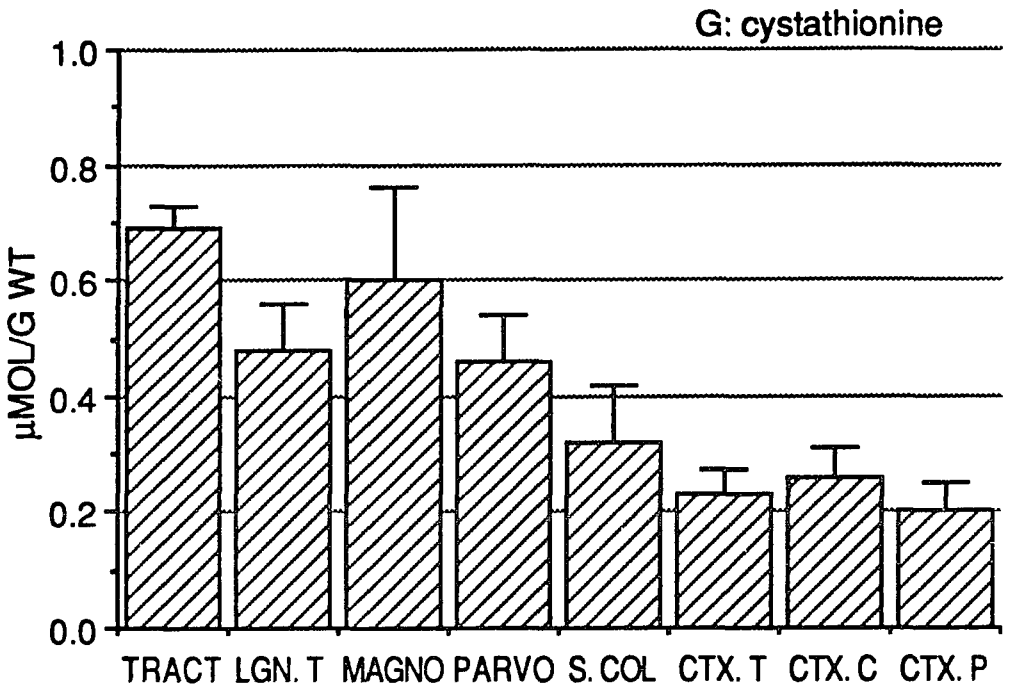


Figure 11 (continued)



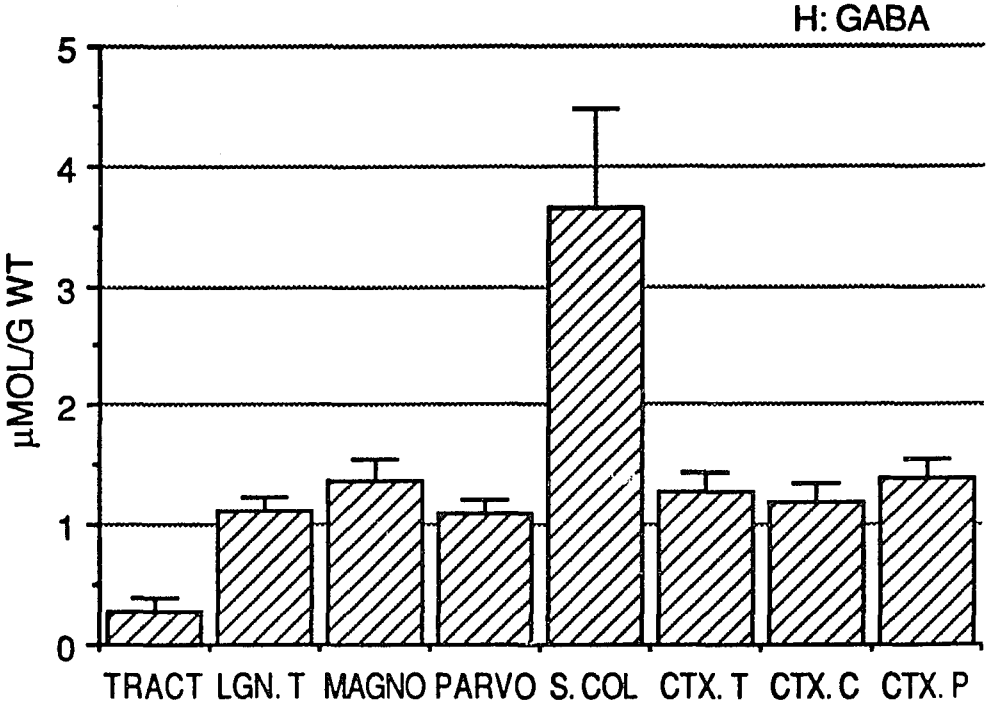


Figure 11 (continued)

3.1.3 EFFECTS OF LESIONS

Optic tract Section

A total of six monkeys was employed to study the effect of right optic tractotomy on the amino acid concentration in structures of the visual system. The LGNd was sampled in all of the animals, and the optic tract, superior colliculus and striate cortex were studied only in monkeys #1035, 1036, 1037 and 1040. The statistical significance between mean differences of absolute values in the control and experimental sides was determined with the Student's paired t-test (see Materials and Methods); differences were also expressed as the percent change from control values, calculated with the formula [(experimental/control)-1]*100. Results are given in Tables 9A-9H; Figs.12A-12H.

In the portion of the optic tract distal to the lesion, i.e., close to the LGNd, only ASP and GLU showed a significant reduction ($p<0.01$) in their mean concentration, which amounted to 50% and 48%, respectively (Table 9A; Fig.12A). GABA levels also were lower, whereas those of TAU, GLN, GLY, ALA and CTH were higher than controls. The increases in ALA and GLY were large (70% and 69%, respectively), and that for ALA reached significance (Table 9A).

In the LGNd (Table 9B; Fig.12B), only GLU levels were significantly reduced, by 12% of control values. ASP and GABA were likewise decreased, but the differences failed to reach significance. Other amino acids showed increases of various

magnitudes, some of which were statistically significant (GLN, CTH), and occasionally large (CTH).

Separate analysis of the magnocellular and parvocellular segments of the LGNd (Table 9C-9D; Fig.12C-12D) indicated a small (11%) but significant decrease in the concentration of GLU in the parvocellular layers; the reduction in the magnocellular division was larger (19%), but failed to reach significance probably due to the high variability in the control values. Although not strictly significant, it is noteworthy that the 26% decrease in ASP levels in the magnocellular layers reached a probability level of 0.056. Cystathionine was the only amino acid with consistently higher levels in both segments of the LGNd after the optic tract section.

In the superior colliculus there was a general decrease in amino acid concentrations, but the probability level of occurring by chance was very high ($p > 0.4$) (Table 9E; Fig. 12E).

In the striate cortex, the measured amino acids decreased in concentration, with the exception of GLY, but none of the differences were statistically significant (Table 9F; Fig.12F). Separate analysis of cortical regions with representations of the central and peripheral visual fields also failed to yield any significant differences (Table 9G-9H; Figs.12G-12H).

TABLE 9
EFFECT OF OPTIC TRACTOTOMY ON THE AMINO ACID CONCENTRATION IN THE MONKEY VISUAL SYSTEM

A: D I S T A L O P T I C T R A C T (N = 4)				
AA	CTRL	EXPM	%CH	P
TAU	2.73 ±0.18	2.82 ±0.33	+ 3	
ASP	2.13 ±0.15	1.06 ±0.12	-50	**
GLU	4.43 ±0.24	2.32 ±0.33	-48	**
GLN	4.79 ±0.56	5.41 ±0.76	+13	
GLY	1.45 ±0.08	2.45 ±0.46	+69	0.076
ALA	0.47 ±0.05	0.80 ±0.08	+70	*
CTH	0.69 ±0.04	0.72 ±0.06	+ 4	
GABA	0.28 ±0.10	0.24 ±0.05	-14	

TABLE 9 (continued)

B: L G N d (T O T A L) (N = 6)				
AA	CTRL	EXPM	% CH	P
TAU	1.92 ±0.19	2.25 ±0.09	+17	
ASP	3.75 ±0.47	3.21 ±0.25	-14	
GLU	6.71 ±0.71	5.88 ±0.54	-12	*
GLN	5.56 ±0.86	6.49 ±0.82	+17	*
GLY	1.30 ±0.46	1.57 ±0.30	+21	
ALA	0.65 ±0.17	0.76 ±0.11	+23	
CTH	0.48 ±0.08	0.70 ±0.07	+46	**
GABA	1.12 ±0.12	0.98 ±0.09	-12	
C: L G N d (M A G N O C E L L U L A R) (N = 6)				
TAU	2.60 ±0.31	2.92 ±0.23	+12	
ASP	3.96 ±0.61	2.92 ±0.21	-26	0.056
GLU	7.04 ±1.19	5.73 ±0.63	-19	0.084
GLN	6.03 ±0.94	6.39 ±0.74	+ 6	
GLY	1.70 ±0.48	1.54 ±0.27	- 9	
ALA	0.72 ±0.20	0.72 ±0.13	0	
CTH	0.60 ±0.16	0.80 ±0.14	+33	*
GABA	1.36 ±0.20	1.15 ±0.07	-15	
D: L G N d (P A R V O C E L L U L A R) (N = 6)				
TAU	1.82 ±0.19	2.15 ±0.09	+18	
ASP	3.71 ±0.48	3.26 ±0.27	-12	
GLU	6.66 ±0.70	5.91 ±0.57	-11	*
GLN	5.49 ±0.88	6.50 ±0.87	+18	*
GLY	1.24 ±0.46	1.57 ±0.31	+27	
ALA	0.63 ±0.21	0.76 ±0.15	+21	
CTH	0.46 ±0.08	0.68 ±0.06	+48	**
GABA	1.09 ±0.12	0.95 ±0.10	-13	

TABLE 9 (continued)

E: S U P E R I O R C O L L I C U L U S (N = 4)				
AA	CTRL	EXPM	% CH	P
TAU	2.25 ±0.39	2.06 ±0.22	- 8	
ASP	3.39 ±0.71	2.94 ±0.33	-13	
GLU	4.69 ±0.97	4.54 ±0.73	- 3	
GLN	5.48 ±1.44	5.04 ±0.79	- 8	
GLY	2.53 ±0.71	1.56 ±0.24	-38	
ALA	0.85 ±0.34	0.43 ±0.10	-49	
CTH	0.32 ±0.10	0.24 ±0.06	-25	
GABA	3.66 ±0.82	3.03 ±0.48	-17	

TABLE 9 (continued)

AA	CTRL	EXPM	% CH	P
F: S T R I A T E C O R T E X (T O T A L) (N = 4)				
TAU	2.44 ±0.35	2.32 ±0.14	- 5	
ASP	3.40 ±0.69	2.45 ±0.41	-28	
GLU	6.80 ±1.41	6.50 ±0.88	- 4	
GLN	5.89 ±1.55	5.75 ±0.59	- 2	
GLY	1.87 ±0.63	1.95 ±0.28	+ 4	
ALA	0.88 ±0.33	0.73 ±0.07	-17	
CTH	0.23 ±0.04	0.19± 0.05	-17	
GABA	1.28 ±0.16	1.18 ±0.12	- 8	
G: S T R I A T E C O R T E X (C E N T R A L)(N = 4)				
TAU	2.26 ±0.40	2.18 ±0.17	- 3	
ASP	3.37 ±0.78	2.60 ±0.46	-23	
GLU	7.03 ±1.42	5.84 ±0.83	-17	
GLN	6.04 ±1.66	5.71 ±0.53	- 5	
GLY	2.35 ±1.06	1.90 ±0.66	-19	
ALA	1.12 ±0.53	0.53 ±0.07	-53	
CTH	0.26 ±0.05	0.23 ±0.07	- 8	
GABA	1.18 ±0.16	1.17 ±0.12	- 1	
H: S T R I A T E C O R T E X (P E R I P H E R A L)(N = 4)				
TAU	2.62 ±0.30	2.46 ±0.18	- 6	
ASP	3.44 ±0.62	3.29 ±0.41	- 4	
GLU	6.58 ±1.42	7.15 ±0.97	+ 9	
GLN	5.73 ±1.62	5.80 ±0.67	+ 1	
GLY	1.39 ±0.31	2.02 ±0.20	+44	
ALA	0.63 ±0.12	0.92 ±0.13	+46	
CTH	0.20 ±0.05	0.15 ±0.03	-29	
GABA	1.39 ±0.17	1.20 ±0.14	-14	

TABLE 9

Effect of optic tractotomy on the amino acid concentration in the monkey visual system

Amino acid concentrations are the mean ±SEM in μmol/g wt. Six monkeys were used in the analysis of LGNd structures, and four monkeys for the remaining structures. The regions analyzed were the portion of the optic tract distal to the lesion, the magnocellular and parvocellular segments of the LGNd, the superior colliculus, and the central and peripheral segments of the striate cortex. The values for the total LGNd (LGN.T) represent the weighted mean of the constituent segments, as defined in Results, Section 3.1.2. Control values (CTRL) are from structures on the left hemisphere, and experimental values (EXPM) are from homologous, paired structures on the opposite hemisphere. Percent change (%CH) is defined as [(EXPM/CTRL)-1]*100. Statistical analysis was made with the Student's paired t-test, at the levels of significance of $p<0.05$ (*), and $p<0.01$ (**); when $0.05<p<0.1$, the numerical value is given in the last column (P); blanks in this column indicate the absence of statistically significant differences.

FIGURE 12

Graphic representation of the effect of optic tractotomy on the amino acid concentration in the visual system

Bars represent the mean amino acid concentration ±S.E.M., expressed in μmol/g wt. Wide hatched and narrow hatched bars indicate the amino acid concentration in the control and experimental sides, respectively. Significant decreases as determined with the Student's paired t-test are indicated at the alpha levels of 0.05 (*) and 0.01 (**). The number of monkeys employed in the analysis of each region is indicated in the corresponding panel. Notations as in Table 9.

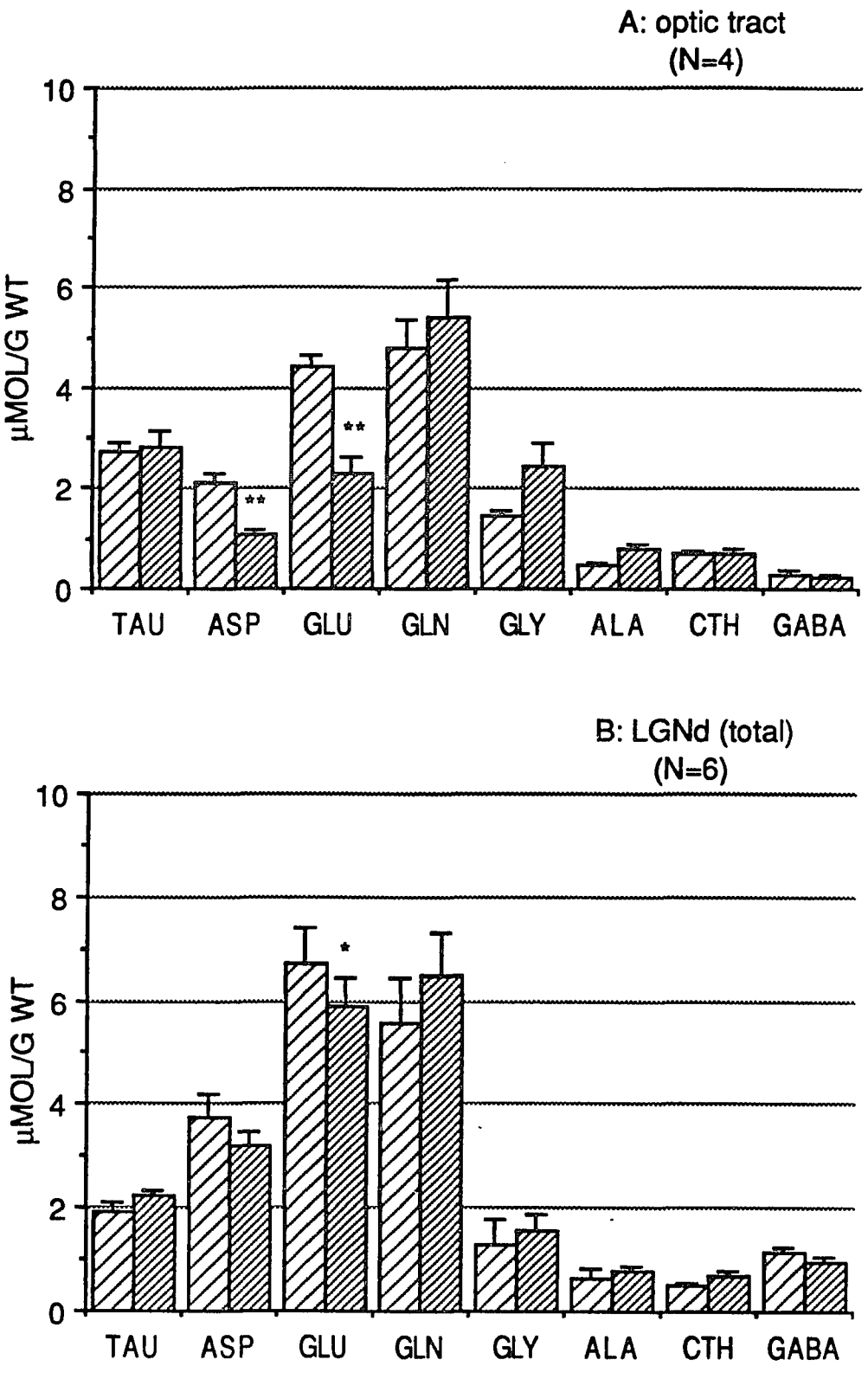

A: optic tract
(N=4)
µMOL/G WT
10
8
6
4
2
0
**
**
TAU
ASP
GLU
GLN
GLY
ALA
CTH
GABA
B: LGNd (total)
(N=6)
µMOL/G WT
10
8
6
4
2
0
*
TAU
ASP
GLU
GLN
GLY
ALA
CTH
GABA

Figure 12

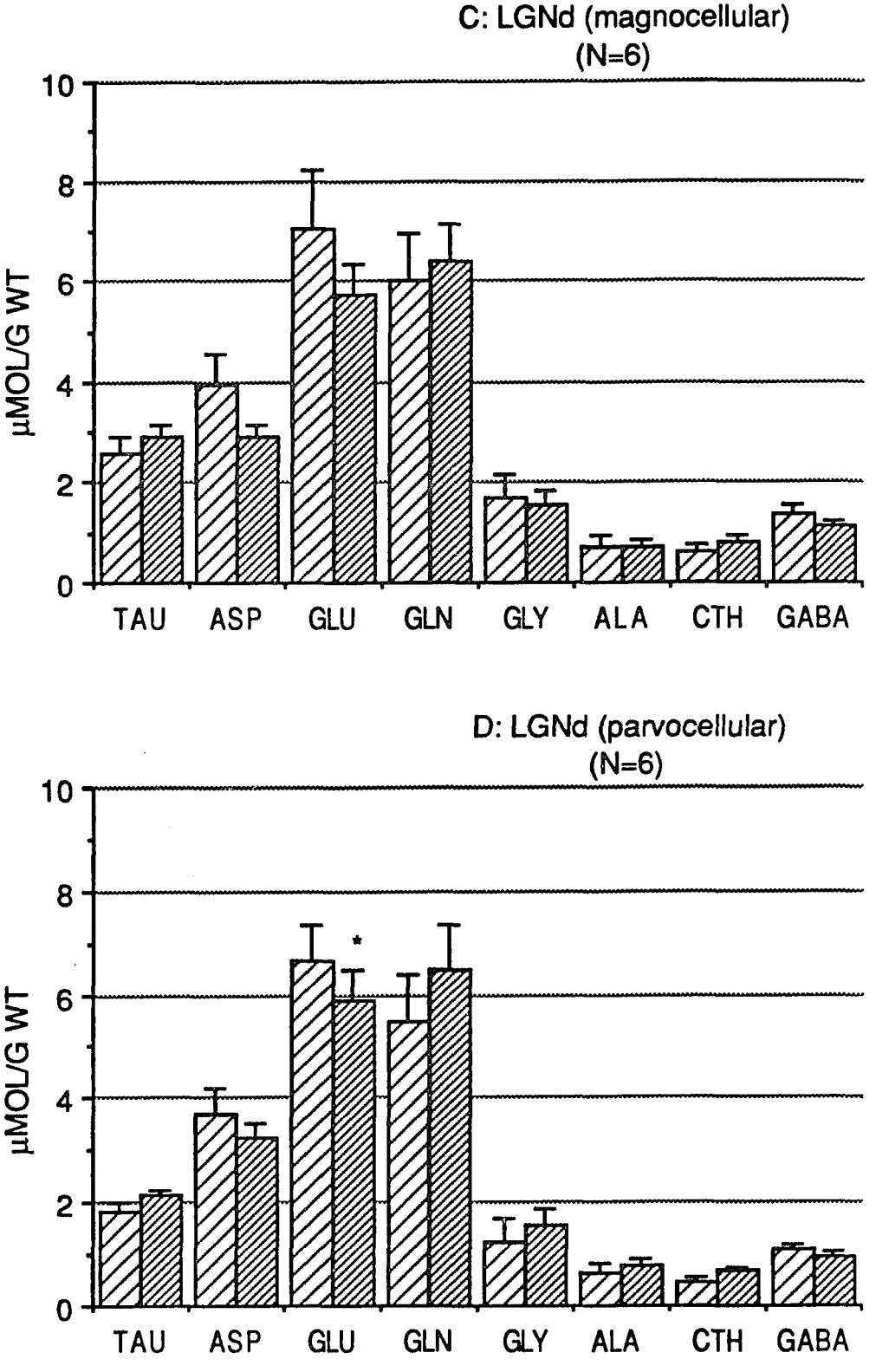
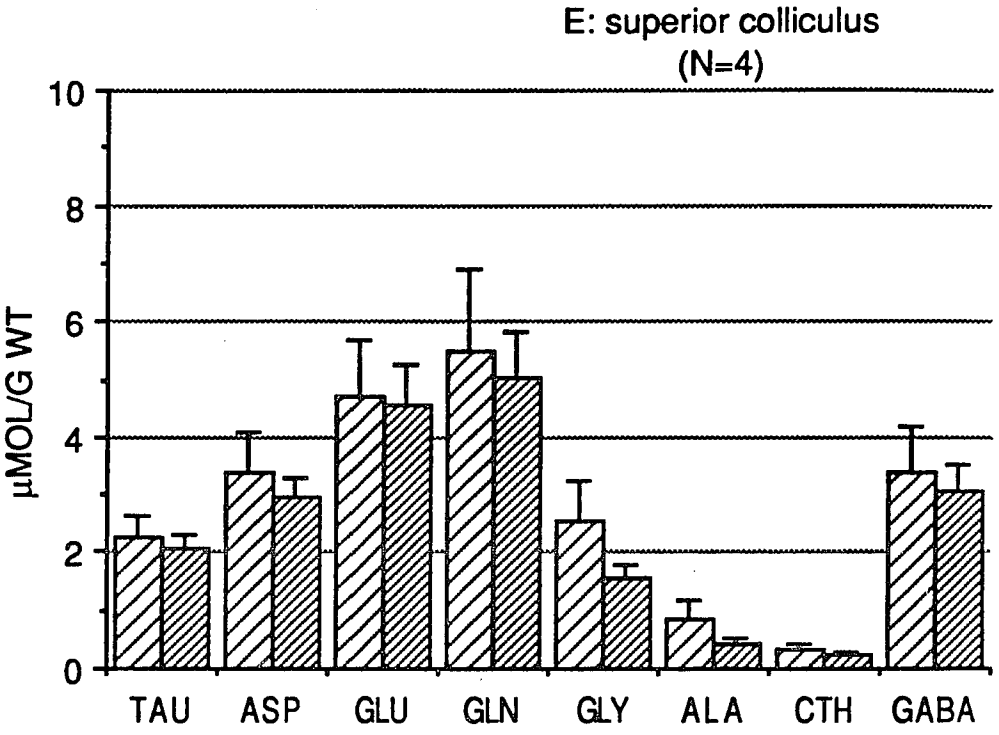


Figure 12 (continued)



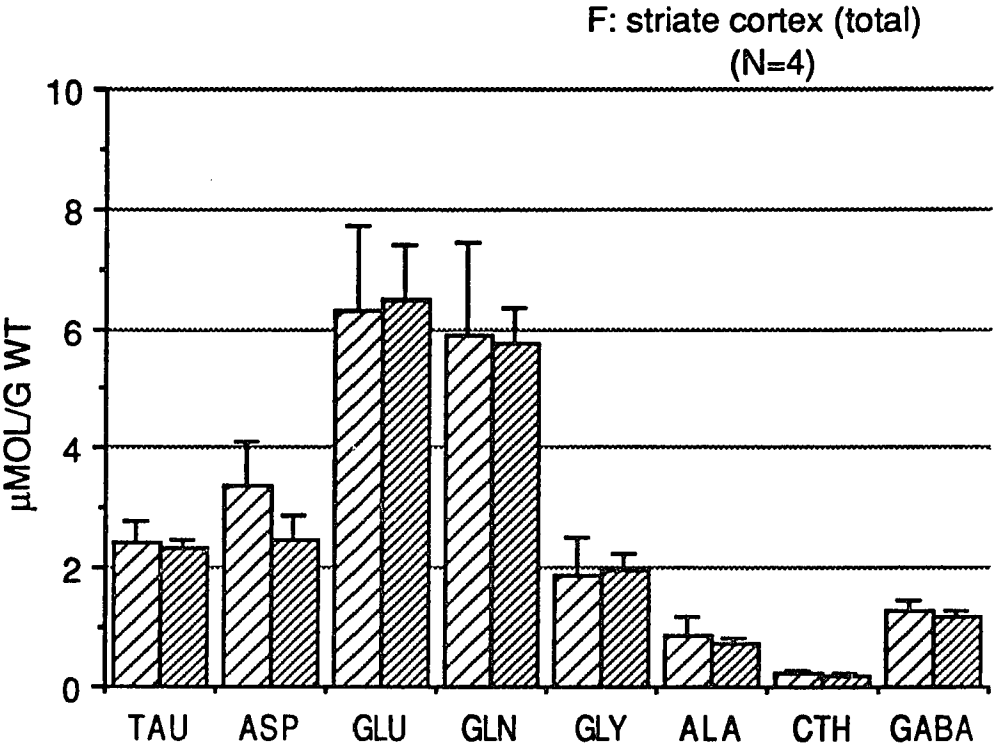


Figure 12 (continued)

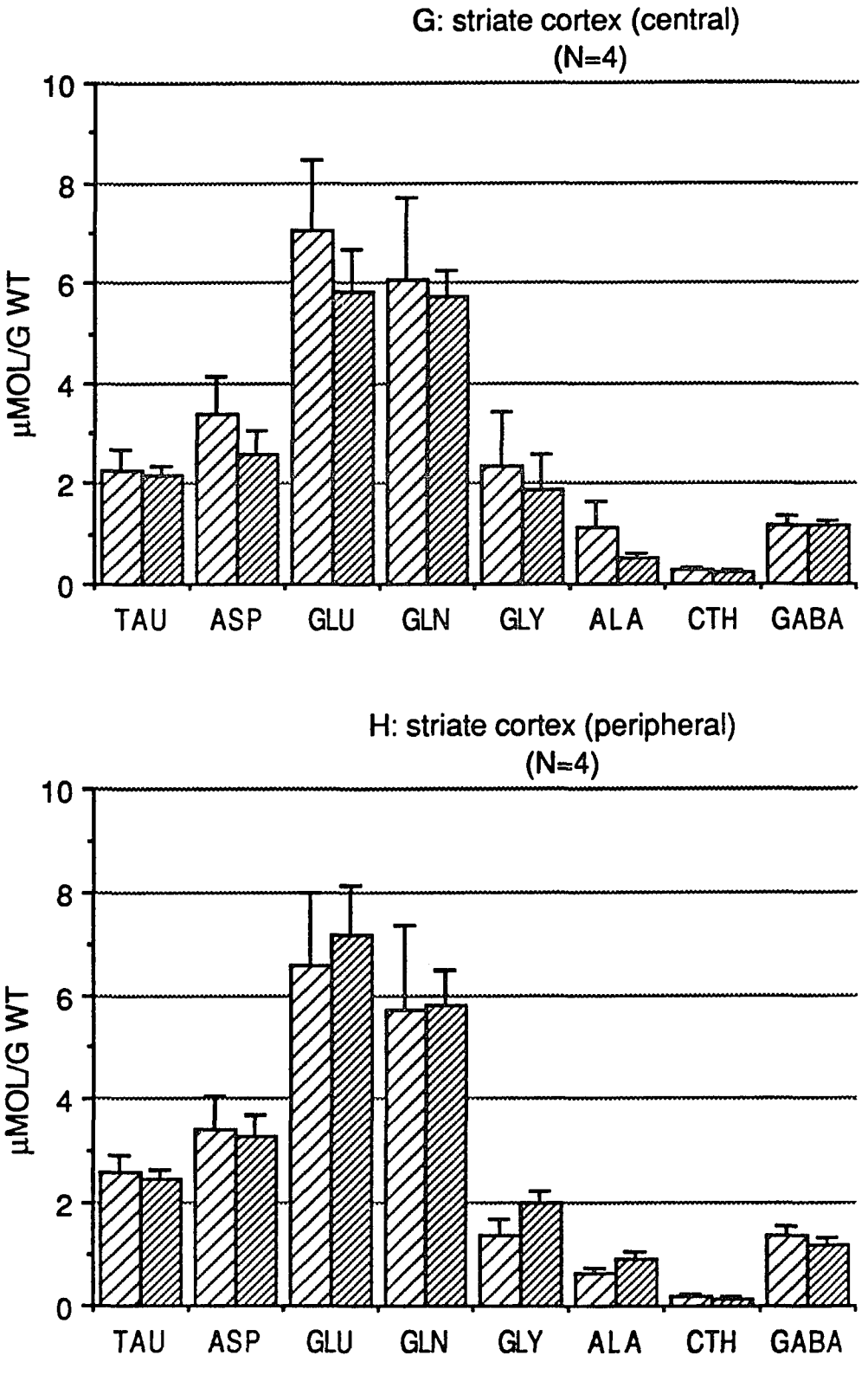


Figure 12 (continued)

Occipital lobectomy

The effect of left occipital lobectomy on the amino acid concentration in the LGNd was examined in two monkeys (#1029 and 1031) after a three-month survival period. Although the LGNd on the operated side appeared shrunken and did not have any discernible lamination, separate samples were taken also from this side approximately 0.5 mm from the ventral surface, designated as "magnocellular", and 0.5 mm from the limit with the white matter capsule in the dorsal region of the nucleus, representing the "parvocellular" component. Since a possible differential shrinkage of both laminae could not be determined, weighted means for the total LGNd (LGN.T) were not obtained.

The results were highly consistent between the two animals (Table 10). Drastic reductions in the concentrations of aspartate and glutamate were observed in both segments of the LGNd after occipital lobectomy; the magnitude of the decreases were in the average 75% for ASP and 66% for GLU. The decreases in alanine and GABA were approximately 50%, and the changes in glutamine and glycine were minimal. The concentration of the two remaining amino acids measured increased after the surgical procedure, especially cystathionine (160%), followed by taurine (56%).

TABLE 10
EFFECT OF OCCIPITAL LOBECTOMY ON THE AMINO ACID CONCENTRATION IN THE LATERAL GENICULATE NUCLEUS

		MONKEY #1029			MONKEY #1031		
AA	REGION	CTRL	EXPM	%CH	CTRL	EXPM	%CH
TAU	MAGNO	3.83	5.10	+33	3.82	4.36	+14
	PARVO	2.13	4.05	+90	2.20	4.08	+85
ASP	MAGNO	4.05	1.44	-64	6.67	1.58	-76
	PARVO	3.58	0.66	-81	4.49	0.97	-78
GLU	MAGNO	10.24	4.44	-57	10.81	4.21	-61
	PARVO	7.46	1.38	-81	8.16	2.85	-65
GLN	MAGNO	8.85	8.32	-6	7.73	6.20	-20
	PARVO	6.27	6.29	0	5.21	5.13	-2
GLY	MAGNO	1.39	1.12	-19	0.88	0.75	-15
	PARVO	0.49	0.81	+65	0.39	0.57	+46
ALA	MAGNO	0.84	0.38	-55	0.61	0.31	-49
	PARVO	0.33	0.18	-45	0.43	0.22	-49
CTH	MAGNO	0.54	1.31	+143	0.67	1.51	+125
	PARVO	0.37	1.12	+203	0.57	1.53	+168
GABA	MAGNO	1.70	0.90	-47	2.14	1.05	-51
	PARVO	0.97	0.48	-50	1.26	0.79	-37

Amino acid concentrations (in μmol/g wt.) were measured in monkeys #1029 and #1031, three months after left occipital lobectomy. (CTRL) represents the right (control) side, and (EXPM) the left (lesioned) side, of the magnocellular (MAGNO) and parvocellular (PARVO) segments of the LGNd. No statistical analysis was performed, due to the small sample size. Percent change (%CH) is defined as [(EXPM/CTRL)-1]*100.

3.2 NAAG AND NASP ANALYSES

3.2.1 NORMAL REGIONAL DISTRIBUTION

The normal regional distribution of NAAG (N-acetyl-aspartylglutamate) and NASP (N-acetyl-aspartate) was studied in the optic tract, LGNd, superior colliculus and striate cortex on the control side (left) of tractotomized monkeys. For a representative chromatogram of the HPLC analysis see Fig.13.

The concentration of NAAG was high in the optic tract (3.86 μmol/g wt), intermediate in the LGNd (2.28 μmol/g wt), low in the superior colliculus (1.00 μmol/g wt), and undetectable (below 0.5 μmol/g wt) in the striate cortex. Significant regional differences are indicated in Table 11. It is apparent that the concentration of NAAG was significantly higher in the optic tract than in the superior colliculus and the striate cortex. Similarly, the levels in the LGNd were higher than in the cortex. Although the magnocellular and parvocellular segments of the LGNd were statistically comparable, only the magnocellular division showed a higher concentration of NAAG when compared to the superior colliculus (see also Fig.14A).

The concentration of NASP was low in the optic tract (4.62 μmol/g wt), intermediate in the LGNd and the superior colliculus (6.44-9.89 μmol/g wt), and high in the striate cortex (approximately 14.0 μmol/g wt) (Table 11). In the latter structure, the concentration of NASP was significantly higher than that in the superior colliculus, LGNd and optic

tract; the concentration of NASP was significantly higher also in the superior colliculus than in the optic tract. No significant differences were observed between the two segments of the LGNd or between the central and peripheral visual field regions of the striate cortex (Table 11; Fig.14B).

NAAG and NASP showed an inverse gradient of concentration in the structures of the visual system. While the concentration of NAAG progressively decreased from the optic tract towards the striate cortex, the concentration of NASP decreased in the opposite direction (Table 11; Fig.15).

FIGURE 13

HPLC chromatogram of NAAG and NASP analysis

The sample is a perchloric acid extract of the monkey LGNd (control side of the magnocellular segment), equivalent to approximately 0.5 mg of wet weight of original brain. Compounds were detected by their U.V. absorbance at a wavelength of 210 nm. Chart speed was 3 mm/min, and the attenuation was 32. Other chromatographic conditions are described in Materials and Methods. A duplicate sample was spiked with NASP and NAAG standards, observing an increase in height of the corresponding peaks.

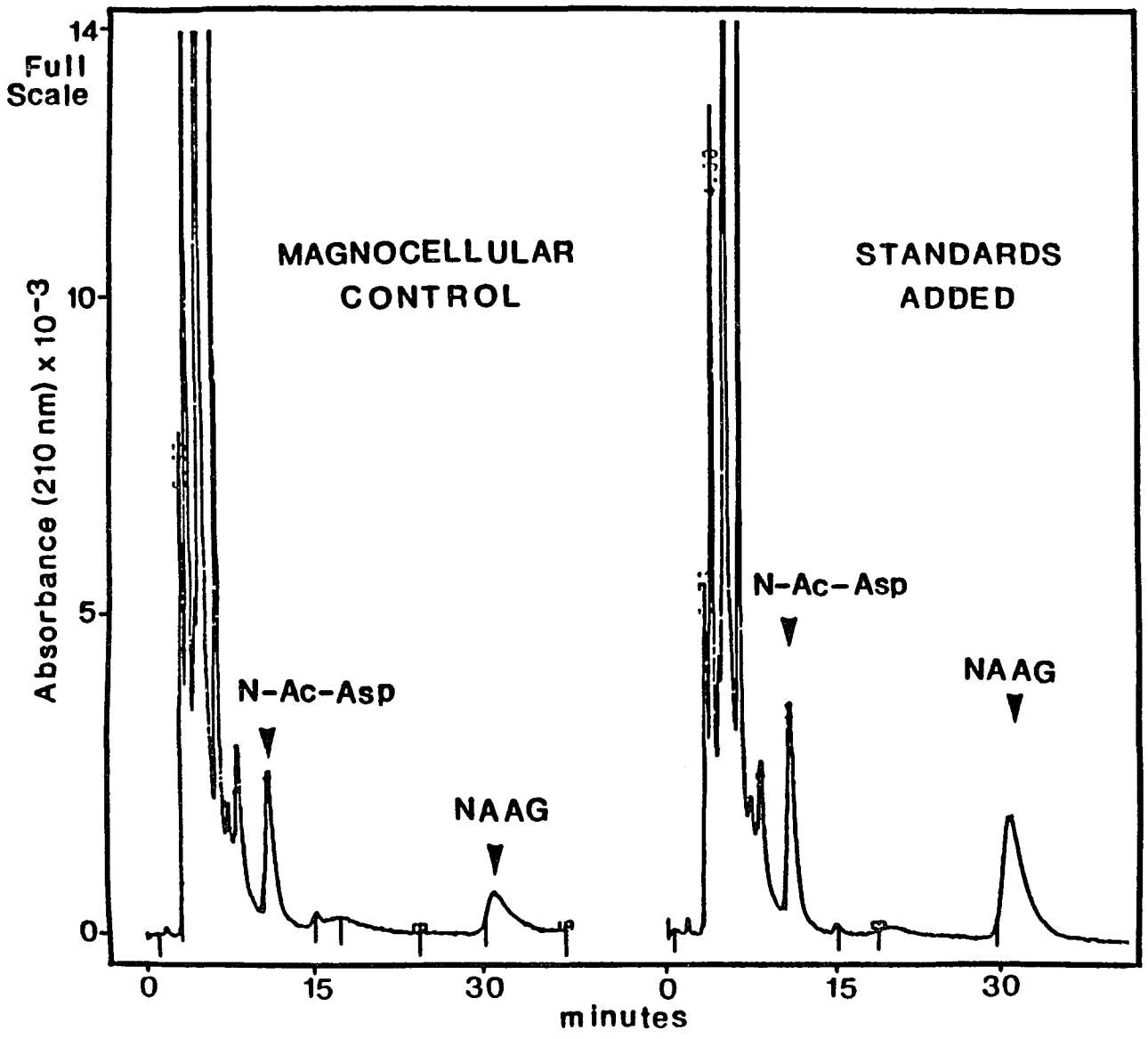

14
Full Scale
10
5
0
Absorbance (210 nm) x 10^{-3}
MAGNOCELLULAR CONTROL
STANDARDS ADDED
N-Ac-Asp
NAAG
N-Ac-Asp
NAAG
0
15
30
0
15
30
minutes

TABLE 11
NORMAL REGIONAL DISTRIBUTION OF NAAG AND NASP
IN THE MONKEY VISUAL SYSTEM

No	REGION	N	CONCENTRATION	DIFF. VS REGION No
			N A A G	
1	TRACT	4	3.86 ±0.91	5, 6, 7, 8
2	LGN.T	6	2.28 ±0.27	6, 7, 8
3	MAGNO	6	3.44 ±0.79	5, 6, 7, 8
4	PARVO	6	2.11 ±0.25	6, 7, 8
5	S.COL	4	1.00 ±0.20	1, 3
6	CTX.T	4	<0.50	1, 2, 3, 4
7	CTX.C	4	<0.50	1, 2, 3, 4
8	CTX.P	4	<0.50	1, 2, 3, 4
			N A S P	
1	TRACT	4	4.62 ±0.92	3, 5, 6, 7, 8
2	LGN.T	6	6.71 ±0.90	6, 7, 8
3	MAGNO	6	8.51 ±1.41	1, 6, 7, 8
4	PARVO	6	6.44 ±0.87	6, 7, 8
5	S.COL	4	9.89 ±2.07	1, 6, 8
6	CTX.T	4	14.40 ±1.88	1, 2, 3, 4, 5
7	CTX.C	4	13.90 ±2.17	1, 2, 3, 4
8	CTX.P	4	14.90 ±1.79	1, 2, 3, 4, 5

TABLE 11

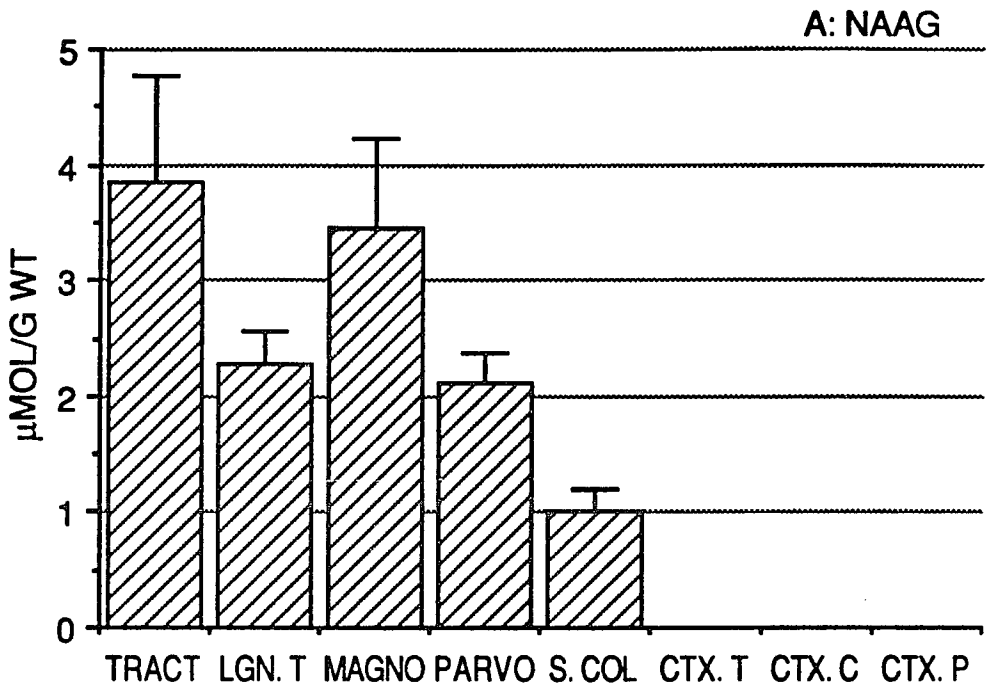
Normal regional distribution of NAAG and NASP in the monkey visual system.

NAAG and NASP concentrations are the mean ±SEM in μmol/g wt. As indicated in column (N), six monkeys were used in the analysis of the LGNd, and four monkeys for the remaining structures. The regions analyzed were the optic tract (TRACT), the magnocellular (MAGNO) and parvocellular (PARVO) segments of the LGNd, the superior colliculus (S.COL), and the central (CTX.C) and peripheral (CTX.P) segments of the striate cortex. The values for the total LGNd (LGN.T) represent the weighted mean of the constituent segments, as defined in Results, Section 3.1.2. The numbers in the last column (DIFF VS REGION No) refer to the numbered regions as in the first column, and indicate statistically significant differences in amino acid concentration; for example, they show that NAAG concentration in the optic tract (region No.1) is significantly different from that in the superior colliculus (region No. 5) and the striate cortices (regions No. 6, 7 and 8). Values of NAAG in the striate cortex were below the level of detection (0.05 μmol/g wt); this latter value was used to determine significant regional differences. Statistical analysis was made with the Bonferroni's multiple comparisons procedure, at the overall alpha level of 0.05, as described in Materials and Methods.

FIGURE 14

Graphic representation of the normal regional distribution of NAAG and NASP in the visual system

Bars represent the mean amino acid concentration ±S.E.M., expressed in μmol/g wt. Six monkeys were used in the analysis of LGNd structures, and four of them for the remaining structures. Notations as in Table 11.


A: NAAG
µMOL/G WT
5
4
3
2
1
0
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P

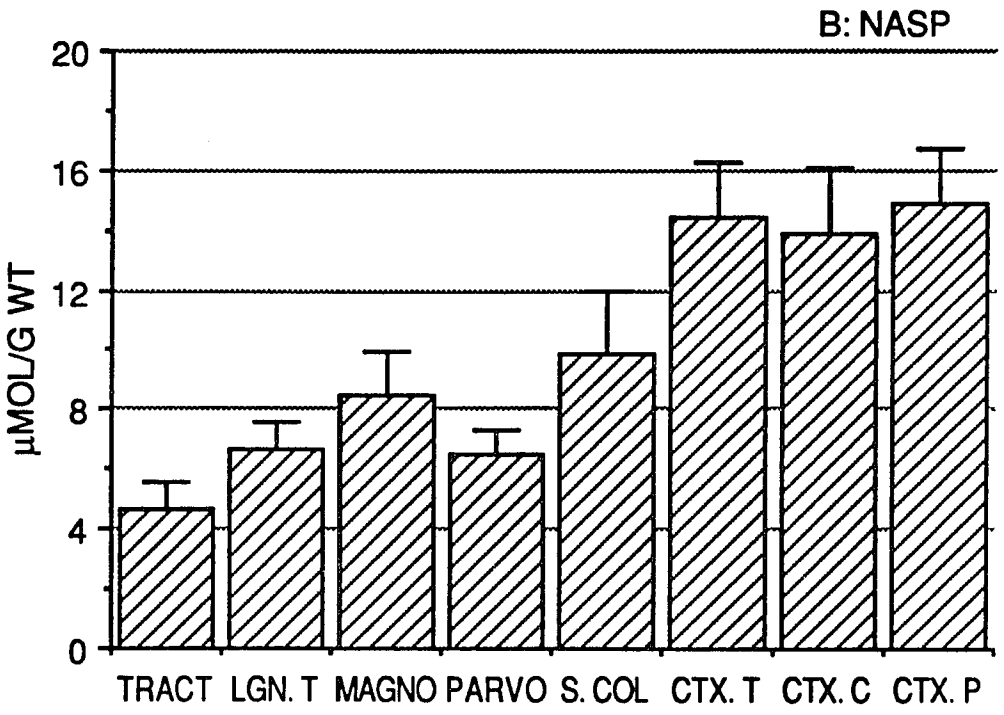

B: NASP
µMOL/G WT
20
16
12
8
4
0
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P

Figure 14

FIGURE 15

Inverse gradient of concentration of NAAG and NASP along monkey visual pathways

While the concentration of NAAG decreases in the rostrocaudal direction to reach undetectable levels in the striate cortex, the concentration of NASP decreases progressively in the opposite direction, from high levels in the striate cortex to low levels in the optic tract.

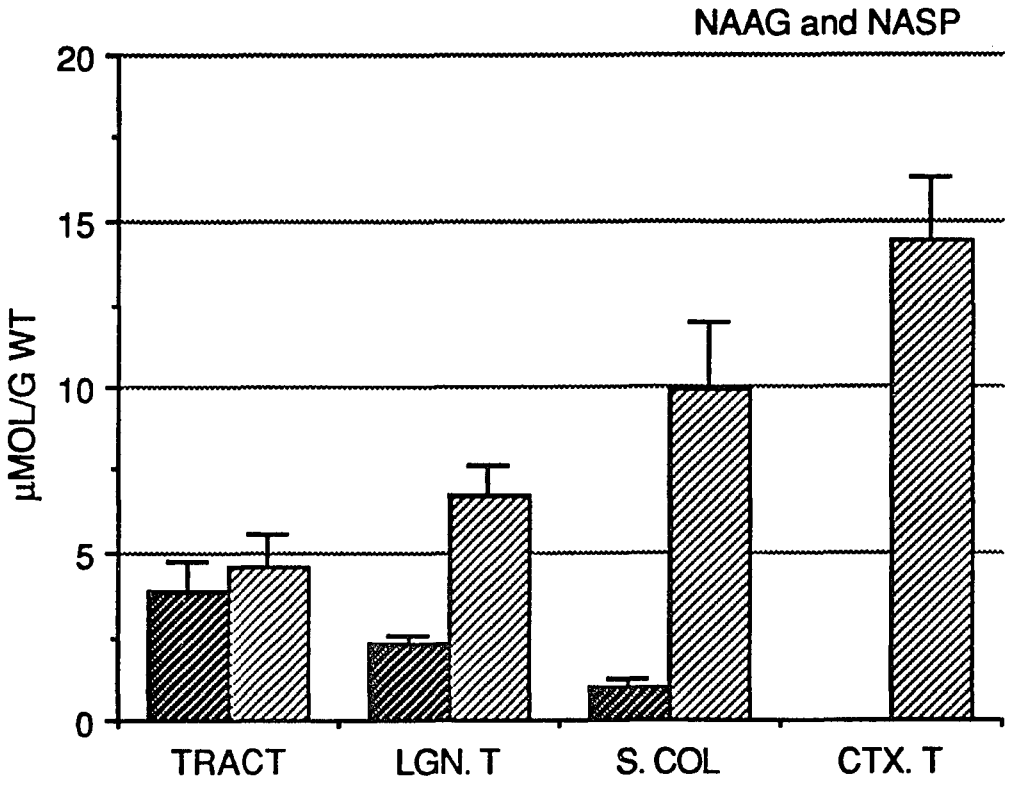


Figure 15

3.2.2 EFFECTS OF OPTIC TRACT SECTION

In the portion of the optic tract distal to the lesion, i.e., close to the LGNd, optic tractotomy produced a dramatic and significant ($p<0.05$) decrease in NAAG concentration, which amounted to 82%. The reduction in NASP was 19%, but not significant (Table 12; Figs.16A-16B).

In the LGNd, the concentration of NAAG decreased also significantly, but less prominently, than in the optic tract (30%). The reduction was significant in both segments on the LGNd, but it was larger in the magnocellular (62%) than in the parvocellular (22%) layers. The effect of optic tractotomy on NASP concentration consisted in an overall significant decrease of 20% for the total LGNd; the reduction, however, was only apparent in the parvocellular segment, where it amounted to 17% (Table 12; Figs.16A-16B).

In the superior colliculus, NAAG concentration was moderate and at the same levels in both the control and experimental sides. NASP levels were normally higher than those of NAAG in this structure, but, similarly to NAAG, they did not decrease significantly after optic tractotomy (Table 12; Figs. 16A-16B).

In the striate cortex, NAAG concentration was below the level of detection in both control and operated sides, so the effect of the lesion could not be determined in this region.

NASP concentration was normally several fold higher than in any other structure of the visual system, and after optic tract section it did not change significantly (Table 12; Figs. 16A-16B).

TABLE 12
EFFECT OF OPTIC TRACTOTOMY ON NAAG AND NASP CONCENTRATION IN THE MONKEY VISUAL SYSTEM

No	REGION	N	CTRL	EXPM	% CH	P
			N A A G			
1	TRACT	4	3.86 ±0.91	0.68 ±0.10	-82	*
2	LGN.T	6	2.28 ±0.27	1.59 ±0.10	-30	*
3	MAGNO	6	3.44 ±0.79	1.31 ±0.14	-62	*
4	PARVO	6	2.11 ±0.25	1.64 ±0.13	-22	*
5	S.COL	4	1.00 ±0.20	1.00 ±0.24	0	
6	CTX.T	4	<0.50	<0.50	ND	
7	CTX.C	4	<0.50	<0.50	ND	
8	CTX.P	4	<0.50	<0.50	ND	
			N A S P			
1	TRACT	4	4.62 ±0.92	3.47 ±0.73	-19	
2	LGN.T	6	6.71 ±0.90	5.37 ±0.58	-20	*
3	MAGNO	6	8.51 ±1.41	5.63 ±0.35	-34	0.083
4	PARVO	6	6.44 ±0.87	5.33 ±0.63	-17	*
5	S.COL	4	9.89 ±2.07	9.29 ±1.50	- 6	
6	CTX.T	4	14.40 ±1.88	11.05 ±1.04	-23	
7	CTX.C	4	13.90 ±2.17	9.44 ±1.47	-32	
8	CTX.P	4	14.90 ±1.79	12.66 ±0.90	-15	

TABLE 12

Effect of optic tractotomy on NAAG and NASP concentration in the monkey visual system

NAAG and NASP concentrations are the mean ±SEM in μmol/g wt. Six monkeys were used in the analysis of the LGNd, and four monkeys for the remaining structures. The regions analyzed were the portion of the optic tract distal to the lesion (TRACT), the magnocellular (MAGNO) and parvocellular (PARVO) segments of the LGNd, the superior colliculus (S.COL), and the central (CTX.C) and peripheral (CTX.P) segments of the striate cortex. The values for the total LGNd (LGN.T) represent the weighted mean of the constituent segments, as defined in Results, Section 3.1.2. Control values (CTRL) are from structures on the left hemisphere, and experimental values (EXPM) are from homologous, paired structures on the opposite hemisphere. Values of NAAG in the striate cortex were below the level of detection (0.05 μmol/g wt). Percent change (%CH) is defined as [(EXPM/CTRL)-1]*100. (ND) means not determined. Statistical analysis was made with the Student's paired t-test, at the alpha level of $p<0.05$ (*); when $0.05<p<0.1$, the numerical value is given in the last column (P); blanks in the this column indicate the absence of statistically significant differences.

FIGURE 16

Graphic representation of the effect of optic tractotomy on NAAG and NASP concentration in the visual system

Bars represent the mean amino acid concentration ±S.E.M., expressed in μmol/g wt. Wide hatched and narrow hatched bars indicate the amino acid concentration in the control and experimental sides, respectively. Significant differences among both sides were determined with the Student's paired t-test, at the alpha level of 0.05 (*). The number of monkeys employed and the structures analyzed are as in Table 12.

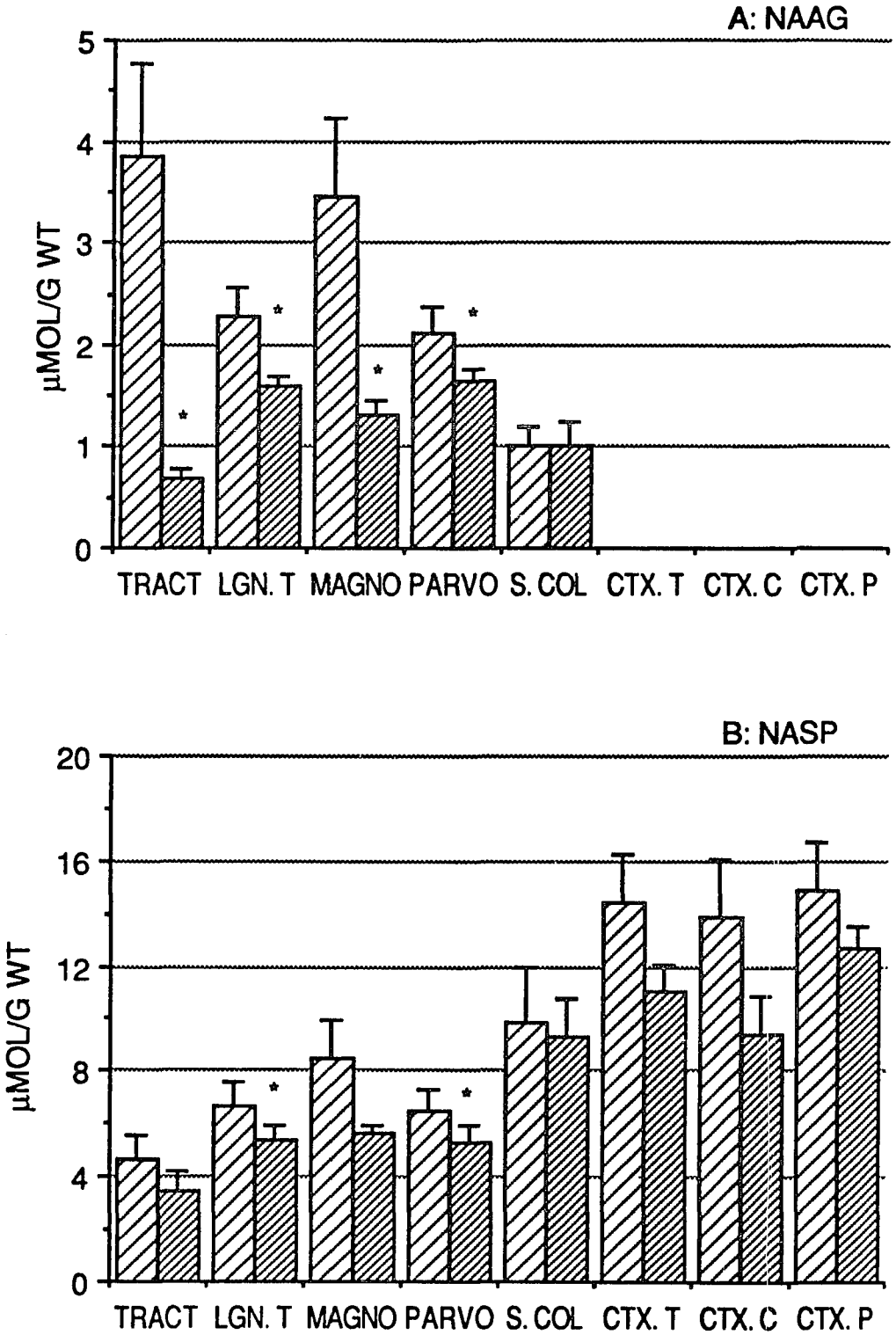

A: NAAG
µMOL/G WT
0
1
2
3
4
5
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P
B: NASP
µMOL/G WT
0
4
8
12
16
20
TRACT
LGN. T
MAGNO
PARVO
S. COL
CTX. T
CTX. C
CTX. P

Figure 16

CHAPTER 5

DISCUSSION

The present investigation described the normal regional distribution of amino acids and NAAG (N-acetylaspartylglutamate) in structures of the monkey visual system, and the results of lesion studies suggested that NAAG, GLU and ASP are neurotransmitter candidates in the retinogeniculate pathways.

Studies on the normal regional distribution were made on structures of the side contralateral to the optic tractotomy, which were considered normal. The animals with occipital lobectomy were not included in this analysis because of the possibility that the hemisphere opposite to the cortical ablation could be affected thorough the interruption of commisural fibers originating in areas 18 and 19, which were encroached by the lesion. Results revealed that, in general, the concentration of amino acids was homogeneous along the visual pathways, including the magnocellular and parvocellular segments of the LGNd (dorsal lateral geniculate nucleus), as well as the areas containing the representations of the central and peripheral visual fields in the striate cortex. Two important exceptions were the significantly higher concentration of GABA in the superficial layers of the superior colliculus, and the decreasing gradient of cystathionine towards the cortex. In addition, there was an

inverse gradient of concentration of NAAG and NASP along the visual pathways.

The candidacy of various substances as neurotransmitters in the retinogeniculate pathways was derived from lesion studies. Optic tract section produced a significant reduction in the concentration of ASP and GLU in the portion of the optic tract distal to the lesion, as well as a small but significant decrease in the concentration of GLU in the LGNd. When examining each segment of this nucleus individually, the change in GLU was only apparent in the parvocellular laminae, but ASP showed a larger reduction that approached significance in the magnocellular segment as well. The most striking change induced by optic tractotomy was in the concentration of NAAG, which decreased by more than 80% in the optic tract, and by 30% in the LGNd. Within this structure, the change was significant in both segments. Finally, effects of the lesion were not detected in the superior colliculus or striate cortex.

Normal Regional Distribution

Amino acids

Levels of amino acids have been previously studied in the visual system of some species (see Table 1 and references therein). However, little information is available for the monkey visual system. For example, there is apparently no data on the amino acid composition of the optic nerve and LGNd

in this species; only the levels of a few amino acids, e.g. GABA and taurine, have been reported in the monkey superior colliculus and striate cortex.

Taurine concentration was highest in the optic tract and lowest in the parvocellular segment of the LGNd. These two concentration extremes differed significantly from each other, but not the intermediate values found in the remaining structures of the visual system. Some possibilities on the significance of this finding will be discussed below, in the section on the effects of optic tract lesion.

Aspartate concentration was significantly lower in the optic tract than in both segments of the LGNd. The reason for this difference is not fully understood, but it may reflect a moderate accumulation of ASP within the geniculate nucleus, probably of extraretinal origin.

Glutamate showed an even distribution along the visual pathways. Johnson and Aprison [1971] found that the concentration of GLU in the optic nerve of the cat was significantly lower with respect to twelve gray matter structures. The authors argued that since free GLU levels in the optic nerve are only slightly higher than those observed in other brain regions where it does not function as a neurotransmitter (ventral root, sympathetic ganglion and distal sensory root), their data suggested that this amino acid may not have a transmitter action in the optic nerve. Furthermore, other investigators have taken into consideration

these results to support their conclusion about the improbable role of GLU as optic nerve transmitter [Tebecis 1973; Tebecis and DiMaria 1972]. However, it should be noted that the concentration of GLU in the optic nerve found in the present study (4.43 μmol/g wt) is high enough to support its role as neurotransmitter. Moreover, its concentration may be considerably higher in retinal terminals, where GLU is possibly compartmentalized into a neurotransmitter pool. In the case of acetylcholine, for example, its free concentration is in the order of 0.3 mM, but when stored in synaptic vesicles, it rises to approximately 200 mM (see Background on Amino Acids). Results of the present study indicate that the concentration of GLU is similar in the four structures of the monkey visual system which were analyzed. Comparable concentrations (within one standard deviation) have been reported for the cat optic nerve [Johnson and Aprison 1971]. However, the concentrations of this amino acid in the superior colliculus and cortical areas were significantly higher in the latter report. The discrepancy may be due to differences in sampling. The authors included entire regions, whereas individual laminae were dissected in the present study. The samples from the superior colliculus were centered on the stratum opticum, and the samples from the striate cortex were centered on the stripe of Gennari. Both of these layers are known to contain the optic fiber input to the region [Schiller 1987; Stone et al. 1979]. In the case of the superior

colliculus, no material was included from the stratum zonale, the most superficial layer, which is known to receive a large input from the visual cortex [Schiller 1987], through a putative glutamergic pathway [Kvale et al. 1983; Kvale and Fonnum 1983; Lund Karlsen and Fonnum 1978]. Therefore, it seems that GLU concentration varies among layers of the superior colliculus and striate cortex, and this could account for the differences in values obtained in the study by Johnson and Aprison [1971] and the present investigation.

Glutamine, glycine and alanine, similarly to glutamate, had homogeneous distributions along the monkey visual pathways. However, cystathionine (CTH) had a progressively decreasing concentration, being highest in the optic tract and lowest in the striate cortex. This observation may point to possible differential roles of CTH in the various regions examined, but no information is available in this regard.

GABA concentration in the stratum opticum and immediately contiguous layers of the normal superior colliculus was significantly higher than in the optic tract, LGNd and striate cortex. Reports in the literature have discussed previously the presence of high levels of GABA in this structure (see Table 1 for references). Study of regional differences of GABA distribution within the superior colliculus itself indicated that the highest values were found in the superficial gray layer, followed by the optic layer and the intermediate gray layer [Okada 1974]. Visual cortex ablation

or optic nerve section did not have any effect on GABA concentration in the superficial gray layer, but the other layers were not analyzed. In the monkey, the superficial layers of the superior colliculus are concerned exclusively with vision [for review see Schiller 1987]. They receive inputs from both retina and visual cortex, and send projections to the pulvinar and parabigeminal nucleus. Retinal inputs to the superior colliculus are topographically organized, and originate from both Y- and W-ganglion cell types [for review see Stone and Dreher 1982]. Whereas strong evidence supports the role of GLU as neurotransmitter of the retinotectal fibers in the pigeon, the evidence is more controversial in the retinotectal fibers in mammals (see Background on Amino Acids, e.g., Lund Karlsen and Fonnum [1978]). Regarding the corticofugal afferents to the superior colliculus, they are probably glutamergic [Kvale et al. 1983; Kvale and Fonnum 1983; Lund Karlsen and Fonnum 1978]. Small interneurons in the superficial gray layer are most likely gabaergic [Mize et al. 1982; Ottersen and Storm-Mathisen 1984a,b]. GABA is also a putative neurotransmitter in the nigrotectal projection [Fonnum et al. 1979]. Anatomically, however, this pathway is described to terminate in the deeper layers of the superior colliculus [Schiller 1987]. In conclusion, GABA found in the superficial collicular layers is most probably confined to interneurons that participate in

inhibitory circuits that generate the varied receptive field properties of collicular neurons.

N-acetyl-aspartyl-glutamate

NAAG concentration was high in the optic tract, moderate in the LGNd, low in the superior colliculus and undetectable in the striate cortex of the monkey (see Table 11 and Fig.14). Conversely, NASP concentration increased progressively from the optic tract towards the striate cortex. The levels of these substances in the LGNd and superior colliculus were similar to those reported in the rat by Anderson et al. [1987]. There are also indications in the literature of an inverse relationship in the rostrocaudal gradients of concentration between NAAG and NASP in different regions of the rat brain [Coyle et al. 1986]. This relationship may reflect a metabolic dependence between the two substances. If NAAG is synthesized from NASP by incorporation of GLU [Cangro et al. 1987], then a high production of NAAG may imply a decrease in the size of the metabolic pool of NASP, assuming the absence of a replenishing pathway for this substance.

Immunocytochemical studies also support the presence of NAAG in the retinogeniculate pathways. Thus, NAAG immunoreactivity was present in retinal ganglion cells, optic tract, and LGNd of the rat, where labeling was present within numerous fibers and puncta, as well as some small cells [Anderson et al. 1986, 1987]. In the cat, NAAG-like

immunoreactivity was observed within the majority of retinal ganglion cells, whether large, medium or small (presumably Y, X and W), of both on-center and off-center types, suggesting a common role for NAAG in all of these cells [Tieman et al. 1987]. However, since NAAG-like immunoreactivity was not found in the retinal ganglion cells of the frog [Kowalski et al. 1987], the possible function of NAAG may not be the same in all vertebrates. It should be noted that staining of geniculate cells in the cat was less intense than that of retinal ganglion cells, and the authors suggested that the NAAG antisera may be reacting in the LGNd with a related peptide, such as the non-acetylated derivative of NAAG.

Recently, NAAG has been proposed as a putative neurotransmitter in motor and sensory systems of several species [see Coyle et al. 1986; Anderson et al. 1986, 1987; Tieman et al. 1987]. Because of the relevance of this dipeptide in the present investigation, a short review of the bases for this postulate is presented next. Biosynthesis of NAAG in spinal sensory ganglia appears to be carried out by incorporation of GLU into N-acetylaspartate, through a ribosomally independent peptide synthetase activity [Cangro et al. 1987]. Glutamine was more efficient than GLU as a precursor for NAAG, a difference that was attributed to the better entry of GLN into neurons, and of GLU into glial cells. Little more is known about the mechanism by which NAAG's physiologic action may be terminated, namely, an enzymatic cleavage into N-acetyl-

aspartate and free GLU, a process which is tightly coupled with a transport system of derived GLU. The peptidase responsible for NAAG degradation was enriched in synaptic plasma membranes, showed a dependence on chloride anions and divalent metal ions such as manganese, and it was also potently inhibited by the excitatory amino acid agonist L-quisqualate [Robinson et al. 1986, 1987]. Regional and ontogenetic differences between NAAG and this peptidase suggested the presence in brain of additional N-acetylated, alpha-linked acidic peptide substrates susceptible to this activity [Blakely et al. 1988]. GLU cleaved from NAAG seemed to be sequestered by a high affinity, sodium- and temperature dependent uptake system, that is enriched in synaptic membranes and is inhibited by veratrine and ouabain, drugs that disrupt the sodium gradient [Blakely et al. 1986].

NAAG shows a high affinity for chloride dependent GLU binding sites [Zaczek et al. 1983], and the interaction is potently inhibited by alpha-amino-phosphonobutyrate (APB). This drug distinguishes a GLU receptor subtype different from NMDA, quisqualate and kainate [Whittemore et al. 1983], and is a selective antagonist of the excitatory action of NAAG in the receptive field of the lateral olfactory tract in the pyriform cortex [Coyle et al. 1986]. Alpha-amino-phosphonovalerate (APV), and alpha-amino-phosphonoheptanoate (APH), antagonists at the NMDA receptor, inhibit the interaction less potently, indicating that the GLU recognition

site for NAAG is probably different from the NMDA, quisqualate and kainate receptors. In another system, such as spinal cord neurons in culture, APV exerts a powerful antagonistic effect on the excitatory action of NAAG, and it is therefore possible that the action of NAAG in this case is mediated through NMDA receptors [Westbrook et al. 1986].

In summary, NAAG is an acidic dipeptide with an uneven distribution in brain, present at concentrations approaching those of GLU or ASP, and exceeding those of most other brain peptides by two or three orders of magnitude. Immunocytochemical studies reveal its presence within several neuronal populations, and the pathways associated with them, including mitral cells of the olfactory bulb and fibers of the lateral olfactory tract, retinal ganglion cells, optic fiber axons, fiber plexuses and small neurons in the LGNd, and in cortical cells and spinal cord neurons. The biosynthesis of NAAG appears to occur via incorporation of GLU into NASP by an enzymatic activity that is ribosomally independent. The enzymatic inactivation of NAAG is carried out by a chloride dependent metallo-peptidase that is potently inhibited by quisqualate. The resultant free GLU is rapidly taken up by a sodium dependent, high affinity uptake system that is tightly coupled to the process of cleavage. Targets of presumed glutamergic pathways, such as pyramidal cells of the pyriform cortex and hippocampus, as well as spinal and cerebellar neurons in culture, are depolarized by NAAG. The

excitatory action of NAAG seems to be mediated in some regions by NMDA receptors, and in others by a GLU receptor subtype other than NMDA, quisqualate or kainate.

Differential composition of the magnocellular and parvocellular divisions of the LGNd

One of the objectives of this project was to determine whether the parvocellular and magnocellular segments of the LGNd differed in their amino acid and NAAG composition. These segments receive parallel, segregated inputs from X- and Y-retinal ganglion cells, respectively [for review see Stone and Dreher 1982]. Since these neuronal types have different sets of anatomical and physiological properties, it is possible that they utilize different neurotransmitters. Separate sampling of the two segments of the LGNd was made possible by the use of the micropunch technique of Palkovits [for review see 1983]. It was also important to count with a sensitive analytical procedure, such as the one provided by HPLC with fluorometric and ultraviolet detection, since the amount of material available was limited. Results of the present study indicate that the magnocellular and parvocellular laminae of the monkey LGNd do not differ in their amino acid, NAAG and NASP composition. Since the Y-type of optic fibers terminate in both the magnocellular segment of the LGNd and the superior colliculus, it is noteworthy that these two structures differ significantly in two main aspects:

(1) GABA is higher in the superior colliculus, and (2) NAAG is higher in the magnocellular component. These differences may reflect the presence of additional gabaergic elements in the superior colliculus (as mentioned above), and of additional NAAG-containing elements in the magnocellular segment of the LGNd. In fact, immunocytochemical studies have revealed the presence of NAAG (+) neurons and fibers in the LGNd of the rat and cat [Anderson 1986, 1987; Tieman 1987].

Conclusions

The results on the normal regional distribution give normative data, so far not available, on the levels of amino acids and NAAG in several structures of the monkey visual system. A few of the substances have a differential distribution along the pathways, and its possible significance was discussed. Within the context of one of the objectives of the present study, it is interesting that no major differences were found between the magnocellular and parvocellular components of the LGNd, implying that retinal terminals may share similar substances in the two segments.

Effects of Optic Tract Section

Tractotomized animals survived for seven days. This time was chosen to obtain full retinal terminal degeneration, and have a minimal glial and transneuronal reaction in the ipsilateral LGNd (see Background for references). The amino

acid and NAAG levels measured in this nucleus after the survival period would reflect their presence in elements other than retinal terminals. The differences with their own paired controls on the contralateral LGNd would then be an indication of the concentrations of these substances within retinal terminals. Although it is possible that some of the differences observed are secondary to metabolic changes induced by the lesion, it must be noted that this limitation may be more theoretical than real. In the majority of the putative glutamergic pathways where a decrease in GLU concentration after interruption has been documented, other criteria, such as uptake, release and identity of physiologic action, have been fulfilled [see Table 12-4 in McGeer and McGeer 1981, and Tables 1-5 in Ottersen and Storm-Mathisen 1986]. This type of objection may not be applicable to NAAG, which probably does not have such a widespread metabolic role as GLU in brain.

Optic tract

In the portion of the optic tract distal to the lesion, tractotomy induced large reductions in the concentrations of ASP (50%), GLU (48%) and particularly of NAAG (82%). These findings have not been reported previously for any species, and emphasize the importance of these substances as neurotransmitter candidates in the retinogeniculate pathways. NAAG, GLU and ASP were also decreased in the LGNd, although

to a considerably lesser extent (see below). The results probably reflect the fact that the optic tract is a more homogeneous structure, containing only optic fibers and glial cells; in contrast, the LGNd is composed of several other elements, such as P-cells, interneurons, and axons and terminals from varied sources, which in the most part are not directly affected by the lesion (see Background section).

Lateral geniculate nucleus

In the LGNd ipsilateral to the optic tract section, significant decreases were found in the levels of NAAG, NASP and GLU. Here again, the most dramatic drop was in the levels of NAAG, which amounted to 30%, suggesting that approximately this portion is present in retinal terminals, and the remaining in other components of the geniculate nucleus. A major reduction (64%) has been reported also in the rat LGNd on the side contralateral to one eye enucleation, after a seven-day survival [Anderson et al. 1987]. The greater magnitude of the deficit, with respect to the present result in the monkey, may be a reflection of the persistence of some normal (uncrossed) fibers contralateral to the enucleation, and the presence of some degenerated terminals from crossed fibers on the side of the enucleation. This anatomic arrangement results in an overestimation of the values in the contralateral LGNd, and an underestimation in the ipsilateral nucleus. Thus, the resulting difference would be greater than

that of the present study, where section of one optic tract resulted in total deafferentation of the LGNd on the same side, and total preservation of the nucleus on the opposite side.

The findings on GLU indicated a small (12%) but significant decrease in its concentration after surgery, suggesting that most of the pool of this amino acid resides outside retinal endings, most probably in neurons and corticogeniculate terminals [Kvale and Fonnum 1983; also see below: Effects of Occipital Lobectomy].

Magnocellular and parvocellular laminae

The present study succeeded in quantifying separately the magnocellular and parvocellular divisions of the LGNd, after optic tract section, thereby providing information on possible differences in neuroactive substances utilized by retinogeniculate pathways of the X- and Y- types. No previous attempts are known that have carried out this kind of analysis.

The significant reduction in NAAG concentration found in the LGNd as a whole on the side of optic tractotomy was also apparent in each segment of this nucleus when measured individually. The deficit was more marked in the magnocellular than in the parvocellular division; this finding is probably due to the fact that the magnocellular laminae contain a higher proportion of optic tract fibers, which were found to

exhibit an even greater decrease in NAAG concentration (see above). These results indicate that this dipeptide may be utilized as a putative neurotransmitter in both the X- and Y-retinogeniculate pathways, known to terminate in the parvocellular and magnocellular segments of the LGNd, respectively.

The results on GLU showed again a small but significant decrease in the parvocellular laminae. A reduction of larger magnitude was obtained in the magnocellular layers, but it only approached statistical significance, probably due to the uncommonly high variability in the values on the control side (Table 9C).

Finally, the findings on ASP are less clear. An almost significant reduction of considerable magnitude (26%) was only found in the magnocellular division. Whether this is a reflection of the higher proportion of optic tract fibers, as in the case of NAAG (see above), it is not certain. Moreover, no significant deficits were found in the parvocellular laminae.

Superior colliculus

The effects of optic tractotomy were not detected in the superior colliculus. In fact, none of the compounds analyzed showed significant decreases on the side of the lesion. A possible reason for this lack of effect could be the longer time course of degeneration in retinotectal terminals as compared to the retinogeniculate endings. This argument is

supported by the greater distance from the tract section to the colliculus than to the geniculate (see Background, and Pecci Saavedra et al. [1971]). The hypothesis is testable by using a preparation of survival time longer than seven days. Some experimental findings, however, may be against this interpretation. It has been observed that terminal degeneration in the monkey superior colliculus was heaviest six days after eye removal [Lund 1972]. This study, however, involved only unilateral eye enucleation, and therefore, any region of the colliculus examined ought to contain both degenerating and normal terminals due to the approximately 50% crossing of fibers in the optic chiasm. Thus, no claims can be made to exclude the possibility that some of the "normal" retinal boutons present may degenerate later on. In fact, it apparently took over three weeks for total disappearance of degenerating optic terminals [Lund 1972].

Another possibility for the difference between the decrements found in the LGNd and the lack of effects in the superior colliculus is that the retinotectal pathway may utilize a transmitter substance not included in the analyses, and not presently known. This viewpoint could be acceptable in the case that retinotectal fibers represent the axons of a discrete population of ganglion cells projecting exclusively to the superior colliculus. There is some evidence, however, indicating that many of them are axon collaterals of retinogeniculate fibers [Bunt et al. 1975]. In the latter instance,

the present negative findings would constitute a violation of Dale's principle [Dale 1935], in as much as they would indicate that different substances could be released at different sites of a single neuron. In spite of its unorthodoxy, this idea cannot be totally ruled out, since there is evidence for quasi-independent synaptic units within single neurons in other central nervous system structures. It has been speculated that some of this units might release different neuroactive substances [Shepherd 1988]. To paraphrase Dale [1935]: "the possibility has at least some value as stimulus for further experiment".

Striate cortex

In the striate cortex, the concentration of amino acids, NAAG and NASP remained unaffected after optic tract section. These negative results may be due to the short survival time, which precluded the occurrence of marked transneuronal changes in the LGNd. This possibility can be tested in preparations with longer survival periods, when transynaptic atrophy could lead to alterations in the terminal fields of geniculate neurons within the striate cortex. In fact, some recent pharmacologic and electrophysiologic evidence suggests GLU and ASP as geniculocortical transmitters [Tsumoto et al. 1986].

Conclusions

The results of optic tract section support the notion that GLU and particularly NAAG are strong neurotransmitter candidates in the retinogeniculate pathways. These substances appeared to be shared by both X- and Y- retinal terminals. Although the role of ASP cannot be ruled out, the present findings do not provide firm evidence for such a function. Finally, no definitive conclusions can be drawn from the lack of effects in the superior colliculus and striate cortex, since it can be due to the short survival time of the preparations, which was mandated by the main objective of the study, namely to investigate the identity of the transmitter(s) in the retinogeniculate pathways.

Effects of Occipital Lobectomy

The purpose of studying the effects of chronic (three-month survival) cortical ablations on the LGNd was to add support to the significance of the reductions found in GLU and ASP after optic tractotomy. Since there is evidence that corticogeniculate terminals [Lund Karlsen and Fonnum 1978] and P-cells [Tsumoto et al. 1986] utilize GLU/ASP as neurotransmitter(s), it was hypothesized that after chronic ablation, resulting in the disappearance of these elements and the relative preservation of retinal terminals and interneurons [Pasik et al. 1973], the combined reductions in these compounds found after optic tractotomy and occipital lobectomy

would approach 100%. This was in fact the case. Whereas optic tract lesion resulted in a 12% decrease in GLU and a 14% decrease in ASP (see above), occipital lobectomies yielded a 66% and 75% reduction, respectively. The minimal difference between the combined values to 100% could be attributed to the noted shrinkage of the LGNd on the side of the ablation, which could account for an overestimation of the concentration values.

The moderate decrease in the levels of GABA probably represents the disappearance of some interneurons and/or terminals from the thalamic reticular nucleus, which degenerate transneuronally after cortical ablation.

CONCLUSIONS

The main objective of the present investigation was to discover the identity of the neurotransmitter(s) in the retinogeniculate pathways of the monkey. This species offered the possibility of analyzing separately the influence from the X- and Y- retinal ganglion cells, which project in a segregated fashion to the parvocellular and magnocellular segments of the LGNd, respectively.

Results of the present investigation strongly support NAAG as neurotransmitter candidate in both retinogeniculate pathways. The role of GLU is also supported by the present data, but the magnitude of the changes was less dramatic,

probably due to the large metabolic pool of this amino acid. The role of ASP cannot be negated, but more experimentation would be needed to verify this possibility.

One criteria of transmitter identification postulates that for a substance to be considered a candidate, its concentration must decrease in the terminal field after interruption of the corresponding afferent pathway. This criterion, when combined with a suitable analytical method such as HPLC with fluorometric or ultraviolet detection, offers two important advantages: (1) large screening capabilities, allowing the simultaneous study, at least in principle, of twenty or more compounds; (2) requirement of small amounts of tissue, which is a major consideration when this factor is limiting. However, this method may be regarded less sensitive than uptake or turnover measurements to detect changes after interruption of a nerve pathway.

The concentration of some amino acids increased in the side ipsilateral to the lesion, after optic tract section or occipital lobectomy. The increases in alanine and glycine can be due to a greater rate of protein degradation than protein synthesis, which may occur as part of the general degenerative process taking place in structures affected by the lesion. The small increases in taurine levels after optic tractotomy in the LGNd cannot be explained as for alanine or glycine, since taurine is not a normal constituent of proteins. The results of increased concentration after the lesion would

suggest that this amino acid is not an important axonal component, in which case the neuroglia would be the remaining source. The fact that taurine was significantly lower in the normal parvocellular layers than in the optic tract (see above on Normal Regional Distribution) would rise the possibility that the proportion of the glial component in the parvocellular layers is lower than in the rest of the geniculate. The large increase in cystathionine observed in the LGNd after optic tractotomy and particularly after occipital lobectomy is more difficult to explain. Its accumulation in brain may result from an imbalance between the cystathionine synthase and the cystathionase reactions, producing an augmented synthesis and/or decreased degradation of this amino acid. Future studies may be designed to assess the activity ratio of these two enzymatic reactions after the lesion.

To establish a substance as a neurotransmitter in a certain pathway in the brain, several anatomic, biochemical, electrophysiologic and pharmacologic criteria must be fulfilled. Future experiments may be directed towards this goal. For example, the visualization of NAAG within synaptic vesicles of retinal terminals at the electron microscope level would add strong support to the present results. The response of geniculate neurons to iontophoretic injections of NAAG needs to be evaluated, as well as its release upon electrical or photic stimulation of the optic nerve.

CHAPTER 6

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