FACULTY OF MEDICINE
DEPARTMENT BRAIN AND BEHAVIOUR

# NEOSTRIATAL AND THALAMIC INTERNEURONS

## THEIR ROLE IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S CHOREA, PARKINSON'S DISEASE AND CATATONIC SCHIZOPHRENIA

*by*

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## CONTENTS

# INTRODUCTION

Approaching the pathophysiology of so-called 'extrapyramidal' diseases is a delicate and heavy task, even moreso if one intends to do this by morphological means.

There exists indeed in the field of 'basal ganglia disorders' a chaotic nomenclature, an enormous divergence of opinions about morphological, biochemical and physiological function of the central grey nuclei and vagueness about the origin of symptoms.

These problems are induced because different research disciplines explored the same brain areas but gave them other 'more functional' names and because experimental animal models were introduced without stable comparative neuroanatomical data.

Before defining the present study objectives - namely counting and sizing of $\pm$ 100.000 nerve cells - (Chapter I-D), I would like to make some remarks on the current technology (Chapter I-A), a general conception about basal ganglia function based on pathology, physiology and biochemistry (Chapter I-D) and the clinical symptomatology of some extrapyramidal diseases with proven or suggested basal ganglia lesions (Chapter I-C).

## Chapter I

# THE BASAL GANGLIA

## A. TERMINOLOGY

– In the human brain, *basal ganglia* mean to the *neuroanatomist* the nucleus caudatus, putamen, claustrum and pallidum externum and internum; but in lower mammals there is only one pallidum, while the 'functional' homologue of the internal pallidum is known as Nucleus Entopeduncularis (Denny Brown, 1962; De Long, 1971). Some include also the Corpus Amygdaleum, connected to Nucleus Accumbens by the stria terminalis.

The *embryologist* will dispute the grouping of those nuclei as 'basal ganglia': the pallidal nuclei, indeed, emerge in embryogenesis from the diencephalon, while claustrum, caudatum, putamen and amygdaleus are telencephalic nuclei (Ariens-Kappers et al., 1960; Richter, 1965).

The *neurophysiologist* suggests the thalamus to belong to the basal ganglia, for functional reasons: the neostriatum (putamen - caudatum) projects to the ventral thalamus (via the pallidum) Dormont et al., 1960; Kuo and Carpenter, 1973). But this is only one projection zone: also the subthalamus, hypothalamus and mesencephalic nuclei (e.g. L. Niger) are known to be closely connected to the basal ganglia. (Jung and Hassler, 1960; Carpenter et al., 1968).

For the *clinician* 'basal ganglia disorders' refer to syndromes with pathological findings in one or several nuclei in the *highly interrelated group of nuclei at the base of the brain* belonging to telencephalon as well as to diencephalon and even mesencephalon (caudatum-putamen-pallidum-thalamus-subthalamus-locus niger) (Vinken and Bruyn, 1968).

– Wilson (1912) introduced the term '*extrapyramidal* motor disease' in contrast to 'pyramidal motor disease' while Jacob (1923) introduced the name '*extrapyramidal system*', indicating what Vogt C. and O. (1920) called the *striatal system*: a system of brain structures so intimately related with corpus striatum that lesions in any part of the system evoke abnormal motor behavior (caudatum - putamen - pallidum - nucleus ruber, subthalamus, niger, interstitialis and Darkschewitschi, and part of the tha-

lamus).
In this concept, the extrapyramidal system is considered responsible for the motor mechanisms, not depending on the pyramidal system.
However, motor mechanisms cannot be delineated anatomically as motor effects are obtained from the whole brain.

Thus the term '*extrapyramidal*' is vague and points only *conventionally* to the *motor syndromes consisting of involuntary movements* as chorea, tremor, athetosis and of *rigidity*. 'Pyramidal' motor disease is then spastic paresis with hyperactive tendon reflexes and so-called pyramidal signs (Babinski, loss of abdominal reflexes).

– The '*corpus striatum*' or '*the striatum*' in neuroanatomical literature sometimes implies caudatum and putamen (also grouped as *neostriatum*) and pallidum externum and internum (the phylogenetically older *paleostriatum*). Some authors refer also to the Nucleus Amygdaleus as *archistriatum*.
The only descriptive name *lenticular nucleus*, indicating putamen + pallidum, is rather abandoned in neuroanatomy but is still in use in pathology e.g. 'hepato-lenticular degeneration'.

– The rostral part of the nucleus caudatus in man may have a homologous function like the much more developed *limbic striatum* in lower mammals (Barbeau, 1973; Stevens, 1973). The limbic striatum (nucleus accumbens, nucleus olfactorius and nucleus of the stria terminalis) together with the septal nuclei are fairly voluminous in animals up to the primate but only minute in man. It is possible that the limbic striatum plays an important role in the pathogenesis of psychotic symptoms (Stevens, 1973).

– The huge diencephalic relay nucleus in man, *the thalamus*, is still what its greek nomination suggests 'a room without windows'. This nucleus is changed considerably in phylogenesis (Gerebtzoff et al., 1973). Experimental data therefore are only roughly attributed to man. The most reliable facts are based on studies in what is called '*the ventrobasal complex*' and the *corpora geniculata* but for these regions great controversies still exist (Mehler, 1971). The descriptive neuroanatomical subdivisions of the human thalamus vary considerably: anglo-saxon authors are rather 'lumpers' while German authors are the so-called 'splitters'. For detailed discussion about thalamic nomenclature, I refer to the results of the international Leuven-symposium 1963 (Dewulf, 1971). Beyond doubt it may be said that there are 9 main formations within the human thalamus: the anterior formation, the lateral formation, the medial formation, the posterior formation (*pulvinar*), the reticular formation (the external Hüelgebiete), the intralaminar formation, the paraventricular formation, the geniculate formation and the epithalamic formation. Grosso modo

the lateral formation corresponds to the '*specific nuclei*', the anterior, medial and posterior formation to the '*associative nuclei*' and the reticular and intralaminar formation (without centrum medianum) to the '*aspecific nuclei*'.

## B. BASAL GANGLIA FUNCTION

A specific function hardly can be assigned to any individual nucleus of the basal ganglia complex.

Indeed, information about functional properties should be gained from anatomical studies (fibre connections), experiments (ablation, stimulation), biochemical evaluation and clinico-pathological observations.
Particular difficulties arise when applying these techniques to the basal ganglia.

*The descriptions of connections* between nuclei as derived from fibre impregnation methods are variable and incomplete, due to technical unreliability (Martin, 1970; Mettler, 1968).

*Experimental studies* in animals are faced primarly with the problem of whether or not those results can be attributed to man: neuroanatomy and certainly clinical behavior in man is rather specific. Furthermore, methodological problems render very controversial results: *lesions* made in one of the basal ganglia will interrupt also fibres to neighbouring structures; *stimulation* studies are frequently performed in the anaesthetized animal; the electrodes must be truly placed in the target without damaging other structures which may be obtained stereotactically, and the given stimulus (electrical-chemical) should be kept from spreading to adjoining structures.
For all these reasons, the findings are disputable.
The most relevant findings are obtained by stereotactic stimulation in man (Brown, 1968).

*Biochemical estimates* of homogenates of certain brain areas give valuable results. Some inconveniences, however, arise from the fact that no true localization of substances can be assigned and that many enzymes or transmitter substances very quickly change post-mortem.
*Clinico-pathological observations*, by which so much of our knowledge of brain function has been acquired, did not disclose a great deal of basal ganglia mechanisms (Martin, 1971). The method indeed depends on the occurrence of circumscribed lesions, and almost all the diseases having symptoms attributed to basal ganglia function are diffuse pathological processes (Parkinson, Chorea, ...). When focal lesions occur, e.g. vascular lesions in

putamen or pallidum; tumours in thalamus, we do not recognize many symptoms resulting from them. Moreover, as will be discussed more extensively below (introduction C), the symptoms occurring in basal ganglia diseases familiar to the clinician are of 'positive' nature (tremor, involuntary movements, ...): they involve an excess of activity: such symptoms hardly can arise from destructive pathology. Pathological examination shows eventually destroyed structures, but it does not reveal which of the surviving regions are responsible for the 'positive' signs.

In spite of those difficulties, a vast literature about experimental and clinico-pathological studies (Jung and Hassler, 1960; Mettler, 1968; Ward, 1968; Laursen, 1963) offers ample evidence that basal ganglia - together with cerebellum - *have a primary role in motor integration and thus in behavior integration*. Behavior indeed will only be disclosed to the observer by means of motor events (Ward Jr., 1968; Horridge, 1968).

While the cerebellum seems to be 'the ballistic clock', controlling rapid movements, the basal ganglia have an integrative role in slow movement, posture, reflex and automatic movements (Kornhüber, 1971; 1974; De Long and Strick, 1974). The suprasegmental control of gamma muscular activity by the basal ganglia is substantially documented (Ward, 1968; Hassler et al., 1960; Hassler, 1972).

The sensory input will reach the neostriatum via the thalamus directly (thalamostriatal fibres) or indirectly (thalamocortical and corticostriatal fibres). Efferent striatal connections influence the motor cortex via pallidum and lateral thalamus. The integrated motor response passes to the spinal motoneuron via the pyramidal tract.

On almost every level (spinal cord - thalamus - striatum - cortex) interneuronal systems take part in the message processing in an inhibitory or facilitatory fashion. The interneuronal system with multiple synaptic contacts, functioning in 'a go or hold' manner according to critical levels, implies the possibility to provide the most adequate response to the multiple stimuli arriving at the same time (Horridge, 1968). This mechanism accounts for the species reflex activity and might explain some strange reactions to unexpected and thus strong stimuli as in paradoxical hypermotricity (kinésie paradoxale) in Parkinson patients.

The morphology of interneurons is still quite speculative. Only in the spinal cord and the cerebellum the anatomy of proven inhibitory interneurons is well established (Eccles, 1967). By analogy, interneurons are described as 'small neurons with multiple amply branched dendrites' of the Golgi type II. Such cells are

very numerous in the neostriatum and the thalamus (Cajal, 1911, 1966; Dewulf, 1971).
Physiological studies showed that cells of these nuclei are inhibitory (Anderson et al., 1964; Eccles, 1966), but the identity of these inhibitory cells and the Golgi type II cells is not unequivocally proven.

Moreover, inhibition or facilitation is bound to specific neurotransmitters. Acetylcholine, known for a long time as transmitter substance in the peripheral nervous system, is also abundantly present in the central nervous system but without definite proof of transmitter function in the brain.

Since the first demonstration by the fluorescence technique of Falck (1962) by the Swedish school (Carlsson et al., 1962; Carlsson, 1959, 1966), catecholamines (dopamine - noradrenaline) and later on indole-amines (serotonine) were suggested to be the central nervous system neurotransmitters. Biochemical estimations indeed showed their incidence among brain regions (Lloyd and Hornykiewicz, 1960; Hornykiewicz, 1966; Lloyd and Hornykiewicz, 1970) and developed the different but interrelated pathways of monoamine metabolism.

In several mammals the fluorescence technique provided morphological description of dopaminergic (nigro-striatal, meso-limbic-tubero-infundibular), noradrenergic ascending dorsal (mesencephalo-cerebello-cerebral) and ventral (mesencephalo-NFB-amygdale) and tryptaminergic (ascending + descending) systems (Anden et al., 1964, 1965, 1966; Dresse, 1967; Nobin and Björklund, 1973; Hökfelt, 1974).
Post-mortem examination of human brains by fluorescence is less complete.

The role of monoamines in neurotransmission within the C.N.S. seems likely: in Parkinson disease, dopamine is drastically decreased (Fahn et al., 1971; Hornykiewicz, 1966, 1971) and L-Dopa administration produces clinical improvement; in psychosis, neuroleptics and antidepressive drugs bring about positive clinical change in behavior and interfere definitely with monoamine metabolism (Carlsson, 1964; Dress, 1967; Cooper et al., 1974).

However, several clinical symptoms in Parkinson disease and psychosis are not substantially influenced by the contemporary treatment. In psychosis and Huntington's Chorea, no significant changes in monoamine brain levels have been demonstrated.

The almost exclusive enthusiasm for monoamines as putative C.N.S. transmitter was gradually extended to other known and proven neurotransmitters, extensively present in the C.N.S.: acetylcholine and $\gamma$-aminobutyric acid (GABA) (Wollemann, 1970; Godfraind, 1975; Johnson, 1972).

GABA is a proven inhibitory synaptic transmitter (Curtis and Watkins, 1960; Krnjevic, 1970; Anden and Stock, 1973) and is found distributed within all basal ganglia in considerable amount (Müller and Langemann, 1962; Fahn and Coté, 1968). In Huntington's Chorea, glutamic acid decarboxylase (G.A.D.)(Bird et al., 1973; Bird and Iversen, 1974) and $\gamma$-aminobutyric acid (GABA) (Perry et al., 1973) are decreased.

Experimental studies in animals showed that GABA-antagonists (bicuculline) instilled in certain regions of the basal ganglia cause a behavior in animals comparable to psychotic states in man (Stevens, 1974).

In Parkinson patients GABA levels are decreased within the basal ganglia (Lloyd and Hornykiewicz, 1973; Rinne et al., 1974). There is some evidence of the existence of GABA-ergic feed-back systems, e.g. strionigral (Kim et al., 1971; Hattori et al.,1973).

In contrast to the morphological localisation of catecholamines made available by fluorescence, no such method exists for GABA. Immunofluorescence might offer new possibilities (Saito et al., 1974).

Analogous to Parkinson disease - where destruction of locus niger neurons and decrease of dopamine was understood later on by the demonstration of the nigro-striatal dopaminergic pathway - the massive loss of neostriatal neurons, mostly Golgi type II neurons, in Huntington's Chorea and the decrease of GABA in this disease may also be correlated.

This coincides with the idea of inhibitory interneurons as being 'small Golgi type II' cells.

Considering basal ganglia disorders as the results - at least partially - of deficiencies in inhibitory systems is quite deductive: it corresponds with the physiological observations of basal ganglia function, with the decrease of GABA in some diseases and with the clinical observation of extrapyramidal syndromes.

## C. CLINICAL SYMPTOMATOLOGY

As pointed out before, destructive lesions within the C.N.S. cannot be accounted for positive symptoms (Martin, 1967). Nevertheless, basal ganglia diseases are characterized to the neuropathologist as presenting more or less circumscribed areas of destruction within the brain, while to the clinician the positive or productive symptomatology is quite specific for each entity. Negative or deficiency symptoms, however, exist definitely in those disorders but these are obscured by the productive signs (Denny-

Brown, 1968).

*Deficiency symptoms* are due to either a loss of function of an active nervous structure or its efferents (primary negative symptoms) or they may result from a positive symptom (secondary negative symptom).

*Productive symptoms* are 'release phenomena': due to the overactivity of a nervous structure, released from the control of another structure.

In *Parkinson disease* and *Huntington's Chorea* negative symptoms are disorders of postural fixation, locomotion, wrightning, phonation and articulation and akinesia (Denny-Brown, 1968). Release symptoms, however, are much more pronounced. In Parkinson disease we find rigidity and tremor. In Huntington's Chorea the involuntary choreic movements predominate but in certain forms muscle tone is seriously influenced.

Those symptoms are the result of 'disinhibition' of certain brain structures.

*Catatonic schizophrenia* - although no definite cerebral damage has been shown - also presents a double symptomatology. There are negative (autism, inactivity) and positive symptoms (hallucinations, catatonic behavior). The schizophrenic syndrome can be looked upon as resulting from loss of inhibition leaving the subject with a multitude of stimuli which cannot be sufficiently integrated. The deficiency of an inhibitory system results in thought and motor disorganization and inappropriate reactions. Autism might be considered as the result of some defence-reaction towards 'the subjective disorganized world'. This conception corresponds to some psychological and electrophysiological data (Bellak, 1970; Stevens, 1973).

Summarizing, basal ganglia disorders (extrapyramidal syndromes, striatal syndromes) are characterized by 'release phenomena': a deficient inhibitory control system could be the basic element in their pathogenesis.

## D. OBJECTIVES OF THE PRESENT STUDY

Within the group of 'basal ganglia' in the human brain, the neostriatum and the thalamus are distinguished from the other nuclei (pallidum - subthalamicus - locus niger - zona innominata and incerta) morphologically by having two nerve cell populations, 'relay cells' and 'interneurons' Golgi type II.

While the 'small neurons' in the neostriatum are well known in classical neuropathology, the 'small neurons' (internuncial cells microneurons) of the thalamus are very little known.

In view of the putative inhibitory role of these microneurons - as described above -, detailed morphological analysis of those structures in extrapyramidal diseases seems of primary importance.

In the present study, a *quantitative evaluation* of the nerve cell population of neostriatum and thalamus has been performed in brains of normal individuals and of those suffering from Huntington's Chorea, Parkinson disease and catatonic schizophrenia.

In order to connect experimentally gained understanding of basal ganglia function with human pathology, a quantitative study of neostriatum in the rat and dog and of neostriatum and thalamus in the monkey was carried out.

## Chapter II

## MATERIALS AND METHOD

### A. MATERIALS

#### 1. Human Brains

*a. Normals:* 'normal brains' are brains from individuals whose anamnestic and autopsy records did not disclose any neurological disease. 8 brains of adults, from 24 yrs to 99 yrs were selected out.

| Code | Age | Sex | Cause of Death |
|---|---|---|---|
| $N_1$ | 24 yrs | male | stab wound |
| $N_2$ | 30 yrs | female | road accident |
| $N_3$ | 39 yrs | male | pleuritis-heart failure |
| $N_4$ | 41 yrs | female | uterusatonie |
| $N_5$ | 47 yrs | male | myocardial infarction |
| $N_6$ | 50 yrs | male | myocardial infarction |
| $N_7$ | 62 yrs | male | gastric neoplasm |
| $N_8$ | 99 yrs | female | pneumonia-heart failure |

The brains $N_5$ and $N_6$ were prepared in our laboratory 'Cellulaire Studie van het Zenuwstelsel' (K.U.L.); the brains $N_1$, $N_2$, $N_3$, $N_4$ $N_7$ and $N_8$ were obtained by the C. and O. Vogt Institut für Hirnforschung (University of Düsseldorf; West-Germany).

*b. Huntington's Chorea:* 7 brains of patients with Huntington's Chorea disease (choreic form) from the collection of the Laboratory for Neuropathology, University of Utrecht, The Netherlands, were studied.
Brief clinical case reports will be given in the section on Huntington's Chorea.

| Code | Age | Sex |
|---|---|---|
| $CH_1$ | 44 | female |
| $CH_2$ | 47 | female |
| $CH_3$ | 58 | female |
| $CH_4$ | 58 | male |
| $CH_5$ | 63 | male |
| $CH_6$ | 63 | male |
| $CH_7$ | 72 | male |

In these cases, the caudatum and the thalamus were studied. The quantitative evaluation of the putamen in Huntington's Chorea was performed on another series of brains and reported previously (Dom et al., 1973).

*c. Parkinson disease:* 5 brains of patients with Parkinson disease, 2 postencephalitic and 3 idiopathic, were chosen from the collection of the C. and O. Vogt Institut für Hirnforschung. These brains were part of the series of Parkinson brains studied by Hassler in order to give his well-known description of the neuropathology of Parkinson disease (Hassler, 1938). A brief clinical case report follows in the section on Parkinson disease.

| Code | Age | Sex | Clinical Form |
|---|---|---|---|
| $P_1$ | 32 | female | postencephalitic |
| $P_2$ | 46 | male | postencephalitic |
| $P_3$ | 57 | male | idiopathic |
| $P_4$ | 68 | male | idiopathic |
| $P_5$ | 71 | female | idiopathic |

*d. Catatonic schizophrenia:* 5 brains of schizophrenics were selected from the collection of the C. and O. Vogt Institut für Hirnforschung. A brief clinical case report will be drawn in the section on catatonic schizophrenia.

These brains were obtained in the era before neuroleptic treatment was in use: no significant biological treatment was given to the patients under consideration.

These cases attributed to the systematic neuropathological study of schizophrenia performed and reported between 1950 and 1965 by the school of Vogt (Hopf, 1959-1954; Bäumer, 1954; Fünfgeld, 1954;

Treff and Hempel, 1958, 1959, 1960, 1962).

| Code | Age | Sex |
|---|---|---|
| $S_1$ | 24 | male |
| $S_2$ | 26 | female |
| $S_3$ | 29 | female |
| $S_4$ | 42 | female |
| $S_5$ | 44 | female |

2. Animals

*a. Rat:* adult white Wistar rats of $\pm$ 250 g were studied. The brains of the anaesthetized animals were fixed by perfusion with formalin 4 % after Ringer-solution via A. Carotis.

Cytometric evaluation in the neostriatum was performed in normal animals and in animals treated with neuroleptic drugs.

The detailed study design will be given in the chapter on experimental parkinsonism.

*b. Dog:* the brains of normal Beagles were studied.
The brains were fixed by perfusion with glutaraldehyde 3.5 % after Ringer-solution via the left cardiac ventricle.
Quantitative measurements were obtained in the neostriatum of Beagles at the age of 3 months, 6 months, 1 year, 2 years and 3 years.

*c. Monkey:* cytometric evaluation of neostriatum and thalamus was obtained from 3 brains of adult Cercopithecus aethiops aethiops. The brains were fixed in Formalin 4 % after decapitation.

The brain material, human and experimental, was embedded in paraffin and 20$\mu$ serial sections were cut. The sections were stained with Cresylviolet (Nissl-Stain).

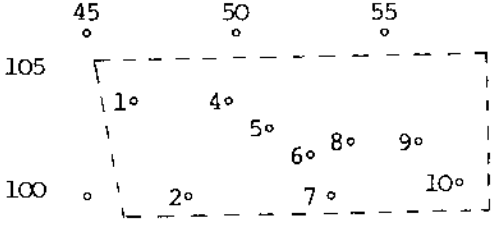
B. METHOD

1. Technique

This study was performed with a cytometric technique originally developed in our laboratory by Dewulf (1971).

Using an ocular micrometer, furnished with rows of circles gradually increasing in diameter, each nerve cell was measured by fitting a circle over the cell so that it did not mask any part of the cell border.

The area to be sized out, was drawn out with the aid of the stage micrometer on the microscope. Points were indicated at random on this schema. The microscope was then adjusted to these points by means of the coördinates of these points on the scheme. By each adjustment, a square of 0.07 $mm^2$ (= 1 unit) real tissue could be studied with the magnification used (Leitz-microscope: 8 x 1.25 x 45 = 450 x).




1) 103-47
2) 100-49
3) 101.5-49.5
4) 103-50
5) 102-51.5
6) 101-52.5
7) 100-53.5
8) 101-54.5
9) 101-56
10) 100-57

At least 300 nerve cells were measured for a given nucleus in each case.
The nerve cell population was then depicted in a percentage distribution curve (neuronal formula), having in absciss the cell diameter in micra and in ordinate the percentage amount of cells per diameter.
To obtain the numerical nerve cell density (the number of cells per $mm^3$) the number of cells per unit (= 0.07 $mm^2$) had to be multiplied by 710 because all sections studied were 0.02 mm thick (20 micra sections) (0.07 x 0.02 x 710 = $\pm$ $1mm^3$).
For each brain, a neuronal formula was worked out in caudatum,

putamen, thalamus anterior, thalamus lateralis, thalamus medialis and in thalamus posterior.
In order to obtain as comparable results as possible between several brains, nerve cell sizing was performed ± at the same level within the different structures. This was made possible by serial topometric macrophotographs every 2 mm throughout the brain $N_6$, prepared according to the method described by Dewulf et al. (1962).
With the help of myelin preparations, the level to be worked out for each human brain could be ascertained by referring to the macrophotographs of $N_6$.
The levels chosen for each structure will be indicated in the sections where normal neostriatum and thalamus cytometry will be discussed.

## 2. Reliability of the technique

The application of quantitative techniques in histology, raises doubts as to the reliability of this measurement technique. There are several sources of error in the measurement of microscopic objects (Weibel and Helias, 1967). These errors may be categorized under two major headings:
1) changes in the tissue during the various procedures of preparation (fixation, dehydration, embedding, cutting of sections) (Bauchot, 1967)
2) errors of measurement proper.

1) The first group, *changes in tissue*, is difficult to correct but for the present study, attention was paid to minimize those changes as much as possible: most of the human brains were obtained from the same laboratory using the same procedure of preparation for many years.

For the normal brains $N_1$, $N_2$, $N_3$, $N_4$ and $N_7$, the shrinkage factor was defined in the C. and O. Vogt Institut für Hirnforschung (Lange and Thörner, 1974): a) on the basis of fresh brain weight and volume of the cerebral hemispheres calculated from serial sections. If one knows that 88 % of brain weight is accounted for by the cerebral hemisphere and that the specific weight of brain substance (white + grey matter) is 1.0365, then the shrinkage factor is:

$$SF_1 = \frac{\text{brain weight} \times 0.88 : 1.0365}{\text{hemisphere volume}}$$

b) on the basis of linear dimensions (brain length, width, height) in formalin 4 % and on the sections after preparation:

$$SF_2 = \frac{\text{length} \times \text{width} \times \text{heigth in formalin}}{\text{length} \times \text{width} \times \text{heigth after preparation}}$$

The shrinkage factor is then:

$$\frac{SF_1 + SF_2}{2}$$

The values obtained are: $N_1$ 2.00
$N_2$ 1.75
$N_3$ 1.70
$N_4$ 1.85
$N_7$ 1.85

The difference is thus very small, proving that the influence of preparation procedures is fairly equal for all brains. This will be demonstrated below in the values of numerical densities obtained.

2) The *errors due to measurement proper* can be reduced by taking some precautions.

Fragmentation of nerve cells by cutting sections can result in sizing the same cell on two sections. The larger the cell and the thinner the section, the greater the error due to fragmentation (Abercrombie, 1946; Koningsmark et al., 1969). This error can be corrected by measuring only those cells which have a nucleolus within the nucleus.

Measuring nerve cells by defining 'length' and 'width' induces subjectivity because the boundary between cell body and dendrites or axon is an arbitrary one. The same difficulty applies to the 'Treffer method' of Haug (1958): are treffer points on nerve cell dendrites or glial branches ?

Our method of circles rules out subjectivity: all nerve cells with a nucleolus were measured throughout the field.
There is only one disadvantage: this method does not characterize the shape of the cell.

In order to evaluate the impact of the inconveniences of neuronal degeneration, a preliminary study on 600 cells in the neostriatum of one case of Huntington's Chorea was performed to compare the results obtained by the circle method and by careful selection of the two cell axes and indication of the cell shape. As published previously the very high correlation between the two methods was statistically proven (Dom et al., 1973).

With the present study, this correlation between the two methods will be shown again: some of our material was worked out before by other authors with the technique of measuring the two main cell axes. The results they obtained coïncide much with our findings.

## Chapter III

# NORMAL HUMAN NEOSTRIATUM

Considering the caudatum and putamen together in neuroanatomy and pathology depends upon the facts that 1) their embryologic origin is from continuous territory: the division is made up only by projection fibres and the relative volume of each varies from species to species in a way that the putaminal volume increases at the expanse of caudatum as one ascends the phylogenetic scale (Harmann and Carpenter, 1950);

2) their cytological composition and connections appear similar.

## A. THE CYTOLOGY OF THE STRIATUM

The cytology of the striatum has been described by a considerable number of authors (Ramón Cajal, 1911; Foix and Nicolesco, 1925; Hunt, 1933; Namba, 1957; Mettler, 1968). In Nissl stain, two distinct nerve cell types are quite obvious: small and middle sized neurons, mostly round, triangular or oval in shape, and large cells, oval, pyramidal or stellate in shape.
In the smaller population, the chromatic substance is granular and evenly distributed throughout the cytoplasm, while the larger neurons have more coarse 'nissl'-chromatin bodies (Photo 1).
In Golgi preparations, the large cells exhibit fewer slender rather smooth dendrites; their axons are tortuated, sparsely branched and difficult to follow (Golgi type I). The smaller neurons show numerous stout, irregular and frequently branched dendrites with many short spines, giving them a brush-like appearance; the axon is smooth but widely branched and distributed (Golgi type II). Some authors (Cajal, 1911; Namba, 1957) still divide up the smaller neuron population in two distinct types on the basis of size and Golgi appearance: the small 'cellules petités à cylindre-axe court' (Cajal) [alpha cells (Namba)] and medium-sized 'cellules moyennes à cylindre-axe court' (Cajal) [beta cells (Namba)].
Recent researchworkers support this view: Rafols (1974) describes in the primate neostriatum two types of Golgi type II cells by means of Golgi-stains and electron microscopy. According to his findings the two types of cells might even contain different neurotransmitter substances.

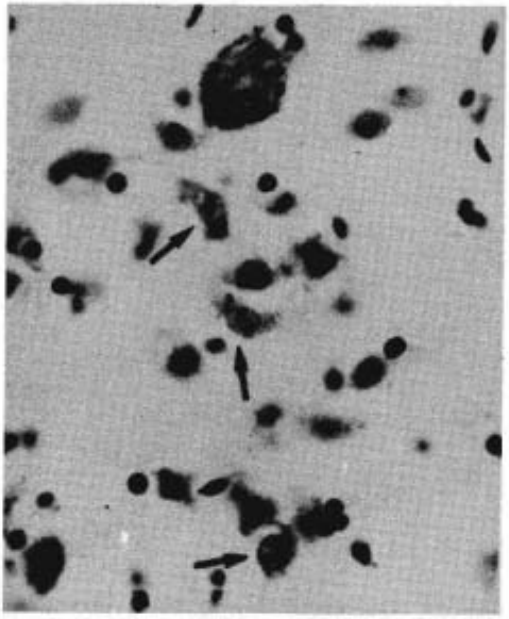


PHOTO I: *Human neostriatum. Microneurons indicated by arrow. Nissl stain. Magnification 500 x.*

## B. QUANTITATIVE CYTOMETRIC VALUES

Quantitative cytometric data of the normal neostriatum are available since the description of Foix and Nicolesco (1925): the ratio large-small neurons was estimated 1-20, a value also quoted by Peele (1961); but those authors do not give any reference to the method used.
Dunlap (1927) made semi-quantitative evaluations of caudatum and putamen: in 6 micron sections, he counted the amount of cells per field ($\pm$ 0.24 $mm^2$) and obtained thus 70 neurons per field for caudatum and 60 neurons per field for putamen. He made no distinction between the two nerve cell types. The same lack of distinction can be ascribed to the values given by Carmann (1966).

Namba (1957) gives results for one human brain: the ratio large-small neurons in neostriatum is between 1:53 and 1:125. His method, however, is biased towards an overestimation of large neurons.

Treff (1964) reported the following values: 35.000 small neurons per $mm^3$ and 690 large cells per $mm^3$, and ratio large-small 1:52. Although he does not give a full description of his method, it is apparent that he counted all neurons with nucleus without considering the nucleolus, which necessarily implies the error of counting only cell fractions (see chapter II).

Tabuchi (1969) made counts in 5 normal brains between the ages of 20 and 45. His method of counting and sizing is not reported. The results obtained were: 29.200/$mm^3$ for small neurons, 110/$mm^3$ large neurons and large-small ratio 1:270. The mean size of small neurons is 13.9 $\mu$ in length and 11 $\mu$ in width.

Schröder (1970), Lange and Thörner (1974) made an extensive quantitative analysis in a series of normal brains. They report in detail the methods used and the corrections to be considered in cytometric studies. They obtained the following values: numerical density for small neurons 21.644/$mm^3$ in man and 23.540/$mm^3$ in woman, for large neurons 132/$mm^3$ in man and 126/$mm^3$ in woman; ratio large-small is 1:175 for the total group. The cell size for the small population is given by the average nucleus diameter 8.6 micron.
Böttcher (1975) reported in the human neostriatum a neuron density of + 19.500/$mm^3$, a large-small ratio 1:60 and an average nucleus diameter of 8.02 micron.

## C. PERSONAL CYTOMETRIC STUDY OF THE NORMAL NEOSTRIATUM

The caudatum and the putamen were studied in 8 normal brains ($N_1 \longrightarrow N_8$):

5 males: $N_1$ (24 yrs), $N_3$ (39 yrs), $N_5$ (47 yrs), $N_6$ (50 yrs) and $N_7$ (62 yrs) and 3 females: $N_2$ (30 yrs), $N_4$ (42 yrs) and $N_8$ (99yrs).

### 1. Regional differences in cytometric values within the neostriatum

As caudatum and putamen are very huge nuclei it seemed worthwile to study initially one brain at *different levels throughout the whole nucleus* in order to check eventual regional differences.

Therefore, in brain $N_5$ the neuronal population of the left caudatum was evaluated every 4 mm (6 levels) and of the left putamen every 2 mm (6 levels). Level 1 of the caudatum and the putamen corresponds to the vertico-transversal section through the middle third of the commissura anterior. Level 6 of the caudatum corresponds to the vertico-transversal section through the commissura posterior, and of the putamen to the section through the maximal development of the locus niger.

The neuronal formula for the small neuron population for caudatum at different levels is depicted in plate I, for putamen in plate II.

The results obtained for numerical densities (number per $mm^3$) of small (micro) and large (macro) neurons, large-small ratio (L:S) and average diameter of microneurons (Ø in micra) is summarized in the following schemes:

*Caudatum*

| | Level | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| NUMERICAL DENSITY | macro | 135 | 206 | 71 | 135 | 71 | 71 |
| | micro | 21.584 | 23.160 | 22.010 | 21.243 | 21.108 | 17.956 |
| | $\frac{L}{S}$ | $\frac{1}{157.5}$ | $\frac{1}{113}$ | $\frac{1}{321}$ | $\frac{1}{155}$ | $\frac{1}{308}$ | $\frac{1}{262}$ |
| | Ø | 10.07 | 9.77 | 9.88 | 9.71 | 9.21 | 9.63 |

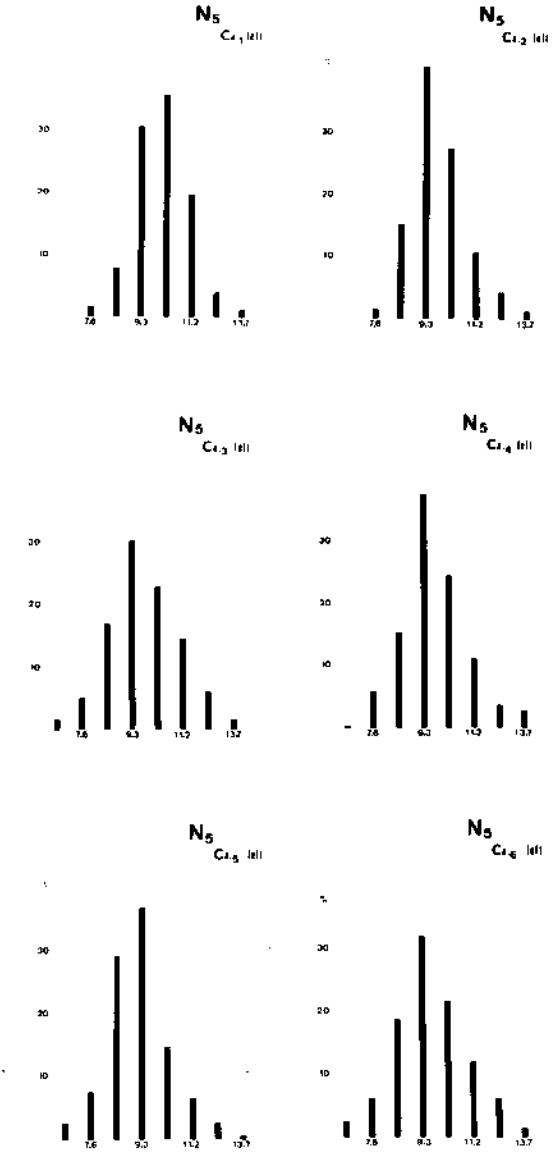



PLATE I: *Micronеuron distribution in the normal caudatum at different levels: in ordinate %, in abscis Ø in micra*

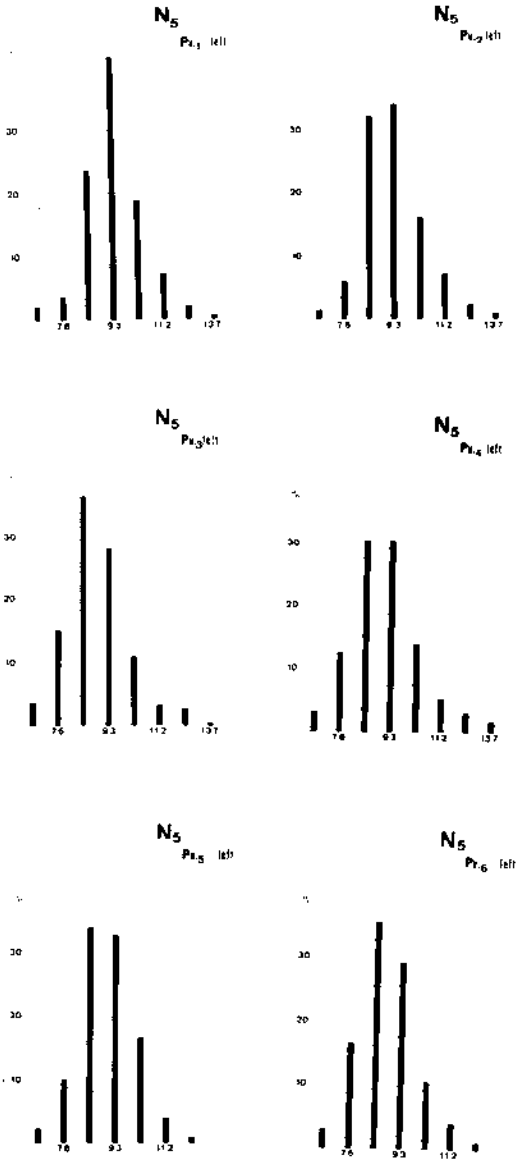



PLATE II: *Microneuron distribution in the normal putamen at different levels: in ordinate %, in abscise Ø in micra*

*Putamen*

| NUMERICAL DENSITY | Level | 1 | 2 | 3 | 4 | 5 | 6 |
|---|---|---|---|---|---|---|---|
| | macro | 135 | 135 | 277 | 135 | 71 | 206 |
| | micro | 23.849 | 22.273 | 20.973 | 19.880 | 17.473 | 18.297 |
| | $\frac{L}{S}$ | $\frac{1}{174}$ | $\frac{1}{162.5}$ | $\frac{1}{76.5}$ | $\frac{1}{145}$ | $\frac{1}{255}$ | $\frac{1}{89}$ |
| | ø | 9.63 | 9.26 | 8.88 | 9.13 | 9.01 | 8.83 |

From those values, it is apparent that the numerical microneuron densities are quite uniform throughout the nuclei, except for the most caudal regions (levels 5 and 6): this is easily understood by the fact that caudally-especially in the putamen- there are more fibre bundles passing through.

Concerning the average diameter and distribution of the microneuron population, the following remarks should be made:

1) The small neurons are somewhat larger in the caudatum than in the putamen.

2) The small neurons tend to decrease in size from rostral to caudal regions in putamen as well as in caudatum.

3) Statistical analysis by Kolmogorov-Smirnov test of the distribution at different levels discloses that the rostral and caudal levels are significantly different.

Plate III gives the cumulative frequency distribution at different levels from rostral ($L_1$) to caudal ($L_6$) within caudatum and putamen: the more caudal, the smaller the microneurons. The full black line depicts the average for $L_1 + L_2$, the dash-line the average for $L_5 + L_6$.

Based on these results, it was concluded that in the different brains only the middle third of the caudatum and putamen (regions corresponding to the levels $L_3$ and $L_4$) needed to be used for cytometric analysis in order to obtain as comparable results as possible.

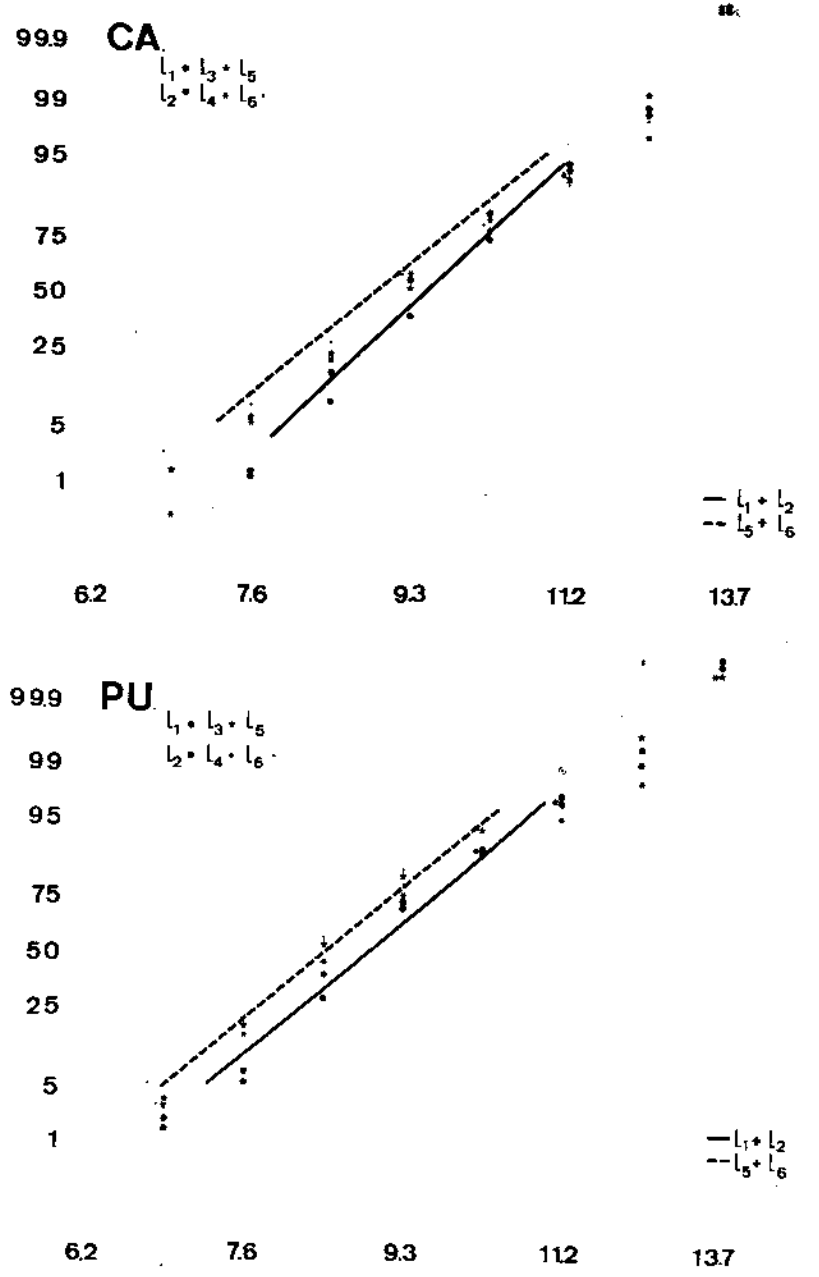



PLATE III: *Cumulative frequency distribution of microneuron population at different levels plotted on probability paper. Ca = caudatum, Pu = putamen. In ordinate %, in abscise Ø in micra*

## 2. Cytometric differences in the neostriatum between left and right hemisphere

In the studies up to now, no mention has been made of *differences in cytometric characteristics between left and right hemisphere*. As our study for all brains was performed in the left (dominant) hemisphere, one might wonder whether the same values are applicable to the other hemisphere. In view of this question, in brain $N_6$ (50 yrs) counting and sizing of the neuron population in caudatum and putamen was obtained bilaterally at the same levels (middle third).

The next table summarizes the numerical values:

*Caudatum*

| | Numerical macro | Density micro | $\frac{L}{S}$ | Ø Average |
|---|---|---|---|---|
| Left | 142 | 20.954 | $\frac{1}{147}$ | 9.41 |
| Right | 142 | 21.371 | $\frac{1}{150}$ | 8.48 |

*Putamen*

| | Numerical macro | Density micro | $\frac{L}{S}$ | Ø Average |
|---|---|---|---|---|
| Left | 213 | 20.954 | $\frac{1}{99}$ | 8.19 |
| Right | 213 | 21.584 | $\frac{1}{100}$ | 8.48 |

The obvious high correlation between the two hemispheres agrees quite well with volume estimations of the neostriatum in man: these also have shown that there is no difference between left and right hemispheres ($\pm$ 10 $cm^3$ in man; $\pm$ 9 $cm^3$ in woman) (Orthner and Sendler, 1969, 1975) (Lange and Thörner, 1974).

## 3. Cytometric evaluation of neostriatum at different ages

Neurocytometric counts in the middle third of left caudatum and putamen *in the normal brains of different age groups* were performed.

The percentage distribution of microneuron populations is shown in plate IV (caudatum) and plate V (putamen).
The evolution with age is visualized on plate VI by the cumulative frequency distributions drawn on probability paper for the 8 brains. It is clear that there exists a progressive decrease in diameter of microneurons with increasing age.

Kolmogorov-Smirnov analysis shows significant difference in size distribution between younger and older brains. Up to the age of 45 the distribution patterns are rather similar. The same applies for the group above 45 years, with exception of $N_8$. Averaging the whole group in order to compare with results obtained in pathological material implies thus serious disadvantage.

As will be seen later on, the schizophrenic brains studied are all in the 20 to 45 age group, while all the choreic brains and all but one of the Parkinson brains in our study fall in the age group above 45 yrs.

Therefore, it seems logical to group together from our material the results of $N_1$ (24 yrs), $N_2$ (30 yrs), $N_3$ (39 yrs) and $N_4$ (42 yrs) (young normals: 20-45 yrs) and of $N_5$ (47 yrs), $N_6$ (50yrs) and $N_7$ (62 yrs) (old normals: 46-65 yrs). The brain $N_8$ (99 yrs) will be excluded from the comparison group because in the pathological cases (Parkinson - Chorea) there is no brain from a patient older than 72 years.

On plate VI, the average distribution for both age groups in both structures is drawn out: full black line for the brains $N_1 \longrightarrow N_4$ (20-45 yrs), dash-line for $N_5 \longrightarrow N_7$ (45-65 yrs).

For the normal brains 20-45 yrs, the numerical values obtained are summarized in the next tables: numerical densities (amount per $mm^3$) for micro- and macroneurons, the L/S ratio (ratio macro/micro neurons) and the average diameter in micro for the microneurons.

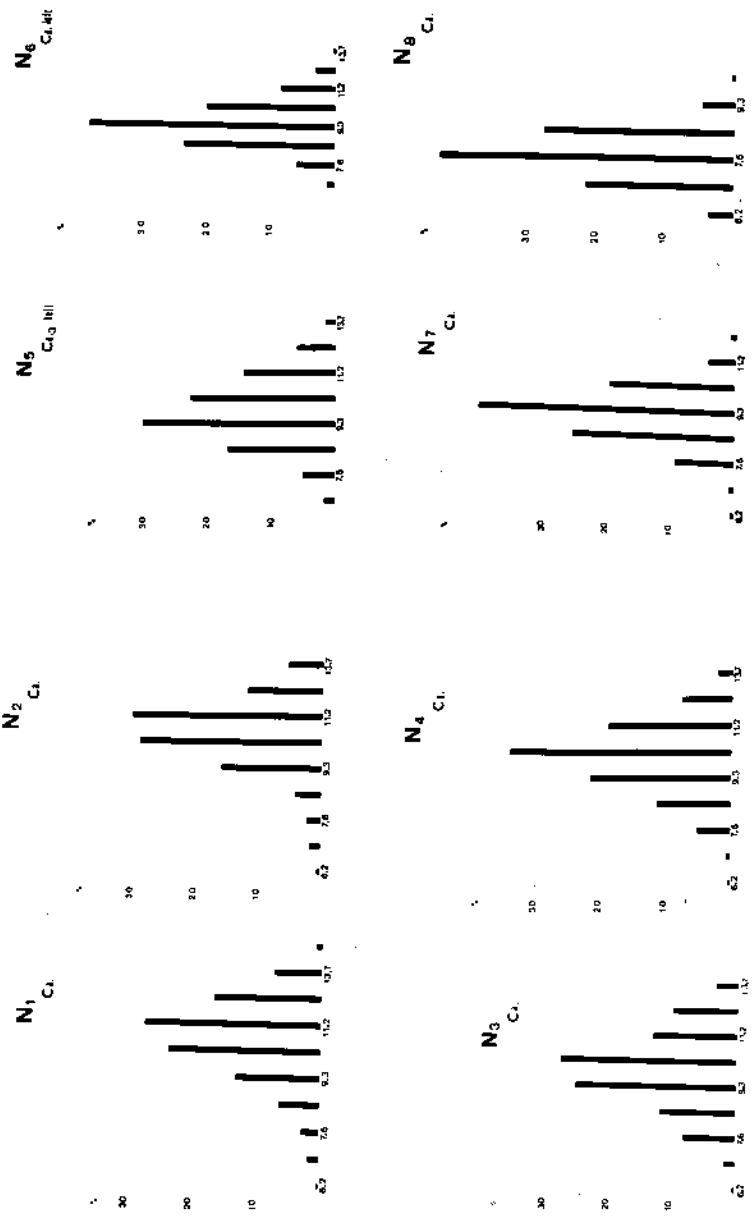



PLATE IV: *Microneuron distribution in the normal caudatum at different ages: in ordinate %, in abscise Ø in micra*

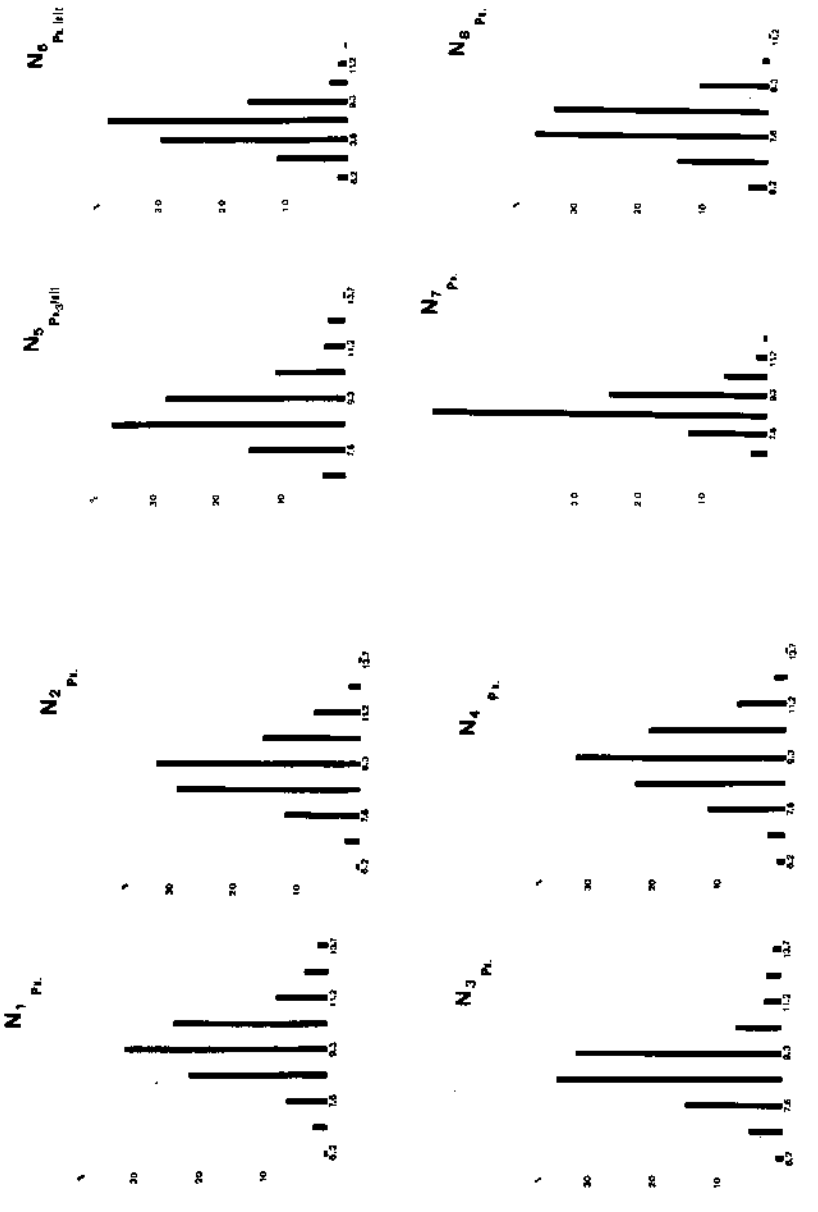


PLATE V: *Microneuron distribution in the normal putamen at different ages: in ordinate %, in abscise Ø in micra*

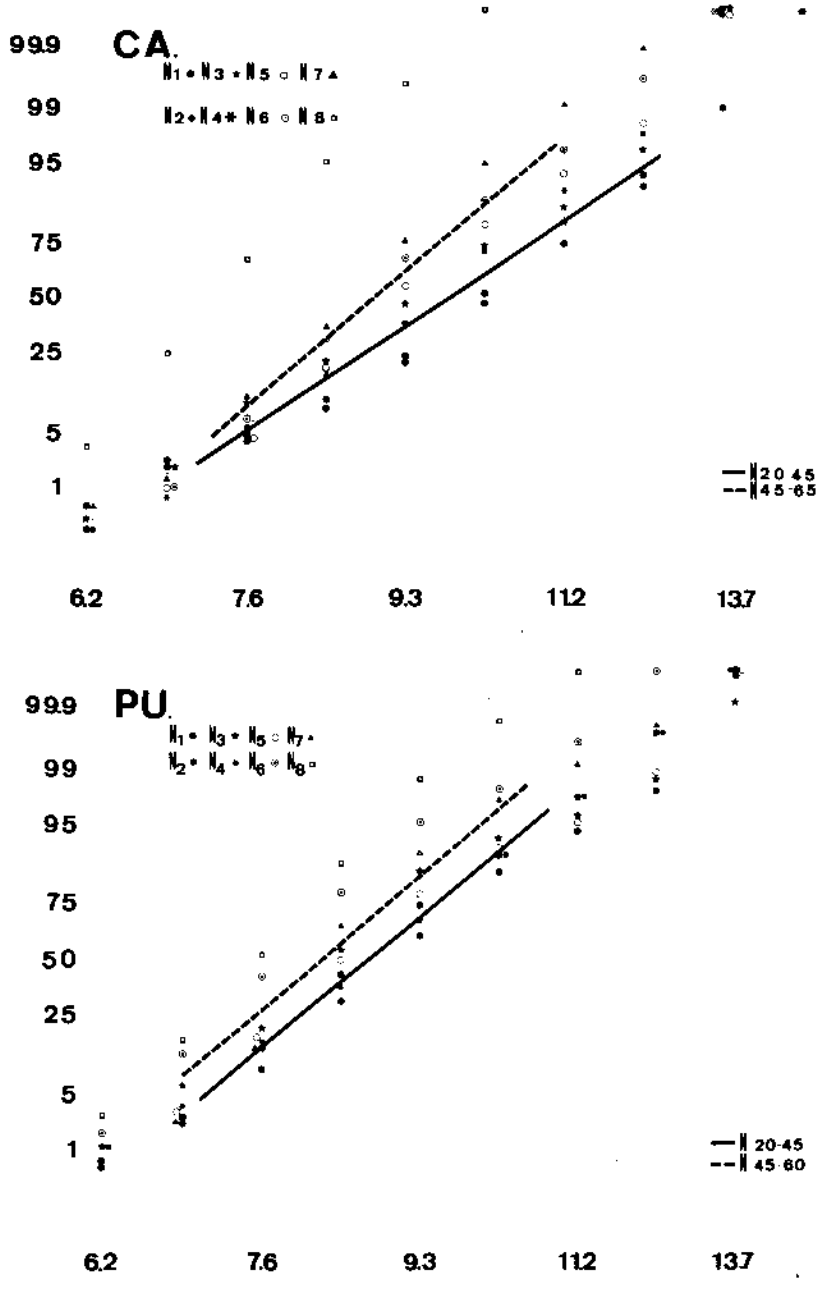



PLATE VI: *Cumulative frequency distribution of microneuron population at different ages plotted on probability paper. Ca = caudatum, Pu = putamen. In ordinate %, in abscissa ø in micra*

*Caudatum*

| | Numerical macro | Density micro | $\frac{L}{S}$ | Average Ø in micra |
|---|---|---|---|---|
| $N_1$ | 270 | 22.663 | $\frac{1}{83}$ | 10.78 |
| $N_2$ | 57 | 20.696 | $\frac{1}{379}$ | 10.63 |
| $N_3$ | 142 | 21.371 | $\frac{1}{150}$ | 9.95 |
| $N_4$ | 57 | 23.153 | $\frac{1}{424}$ | 10.08 |
| AVERAGE | 132 | 21.971 | $\frac{1}{217}$ | 10.38 |

*Putamen*

| | Numerical macro | Density micro | $\frac{L}{S}$ | Average Ø in micra |
|---|---|---|---|---|
| $N_1$ | 163 | 19.953 | $\frac{1}{119}$ | 9.47 |
| $N_2$ | 220 | 21.193 | $\frac{1}{97}$ | 9.10 |
| $N_3$ | 220 | 23.075 | $\frac{1}{106}$ | 8.80 |
| $N_4$ | 220 | 23.756 | $\frac{1}{109}$ | 9.19 |
| AVERAGE | 206 | 21.894 | $\frac{1}{107}$ | 9.13 |

For the normal brains 45-65 yrs the values obtained are shown in the following tables:

*Caudatum*

| | Numerical Density macro | micro | $\frac{L}{S}$ | Average Ø in micra |
|---|---|---|---|---|
| $N_5$ | 139 | 22.585 | $\frac{1}{165}$ | 9.76 |
| $N_6$ | 142 | 20.945 | $\frac{1}{147}$ | 9.41 |
| $N_7$ | 64 | 25.106 | $\frac{1}{203}$ | 9.16 |
| AVERAGE | 115 | 22.878 | $\frac{1}{203}$ | 9.43 |

*Putamen*

| | Numerical Density macro | micro | $\frac{L}{S}$ | Average Ø in micra |
|---|---|---|---|---|
| $N_5$ | 206 | 20.427 | $\frac{1}{99}$ | 9.01 |
| $N_6$ | 213 | 20.954 | $\frac{1}{99}$ | 8.48 |
| $N_7$ | 142 | 26.412 | $\frac{1}{121}$ | 8.67 |
| AVERAGE | 187 | 22.597 | $\frac{1}{121}$ | 8.72 |

- The numerical densities and the L/S ratio are not significantly different between the two age groups. There exists,however, a definite decrease in microneuron diameter with increasing age, as was already evident by the distribution curves.

- In both age groups, there are significant differences between putamen and caudatum:
  a) The macroneuron density seems $\pm$ 62 % higher in the putamen

than in the caudatum.
As the microneuron population is identical, the L/S ratio in the caudatum is lower than in the putamen.

b) The average microneuron diameter is ± 10 % smaller in the putamen than in the caudatum.

These differences are not mentioned in previous cytometric studies of the striatum and might explain some discrepancies in results between the most recent publications (e.g. L/S ratio 1/270 by Tabuchi (1969), 1/52 by Treff (1964), 1/175 by Schröder (1970) Lange and Thörner (1974), 1/60 by Bøttcher (1975).
The results obtained by the coworkers of Hopf (Schröder, Lange and Thörner), with other techniques, correspond with our results, if one does not make the distinction between age groups or between caudatum and putamen: our overall values compared to those of the Hopf-school are given below:

| | microneuron-density | macroneuron-density | L/S ratio | average Ø microneurons |
|---|---|---|---|---|
| Hopf | 22.590 | 129 | 1/175 | 8.62 micra (nucleus) |
| Present study | 22.635 | 160 | 1/162 | 9.43 micra (nucleus) |

This close correlation is the more interesting because most of the brains in our study were part of the brain material used by Lange and coworkers, but the latter authors used a different quantitative technique.
It seems important to discern caudatum and putamen in cytometric studies and to choose comparable age groups in neuropathological studies, because these morphological differences may correspond to some functional differences between putamen and caudatum, cited in the literature (Szabo, 1962; Ward, 1968; Laursen, 1963) and neuropathological study could reveal damage to one of either structures specifically.

## 4. Concluding summary from this study of the normal neostriatum

1. The numerical neuron densities (macro- and microneurons) are rather uniform throughout putamen and caudatum: ± 22.000 microneurons per $mm^3$ in putamen and caudatum; ± 125 macroneurons per $mm^3$ in caudatum; ± 195 macroneurons per $mm^3$ in putamen.

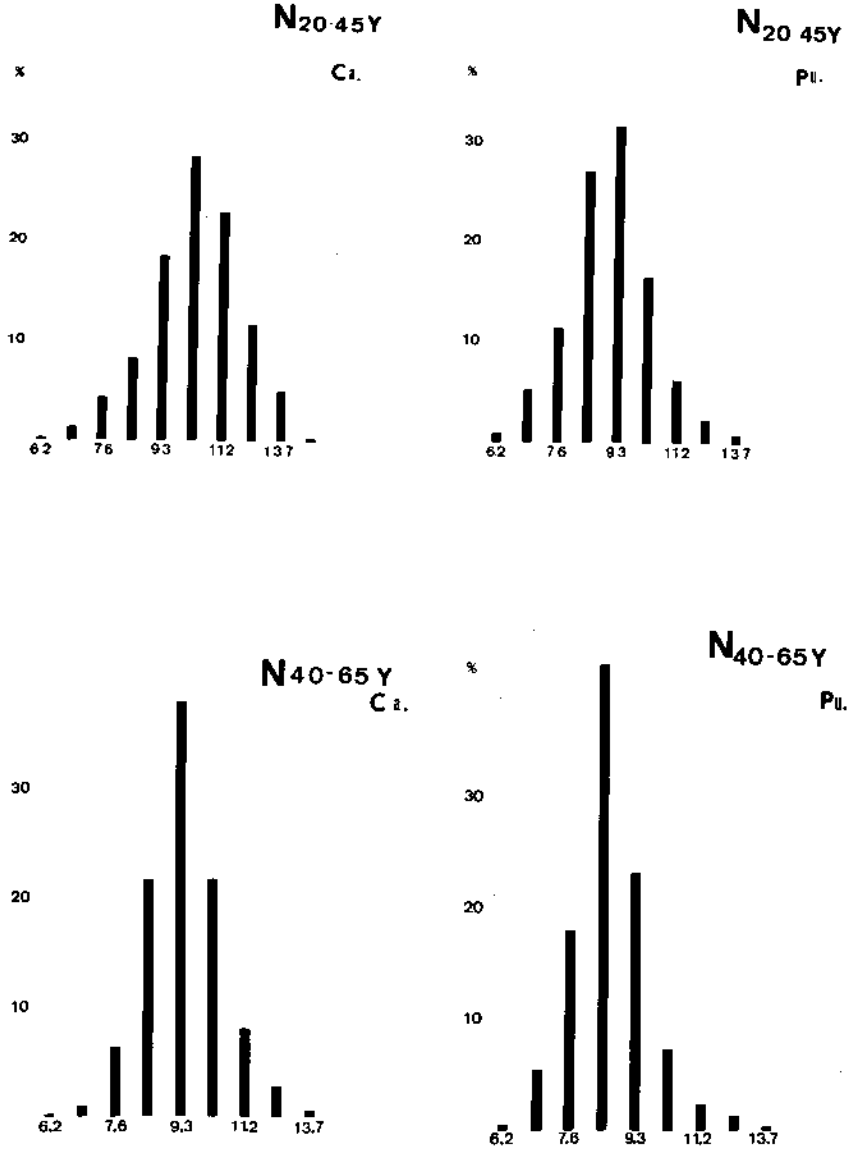



PLATE VII: *Average distribution of microneurons in caudatum (Ca) and putamen (Pu) for young (20-45 yrs) and old (40-65yrs) normal brains. In ordinate %, in abscise Ø in micra*

2. The average diameter of microneurons decreases slightly from rostral to caudal both in putamen and caudatum: in caudatum from 10.07 to 9.63 micra; in putamen from 9.63 to 8.83 micra.

3. There is no difference in numerical densities, L/S ratio and average diameter between left and right hemisphere.

4. The L/S ratio is smaller in caudatum (1:210) than in putamen (1:115) because the macroneuron density is lower in caudatum than in putamen.

5. The average microneuron diameter is ± 10 % smaller in the putamen (± 8.92 micra) than in the caudatum (± 9.9 micra).

6. With increasing age (24 to 99 yrs), there is no difference in caudatum and putamen for numerical densities (micro- and macroneurons) nor L:S ratio. There is, however, a gradual decrease of average diameter of microneurons with increasing age. Therefore, plate VII gives the average percentual distribution of Golgi type II microneurons in caudatum and putamen for two age groups: adults 20-45 yrs and adults 45-65 yrs.

## Chapter IV
## NORMAL HUMAN THALAMUS

The thalamus in the human brain is a large diencephalic nucleus composed of several subgroups (formations of nuclei).

It is out of the scope of the present study to give a review of the vast literature about the cytoarchitectonics and functional organization of different thalamic regions: in that respect, some major studies can be consulted (Vogt: Thalamusstudien I-III, 1941; Dekaban, 1953, 1954; Feremutsch and Simma, 1954, 1955; Hassler, 1959; Hopf, 1965; Hassler and Stephan, 1966; Dewulf, 1971; Van Büren and Börke, 1972; Gerebtzoff et al., 1973).

In view of the present study objectives, two subjects will be discussed more amply: 1) Thalamic microneurons
2) Quantitative evaluation of the thalamus and its neuron population.

### A. THALAMIC MICRONEURONS

#### 1. Literature review

Almost all thalamic nuclei harbour two types of nerve cells: in a descriptive way, they may be called macro- and microneurons.

von Monakow (1895) first mentioned the presence of 'Schaltzellen' in the human thalamus. The 'petits neurones à cylindre-axe court' of Cajal (1911) are apparently the homologues of the microneurons. Lemieux (1953) mentioned that the 'small internuncial thalamic nerve cells' were load up with fat substance in a case of amaurotic idiocy.

Powell (1952), Waddy and McLardy (1956) and McLardy (1963) spoke about thalamic 'microneurons' surviving after cortical ablation and the latter even gave a quantitative estimation amonst different thalamic formations: 1 to 6 in the lateral nucleus, 1 to 3 in anterior and medial nuclei.

Hassler (1959) called the small thalamic neurons 'internuncial cells'; in addition to the characteristic appearance of those cells in Nissl stain, he gave for all thalamic subdivisions the adjective 'few, many, very numerous' according to the prevalence in microscopic examination.

Scheibel and Scheibel (1966) showed with the Golgi technique the presence of Golgi type II cells in the ventrobasal complex of the thalamus and in the centrum medianum.

With Golgi technique and electron microscopy, Tömbol (1967) described 'short neurons' in the specific thalamic nuclei of the cat. Studying the synaptic architecture of the medial geniculate body in the monkey, Majorossy et al. (1968) reported 17 % of microneurons present.
Dewulf et al. (1969-1971) performed a systematic quantitative evaluation of the neuron population in most nuclei of one human thalamus. The typical morphological appearance of microneurons in Nissl stained sections was stressed. Moreover, it was shown that the amount of microneurons (internuncial cells) varies among nuclei from 5 % of the total neuron population (N. Genic. Lat.) to more than 50 % of the total population (N. Medialis). Detailed results are reported in the topometric atlas of human thalamus edited by Dewulf (1971).
Ralston and Herman (1969) described the fine morphology of the ventrobasal thalamus in the cat: they found 20 % of Golgi type II microneurons.
Martin (1970) insisted upon the meaning of microneurons in the centrum medianum in different abiotrophies, while Varella (1969) studied some intralaminar thalamic nuclei in relation to his evaluation of 'the reticular system of the brain'.
Mathers (1972) reported 30 % of microneurons in the pulvinar of the Squirrel monkey.

Dom and Dewulf (1969) and Dom (1973) differentiated by means of serial quantitative analysis in the N. Dorsalis Superficialis Thalami (N. Lateralis Dorsalis), two parts: a dorsomedial part having 40 % of microneurons and a ventrolateral part having 25 % of microneurons. In animals up to the primate, there are indeed two subdivided parts in this nucleus. In man, it was shown that the N. Dors. Superf. did have projections to the limbic cortex, besides projections to the parietal and temporal cortex (Yakowlev et al., 1966). Dom (1973) proposed the dorsomedial part of the nucleus to be responsible for the limbic projections because the N. Anterior - known to be part of the limbic system - has evenso 35 % of microneurons.
This was confirmed by Mikol and Brion (1975) by pathological and experimental studies.

Somoggi et al. (1973) cited 20 % of Golgi type II neurons in the anterodorsal thalamic nucleus of the cat. In the lateral geniculate body of the monkey, Le Vay (1971) found 10 % of microneurons.
In their extensive atlas of the thalamus, von Büren and Börke (1972) do not distinguish between micro- and macroneurons, but the cytometric graphs show nicely the differential prevalence of neurons with a diameter of ± 10 micron among thalamic nuclei. Gerebtzoff et al. (1973) made an excellent review of the structural, functional and cytochemical organization of the mammalian thalamus: they insist upon the consistency of findings concerning the thalamic microneuron population in spite of using different techniques (Golgi - Nissl stain - electron microscopy). Those authors state that the most complex study in man by Dewulf and collaborators has never been contradicted by the results of others. Martin (1974) reported that the thalamic microneurons seemed to be spared selectively in Wernicke's encephalography.

Dom et al. (1976) showed that there exists a selective loss of microneurons within the ventrolateral thalamus in Huntington's Chorea. The possible relationship with GABA deficiency was discussed.

## 2. Morphology

*In Nissl stain*, the small neurons have a characteristic appearance. Their size is much closer to glial cells than to macroneurons (Photo 2a). The shape is mostly triangular, sometimes oval or even bipolar.

They have a round, rather dense nucleus. The nucleolus may be present but is sometimes 'drawn out by two semi-lunar shaped chromatin block' as described by Hassler (1959).
Their 'honeycombed' cytoplasm is fairly reduced in volume, mostly without well identified granular chromatin blocks: the Nissl substance is evenly distributed throughout. The cell branches tortuated with hooky spines or secondary branches sometimes may be followed a fair distance towards the contact zone with macroneurons (Photo 2b,c).

The microneurons are present throughout the whole nucleus but in N. Anterior and N. Medialis, they tend to form small clusters of 3 to 5 neurons; this phenomenon is not apparent in the lateral or the posterior nuclei.

*In Golgi-studies* (Tömbol, 1967; Scheibel and Scheibel, 1966; Somogyi et al., 1973), the cell body of the small neurons is spheroïd or ovoïd in shape with few (2-4) main dendrites. The

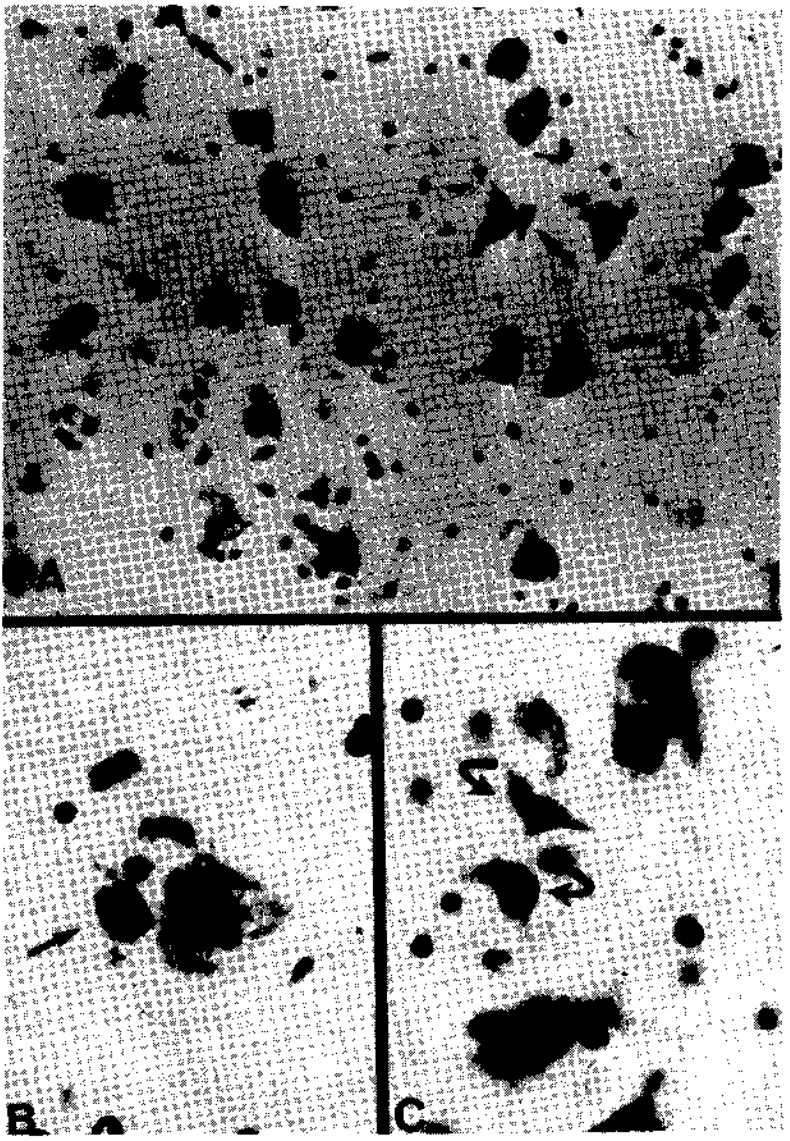



PHOTO II: *Human thalamus anterior. Microneurons indicated by arrow. Nissl stain. Magnification A 250 x; B and C 1000 x.*

course of the dendrites is slightly wavy having irregular branches and rather long spine-like processes terminating in spherical thickenings. At the apical parts of the dendrites the processes become more numerous and larger giving a bushy appearance. The dendrites of the Golgi type II cells are considerably longer (250-400 μ) than those of thalamocortical relay neurons (200 μ). The axon is thin, smooth and immediately arborizes profusely in the neighbourhood of the cell.

*Electron microscopical* description is rather limited (Tömbol, 1967; Pappas et al., 1966; Mathers, 1972): the small cell type has a dense plasma structure, with irregular endoplasmic cisterns and large, light, sparsely cristated mitochondria.
No specific synaptic relations to macroneurons are show as yet.

## 3. Role of thalamic microneurons

The small neurons of the thalamus, having specific morphological characteristics, behave differently from the 'relay' macroneurons.

It is stressed that the small neurons persist after cortical ablation. Therefore, together with the Golgi description of being short axoned, they are currently viewed as 'internuncial cells', 'Schaltzellen', 'interneurons'. Some workers have some reservations about this view because in certain instances, there is a degeneration in animals after long-standing cortical ablation (Hassler, 1964) and because no short axoned cells were observed with Golgi technique in the thalamic reticular nucleus (Scheibel, 1966).

This controversy can be explained by the following facts:
1) thalamic Golgi type II cells are found in man, primates and carnivores (cat-dog) but not in small rodents (rat-mice). Moreover, the amount in the associative thalamic nuclei of the dog is much lower than in primates and man (personal observation).

2) in some thalamic nuclei e.g. the N. Reticularis, the microneurons make out only 10 to 15 % of the total neuron population (Dewulf et al., 1969).

Indeed, the prevalence rate amongst different thalamic nuclei in man is well systematized: aspecific nuclei ± 15 %, specific nuclei ± 25 % (except C. Genicul. Lat. 5 %) and associative nuclei ± 35-45 % (Dewulf et al., 1969-1971). Almost the same distribution is found in the monkey (Dom, present study, chapter VIII).

In pathology, the microneurons sometimes react in distinct opposition to the macroneurons: some diseases seem to spare selectively microneurons (Gayet-Wernicke disease, Martin, 1974) while other disorders attack them preferentially (Huntington's Chorea: Dom et al., 1976).

Electrophysiological studies of the thalamus (Andersen and Eccles, 1962; Andersen and Sears, 1964) demonstrated cells having a pre- and postsynaptic inhibitory action. Eccles (1966) demonstrated that in the ventrobasal thalamus 24 % of the total neuron population has 'inhibitory' characteristics. It might be stressed that in the ventrobasal thalamus, we found 25 % of microneurons.

Summarizing, the microneurons seem to be 'interneurons', having a fundamental role in integration of higher nervous functions. Their inhibitory action still is to be proven.

## B. QUANTITATIVE EVALUATION OF THE THALAMIC NEURON POPULATION

The variation in size of the global thalamus and of thalamic nuclei among normal brains has been reported in most stereotactic atlasses of the brain (Talairach et al., 1957; Schaltenbrand and Bailey, 1959; von Büren and Börke, 1972).

Detailed volumetric analysis of thalamic nuclei in different mammals was presented by Hopf (1964-1965).

Cytometric studies of the thalamic neuron population, however, is rather scarce.
The quantitative studies carried out by the Vogt school around 1950 comparing thalamus in normal brains and schizophrenics are well known (Treff and Hempel, 1955, 1958, 1959, 1962; Funfgeld, 1954; Bäumer, 1954; von Buttlar-Brentano, 1954). These studies gave only numerical values without cytometry and were biased towards subjective interpretation as will be discussed in the chapter on schizophrenia (chapter VII).

von Büren and Börke (1972) made cytometric studies in different human thalamic nuclei but did not distinguish between micro- and macroneurons.

Systematic cytometric evaluation of one normal human thalamus has been performed by Dewulf and collaborators (Dewulf et al., 1969-1971; Dom et al., 1969-1973-1975; Martin, 1970; Varela, 1969). Those results for one brain had to be extended to several cases.

In the present study, the evaluation of the thalamic population of 5 normal human brains of different ages is shown. The thala-

mus of three adult monkeys was also analysed in view of experimental studies; the values obtained in the monkey will be reported in chapter VIII.

## C. PERSONAL CYTOMETRIC STUDY OF THE NORMAL HUMAN THALAMUS

The thalamus is studied in the cases $N_1$ (24 yrs), $N_2$ (30 yrs), $N_4$ (42 yrs), $N_5$ (47 yrs) and $N_7$ (62 yrs); $N_1$, $N_5$ and $N_7$ are male; $N_2$ and $N_4$ female.

The main purposes of the study are: 1) confirmation in several normal adult brains of different age groups the incidence of microneurons among thalamic regions with eventual age variability 2) to obtain reference values for the study of basal ganglia disorders.

### 1. Methodological remarks

The study of almost all individual nuclei of one thalamus - performed by Dewulf and collaborators - took several years for 6 researchworkers. It would be almost unfeasible to repeat this for a series of normal and pathological brains.

As the obtained results of the study mentioned showed that the percentage of microneurons for the subnuclei of a given thalamic formation was uniform (e.g. lateral group 25 %, anterior 30-35 %, medial 40-45 %, posterior 35-40 %, intralaminar, reticular, paramedian $\pm$ 15 %) and because the aim of the present study primarily is concerned with microneuron incidence, it was decided to perform cytometric counts for all brains (normal + pathological) in one main nucleus of the four best delineated thalamic formations: anterior, medial, lateral and posterior.

For the Formatio Anterior, counting and sizing was performed in the middle third of the Nucleus Antero principalis.
For the Formatio Medialis, the middle third of the Nucleus Mediocaudalis was chosen.
For the Formatio Posterior, the middle third of the Nucleus Pulvinaris medialis was studied.
For the huge Formatio Lateralis, counts of dorsal and ventral regions were assembled in the Nucleus Dorsalis Intermedius and the Nucleus Ventralis Oralis.

Within every single region for each brain at least 300 nerve cells were sized. Sampling was done as described in chapter II: drawing out the region and adjusting the microscope to random points on the scheme.

## 2. Comparison of left and right hemisphere

In all cases the left hemisphere was evaluated and it seemed worthwile to check eventual discrepancies between left and right thalamus.

In this way, for one case ($N_5$) bilateral counting and sizing was performed.
The next table shows the numerical values.

| | | NUMERICAL DENSITY | | AVERAGE Ø in micra | | % MICRO |
|---|---|---|---|---|---|---|
| | | macro | micro | macro | micro | |
| Thalamus Anterior | L | 6.930 | 3.614 | 17.34 | 8.23 | 34.29 |
| | R | 7.270 | 3.209 | 16.69 | 8.30 | 30.65 |
| Thalamus Medialis | L | 5.595 | 4.331 | 15.82 | 7.8 | 43.53 |
| | R | 5.801 | 4.352 | 15.37 | 7.76 | 42.86 |
| Thalamus Lateralis | L | 3.550 | 1.214 | 17.25 | 8.62 | 25.50 |
| | R | 3.486 | 1.257 | 17.22 | 8.80 | 26.58 |
| Thalamus Posterior | L | 6.656 | 3.976 | 16.57 | 8.58 | 37.40 |
| | R | 6.568 | 3.337 | 16.20 | 8.76 | 33.70 |

From those data, it is apparent that there is no significant difference in numerical densities; thus also the percentage of microneurons is quite comparable.
The average diameter of microneurons and the distribution are ± equal bilaterally.
The only difference noted is visualized in plate VIII displaying cumulative frequency distributions of macroneurons with statistical analysis by Kolmogorov-Smirnov test: *the macroneurons in the left hemisphere are always slightly larger than on the right side, except for the thalamus lateralis.*

## 3. Evolution with age in the adult human thalamus

As will be shown by the tables and the plates below, there are only slight changes due to age in the cytometric values obtained.

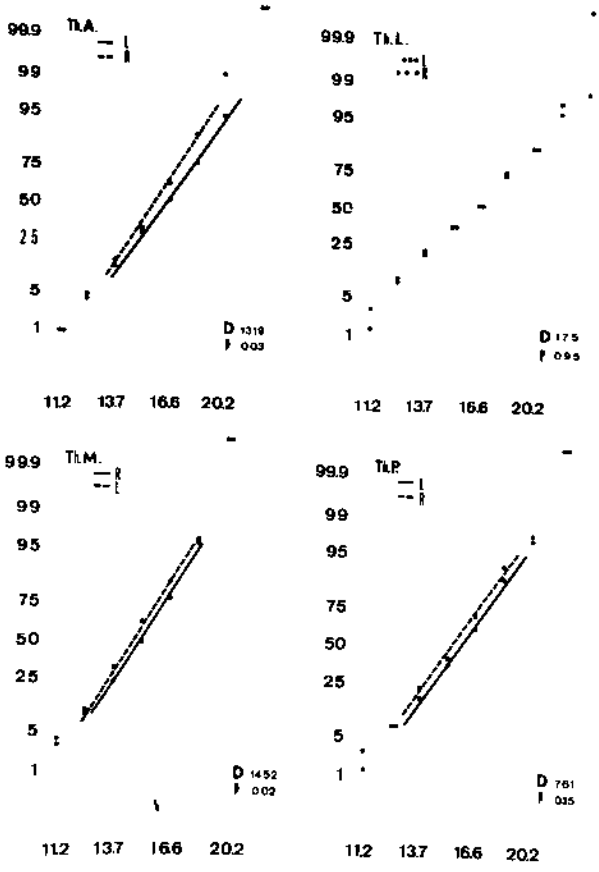



PLATE VIII: *Cumulative frequency distribution of macroneurons in the four thalamic regions, left compared to right. In ordinate %, in abscise Ø in micra.* $D = D_{max}$, *p = probability according to Kolmogorov-Smirnov analysis*

The minimal tendency towards decrease of diameter with increasing age is much less obvious than in the striatum.
However, in view of forming age groups as comparable as possible with our pathological material, an average distribution was calculculated for the brains from 20 yrs to 45 yrs (to be compared with the schizophrenics) and for the brains from 40 yrs to 65 yrs (to be compared with Parkinson and Huntington brains). In this way, the brain $N_4$ (42 yrs) was examined in both groups.

*a. Thalamus Anterior*

Plate IX shows the percentage distribution for all cases and the average distribution for both age groups.
The next tables give the results of numerical densities (number/$mm^3$), average Ø in micra and the percentage of microneurons.

| TH.A. 20yrs-40yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_1$ | 6.177 | 3.522 | 17.36 | 8.44 | 36.30 |
| $N_2$ | 6.792 | 4.260 | 18.08 | 9.14 | 38.54 |
| $N_4$ | 6.866 | 3.145 | 17.26 | 8.73 | 31.44 |
| AVERAGE | 6.612 | 3.642 | 17.57 | 8.79 | 35.54 |

| TH.A. 40yrs-65yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_4$ | 6.866 | 3.145 | 17.26 | 8.73 | 31.44 |
| $N_5$ | 6.930 | 3.614 | 17.34 | 8.23 | 34.29 |
| $N_7$ | 7.100 | 3.216 | 17.90 | 8.32 | 31.20 |
| AVERAGE | 6.965 | 3.325 | 17.52 | 8.44 | 32.11 |

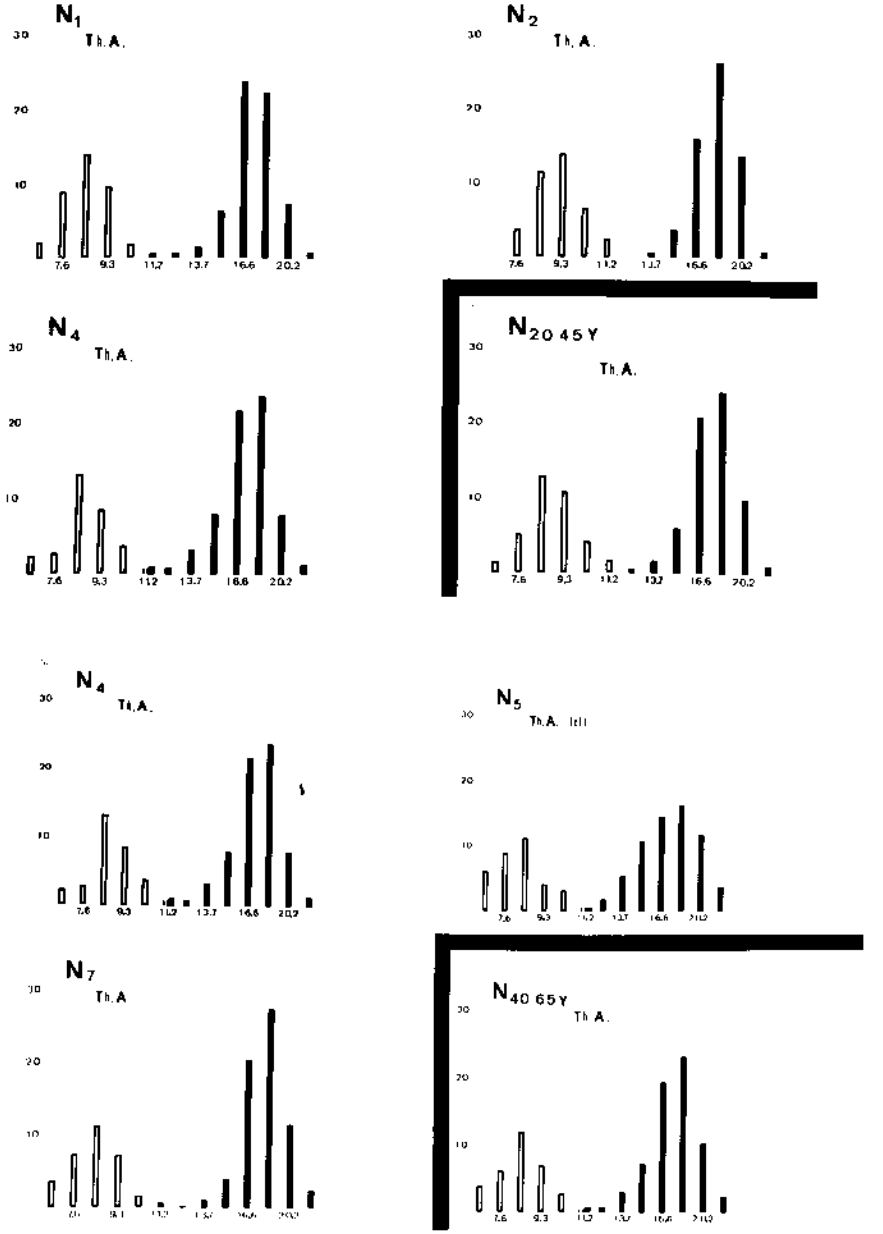



PLATE IX: *Micro- (white bars) and macroneuron (black bars) distribution in the normal anterior thalamus at different ages, and average distribution for young (20-45yrs) and old (40-65yrs) normals*

*b. Thalamus Medialis*

Plate X shows the percentage distribution for both age groups.

In the next tables, the numerical results are summarized for both age groups.

| TH.M. ~~40yrs-65yrs~~ | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_1$ | 5.538 | 4.544 | 15.69 | 8.88 | 45 |
| $N_2$ | 5.893 | 5.751 | 17.89 | 9.08 | 49.4 |
| $N_4$ | 5.467 | 4.686 | 16.46 | 8.87 | 44.6 |
| AVERAGE | 5.633 | 4.993 | 16.71 | 8.96 | 46.52 |

| TH.M. 40yrs-65yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_4$ | 5.467 | 4.686 | 16.46 | 8.87 | 44.60 |
| $N_5$ | 5.595 | 4.331 | 15.82 | 7.8 | 43.53 |
| $N_7$ | 5.869 | 4.165 | 16.59 | 8.78 | 41.50 |
| AVERAGE | 5.643 | 4.394 | 16.31 | 8.52 | 43.19 |

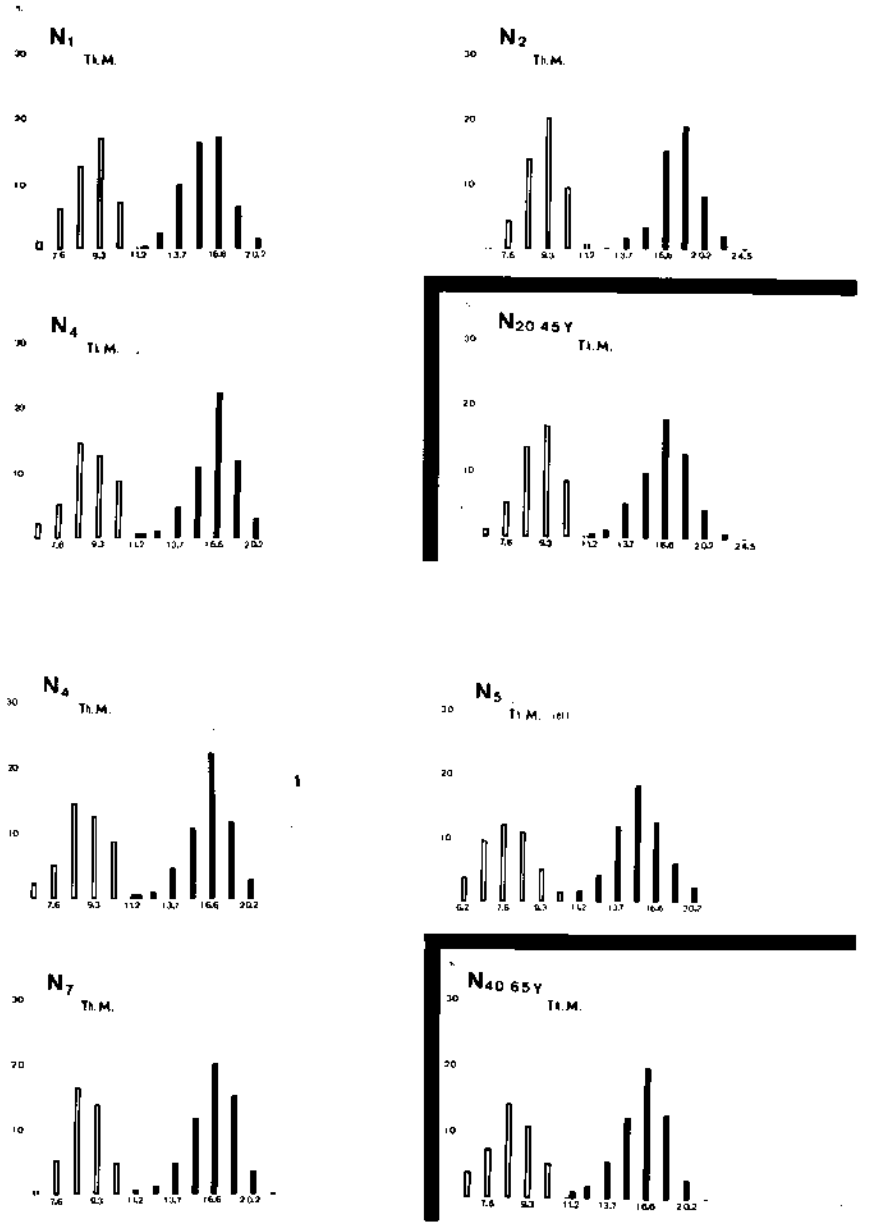



PLATE X: *Micro- (white bars) and macroneuron (black bars) distribution in the normal medial thalamus at different ages, and average distribution for young (20-45yrs) and old (40-65yrs) normals*

*c. Thalamus Lateralis*

Plate XI depicts the neuronal percentage distribution for all cases.

The next tables summarize the numerical characteristics of the two thalamic neuron populations.

| TH.L. 20yrs-45yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø in micra macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_1$ | 3.248 | 1.012 | 17.86 | 8.74 | 23.75 |
| $N_2$ | 3.550 | 1.171 | 17.77 | 8.65 | 24.77 |
| $N_4$ | 3.106 | 1.101 | 18.35 | 8.80 | 26.2 |
| AVERAGE | 3.301 | 1.095 | 17.89 | 8.72 | 24.86 |

| TH.L. 40yrs-65yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø in micra macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_4$ | 3.106 | 1.101 | 18.35 | 8.80 | 26.2 |
| $N_6$ | 3.550 | 1.214 | 17.25 | 8.62 | 25.5 |
| $N_7$ | 3.621 | 1.367 | 17.98 | 8.75 | 27.4 |
| AVERAGE | 3.426 | 1.227 | 17.80 | 8.72 | 26.34 |

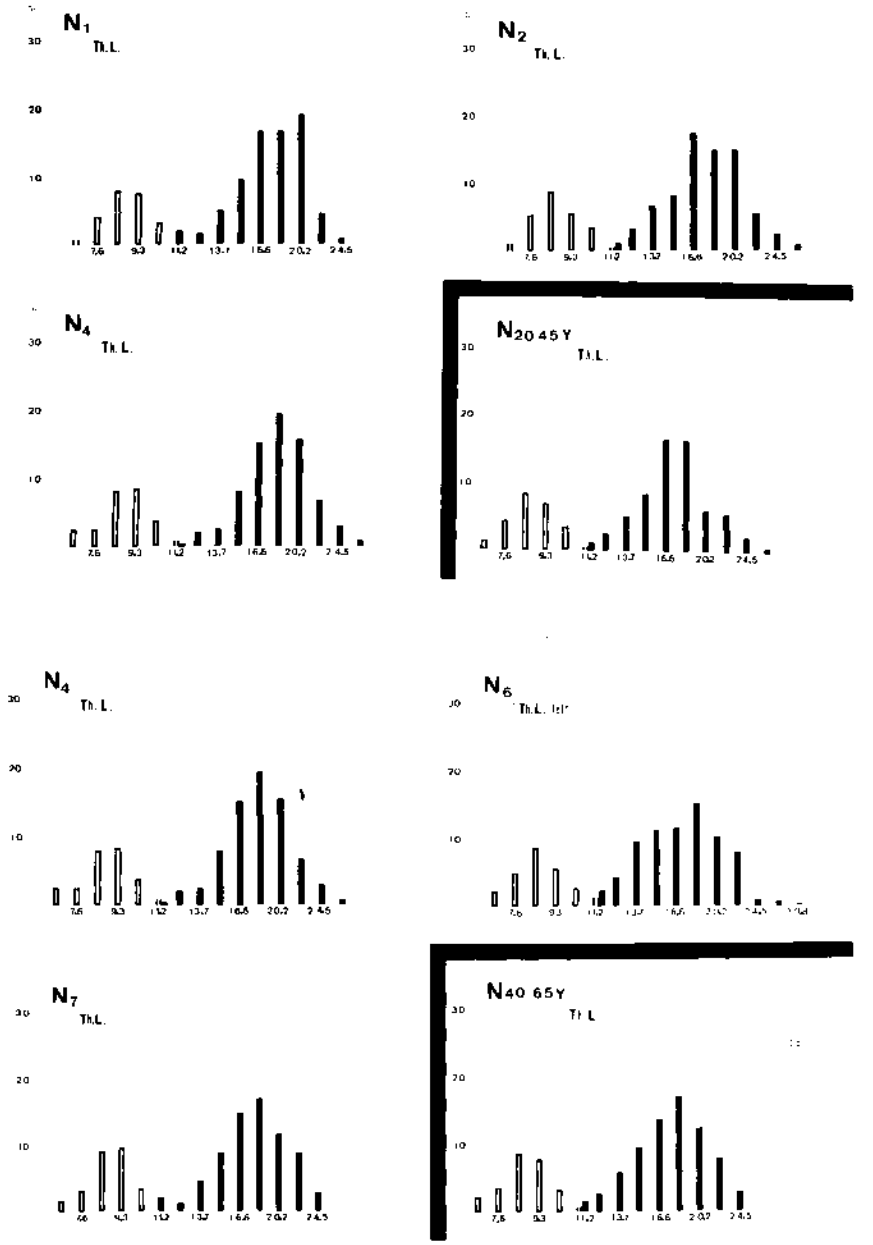



PLATE XI: *Micro- (white bars) and macroneuron (black bars) distribution in the normal lateral thalamus at different ages, and average distribution for young (20-45yrs) and old (40-65yrs) normals*

*d. Thalamus Posterior*

Plate XII shows the percentage neuronal distribution for both age groups.

In the following tables, the numerical densities and average diameters are given for the different cases in both groups.

| TH.P. 20yrs-45yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø in micra macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_1$ | 7.618 | 4.686 | 15.52 | 8.39 | 38.08 |
| $N_2$ | 4.934 | 3.905 | 17.27 | 8.9 | 44.17 |
| $N_4$ | 6.440 | 4.047 | 17.19 | 8.34 | 38.6 |
| AVERAGE | 6.331 | 4.213 | 16.47 | 8.49 | 39.52 |

| TH.P. 40yrs-65yrs | NUMERICAL DENSITY macro | micro | AVERAGE Ø in micra macro | micro | % MICRO |
|---|---|---|---|---|---|
| $N_4$ | 6.440 | 4.047 | 17.19 | 8.34 | 38.6 |
| $N_5$ | 6.656 | 3.976 | 16.57 | 8.58 | 37.4 |
| $N_7$ | 6.766 | 3.642 | 16.37 | 8.50 | 35 |
| AVERAGE | 6.621 | 3.888 | 16.69 | 8.48 | 37.04 |

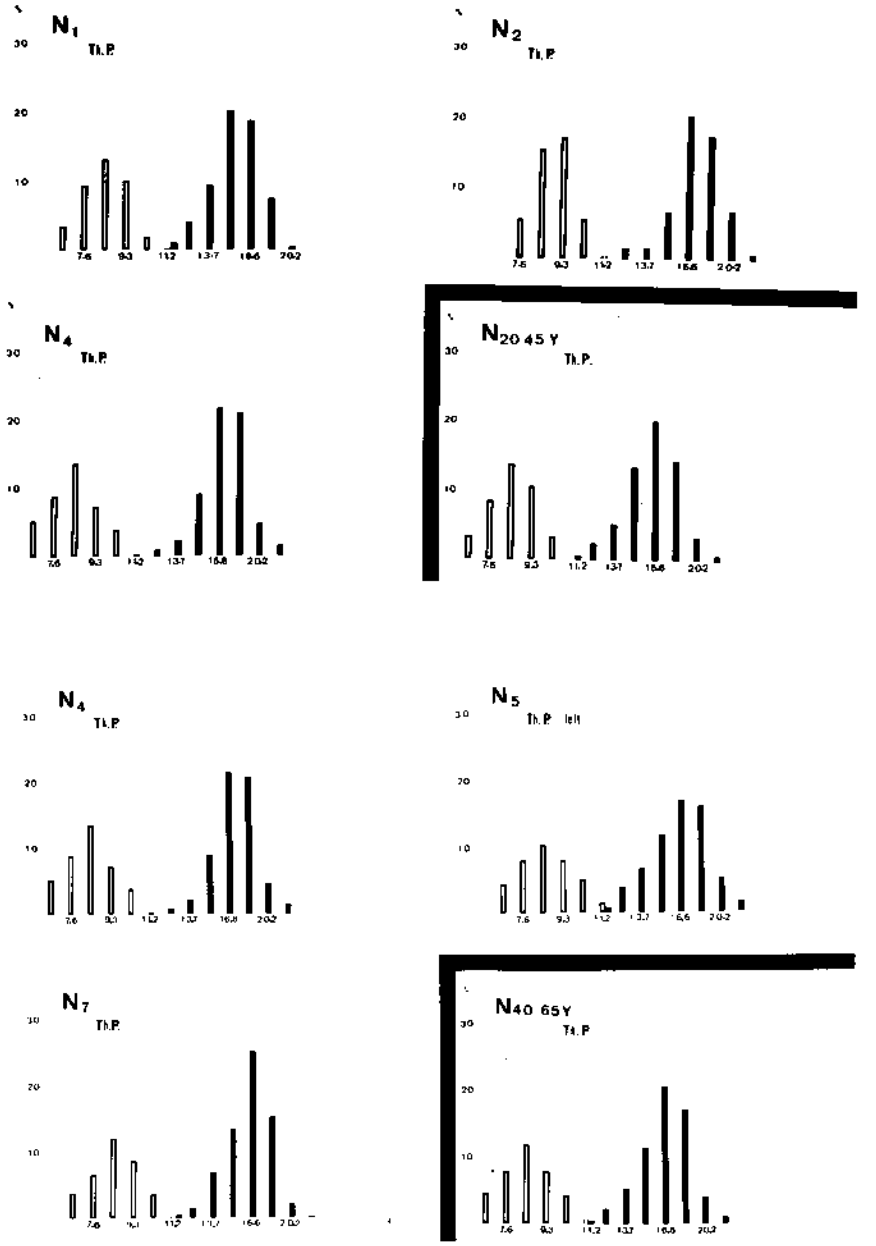



PLATE XII: *Micro- (white bars) and macroneuron (black bars) distribution in the normal posterior thalamus at different ages, and average distribution for young (20-45yrs) and old (40-65yrs) normals*

## 4. Summary of cytometric values in the human thalamus

1. The thalamic neuron population in the normal adult human brain is composed of *two distinct populations:* the macro- and the microneurons.

   a) *the macroneurons*: - in the thalamus anterior (N. Anteroprincipalis) have an average diameter of ± 17.5 micron and are rather densely arranged (± 6.750 neurons/$mm^3$)
   - in the thalamus medialis (N. Mediocaudalis) have an average diameter of ± 16.5 micron and their density is a little less (± 5.600 neurons/$mm^3$)
   - in the thalamus lateralis (N. Vent.Or.+ Dors.Im.) have an average diameter of ± 17.85 micron and are loosely arranged throughout the nucleus (± 3.350/$mm^3$)
   - in the thalamus posterior (N.Pulv.Med.) have an average diameter of ± 16.5 micron and a density between that of thalamus anterior and medialis (± 6.450/$mm^3$)

   b) *the microneurons*: have an almost equal size in all thalamic regions: the average diameter is ± 8.65 micron. Their incidence in the thalamus anterior is 30-35 % (± 3.450/$mm^3$), in the thalamus medialis 40-50 % (± 4.650/$mm^3$), in thalamus lateralis ± 25 % (± 1.150/$mm^3$) and in thalamus posterior 35-40 % (± 4.000/$mm^3$).

2. Both micro- and macroneurons in the thalamus do not change much between the age of 20 yrs to 65 yrs: their size tends to decrease a little (statistically insignificant) and it is possible that the percentage of microneurons also decreases slightly.

3. The macroneurons are slightly larger in the left than in the right thalamus.
   The microneurons are bilaterally equal in size.

## Chapter V

## HUNTINGTON'S CHOREA

Hereditary chorea is a disease with a dominant trait characterized by its most striking feature, the involuntary 'choreic' movements. Although known since 1842 (Dunglison, 1842), it was named after the 'descriptio princeps' of George Huntington in 1872. Ever since, hundreds of scientific papers have been devoted to this incapacitating disease (Centennial Bibliography, Bruyn et al., 1974).

Nevertheless, the cause of the disease is as yet unknown. The proven decrease in G.A.D. (Bird, 1973) and GABA (Perry, 1973) in this disorder might lead to better understanding and eventual therapy.

### A. NEUROPATHOLOGICAL STUDIES

Neuropathological studies are numerous and well documented. Although until ca. 1910 some authors mention the lesions of the caudatum in choreic brains (Harbinson, 1880), most reports favour 'a chronic disseminated encephalitis'. Anglade (1906), Jelgersma (1908), but mostly Vogt and Vogt (1920) and Bielschowsky (1922) in their brilliant studies on the striate system stressed the gross atrophy of the caudatum and the putamen to be always present in chorea disease (Photo 3).

The contemporary neuropathological spectrum of Huntington's Chorea might be summarized as follows: beside nerve cell loss in cerebral and cerebellar cortex in longstanding illness, the main lesions are confined to the 'basal ganglia': extreme loss of Golgi type II cells in the neostriatum (Photo 4), variable neuron loss and gliosis in surrounding nuclei as pallidum, locus niger, thalamus, n. subthalamicus.

### B. QUANTITATIVE CYTOMETRY OF BASAL GANGLIA IN HUNTINGTON'S CHOREA

— In order to get a more exact idea of the *neostriatal nerve cell loss*, quantitative studies were performed. In 1927, Dunlap

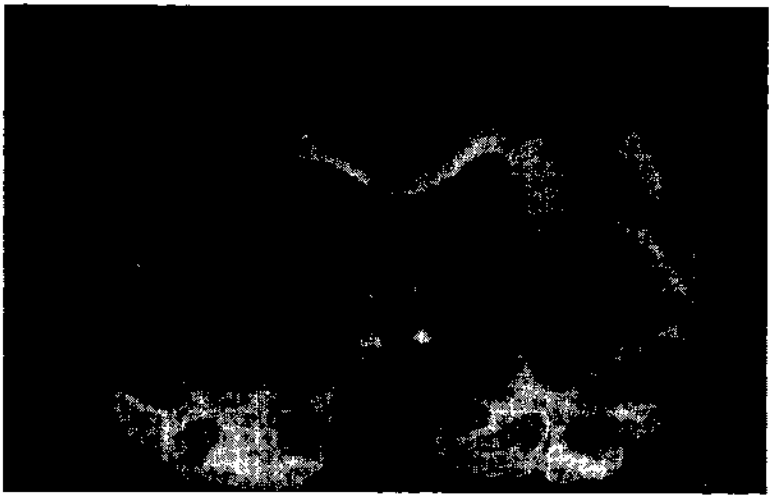


PHOTO III: *Coronal section through the brain of Huntington's Chorea. Note the atrophy of the caudatum and the ventricular dilatation.*

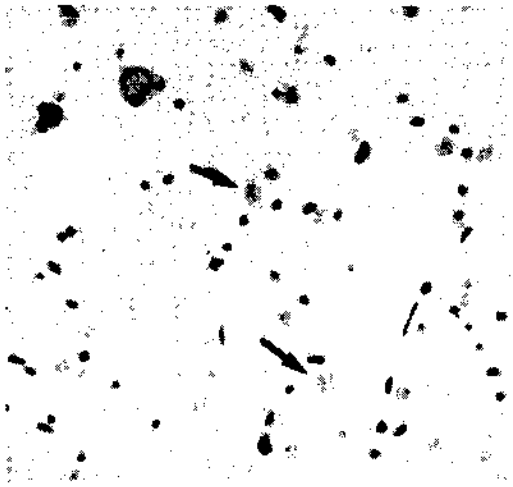


PHOTO IV: *Neostriatum in Huntington's Chorea. Note atrophy of microneurons (arrows). Nissl stain. Magnification 310 x.*

found a decrease of nerve cells from 60/field in the normal brain to 15/field in chorea brains.

von Santhà (1931) studied one chorea brain and reported a 90 % loss in caudatum and a 40 % loss in putamen. Moreover, he states the macroneurons to be spared, a thesis already formulated by Hunt (1971) and confirmed by Hallervorden (1957). The latter author insisted on a more pronounced loss in caudatum than in putamen. Lange and Thörner (1974), however, found by volume estimations of caudatum and putamen in chorea always the same proportional atrophy.

McCaughey (1961) obtained a decrease of neostriatal small neurons from 30/unit in normals to 6/unit in chorea.

Schröder (1970) made extensive cytometric studies in the neostriatum and reported a small neuron loss of 70 %.

Dom et al. (1972-1973) evaluated the putamen in different forms of chorea and reported that the small neuron loss in subchorea was ± 20 %, in prechorea + 65 %, in akinetic adult chorea ± 70 %, in choreiform adult chorea ± 80 % and in one case of juvenile rigid chorea + 90 %. The macroneurons in all forms of chorea appeared to be spared except in the case of juvenile rigid chorea.

- The nerve cell loss of *pallidum* in qualitative neuropathological studies is controversial: although most authors (Margulies, 1914; Spielmeyer, 1926; Hallervorden, 1957; Burch et al., 1968) find a definite loss of neurons, some incline to the interpretation that this neuron loss is only secondary to the neostriatal cell atrophy.

  Quantitative studies, apart from the semi-quantitative data of Dunlap (1927) indicating a loss from 5/unit to 3/unit, were performed by the collaborators of Hopf: Lange and Thörner (1974) found a neuron loss of 45 %. By those authors this loss is considerated a primary lesion rather than a transneuronal atrophy.

- For the *Nucleus Subthalamicus*, nerve cell loss is repeatedly described (Alzheimer, 1911; Vogt, 1920; Casper, 1930; Marburg, 1949). Quantitative data are only available from the study of Lange and Thörner (1974): 25 % of neurons are lost.

- *The thalamus* in Huntington's Chorea is rather incompletely examined - as in many disorders because of its complicated structure -. The qualitative pathological studies provide only vague and inconsistent descriptions: the macroneurons in all thalamic regions are sometimes reported to be a little shrunken

or loaded with lipofuscin. Microneurons are only mentioned in the study of the centrum medianum by Martin (1970): the microneurons are much more atrophied than the macroneurons. Quantitative data about the lateral thalamus in Huntington's Chorea are reported in our study on 7 cases (Dom et al., 1976): the microneuron loss is more than 50 % while the macroneurons are only slightly shrunken without definite loss.

## C. PERSONAL STUDY OF NEOSTRIATUM AND THALAMUS IN HUNTINGTON'S CHOREA

- In 1972 a systematic study of the putamen in different forms of Huntington's Chorea was performed (Dom et al., 1973). In that study, the numerical densities, the L/S ratio and the average diameter of the microneurons in the putamen were determined in 4 normal brains, 3 brains of choreiform adult chorea, 2 brains of akinetic adult chorea, 2 brains of subchorea, 1 brain of prechorea and 1 brain of juvenile rigid chorea (Westphall variant). Detailed description of the analysis may be consulted in the previously cited report. To summarize, the following results were achieved:
  1) loss of microneurons: in choreiform adult chorea: $\pm$ 80%
     in akinetic adult chorea : $\pm$ 70%
     in subchorea : $\pm$ 20%
     in prechorea : $\pm$ 65%
     in juvenile rigid chorea : $\pm$ 90%
  2) macroneurons: appeared unaffected except in the juvenile rigid chorea brain
  3) average diameter of surviving microneurons decreased in subchorea, prechorea and akinetic adult chorea but surprisingly increased in choreiform adult chorea and juvenile rigid chorea.

- In 1976, the ventrolateral thalamus in 7 brains of adult choreiform choreics was cytometrically studied (Dom et al., 1976). Summarizing, the microneurons were lost rather selectively ($\pm$ 50%) while the macroneurons showed a slight decrease in diameter without significant loss. The average diameter of microneurons was definitely decreased. More details can be found in the previous report.

- At present, the cytometric studies were performed in caudatum and thalamus anterior, medialis and posterior in 7 brains of choreiform adult choreics.

1. Clinical case histories

- Case I: Mrs. Ti... Be... H: °1913 - $^{+}$1957:

Her mother suffered from chorea. Mrs. Ti... completed primary school and highschool education. Married in 1939 and had two children. In 1945, she became depressed, bizarre and sometimes violent. In 1946, first admission to a psychiatric hospital. No neurological signs were observed and diagnosis of anxiety-depression was made. In 1949, first signs of involuntary movements in all 4 limbs and tongue. In 1951, admission to psychiatric hospital because of mental deterioration and irritability. Choreatic movements increased gradually. In 1957, restlessness, fever and coma preceded death.

- Case II: Mrs. Pl... J: °1913 - $^{+}$1960:

Her father and 2 sisters had chorea. Mrs. P... showed bilateral congenital hipdislocation and a talipes equinus on the left side. She completed primary school education and thereafter worked as a servant. Progressively, she became schizoid and had to be admitted to a psychiatric hospital because of attempted suicide in 1949. Back home in 1950, she lived an isolated life and began to talk to herself. A second hospital admission in 1951 was made necessary because of restlessness, bizarre feelings and dysphagia. In 1952, mental deterioration and choreic movements firstly appeared. She died in cachexia in 1960.

- Case III: Mrs. Ve...Dij...A: °1900 - $^{+}$1958:

Her father and 1 sister had chorea. Up to the age of 40 yrs, no abnormalities were noted. In 1940, she became restless, irritable and acquired the first involuntary movements. In 1948, because of a suicide attempt, she was admitted to the psychiatric hospital. Gradual mental deterioration and increase of choreic movements appeared. In 1956, she became more and more difficult, irritable and disoriented. Therefore, in 1958 a serpasil treatment was started: patient died suddenly.

- Case IV: Mr. Rij...D: °1906 - $^{+}$1964:

His mother, 1 brother and 2 sisters had chorea. Mr. R... completed primary school and afterwards worked at home. In 1934, he became gradually catatonic, stuporous, negativistic, autistic and in 1938 he was admitted to the psychiatric hospital with diagnosis of schizophrenia. From 1940 to 1957, he lived withdrawn at home. In 1957, choreatic movements appeared and also dysarthria. He was admitted to the hospital where he progressively deteriorated. He died in cachexia in 1964.

- Case V: Mr. T...J: °1894 - $^{+}$1956:

His mother and 4 sisters were choreic. Mr. R... was a skilled labourer and a good husband until 1950. Then he presented choreatic movements and became severely depressed. He became alcoholic. In 1956, he was admitted to a psychiatric hospital in poor condition: dehydration, cachexia. Three weeks later he died.

- Case VI: Mr. H...C: °1906 - $^{+}$1963:

His father and 1 brother were choreic. Around the age of 20yrs, Mr. H... became socially unadaptable: alcoholism, fighting, lying. In 1952, he was admitted to a psychiatric hospital with the diagnosis 'psychopathy'. In 1955, he had problems with equilibrium and occasional choreatic movements. In 1959, he mentally deteriorated and chorea became more and more pronounced. He died in cachexia because of dysphagia.

- Case VII: Mr. V...J: °1892 - $^{+}$1964:

His mother had Huntington's Chorea. After primary school, he became labourer. He married in 1923. In 1939, he became choreic and experienced epileptic seizures. In 1958, he had to be admitted to a psychiatric hospital due to severe chorea, with dysarthria and memory disturbances. He still had epileptic seizures. Since 1960, he deteriorated progressively and he died cachectic in 1964.

## 2. Cytometric results

Plates XIII, XIV, XV, XVI, XVII, XVIII and XIX demonstrate for each case the percentual neuronal distribution among cell size (in micra) in Caudatum (Ca) and Thalamus Anterior (Th.A.), Medialis (Th.M.) and Posterior (Th.P.). The caudatum was measured in all cases. The thalamus was not evaluated in case IV and VI. In case VII, only the medial and posterior thalamus could be worked out.

### *a. Nucleus Caudatus in Huntington's Chorea*

Cytometric evaluation was performed in the middle third of the caudatum.

The next table gives the numerical results (numerical densities, large-small ratio, and average Ø microneurons) for each case as well as the average for the 7 cases.

# CASE 1

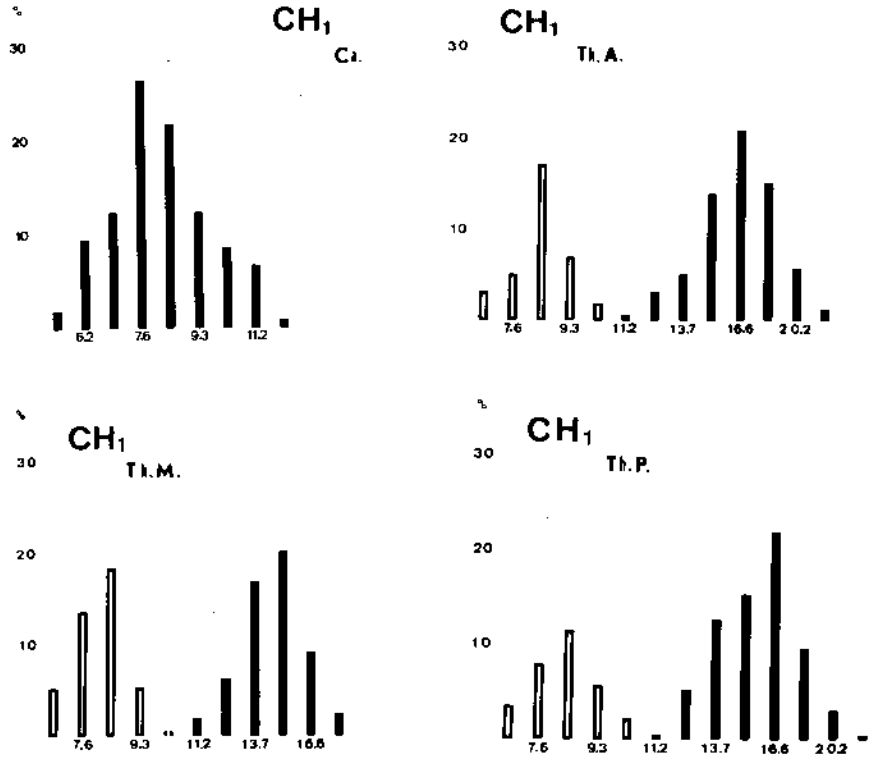



PLATE XIII: *Microneuron distribution in caudatum (Ca - black bars) and micro- (white bars) and macroneuron (black bars) distribution in the thalamus anterior (Th.A.), medialis (Th.M.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

# CASE 2

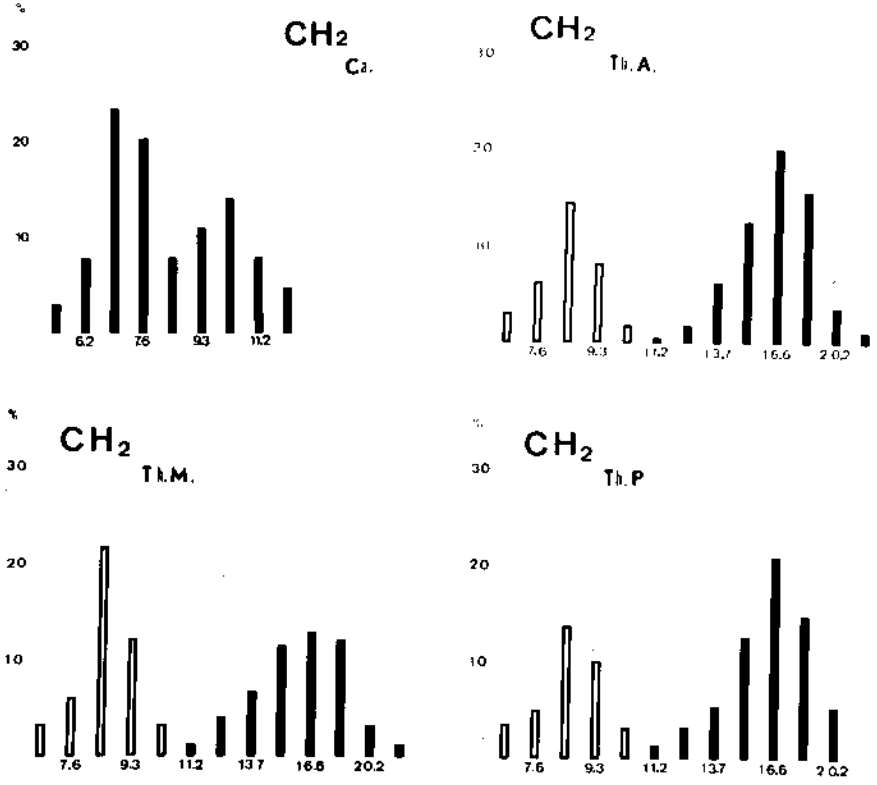



PLATE XIV: *Microneuron distribution in caudatum (Ca - black bars) and micro- (white bars) and macroneuron (black bars) distribution in the thalamus anterior (Th.A.), medialis (Th.M.) and posterior (Th.P.). In ordinate %, in abscis Ø in micra*

# CASE 3

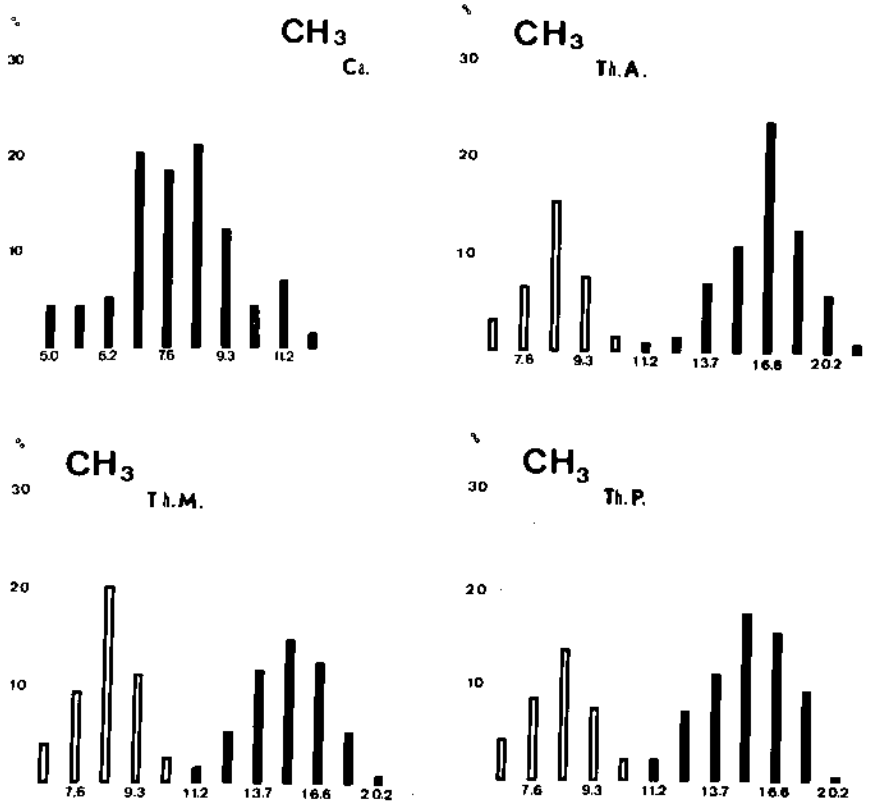



PLATE XV: *Microneuron distribution in caudatum (Ca - black bars) and micro- (white bars) and macroneuron (black bars) distribution in the thalamus anterior (Th.A.), medialis (Th.M.) and posterior (Th.P.). In ordinate %, in abscissa Ø in micra*

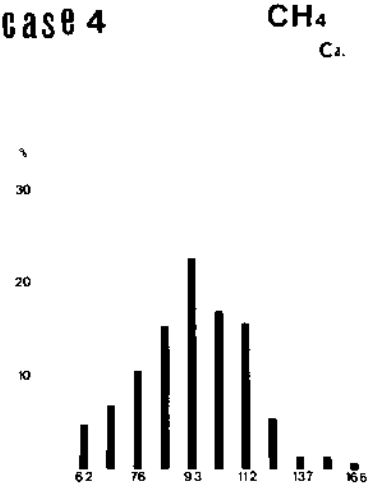



PLATE XVI: *Microneuron distribution in the caudatum. In ordinate %, in abscis Ø in micra*

# CASE 5

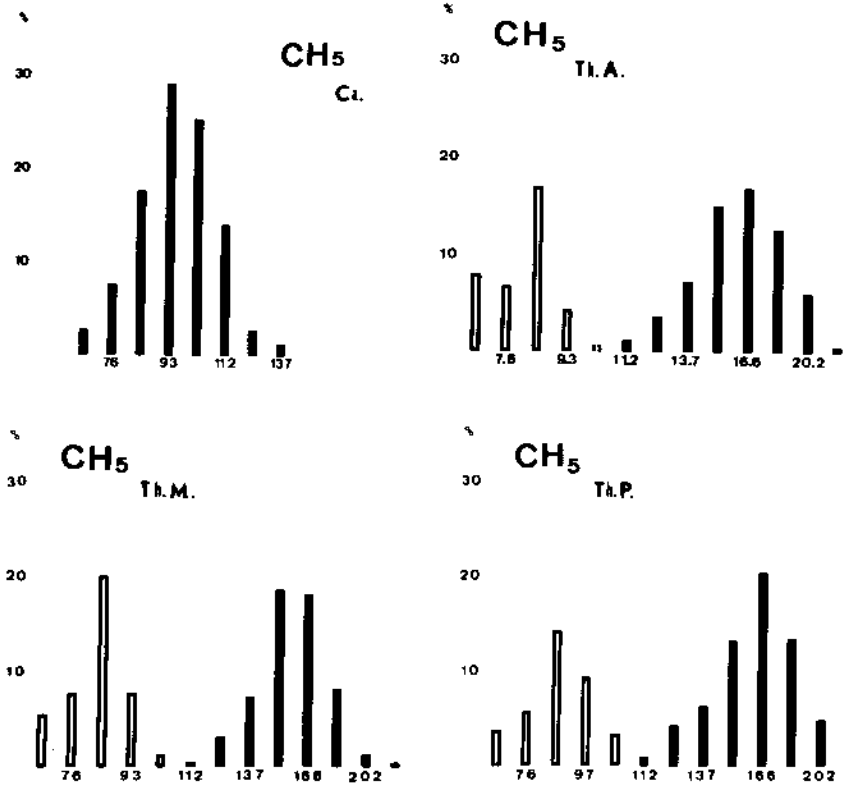



PLATE XVII: *Microneuron distribution in caudatum (Ca - black bars) and micro- (white bars) and macroneuron (black bars) distribution in the thalamus anterior (Th.A.), medialis (Th.M.) and posterior (Th.P.). In ordinate %, in abscis Ø in micra*

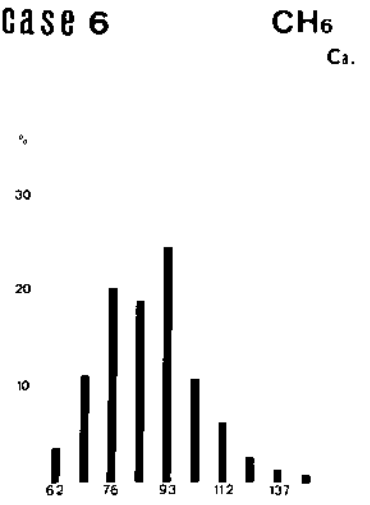



PLATE XVIII: *Microneuron distribution in the caudatum. In ordinate %, in abscise Ø in micra*

# CASE 7

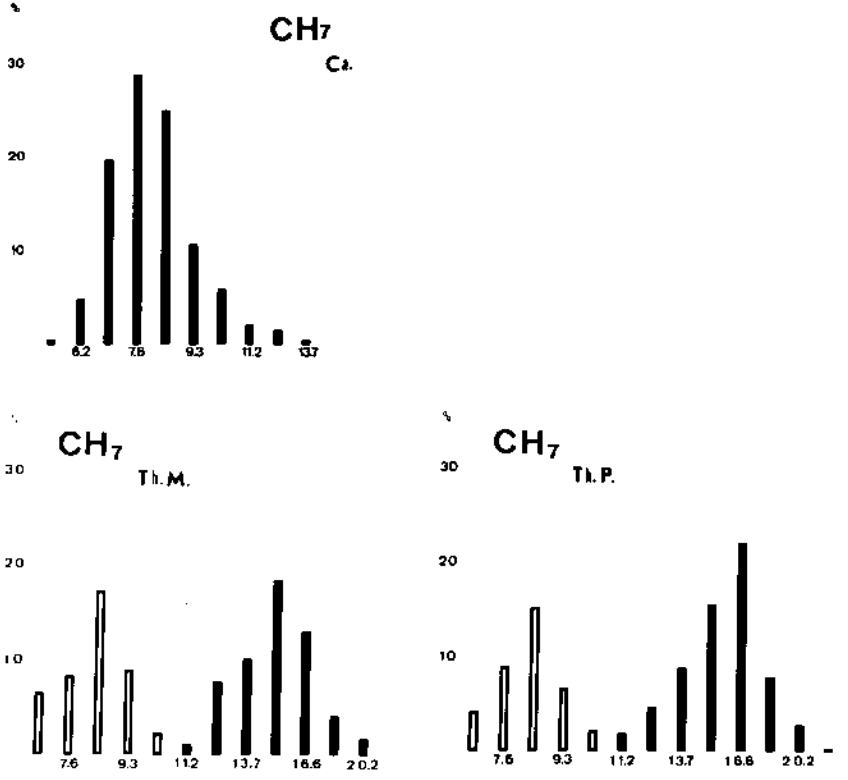



PLATE XIX: *Microneuron distribution (black bars) in caudatum (Ca) and micro- (white bars) and macroneuron distribution in thalamus medialis (Th.M.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

| CAUDATUM C.H. | NUMERICAL DENSITY macro | micro | L/S RATIO | AVERAGE micro |
|---|---|---|---|---|
| $CH_1$ | 92 | 5.013 | $\frac{1}{53}$ | 8.22 |
| $CH_2$ | 0 | 2.066 | 0 | 8.36 |
| $CH_3$ | 28 | 3.209 | $\frac{1}{113}$ | 7.99 |
| $CH_4$ | 114 | 8.816 | $\frac{1}{75}$ | 9.47 |
| $CH_5$ | 35.5 | 11.750 | $\frac{1}{331}$ | 9.58 |
| $CH_6$ | 43 | 4.608 | $\frac{1}{114}$ | 8.80 |
| $CH_7$ | 21 | 3.791 | $\frac{1}{187}$ | 8.09 |
| AVERAGE | 48 | 5.608 | $\frac{1}{118}$ | 8.91 |

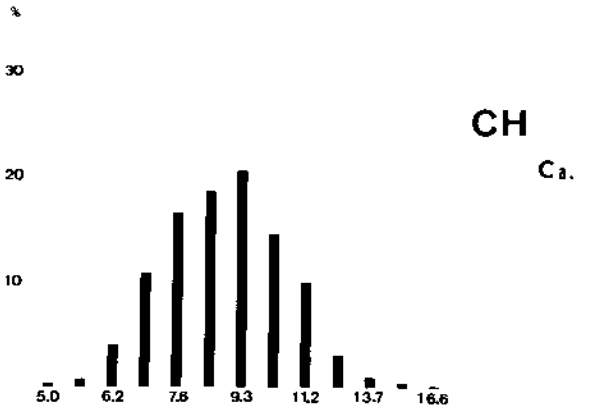
Comparing these average results with the results obtained in the caudatum of normals brains $N_{45yrs-65yrs}$ (chapter III):

a- the microneuron loss is $\pm$ 75 % ($22.878/mm^3 \longrightarrow 5.608/mm^3$). This is quite comparable to the loss found in the putamen: $\pm$ 80 % (Dom et al., 1973).

b- the macroneuron population seems to be damaged too: ($115/mm^3 \longrightarrow 48/mm^3$). Applying the Mann-Whitney U-test, this loss is hardly significant ($U=1$; $p < 0.02$) but taking into account that tissue atrophy should produce an increase, maybe it denotes anyhow a slight loss of macroneurons.
In the putamen this loss of macroneurons was not found.

c- the average diameter of microneurons is 8.91 micron. Compared with the diameter found in normal brains 40-65 yrs, 9.43 micron, there is no significant change ($U=4$; $p < 0.1$). In the putamen, an increase in diameter was found in choreiform chorea: 9.16 $\mu$ instead of 8.72 $\mu$ in normals.

d- the percentage distribution is statistically (Kolmogorov-Smirnov $D_{max} = 24.9$, $p < 0.001$) different from the normal population (Plate XX): the larger cells of the population are better preserved than the smaller ones.




*Average microneuron distribution in caudatum of Huntington brains*

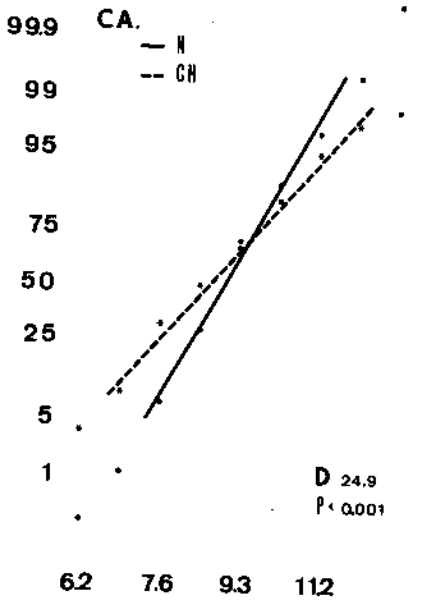



PLATE XX: *Cumulative frequency distribution of microneurons in caudatum of Huntington brains (----) compared to normal (——). In ordinate %, in abscis Ø in micra*

*b. Thalamus in Huntington's Chorea*

The study of the lateral thalamus in all 7 cases was published earlier (Dom et al., 1976).

The Anterior Thalamus (n. Anterior Principalis) is studied in $CH_1$, $CH_2$, $CH_3$ and $CH_5$.
The Medial Thalamus (n. Mediocaudalis) is analyzed in $CH_1$, $CH_2$, $CH_3$, $CH_5$ and $CH_7$.
The Posterior Thalamus (n. Pulv. Med.) is examined in $CH_1$, $CH_2$, $CH_3$, $CH_5$ and $CH_7$.

1° Thalamus Anterior

The numerical values are summarized in the next table:

| TH.A. C.H. | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $CH_1$ | 7.384 | 3.834 | 16.62 | 8.42 | 34.18 |
| $CH_2$ | 7.313 | 3.834 | 16.58 | 8.42 | 34.39 |
| $CH_3$ | 8.307 | 4.473 | 16.59 | 8.38 | 35.00 |
| $CH_5$ | 7.455 | 4.260 | 16.31 | 8.04 | 36.36 |
| AVERAGE | 7.615 | 4.100 | 16.53 | 8.31 | 34.98 |

Considering the values of the normal population 40yrs-65yrs (chapter IV) the micro- and macroneuron density in Huntington's Chorea is increased, but the ratio micro/macro is unchanged so that the microneurons still make out $\pm$ 35 % of the total population.
The average diameter of microneurons is unchanged while the average Ø of macroneurons is slightly decreased:
17.52 $\mu$ (normal) $\longrightarrow$ 16.53 $\mu$ (chorea).

2° Thalamus Medialis

The numerical values are given in the following table:

| TH.M. C.H. | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $CH_1$ | 8.378 | 6.390 | 14.63 | 8.07 | 43.27 |
| $CH_2$ | 5.609 | 4.899 | 16.21 | 8.54 | 46.62 |
| $CH_3$ | 8.307 | 7.526 | 15.15 | 8.42 | 47.53 |
| $CH_5$ | 9.017 | 6.603 | 15.85 | 8.27 | 42.27 |
| $CH_7$ | 6.745 | 5.183 | 15.25 | 8.30 | 43.45 |
| AVERAGE | 7.611 | 6.120 | 15.36 | 8.31 | 44.63 |

Considering the values of the normal population 40yrs-65yrs (chapter IV): - the densities of micro- and macroneurons are increased but the ratio micro/macro is unchanged: 44.6 % are microneurons

- the average diameter of microneurons is unchanged. The diameter of macroneurons increases slightly.

3° Thalamus Posterior

The numerical values are summed up in the next table:

| TH.P. C.H. | NUMERICAL DENSITY macro | micro | AVERAGE Ø macro | micro | % MICRO |
|---|---|---|---|---|---|
| $CH_1$ | 5.574 | 2.485 | 15.81 | 8.31 | 30.84 |
| $CH_2$ | 6.284 | 3.515 | 16.43 | 8.54 | 35.87 |
| $CH_3$ | 6.887 | 3.905 | 15.80 | 8.32 | 36.18 |
| $CH_5$ | 6.852 | 3.870 | 16.28 | 8.51 | 36.09 |
| $CH_7$ | 7.100 | 4.154 | 15.83 | 8.3 | 36.91 |
| AVERAGE | 6.539 | 3.586 | 15.93 | 8.40 | 35.18 |

Considering the values of the normal population 40yrs-65yrs (chapter IV): - micro- and macroneurons densities are identical
- average diameter of microneurons: no change
- average diameter of macroneurons: slight decrease

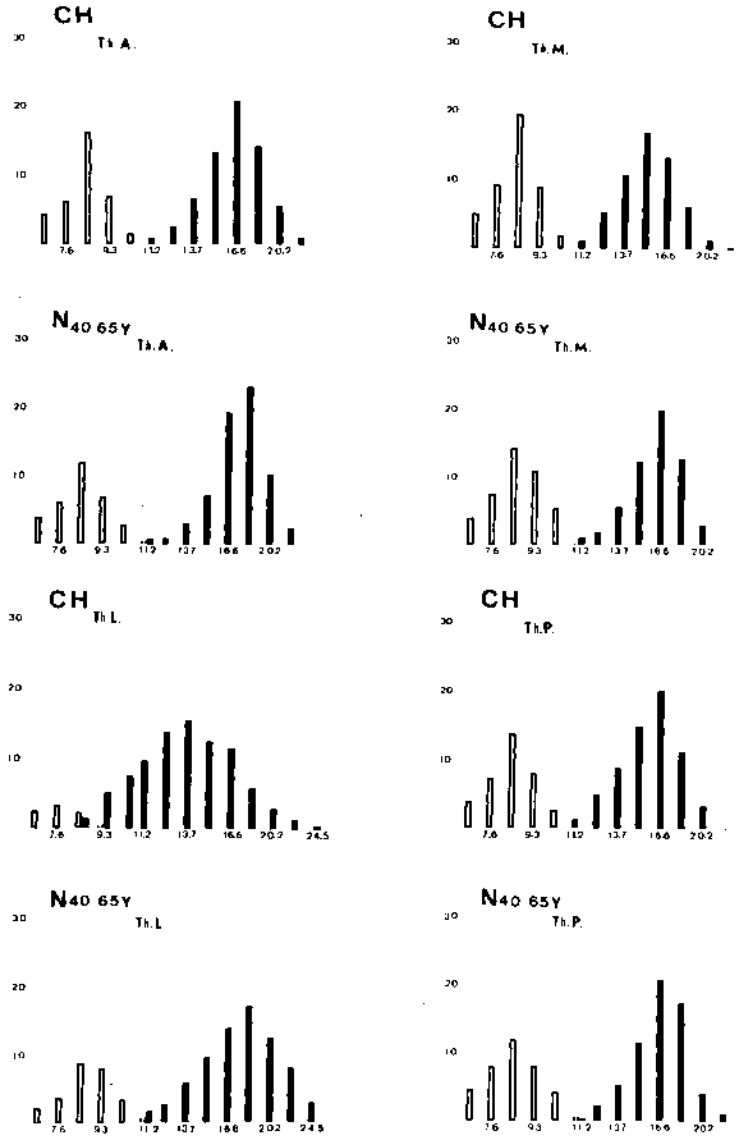
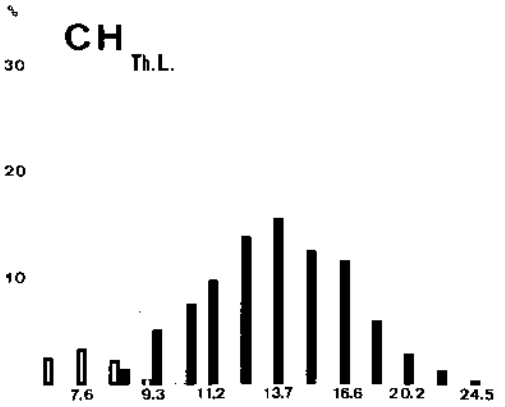



PLATE XXI: *Average micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.) of Huntington brains (CH) compared to normals ($N_{40-65yrs}$). In ordinate %, in abscise Ø in micra*




*Average micro- (white bars) and macroneuron (black bars) distribution in thalamus lateralis (Th.L.) of Huntington brains*

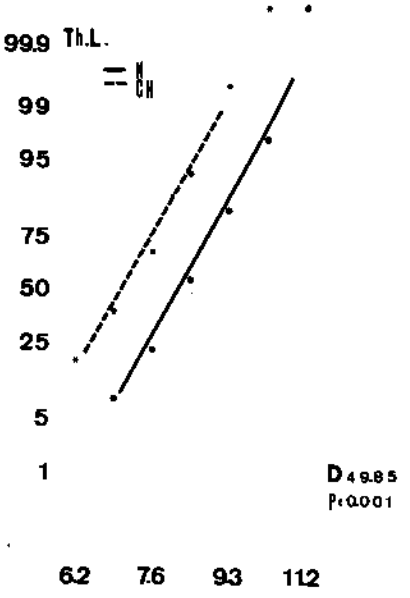



PLATE XXII: *Cumulative frequency distribution of microneurons in thalamus lateralis of Huntington cases (----) compared to normals (——). In ordinate %, in abscissa ø in micra*

### 3. Summary of cytometric results in neostriatum and thalamus in Huntington's Chorea

In *putamen and caudatum*, there is a severe loss of microneurons: 75 % in caudatum, 80 % in putamen. The average diameter of microneurons in the caudatum, however, is unaltered and little increased in the putamen. This seems to imply a better preservation of the larger cells in the Golgi type II population. The macroneurons are preserved in the putamen, but in the caudatum there exists a certain loss of macroneurons in the cases studied.

In *the thalamus*, the microneurons are unchanged in the anterior, medial and posterior regions but are severely demaged in the ventrolateral thalamus: there is a loss of more than 50 %. Plate XXI depicts the average percentage distribution in the four thalamic regions of Huntington cases compared with the normal distribution: the drastic decrease of microneurons in the lateral thalamus of Huntington cases is apparent. The average diameter of microneurons is also decreased only in the lateral complex, but unchanged in the other regions: in the lateral region the diameter is 7.39 $\mu$, compared with 8.72 $\mu$ in the normal brains (Man-Whitney U-test: U=0, $p < 0.01$). The percentage incidence of microneurons in anterior (30 %), medial (44 %) and posterior (35 %) thalamus is unchanged but in the lateral thalamus, there are only 11% of microneurons instead of 25 % in normal brains. Plate XXII shows the average percentage neuronal distribution in thalamus lateralis of Huntington's Chorea cases and the cumulative frequency distribution of microneurons compared with normal. The difference in distribution is marked and statistically significant (Kolmogorov: $D_{max}$: 49.85; $p < 0.001$).
The macroneurons in all thalamic nuclei are shrunken in comparison with normal brains, but this shrinkage is most pronounced in the lateral nuclei: 13.92 $\mu$ instead of 17.8 $\mu$ (U=0; $p < 0.01$). There is, however, no definite macroneuron loss in the thalamus.

## D. ROLE OF GOLGI TYPE II CELLS IN THE PATHOPHYSIOLOGY OF HUNTINGTON'S CHOREA

1° - The role of the neostriatum and the ventrolateral thalamus in motor control and muscle tone is amply evident (Laursen, 1963; Hornykiewicz, 1971; Ward, 1968).

Loss of integrative interneurons in these structures must produce disproportionate motor response.
The neostriatum is considered to have an inhibitory action on the pallidum, which in turn activates the ventrolateral thalamus.
The ventrolateral thalamus, receiving evenso cerebellar input and

having excitatory action on -motorneurons and inhibitory action on -motorneurons, influences the cortical motor response (Barbeau, 1975; Ward, 1968).

Loss of microneurons in the neostriatum in Huntington's Chorea will diminish its inhibitory action on the pallidum. The consequent overexcitation - by the pallidum - of the ventrolateral thalamus should result in uncoordinated movements (because of influence on the cortex) and akinesia because of inhibition of -motorneurons. The loss of inhibitory microneurons in the ventrolateral thalamus itself could produce lack of inhibition on -motorneurons explaining the ill defined tonus changes in chorea.

2° - In Huntington's Chorea it was shown that glutamic acid decarboxylase (G.A.D.) (Bird and Iversen, 1973) and Gama Butyric Acid (GABA) (Perry et al., 1973) is decreased in the basal ganglia. These substances are substantially present in the normal neostriatum and thalamus. The massive loss of neostriatal microneurons in Huntington's disease has been related to this GABA-decrease implying that the Golgi type II cells are GABA-ergic.

In the normal ventrolateral thalamus the amount of microneurons found in our quantitative study (25 %) coincides nicely with the amount of pre- and postsynaptic inhibitory cells found by Eccles (24 %)(1966). Eccles inclines to the hypothesis that the thalamic microneurons are also GABA-ergic (Personal communication, 1975).

A primary defect in GABA-metabolism, however, hardly could be assigned responsible for chorea disease because not all thalamic microneurons in anterior, posterior and medial regions are diseased, as in Parkinson disease GABA is also decreased (Rinne et al., 1974) and because in experimental work, GABA deficiency produces psychotic-like symptomatology (Stevens, 1974; Roberts,1972).

But in Parkinson disease only one dopaminergic pathway is defect (nigrostriatal pathway) leaving other dopaminergic circuits intact. The same phenomenon might apply to GABA-systems in Huntington's Chorea.

3° - The decrease of one neurotransmitter substance should not be regarded as an isolated phenomenon. There are certainly interrelated *imbalances* between several transmitter substances and moreover the denervation of nerve structures might produce *hypersensitivity* to normal amounts of a transmitter (Ungerstedt, 1971).

In this regard, Barbeau (1972-1975) insists upon the dopamine-acetyl-choline imbalance in Huntington's Chorea and Parkinson

disease. The neostriatum indeed is under the inhibitory influence of the nigral dopamine input. Acetylcholine and its vital enzymes - cholineacetylase and cholinesterase - are found in the neostriatum. Olivier et al. (1970) described a cholinergic strio-nigral pathway, thus balancing the dopaminergic nigro-striatal pathway (Anden et al., 1964). The localisation of acetylcholine in the neostriatum is not known. The macroneurons might be tentatively pointed but in view of the findings of the present study, it could be proposed that the larger cells of the Golgi type II cells (middle-sized cells of Cajal, 1911) are cholinergic. Rafols (1974) differentiated two types of Golgi type II cells having different transmitter vesicles in their cytoplasm. The larger type was considered to be 5 to 10 % of the total microneuron population. As has been shown in our cytometric study of the neostriatum in Huntington's Chorea, the larger microneurons seem to be better preserved, thus accounting for the fact that the average diameter of microneurons does not decrease compared with normal values.
Bird and Iversen (1974) showed that cholineacetylase was decreased only in 50 % of choreic cases.

If these cholinergic neurons normally are under the control of the GABA-ergic cells, loss of these cells in Huntington's Chorea realises a state of denervation thus probably accounting for the *'denervation supersensitivity'* to dopamine (Klawans et al., 1972). The beneficial effect of dopamine receptor blockers (neuroleptics) on choreic movements are well established; contrarely, L-dopa loading has been proposed as a test of early detection in chorea patients 'at risk'.

4° - In contrast to the 'choreiform' types of Huntington's Chorea, akinetic adult types - with almost no involuntary movements - and juvenile rigid types are well known.

Our study of the putamen in 2 cases of akinetic adult chorea and one case of juvenile chorea (Dom et al., 1973) is too limited to draw firm conclusions. Moreover, neither the caudatum nor the thalamus were examined in those cases. Some interesting findings unlike the choreiform cases might be mentioned. In the akinetic adult, the atrophy of Golgi type II cells being almost as severe as in the choreiform type, no preservation of larger microneurons was observed. In that type of the disease, the dopamine supersensitivity to 'cholinergic' (?) microneurons cannot be present, thus producing less movement disorder.

In the juveline rigid type, the preservation of larger 'cholinergic' (?) microneurons does exist in the striatum, but the ventrolateral thalamus was not examined. If one supposes preservation of microneurons in that structure, the overexcitation by loss of neostriatal inhibition could lead to rigidity by stimulation of $\alpha$-motorneurons and inhibition of $\gamma$-motorneurons (Hassler, 1972).

## Chapter VI

## PARKINSON'S DISEASE

In 1817, James Parkinson described the 'Shaking Palsy' disease. It is a degenerative nervous system disease of unknown origin characterized by its main clinical features: resting tremor, rigidity and hypo- or akinesia. The prevalence rate is $\pm 2.5^{\circ}/_{oo}$ without definite heredofamilial impact (Rondot and De Recondo, 1973).

The dopamine depletion of the striatum in Paralysis Agitans (Ehringer and Hornykiewicz, 1960) is due to nerve cell loss in the locus niger (Tretiakoff, 1919; Hassler, 1938): the nigrostriatal dopaminergic pathway (Anden, 1964) is interrupted. This dopamine depletion plays an important role in the physiology of tremor, akinesia and rigidity, as shown in experimental work (Poirier and Sourkes, 1965; Poirier et al., 1972) and as shown by chronic neuroleptic treatment (Delay and Deniker, 1961).

Besides this idiopathic 'disease of Parkinson', other Parkinsonian syndromes were described: Postencephalitic Parkinson syndrome (Von Economo, 1917), vascular Parkinsonian syndromes, toxic Parkinsonian syndromes, e.g. by neuroleptics (Delay and Deniker, 1968).

### A. NEUROPATHOLOGY OF PARKINSON'S DISEASE

The anatomical lesions related to the Parkinsonian syndrome are documented by several excellent papers (Tretiakoff, 1919; Foix and Nicolesco, 1925; Hassler, 1938; Hallervorden, 1957; Escourolle et al., 1971).

– The main pathological features are:

1- changes of pigmented brain stem nuclei: *locus niger* and *locus coeruleus*. The nerve cell loss in these structures is proportional to the clinical picture. The neuromelanin pigment decreases: one finds the pigment free in the tissue or within the gitter cells. There exists a reactional gliosis and sclerosis (Photo 5).

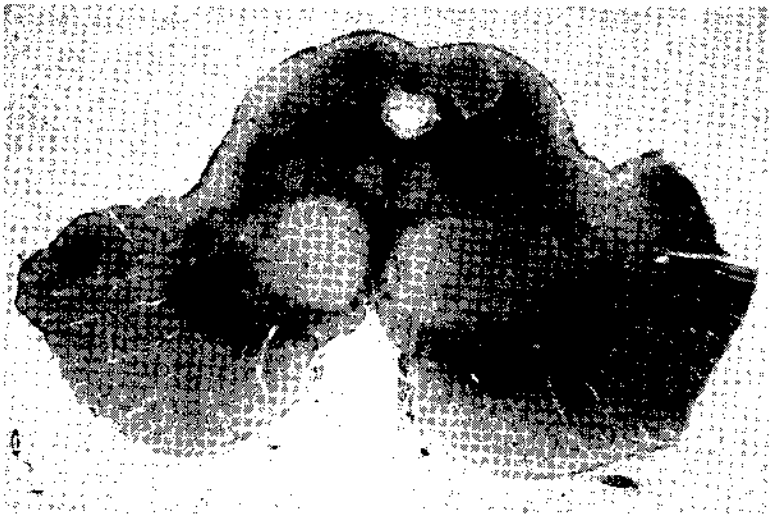


PHOTO V: *Mesencephalon in Parkinson's disease. Note gliosis in locus niger. Holzer stain.*

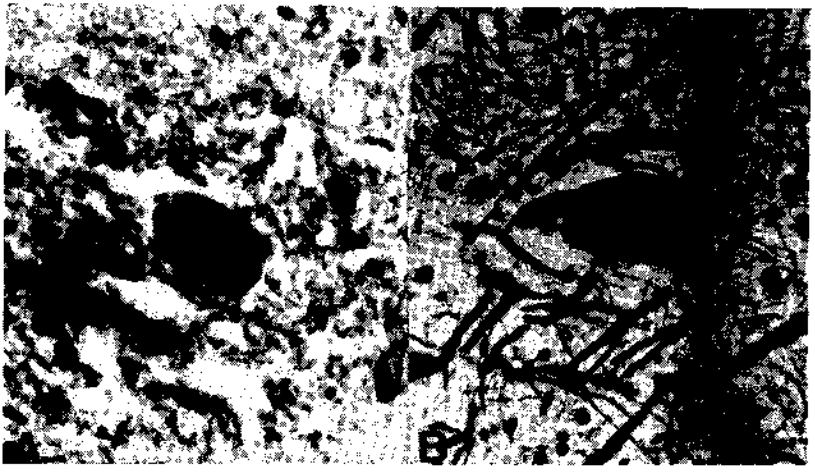


PHOTO VI: *A. Lewy body in neuron of locus niger. H.E; stain.*
*B. Neurofibrillary tangle in neuron of locus niger. Jabonero-silverimpregnation. Magnification 1000 x.*

2- *Occurrence of Lewy bodies:* Lewy bodies are oval or rounded intraneuronal intracytoplasmic corpuscles with a dense core surrounded by a lighter zone (Photo 6a). The exact nature of those bodies is unknown: they contain sphingomyelin but no DNA, carbohydrates, amyloid substance, iron or lead (Den Hartog Jager, 1969). Electron microscopically, they have a fibrillar matrix, more or less dense, with imprecise boundaries.

Lewy bodies are mainly found in pigmented brain structures - thus they maybe related to neuromelanin metabolism - but not exclusively: also nucleus subthalamicus, reticular brain stem formation and medulla might contain Lewy bodies (Den Hartog Jager and Bethlem, 1960).

Lewy bodies are rather specific to the idiopathic form of the disease, although they have been encountered in Postencephalitic Parkinson (Hallervorden, 1933; Lipkin, 1959) and even in normal old aged people (Beheim-Schwarzbach, 1952).

3- *Neurofibrillary degeneration* of neurons: better demonstrated by argentic impregnation. Neurofibrillar tangles are characterized by thickening of neurofibrils in the form of a sphere or a basket (Photo 6b).
By electron microscopy, the tangles are composed of tubular fibrils with a diameter of $\pm$ 200 Å.

Neurofibrillary degeneration is mainly confined to the Postencephalitic Parkinson Syndrome but is not specific for this syndrome: common senescence, Alzheimer disease and other entities may also show this type of nerve cell change.

The neurofibrillary changes are most numerous in locus niger and coeruleus, reticular formation of the brain stem and in the cortex.

— Accessory pathological findings in Parkinson disease are:

- Gliosis and slight nerve cell loss in the brain stem reticular formation
- Nerve cell loss, lipofusin accumulation, gliosis and calcium deposites in the globus pallidus
- Variable but always slight neuron atrophy within the neostriatum and the thalamus.

## B. QUANTITATIVE CYTOMETRY OF BASAL GANGLIA IN PARKINSON'S DISEASE

Quantitative studies in Parkinson disease are rather scarce. In 1963, Pakkenberg reported cytometric analysis of the nerve cell population in the Globus Pallidus of 10 Parkinson brains compared with the results in 10 normal brains. He found no numerical difference between the two groups of brains: 588 neurons per $mm^3$ in normals, 611 neurons per $mm^3$ in Parkinson diseased brains. He insisted, however, upon a significant decrease in RNA content of cytoplasm and nucleolus in Parkinson Pallidum as evidenced by cytophotometry.

In 1965, Pakkenberg and Brody made nerve cell counts in the substantia nigra of Parkinson patients and normals. They found a decrease of 66 % of melanincontaining nerve cells and a decrease of 39 % of melanin-free neurons in the substantia nigra of Parkinson brains compared with the values of their normal controls.
In 1969, Sabuncu found nerve cell loss in the lateral and medial pallidum of Parkinson brains.
In 1972, Dom, Baro and Brucher studied the putamen of three idiopathic Parkinson brains: they found no difference in numerical nerve cell density and nerve cell diameter as compared to four normal brains.

In 1974, Tolppanen performed neuron counts in the substantia nigra of Parkinson patients: he confirmed the nerve cell loss in substantia nigra.
In 1975, Bøttcher found no neuron loss in the neostriatum in Parkinson disease: numerical density $\pm$ 20.000/$mm^3$ for microneurons and a normal nucleus diameter of microneurons: 8.02 $\mu$.

## C. PERSONAL STUDY OF NEOSTRIATUM AND THALAMUS IN PARKINSON DISEASE

Five brains of patients showing a Parkinson syndrome, two postencephalitic and three idiopathic, were selected from the series of brains studied by Hassler (1938).

### 1. Clinical case histories

Case I: $^{\circ}1902 - {}^{+}1934$:
In 1923, this lady developed a sudden high fever, shivering and severe headache. She progressively fell into a deep sleep lasting 3 weeks. Thereafter, she complained of diplopia and drowsiness. Three years later, after a pregnancy, she presented the first signs of Parkinsonian rigidity and tremor. At the age of

29 yrs, she showed rigidity of all limbs and neck, tremor in left arm and leg, mask-like facies, tremor of the tongue, loss of automatic movements, dysarthria and occasional oculogyric crises. At the age of 32 yrs, the tremor was bilaterally present. She died of lungoedema following pneumonia.

Case II: °1884 - +1930:
This man suffered from encephalitis lethargica at the age of 35 yrs. Seven years later, he showed the first symptoms of Parkinsonism. Over a period of 4 years until his death due to circulatory failure, he developed severe rigidity of all limbs, head and trunk, bilateral tremor which was slightly more pronounced on the right side, akinesia and staggering gait and blepharospasms.

Case III: °1877 - +1934:
During world war I, this man first exhibited tremor of all 4 limbs, dysarthria and gait disturbances. Progressively, he acquired severe trunk and limb rigidity, diffuse tremor, pill roll movements and akinesia. He walked bent forward taking small steps. He showed atrophy of the small hand muscles. He became completely bedridden and died at the age of 57 yrs due to decubitus, pyelonephritis and pneumonia.

Case IV: °1864 - +1932:
At the age of 57 yrs this man noted tremor in the left arm. A few months later, the tremor spread to the right arm, incapacitating him to such a degree that he had to abandon his work. Over a period of several years, he became very rigid with mask-like facies, propulsion in gait, loss of automatic movements, tremor of head, tongue and four limbs.
He presented hyperhidrosis and atrophy of hand musculature. At the age of 68 yrs, without signs of dementia, he died of severe bronchopneumonia.

Case V: °1864 - +1935:
This woman was in good health up to the age of 67 yrs. At that time, a slight tremor of the right hand occurred. The tremor progressed to right arm and right leg, accompanied by rigidity. Later on, tremor of tongue and left arm and leg and onset of dysarthria and gait disturbance. A few months before death at the age of 71 yrs, she became disoriented and confused.

## 2. Cytometric results

Nerve cell counting and sizing was performed separately in caudatum and putamen, thalamus anterior, thalamus medialis, thalamus lateralis and thalamus posterior.

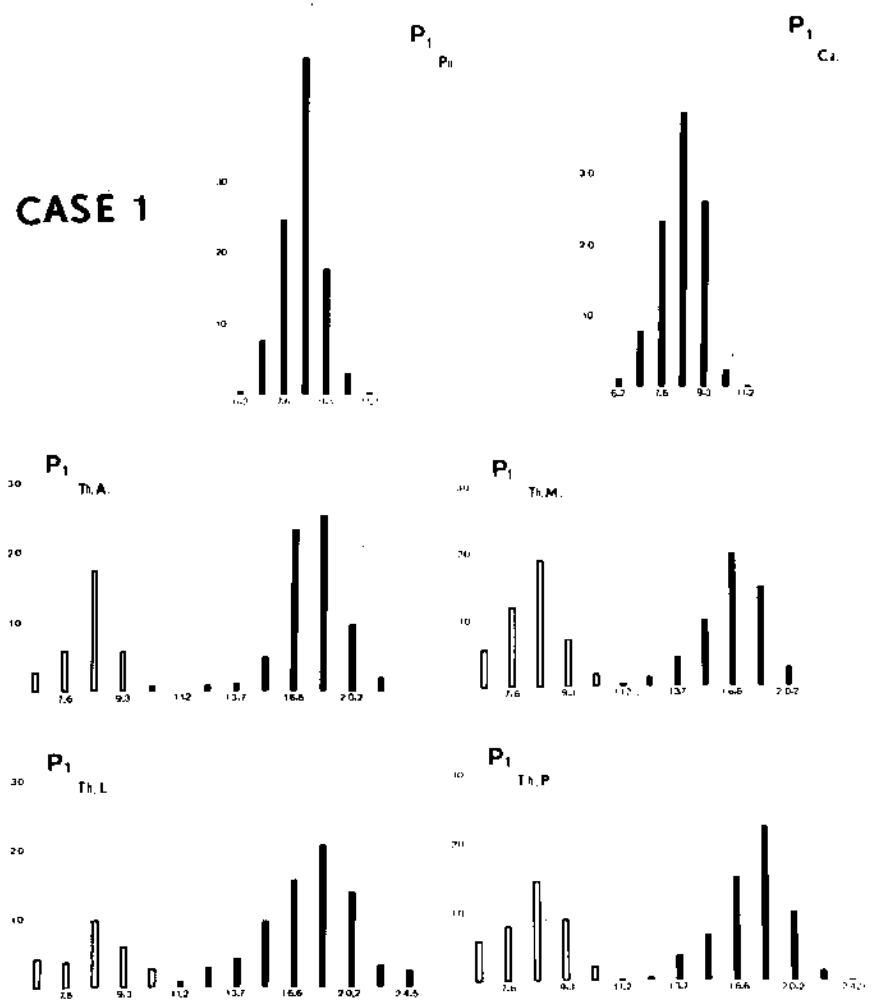



PLATE XXIII: *Microneuron distribution in putamen (Pu) and caudatum (Ca)(black bars), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

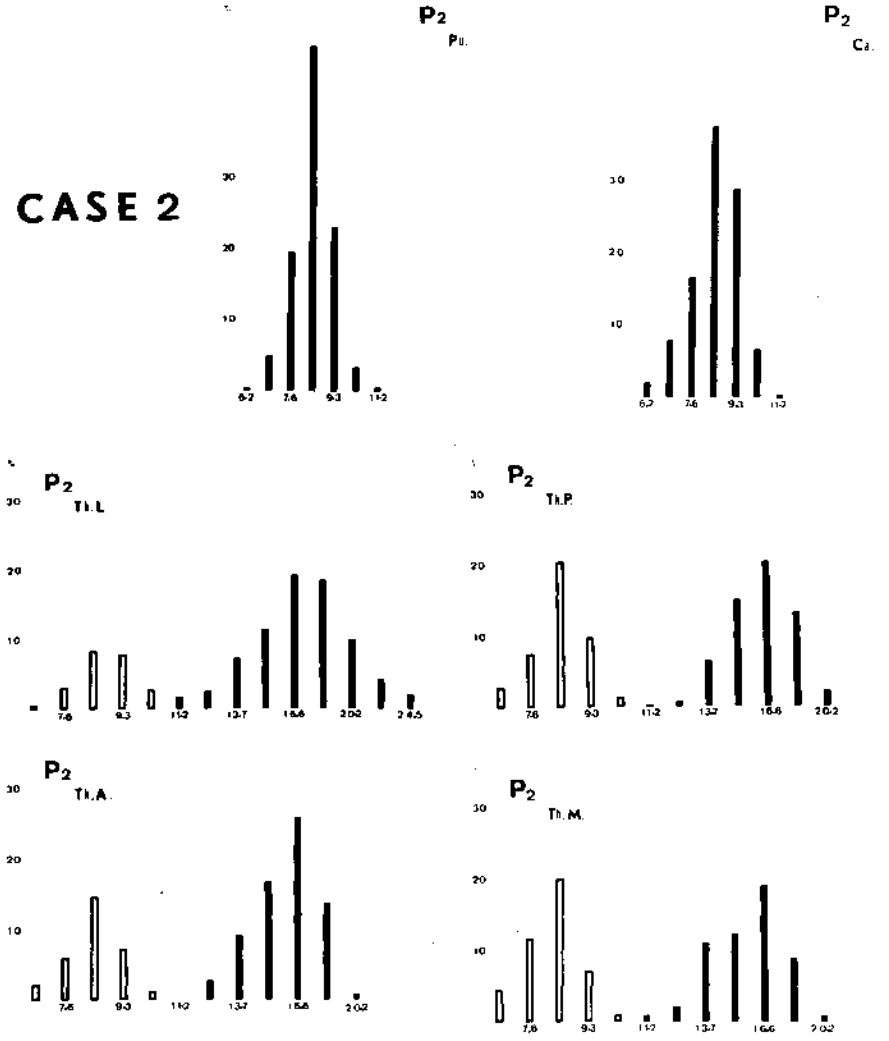



PLATE XXIV: *Microneuron distribution in putamen (Pu) and caudatum (Ca)(black bars), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

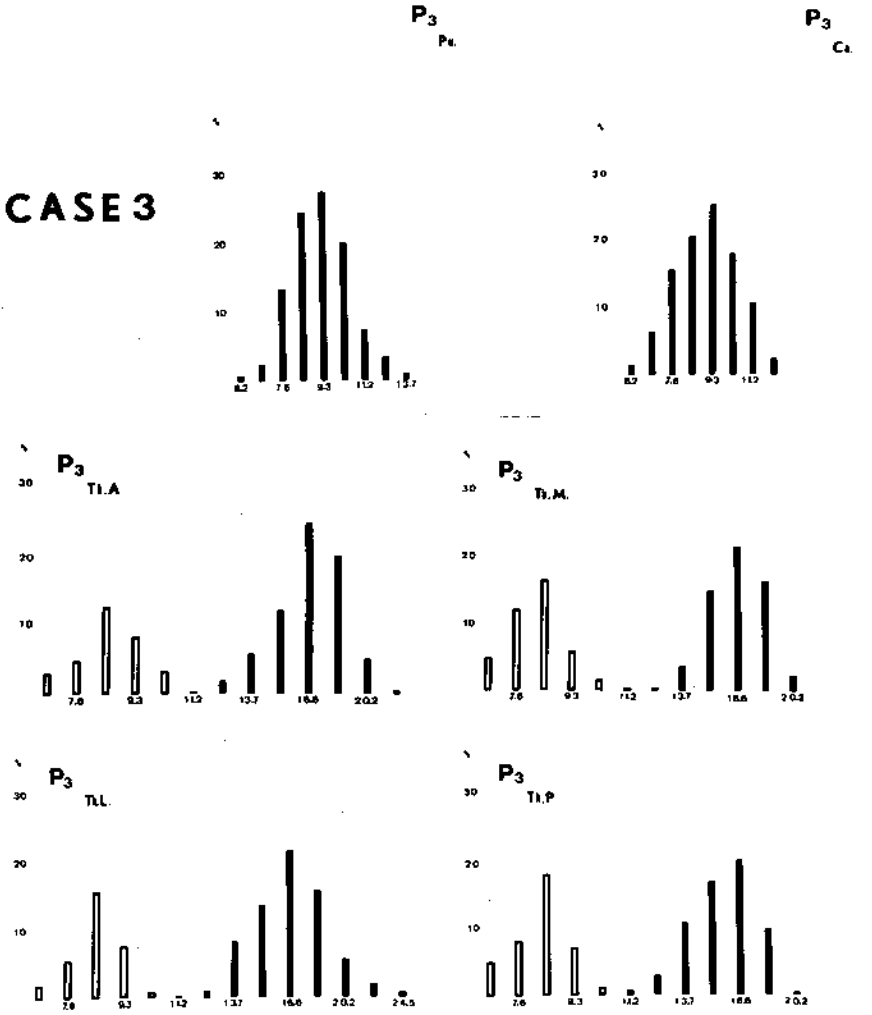



PLATE XXV: *Microneuron distribution in putamen (Pu) and caudatum (Ca)(black bars), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscies Ø in micra*

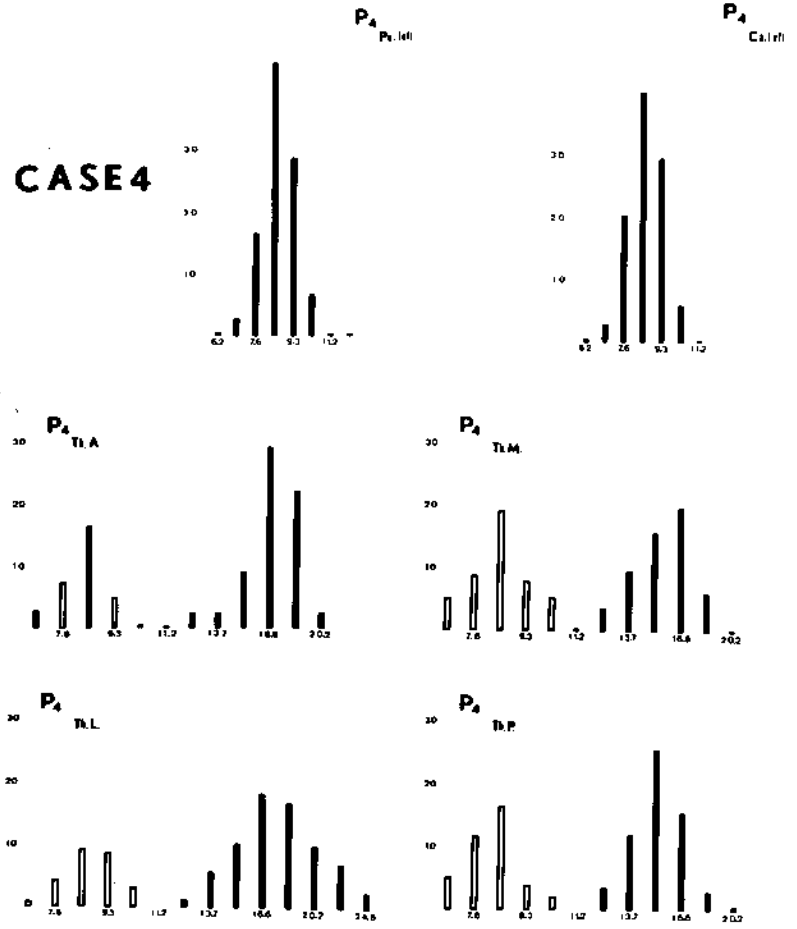



PLATE XXVI: *Microneuron distribution in putamen (Pu) and caudatum (Ca)(black bars), and micro- (white bars) and maroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscissa Ø in micra*

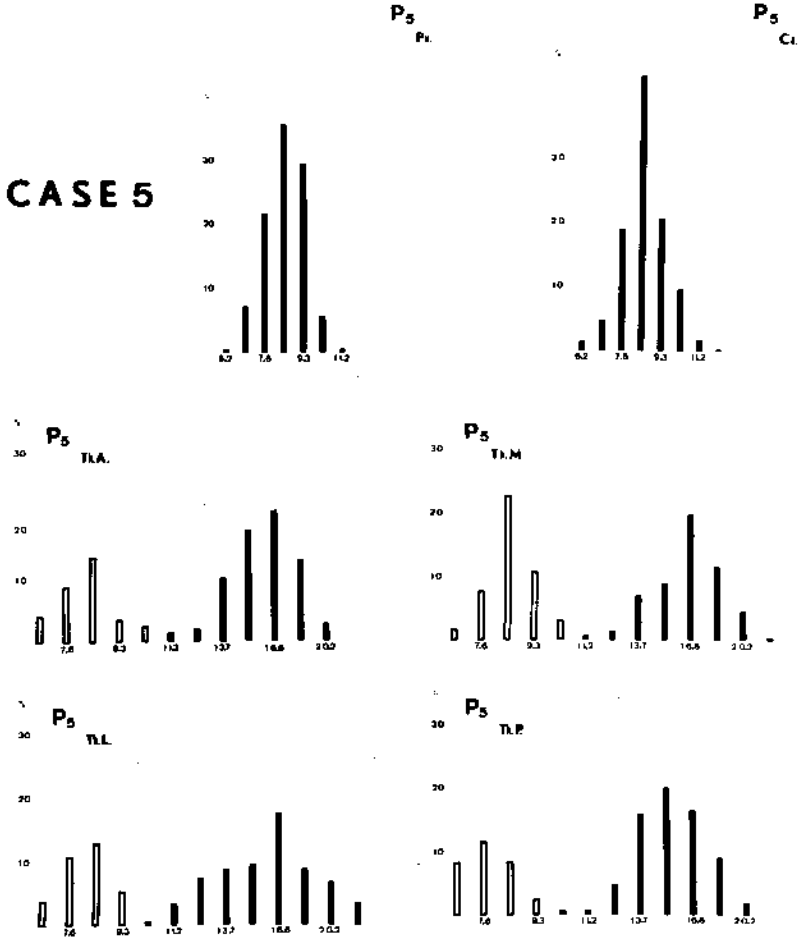



PLATE XXVII: *Microneuron distribution in putamen (Pu) and caudatum (Ca)(black bars), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscissa ø in micra*

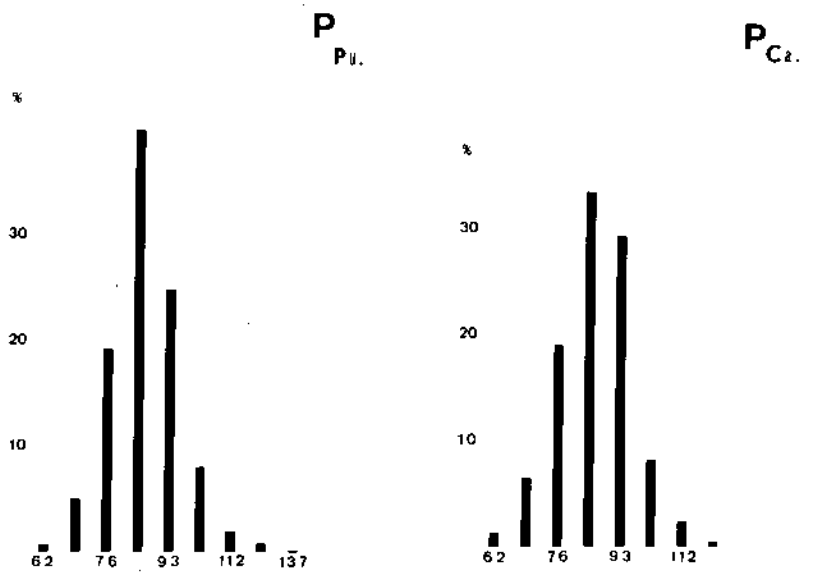
For each case the neuronal formula (percentage distribution among cell size) in those regions is depicted on a separate plate: $P_1$ = plate XXIII, $P_2$ = plate XXIV, $P_3$ = plate XXV, $P_4$=plate XXVI, $P_5$ = plate XXVII.

*a) the neostriatum*

The following tables summarize the numerical values for caudatum and putamen.

| CAUDATUM P | NUMERICAL DENSITY macro | micro | large/small ratio | AVERAGE Ø in micra |
|---|---|---|---|---|
| $P_1$ | 57 | 26.695 | 1/489 | 8.34 |
| $P_2$ | 107 | 24.353 | 1/223 | 8.49 |
| $P_3$ | 163 | 25.205 | 1/154 | 9.08 |
| $P_4$ | 107 | 26.488 | 1/243 | 8.56 |
| $P_5$ | 57 | 27.030 | 1/495 | 8.59 |
| AVERAGE | 98 | 25.953 | 1/264 | 8.61 |

| PUTAMEN P | NUMERICAL DENSITY macro | micro | large/small ratio | AVERAGE Ø in micra |
|---|---|---|---|---|
| $P_1$ | 57 | 26.270 | 1/482 | 8.27 |
| $P_2$ | 106.5 | 21.243 | 1/195 | 8.43 |
| $P_3$ | 163 | 25.844 | 1/158 | 9.23 |
| $P_4$ | 106.5 | 26.270 | 1/240 | 8.61 |
| $P_5$ | 57 | 21.570 | 1/395 | 8.48 |
| AVERAGE | 98 | 24.239 | 1/247 | 8.61 |




*Average microneuron distribution in putamen and caudatum*

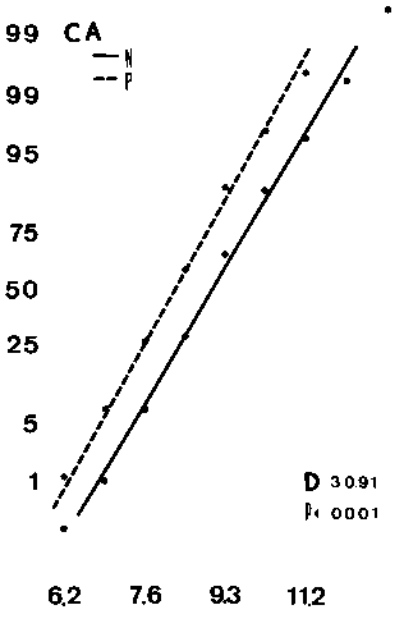



PLATE XXVIII: *Cumulative frequency distribution in caudatum of Parkinson (----) compared to normal (——). In ordinate %, in abscise Ø in micra*

Comparing these results with the values obtained in the neostriatum of normal brains above 45 yrs of age (chapter III), the putamen in Parkinson disease does not show significant differences in microneuron population with the normal brains. In the caudatum, however, the microneurons appear to be statistically significantly smaller: 8.61 micra in diameter compared to 9.34 micro in normals ($u = 0$; $p = 0.018$: Mann-Withney U-test).
Plate XXVIII shows the average distribution of microneurons in caudatum and putamen of Parkinson brains (above) and the cumulative frequency distribution of microneurons in caudatum compared to the normal distribution: the distribution pattern is statistically different (Kolmogorov-test).

*b) the thalamus*

The numerical values obtained in thalamus anterior, medialis, lateralis and posterior are summarized in the next tables:

*Thalamus Anterior*

| | NUMERICAL DENSITY | | AVERAGE Ø IN MICRA | | % |
|---|---|---|---|---|---|
| | macro | micro | macro | micro | Micro |
| $P_1$ | 7.810 | 3.834 | 17.72 | 8.31 | 32.9 |
| $P_2$ | 6.141 | 2.804 | 16.05 | 8.40 | 31.5 |
| $P_3$ | 7.171 | 3.266 | 16.75 | 8.54 | 31.5 |
| $P_4$ | 7.860 | 3.692 | 16.79 | 8.23 | 32.9 |
| $P_5$ | 6.674 | 3.266 | 16.00 | 8.20 | 32.9 |
| AVERAGE | 7.131 | 3.372 | 16.65 | 8.36 | 31.9 |

*Thalamus Medialis*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % Micro |
|---|---|---|---|---|---|
| $P_1$ | 7.491 | 6.355 | 16.57 | 8.21 | 45.9 |
| $P_2$ | 7.292 | 5.772 | 15.79 | 8.22 | 44.1 |
| $P_3$ | 6.816 | 4.665 | 16.58 | 7.17 | 40.5 |
| $P_4$ | 6.390 | 4.920 | 15.57 | 8.32 | 43.5 |
| $P_5$ | 7.292 | 6.248 | 16.53 | 8.54 | 46.2 |
| AVERAGE | 7.056 | 5.592 | 16.29 | 8.28 | 44.1 |

*Thalamus Lateralis*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % Micro |
|---|---|---|---|---|---|
| $P_1$ | 3.692 | 1.349 | 17.73 | 8.42 | 27.0 |
| $P_2$ | 4.196 | 1.249 | 17.30 | 8.74 | 23.1 |
| $P_3$ | 3.710 | 1.722 | 16.79 | 8.42 | 31.5 |
| $P_4$ | 3.550 | 1.314 | 17.92 | 8.71 | 27.0 |
| $P_5$ | 2.322 | 1.179 | 16.24 | 8.13 | 33.7 |
| AVERAGE | 3.494 | 1.363 | 17.28 | 8.49 | 28.4 |

*Thalamus Posterior*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % Micro |
|---|---|---|---|---|---|
| $P_1$ | 5.325 | 3.479 | 15.81 | 8.29 | 39.4 |
| $P_2$ | 5.787 | 4.154 | 16.32 | 8.41 | 41.8 |
| $P_3$ | 6.170 | 3.969 | 15.71 | 8.24 | 39.0 |
| $P_4$ | 6.993 | 4.544 | 15.26 | 8.13 | 39.4 |
| $P_5$ | 6.688 | 3.074 | 15.47 | 7.78 | 31.5 |
| AVERAGE | 6.193 | 3.844 | 16.14 | 8.22 | 38.95 |

Comparing these results with the normal values obtained in the brains of the 40 yrs to 60 yrs age group (chapter IV), and examining plate XIX showing the overall percentual neuronal distribution in the 4 thalamic regions discloses no definite nerve cell loss: the numerical densities and the percentage of microneurons are not decreased in the Parkinson brains.

There seems to exist, however, a microneuron pathology in medial and posterior thalamus; while the decrease of the mean diameter in the medial thalamus is not statistically significant, the decrease of average diameter in the posterior thalamus is: 8.22 micra against 8.48 micra in normals ($u=1$; $p=0.036$, Mann-Withney U-test).

Plate XXX visualizes the difference in distribution pattern of microneurons in Parkinson disease compared with normal: statistical analysis by Kolmogorov-Smirnov test gives significant differences for the medial regions ($p = 0.02$) and to a lesser degree for the posterior thalamus ($p = 0.08$).

The macroneuron population does not show significant difference from normals except for the thalamus posterior: there exists a definite atrophy: 16.69 micra $\longrightarrow$ 16.14 micra ($u=0$; $p=0.018$).

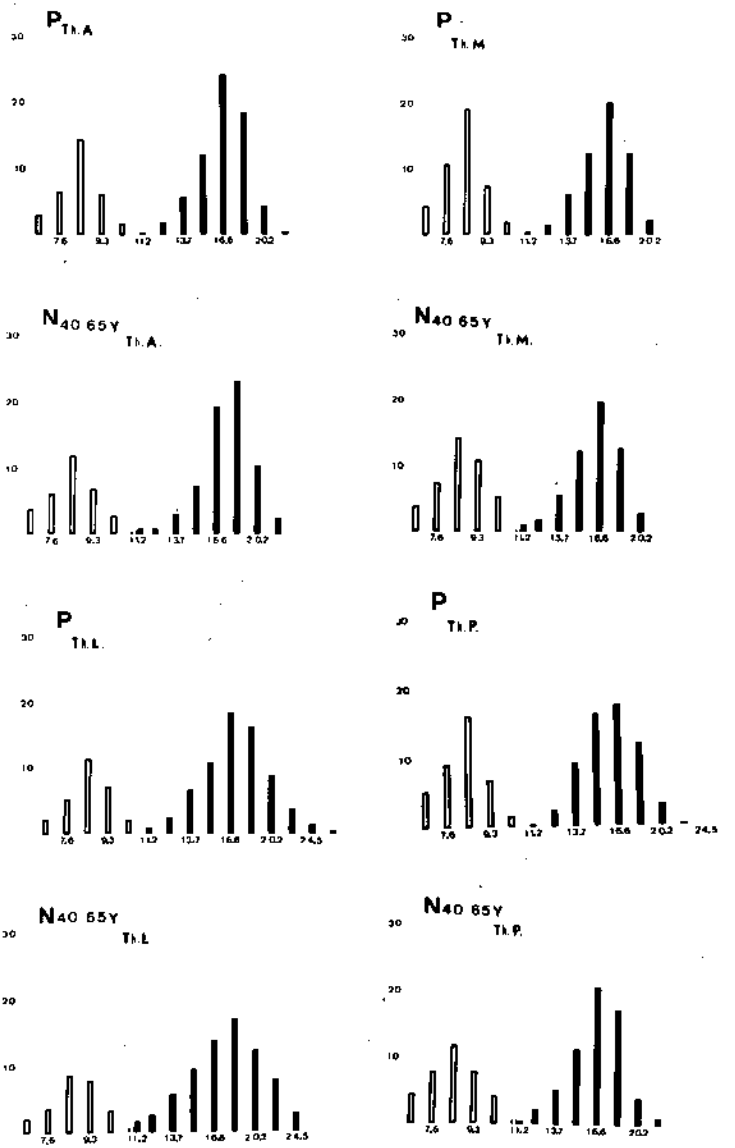



PLATE XXIX: *Average micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.) of Parkinson brains (P) compared to normals (40-65yrs). In ordinate %, in abscise Ø in micra*

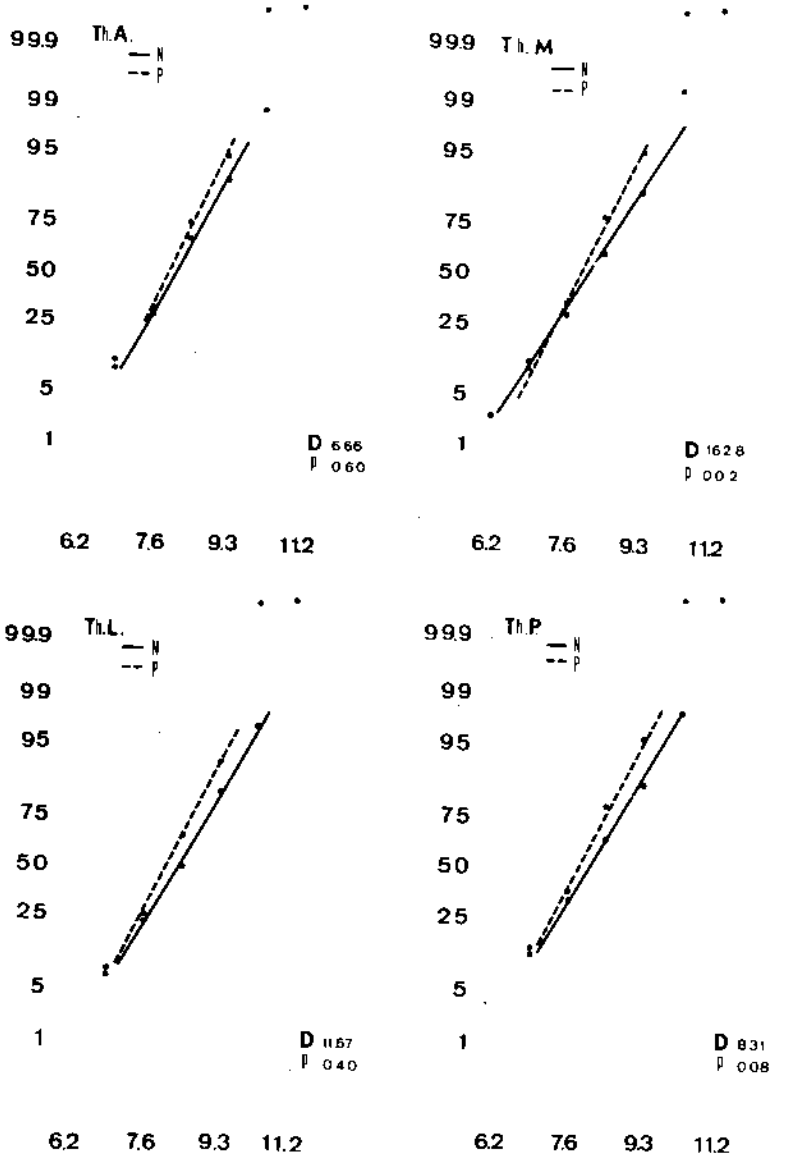



PLATE XXX: *Cumulative frequency distribution of microneurons in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.) in Parkinson brains (----) compared with normals (——). In ordinate %, in abscise Ø in micra*

## 3. Summary and discussion of findings in neostriatum and thalamus in Parkinson disease

a) In the *putamen*, no change in microneuron population was detected.

In the *caudatum*, there is no microneuron loss but a definite atrophy (or hypotrophy ?) of microneurons: the average diameter is significantly decreased ($9.43\,\mu \longrightarrow 8.61\,\mu$).

In the *thalamus*, anterior and lateral regions are unchanged. In the posterior and mostly in the medial thalamus, microneurons are smaller compared with normal brains ($8.48\,\mu \longrightarrow 8.22\,\mu$) without microneuron loss. In the posterior thalamus, the macroneurons also seem to be slightly atrophied ($16.69\,\mu \longrightarrow 16.14\,\mu$).

b) The present study confirmed our findings in the putamen of 3 cases of idiopathic Parkinson syndrome published earlier (Dom et al., 1972). Our results are partially in agreement with the findings of Bøttcher (1975): there is no neuron loss in the neostriatum. Unlike Bøttcher we could demonstrate a decrease of diameter of microneurons only in the caudatum: Bøttcher did not distinguish between caudatum and putamen which might explain the discrepancy.

c) In contrast to the normal appearance of the putamen, the caudatum appears to be changed in Parkinson brains. Although histologically almost identical, caudatum and putamen have distinctly separate connections (Kemp and Powell, 1970; Mettler, 1968). It is therefore not surprising to find selective damage to either structure in some disease.

d) The meaning of the decrease in diameter of the Golgi type II neurons in the caudatum in Parkinson disease is not immediately clear. It could be interpreted as secondary to the known decrease in dopaminergic input from the nigro-striatal pathway: it should thus be some 'dysfunctional' hypotrophy (Ehringer and Hornykiewicz, 1960). It is noteworthy that, unlike the findings in Huntington's Chorea, all microneurons get smaller: there is no preservation of the bigger cells, maybe because it is a secondary atrophy.

e) The thalamic changes are rather restricted to the microneurons Golgi type II in medial and posterior (associative) regions: there is no cell loss but a slight atrophy. More remarkable is that the lateral thalamus (motor pathways), in contrast to Huntington's Chorea, is spared.

f) The changes to Golgi type II neurons in caudatum and thalamus in Parkinson disease might be related to the GABA-decrease in this disease, reported in 1975 by Rinne et al. (1975).

## D. SYMPTOMATIC PARKINSONISM: INFLUENCE OF NEUROLEPTICS ON NEOSTRIATAL NEURONS: EXPERIMENT IN RATS

Ever since the use of neuroleptics in clinical psychiatry, it was noted that all products having antipsychotic action could produce a Parkinsonian syndrome (Deniker, 1961).

Neuroleptics indeed have a sound influence on monoamine metabolism especially on dopamine turn-over by blocking dopamine receptors of the postsynaptic cell (Corrodi et al., 1967).

At present, no morphological changes within the neostriatum (postsynaptic neurons of the strio-nigral dopaminergic pathway) due to neuroleptic treatment have been described. A quantitative study is also not yet reported.

Therefore, the following experiment in the rat was performed.

### 1. Experimental design

Male adult Wistar rats were used. All animals weighed approximately 250 g.

All rats were kept in the same environmental and nutritional conditions throughout the experiment.
During five consecutive days, all animals received a subcutaneous injection: 3 rats placebo, 3 rats haldol (butyrophenone), 3 rats chlorpromazine (phenothiazine), 3 rats pimozide (long-acting drug).
The dose of the neuroleptic drug injected daily for those 5 days was the minimal dose giving inhibition in the autostimulation test (Olds and Travis, 1960; Dresse, 1966): Haldol: 1.25mg/kg s.c. Chlorpromazine 2.5mg/kg s.c.; Pimozide 2.5mg/kg s.c.
For two days, after those five days, the rats did not receive any medication and they were killed on the seventh day in aneasthesia by in situ brain perfusion with formalin 4 % after Ringer solution.

After paraffin embedding, 20 micra serial sections were cut. Every 25th section was stained with cresylviolet.

Cytometric evaluation was performed throughout the striatum of all animals.

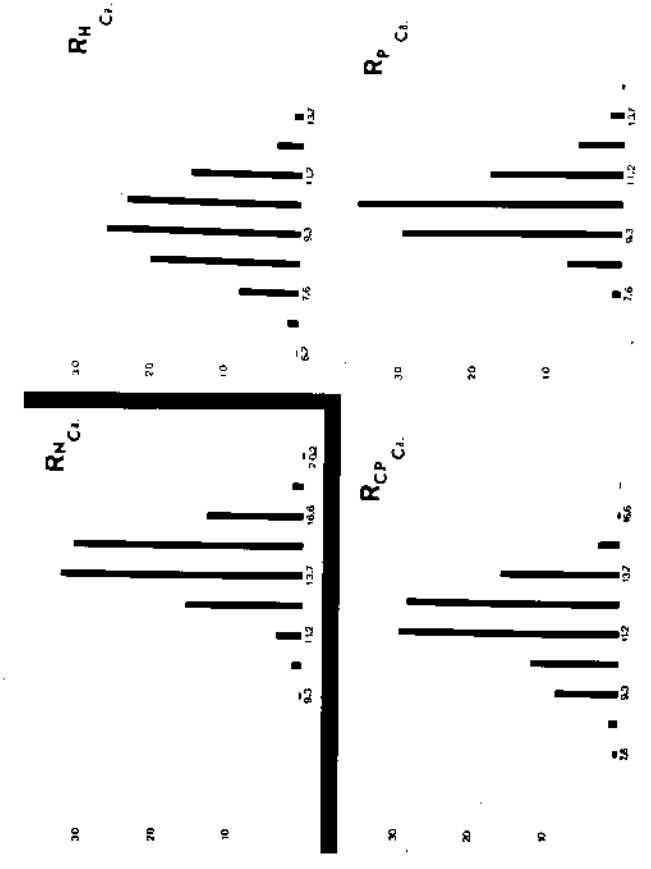



PLATE XXXI: *Average neuron distribution in the caudatum of normal rats ($R_N$), rats treated with Haldol ($R_H$), with Chlorpromazine ($R_{CP}$) and with Pimozide ($R_P$). In ordinate %, in abscise Ø in micra*

## 2. Cytometric results

Picture XXXI gives the average percentage neuronal distribution for the four groups: $R_N$ = placebo group, $R_H$ = Haldol treated, $R_{CP}$ = chlorpromazine treated, $R_p$ = pimozide treated animals.

In the untreated rats, the average diameter of neostriatal neurons is 14 micra with a spread from 9.3 micra to 20.2 micra.

The Haldol treated animals present a drastic decrease in diameter of neostriatal neurons: the average diameter is 9.6 micra with a spread of 6.2 micra to 14.6 micra.

The chlorpromazine treated group shows evenso a decrease in diameter of neostriatal neurons but much less pronounced: the average diameter is 11.8 micra with a spread of 7.6 micra through 18.2 micra.

The pimozide treated rats present neostriatal changes quite close to the Haldol treated animals: the average diameter is 10 micra with a spread of 7.6 micra through 14.6 micra.

## 3. Discussion of results

Although the doses of the drug administered were low and only for 5 days, the influence on the morphology of the neostriatal neurons is fairly considerable.

The changes are mostly pronounced with drugs of the butyrophenone group: this finding is not surprising because these drugs are well known to produce Parkinsonism in humans even at modest treatment dosis. These effects are less spectacular with the phenothiazines used in our experiment: those substances produce in humans primarely sedation and only with higher dosis Parkinsonian symptoms.

The meaning of the decrease in diameter of neostriatal neurons by neuroleptics is speculative at present. Apart from being smaller, the appearance of the neurons is normal in Nissl stain. In order to obtain further information, the above experiment is repeated and pieces of the neostriatum are taken for evaluation by electron microscopy: this study is still going on.

The neostriatal neurons are the postsynaptic cells to the nigrostriatal dopaminergic pathway. Neuroleptics are dopamine receptor blockers (Anden et al., 1970). The decrease in diameter of those neurons could mean fewer receptor sites.

## Chapter VII
## CATATONIC SCHIZOPHRENIA

Schizophrenia is easier to diagnose than to define.
It is a symptom complex occurring in young people, consisting mainly of disturbances in thinking and feeling, detachment from reality (withdrawal) and often hallucinations and bizarre behavior, with a chronic progressive or episodic course (Bleuler, 1968).

Although the aetiology, pathogenesis and pathophysiology is unknown at present, the consistency of symptoms, varying only slightly over time and among cultures (Sanua, 1969), the high prevalence in biological relatives (Rosenthal et al., 1968; Wender et al., 1968; Kety et al., 1974) and the response to treatment (May, 1968) may signify 'chronic schizophrenia' to be a disease entity, a dysfunction of the central nervous system.

In spite of numerous ancient and recent investigations (Marjerrison and Keogh, 1971; De Bault et al., 1973; Torrey and Peterson 1973-1974), any relationship between the occurrence of schizophrenic symptoms and any demonstrable alteration of structure or function of the central nervous system has still to be demonstrated. Therefore, schizophrenia is thought by many clinicians to be a functional disorder, the product of environmental stress, without alteration in neuronal structure or physiology.

As the diagnosis is based almost exclusively on clinical symptoms, there exists no clearcut delineation of the syndrome.
Nevertheless, over 80 % agreement has been found regarding cases in which experienced clinicians state that they are certain of the diagnosis (Hordern et al., 1968; Kety et al., 1974).

The great consistency among different authors in enumerating the symptoms of schizophrenia is evident, but the emphasis placed upon them individually varies considerable: some insist upon positive phenomena (hallucinations, delusions) (Schneider, 1959), others state the more negative symptoms to be fundamental (autism, impaired affect) (Bleuler, 1950) while others simply enumerate critical symptom categories (A.P.A., 1968; Feighner et al.,1972).

In classical literature, subtypes of schizophrenia are delineated: hebephrenic, catatonic, paranoid and simple (Kraepelin, 1925). These conotations are merely descriptive, ascertaining little value to prognosis or choice of therapy. Hebephrenia applies to the case starting early (17-18 yrs), insidious, with bursts of poor and naive hallucinations with very progressive deterioration. Paranoid schizophrenia refers to cases with late onset (+ 35 yrs) presenting primarly a poorly organized delusional system. The simplex form means an evolution towards autism and withdrawal almost without positive symptoms (hallucinations, psychomotor excitation).
Catatonic schizophrenia, starting in the early twenties, represents the full spectrum of schizophrenic thinking and behavior disturbances with psychomotor excitation versus negativism and catalepsy, with hallucinatory manifestations and self destruction versus autistic withdrawal. It takes a chronic course, progressive or episodic.

Regarding prognosis, the schizophrenic syndrome in recent American literature is divided into 'process' and 'reactive' schizophrenia (Hantor et al., 1966); 'process' or chronic schizophrenia is insidious in onset in a pre-existing schizoid personality (shy, oversensitive, avoiding competition, eccentric and day-dreaming, unable to express emotions, poor social and sexual adjustment) without clear precipitating factor to psychosis; 'reactive' schizophrenia suddenly appears, in a person without pre-existing schizoid personality, at the occasion of obvious precipitating circumstances (divorce - leaving home). European literature refers to 'reactive schizophrenia' as 'bouffées délirantes', 'rand- or degeneratiepsychosen' (Rumke, 1967).

This division (process-reactive) seems more and more questionable: the reactive form can hardly be regarded as true schizophrenia because this type of psychotic symptomatology occurs in many other circumstances: sleep deprivations, encephalitis, ...
Moreover, in their excellent study on heredity in schizophrenia, Kety et al. (1968-1974) found no heredity in 'acute' or 'reactive' schizophrenia according to American literature, while in true chronic schizophrenia, the incidence of the disease in biological relatives was significantly higher than in adoptive relatives. They concluded that acute reactive schizophrenia should not be named schizophrenia.

## A. NEUROPATHOLOGICAL STUDIES IN SCHIZOPHRENIA

- The number of attempts to define histologically brain lesions of aetiological significance in schizophrenia are very numerous. At present, however, no anatomopathological substrate can be described as being specifically related to the psychopathology of the schizophrenic syndrome.

- A detailed analysis of the literature concerning brain changes in schizophrenia reported but afterwards refuted can be consulted in the 'Handbuch der speziellen pathologischen Anatomie' (Peters, 1956), in the monograph 'Schizophrenia - Somatic Aspects' (Richter, 1957) and in 'Psychiatrie der Gegenwart - III. Grundlagenforschung zur Psychiatrie' (Peters, 1967).

- Many types of changes were proposed throughout but seemed to be aspecific: cortical non-cellular lacunae (Wernicke, 1900), diffuse cortical cell loss (Winkelman, 1952), demyelination (Roland and Mettler, 1949), metachromatic bodies (Buscaino, 1929), inclusion bodies (Papez and Papez, 1954), decrease of intra-cellular nucleic acids and nucleoproteins (Hyden and Hartelius, 1948), macro- and microglia changes (Pope, 1952), lipid dystrophy (Peters, 1956), enzyme changes (Pope, 1952).

- More interest has to be paid to the ample studies of the German C. and O. Vogt-school: between 1950 and 1964, several papers appeared by Hopf, Fünfgeld, Bäumer, Hempel, Treff. In a series of schizophrenic and control brains, the basal ganglia (neostriatum-pallidum), the thalamus and the N. Basalis (substantia innominata) were thoroughly studied. Seven steps of 'Dwarf cells' (Schwundzellen) were described in all these nuclei. The steps I through III are considered to be of no pathological value. But the more advanced steps IV to VII seemed to the authors characteristic of schizophrenia: the catatonic schizophrenic brains contained many more clusters of 'dwarf cells', especially in the mediodorsal nucleus of the thalamus. All authors mentioned that catatonic schizophrenia cases showed many more changes in striatum and in thalamus, than the hebephrenic or paranoid schizophrenia cases showed. The results of those extensive studies are criticized on qualitative grounds by Peters (1967) and Hueck (1954): the neuron changes described can hardly be specific because agonal stress and also preparation of the brain material must influence the cell appearance in Nissl stain.
  Moreover, the cytoplasm being thoroughly changed, the nucleus is often shown to be normal together without any glial or mesodormal reaction: that this is a chronic lesion, in Peters opinion, is inconceivable.

- Anyhow, some pathology in the basal ganglia and thalamus in schizophrenia would not be surprising, not only on the base of the symptomatology, but because certain findings in neuropathology and neuroradiology favour this opinion. Indeed, in contrast to poor results in 'idiopathic' schizophrenia, many neurological conditions - with definite brain pathology - may present a 'schizophrenic clinical symptomatology' such as CO-intoxication (Roeder-Kutsch, 1941), subacute sclerotizing panencephalitis, tumours around IIIrd ventricle (Malamud, 1971), demyelinating diseases, etc. ... .
  Well-documented neuroradiological studies of large series of chronic schizophrenic brains also showed definite enlargement of the IIIrd ventricle (Hüber, 1967; Nagy, 1967).

## B. QUANTITATIVE BRAIN CELL COUNTS IN SCHIZOPHRENIA

In view of the reported diffuse cortical cell loss in schizophrenia at the beginning of this century, Dunlap (1942) carried out systematic cell counts: he did not note any difference in neuron density between schizophrenic and control brains. Rowland and Mettler (1949) criticized previous published reports on 'cortical cell loss' in schizophrenia and made cell counts on prefrontal cortex biopsies of schizophrenics: they found no difference with control brains.

On serial sections of eight schizophrenic brains, Nagasaka (1925) studied striatum, pallidum, thalamus, locus niger and nucleus dentatus. He stated that the macroneurons (Golgi type I) in the striatum were dropped out and that 2/3 of the locus niger appeared depigmented. The method used by this author is not known.

The most systematic quantitative studies in schizophrenia are those of the Vogt-school on the thalamus, reported from 1950 to 1962. Those authors insisted upon the prevalence of 'dwarf cells' especially in the medio-dorsal nucleus of the thalamus.
Some criticism on qualitative grounds have been cited above. Moreover, the quantitative method used implied certain bias, because counting of cells is performed on the basis of qualitative, thus subjective, classification of nerve cells in eight groups from 'normal' (step 0) to 'dwarf cells' (step VII). Cell counting was done with a magnification 210 times: this way, the microneurons (Golgi type II) could not be evaluated. It should also be pointed out that the Vogt school studied the anterior, lateral and medial thalamus: the posterior thalamus (pulvinar) was not included.

David (1957), Dastur (1959) and Peters (1967) insisted upon the value of quantitative studies in schizophrenia, provided 'objective' parameters are used.

More recently, Colon (1972) performed cell counting in the cortex of 3 schizophrenic brains compared with normal control values: he found cell loss in layer III and V. The method used is fairly controled but the changes found are difficult to interpret: the schizophrenics were rather old and were treated with neuroleptic drugs.

## C. PERSONAL CYTOMETRIC EVALUATION OF NEOSTRIATUM AND THALAMUS IN SCHIZOPHRENIA

All quantitative studies performed up to now were only cell counting studies. No cell sizing evaluation has been done.

With our cytometric techniques described above (chapter II), objective measurements can be obtained: the size of all nerve cells containing a nucleolus is obtained. Special attention is paid to the microneurons Golgi type II in the thalamus, never studied in schizophrenia.

We made our study on the material used by the Vogt-school (Fünfgeld, Hopf, Bäumer, Van Butlar-Brentano, Hempel, Treff). Nerve cell sizing was performed in the caudatum, putamen, thalamus anterior, thalamus medialis, thalamus lateralis and thalamus posterior.

### 1. Clinical case histories

Five catatonic schizophrenic cases were selected from the brains of the C. and O. Vogt-Institut für Hirnforschung. All cases have a history positive for schizophrenia among their biological relatives; all cases did not have any significant biological treatment (Electroconvulsion - Sakel treatment - neuroleptics).

Case I: $^{\circ}1909 - {}^{+}1933$

The grandmother of this student died in a psychiatric institute. He was always somewhat withdrawn. In 1931, he started to express strange thoughts and was admitted to a psychiatric institute some weeks later. He was restless, kneeled and banged his head against the wall and the floor and felt later on in a negativistic mutism with masklike face. These episodes recurred alternating with stereotyped hyperkinesis. Sometimes he laid in a cataleptic state in bed. The cause of death was a large tuberculous abscess of the femur.

Case II: °1904 - $^{+}$1930
This female, a professional store-keeper, was a good student. At the age of about 18 she became less concentrated and imagined she was being persecuted. She became more and more autistic and refused to eat. In 1923, she was admitted to a psychiatric hospital exhibiting catalepsy, negativism and bizarre postures. For months she remained almost mute and negativistic with closed eyes. In 1926, she became confused and restless with hallucinations, laughing and episodes of sudden aggression and destructive behavior.
In december 1927 she developed high fever and breathing difficulties and died 3 days later.

Case III: °1902 - $^{+}$1931
Three aunts of this girl, a bookbinder, were psychotic. In 1922, she experienced a nervous breakdown without known precipitating circumstances. She recovered well until 1925. Then she became restless and withdrawn with spells of head banging: she had to stop working. In 1927, she became severely anxious and was admitted to hospital. She was negativistic, hallucinating, bizarre. Sometimes she became cataleptic with stereotyped movements and self destructive behavior.
In 1931, she died of heart failure.

Case IV: °1888 - $^{+}$1930
This housewife was initially admitted to the psychiatric institute in 1924 because of severe anxiety, hallucinations and disorganisation. She showed grimacing, aggression, self destruction, exhibitionism and regression. She became, at times, mute and negativistic and at other times highly active and hyperkinetic. Many episodes of catalepsy were noted.
In 1930, she died by bronchopneumonia.

Case V: °1886 - $^{+}$1930
The father of this lady, an employee, committed suicide. She was an average student, very cheerful and pleasant. At the age of twenty, she became depressed with suicidal ideas and episodes of confusion. She recovered after a few months but showed a different behavior: she argued frequently with neighbours. She experienced hallucinations and imagined she was being poisoned. She became aggressive and restless and was admitted to hospital. She showed stereotyped behavior, self mutilation, mutism and negativism.
In 1930, she became extremely hyperkinetic and died of dehydration.

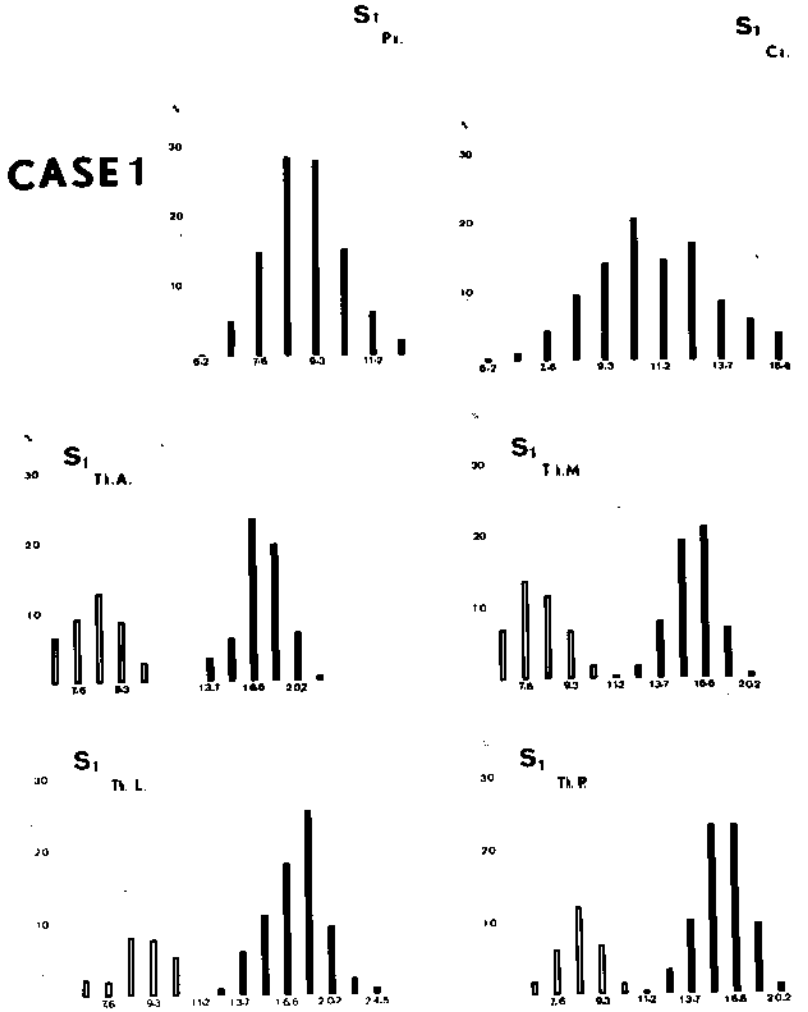



PLATE XXXII: *Microneuron distribution in putamen (Pu) and caudatum (Ca), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior.(Th.P.). In ordinate %, in abscise Ø in micra*

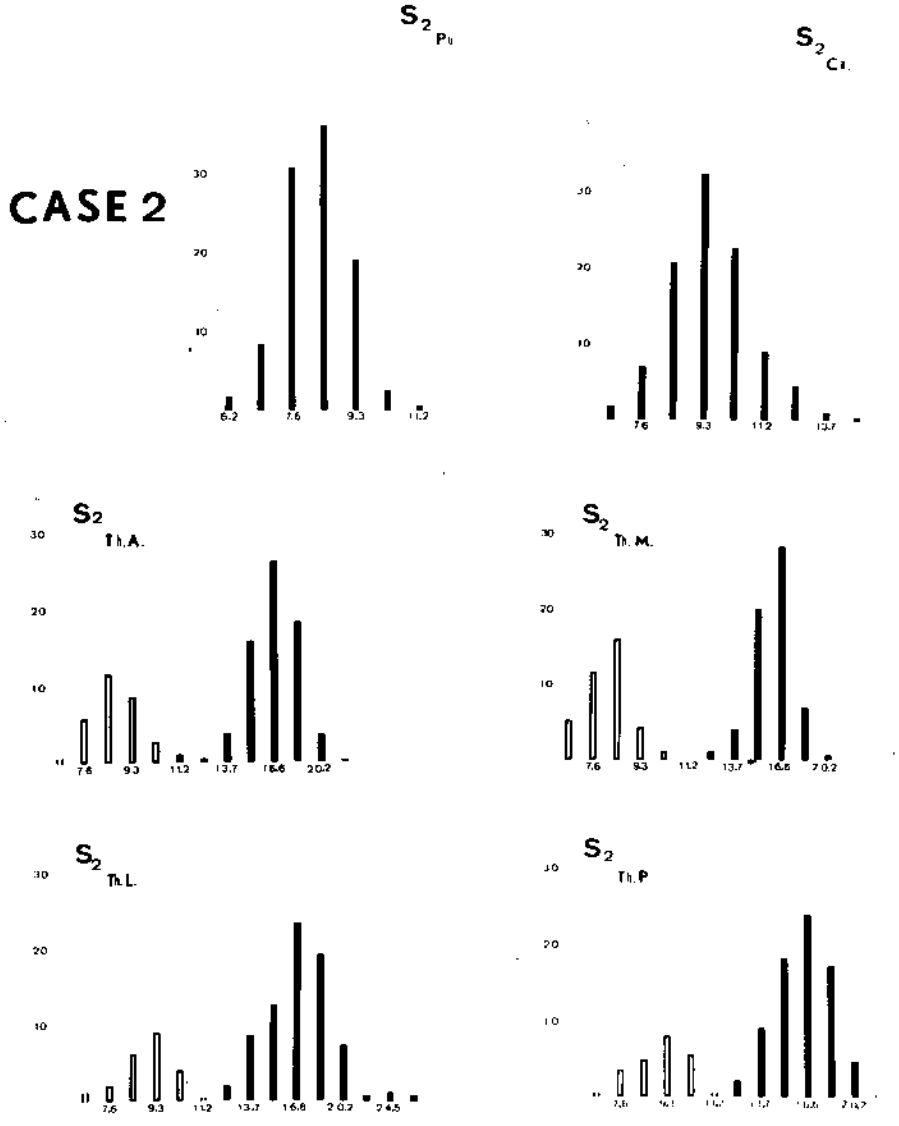



PLATE XXXIII: *Microneuron distribution in putamen (Pu) and caudatum (Ca), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

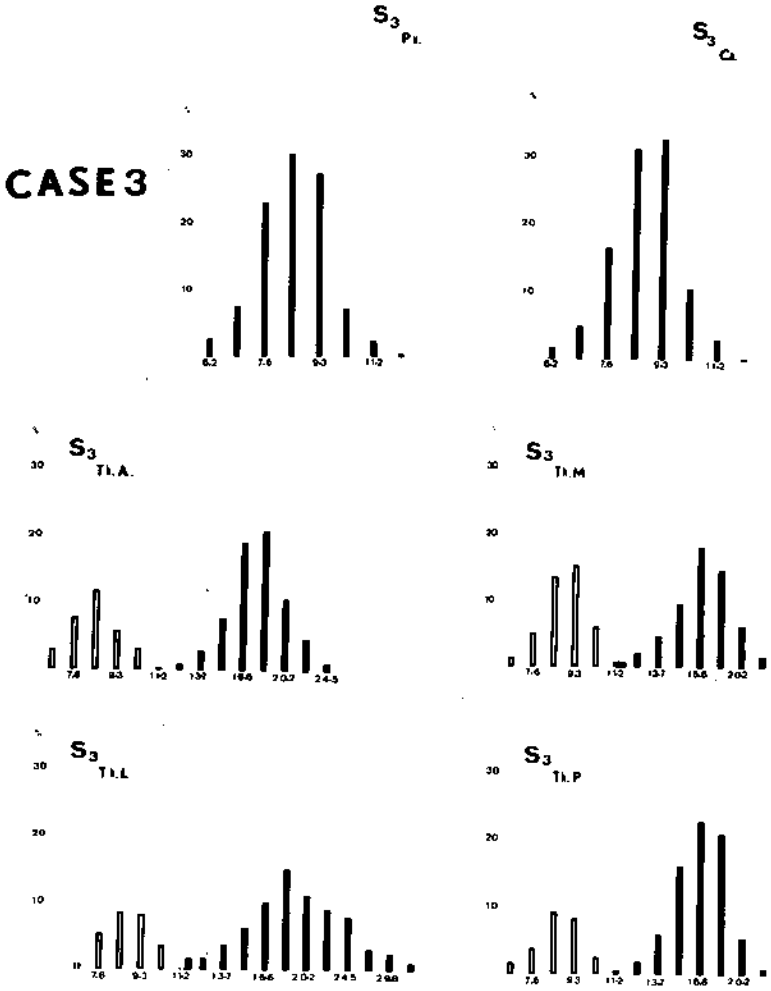



PLATE XXXIV: *Microneuron distribution in putamen (Pu) and caudatum (Ca), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

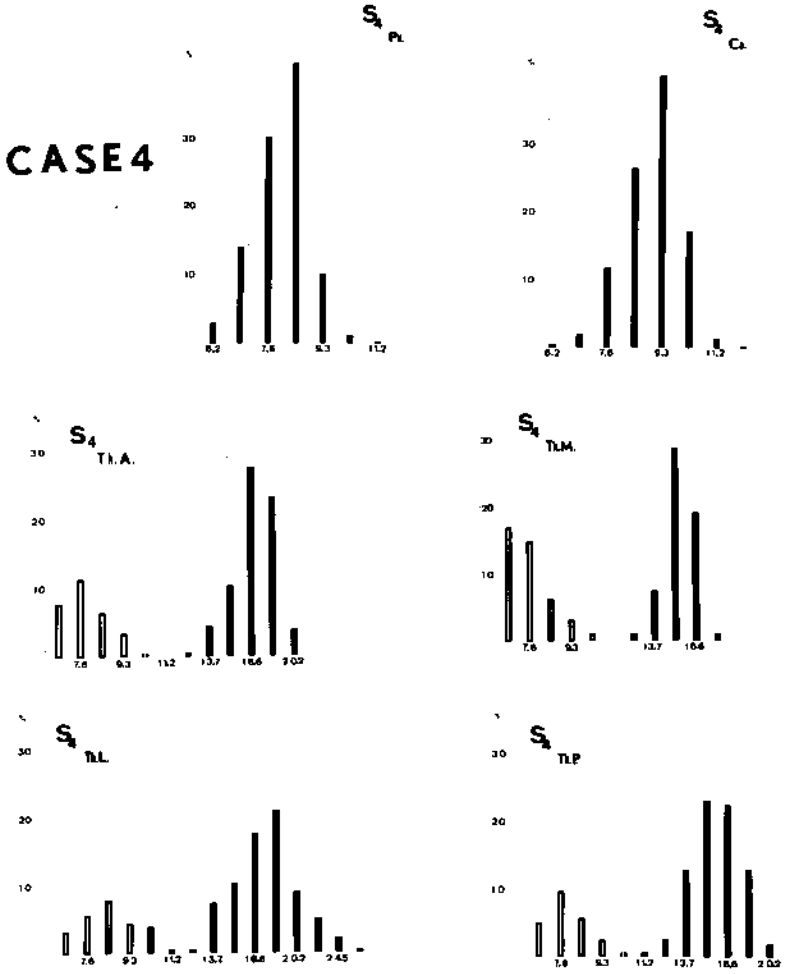



PLATE XXXV: *Microneuron distribution in putamen (Pu) and caudatum (Ca), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.) medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscis Ø in micra*

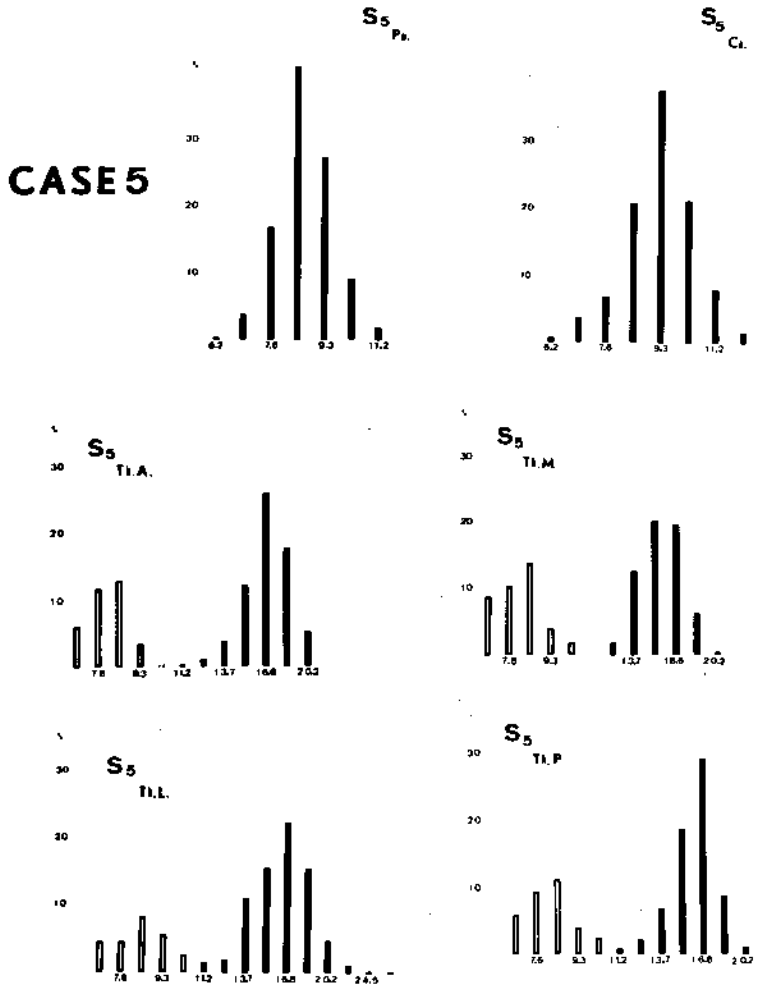



PLATE XXXVI: *Micronеuron distribution in putamen (Pu) and caudatum (Ca), and micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior(Th.A.) medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.). In ordinate %, in abscise Ø in micra*

## 2. Cytometric results

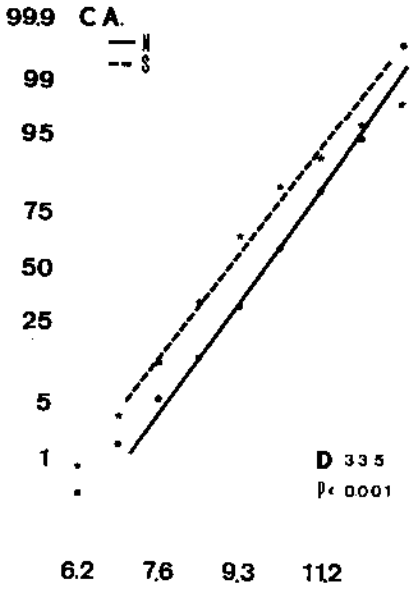
Plates XXXII to XXXVI show the neuronal formulae (percentage distribution) for each case in each nucleus (Ca = caudatum, Pu = putamen, Th.A. = thalamus anterior, Th.M. = thalamus medialis, Th.L. = thalamus lateralis, Th.P. = thalamus posterior).

### *a) The neostriatum*

The numerical values obtained are summarized in the following tables:

*Caudatum*

| | NUMERICAL DENSITY macro | micro | LARGE/SMALL RATIO | AVERAGE Ø IN MICRA micro |
|---|---|---|---|---|
| $S_1$ | 270 | 22.940 | 1/84 | 11.09 |
| $S_2$ | 270 | 21.193 | 1/78 | 9.52 |
| $S_3$ | 106 | 22.663 | 1/205 | 8.72 |
| $S_4$ | 163 | 20.973 | 1/125 | 8.98 |
| $S_5$ | 185 | 19.191 | 1/105 | 9.27 |
| AVERAGE | 199 | 21.392 | 1/119 | 9.51 |




*Cumulative frequency distribution of microneurons in caudatum of schizophrenics (----) compared with normals (——)*

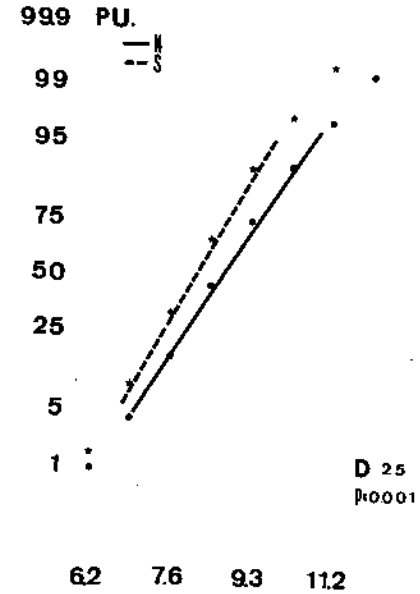



PLATE XXXVII: *Idem in putamen*

*Putamen*

| | NUMERICAL DENSITY macro | micro | LARGE/SMALL RATIO | AVERAGE Ø IN MICRA micro |
|---|---|---|---|---|
| $S_1$ | 163 | 21.137 | 1/129 | 8.99 |
| $S_2$ | 107 | 23.977 | 1/219 | 8.21 |
| $S_3$ | 220 | 22.720 | 1/104 | 8.49 |
| $S_4$ | 110 | 18.403 | 1/168 | 7.98 |
| $S_5$ | 220 | 24.083 | 1/110 | 8.65 |
| AVERAGE | 164 | 22.064 | 1/135 | 8.47 |

Comparing these averages with the average results in the young normal group 20-45 yrs (chapter III), the numerical density (micro- and macroneurons per $mm^3$) and the L/S ratio (large = macroneurons/small = microneurons ratio) in schizophrenic neostriatum are not different from control brains. The average microneuron diameter, however, seems smaller in the schizophrenic putamen and even in the caudatum compared with normal controls: in putamen 8.47 $\mu$ against 9.13 $\mu$ and in caudatum 9.51 $\mu$ against 10.38 $\mu$.
Statistical evaluation of these numerical results by Mann-Withney U-test shows for the putamen a p-value of 0.016 and for the caudatum p = 0.095.
This phenomenon is most easily seen by comparing the average distributions in caudatum and putamen for schizophrenia against controls: plate XXXVII shows cumulative frequency distributions on probability paper.
Kolmogorov-Smirnov test applied to those distributions proves a very significant difference in caudatum as well as in putamen: $p < 0.001$.

*b) The thalamus*

Neuron sizing is performed in thalamus anterior, in thalamus medialis, in thalamus lateralis, in thalamus posterior of each case. The regions within each compartment measured are indicated in chapter IV, the normal thalamus. The micro- (white bars) and macroneuron (black bars) population for each nucleus is

depicted for each case in plates XXXII to XXXVI.

The numerical values obtained are summarized in the following tables:

*Thalamus anterior*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % micro |
|---|---|---|---|---|---|
| $S_1$ | 7.810 | 5.204 | 17.32 | 8.28 | 40.01 |
| $S_2$ | 7.711 | 3.408 | 16.69 | 8.71 | 30.66 |
| $S_3$ | 7.838 | 3.280 | 17.96 | 8.45 | 29.5 |
| $S_4$ | 6.724 | 2.840 | 16.93 | 7.80 | 29.72 |
| $S_5$ | 6.504 | 3.380 | 16.79 | 7.95 | 34.19 |
| AVERAGE | 7.317 | 3.622 | 17.29 | 8.39 | 32.00 |

*Thalamus medialis*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % micro |
|---|---|---|---|---|---|
| $S_1$ | 8.023 | 5.588 | 15.77 | 8.09 | 41.04 |
| $S_2$ | 7.490 | 4.686 | 16.06 | 8.09 | 38.48 |
| $S_3$ | 8.165 | 5.936 | 16.89 | 8.89 | 42.00 |
| $S_4$ | 3.834 | 2.840 | 15.42 | 7.59 | 42.55 |
| $S_5$ | 6.617 | 4.146 | 15.58 | 8.00 | 38.52 |
| AVERAGE | 6.825 | 4.639 | 16.10 | 8.30 | 40.49 |

*Thalamus lateralis*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % micro |
|---|---|---|---|---|---|
| $S_1$ | 3.940 | 1.349 | 17.40 | 8.84 | 25.50 |
| $S_2$ | 3.301 | 994 | 16.96 | 8.96 | 23.14 |
| $S_3$ | 4.594 | 1.683 | 16.81 | 8.74 | 26.84 |
| $S_4$ | 2.325 | 799 | 17.64 | 8.44 | 25.57 |
| $S_5$ | 3.564 | 1.207 | 16.39 | 8.36 | 25.28 |
| AVERAGE | 3.538 | 995 | 17.59 | 8.68 | 25.47 |

*Thalamus posterior*

| | NUMERICAL DENSITY macro | micro | AVERAGE Ø IN MICRA macro | micro | % micro |
|---|---|---|---|---|---|
| $S_1$ | 7.505 | 2.961 | 15.76 | 8.44 | 28.28 |
| $S_2$ | 6.553 | 1.988 | 16.40 | 9.04 | 23.26 |
| $S_3$ | 8.354 | 2.840 | 16.69 | 8.64 | 25.37 |
| $S_4$ | 5.630 | 1.754 | 15.84 | 7.87 | 23.72 |
| $S_5$ | 6.056 | 2.911 | 15.97 | 8.12 | 32.45 |
| AVERAGE | 6.891 | 2.491 | 16.19 | 8.47 | 27.00 |

Comparing these results with the values obtained in the control group of young normals 20-45 yrs (chapter IV), all values obtained in the *thalamus lateralis* in schizophrenic brains are within normal limits.

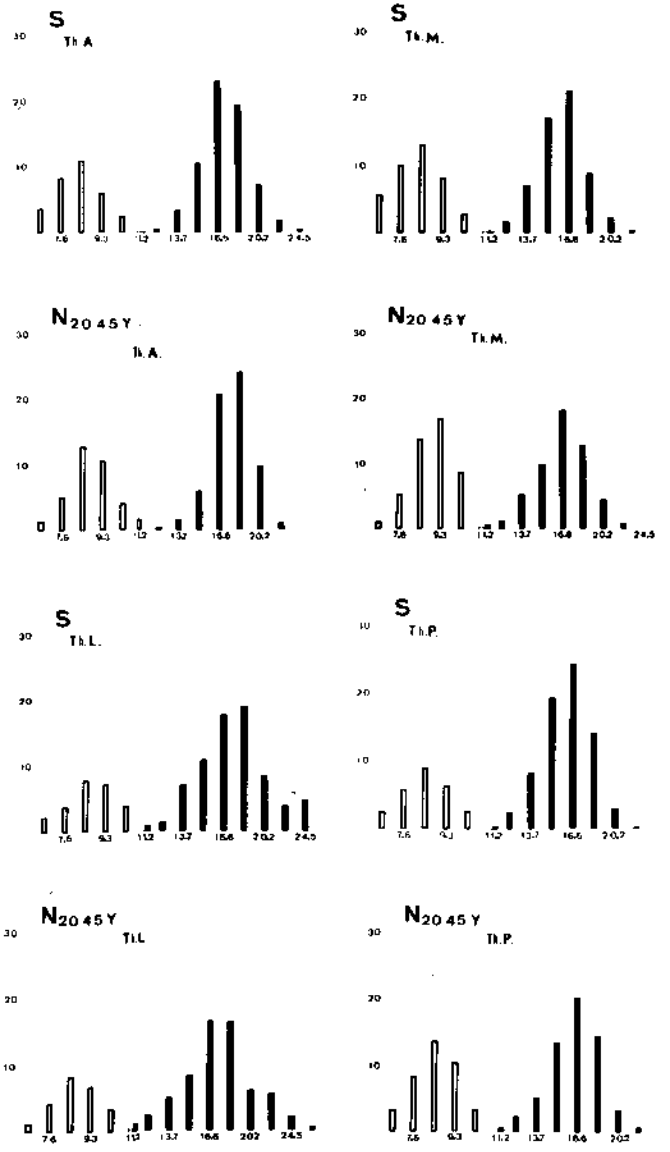



PLATE XXXVIII: *Average micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior (Th.A.), medialis (Th.M.), lateralis (Th.L.) and posterior (Th.P.) in schizophrenics (S) compared with normals (20-45yrs). In ordinate %, in abscise Ø in micra*

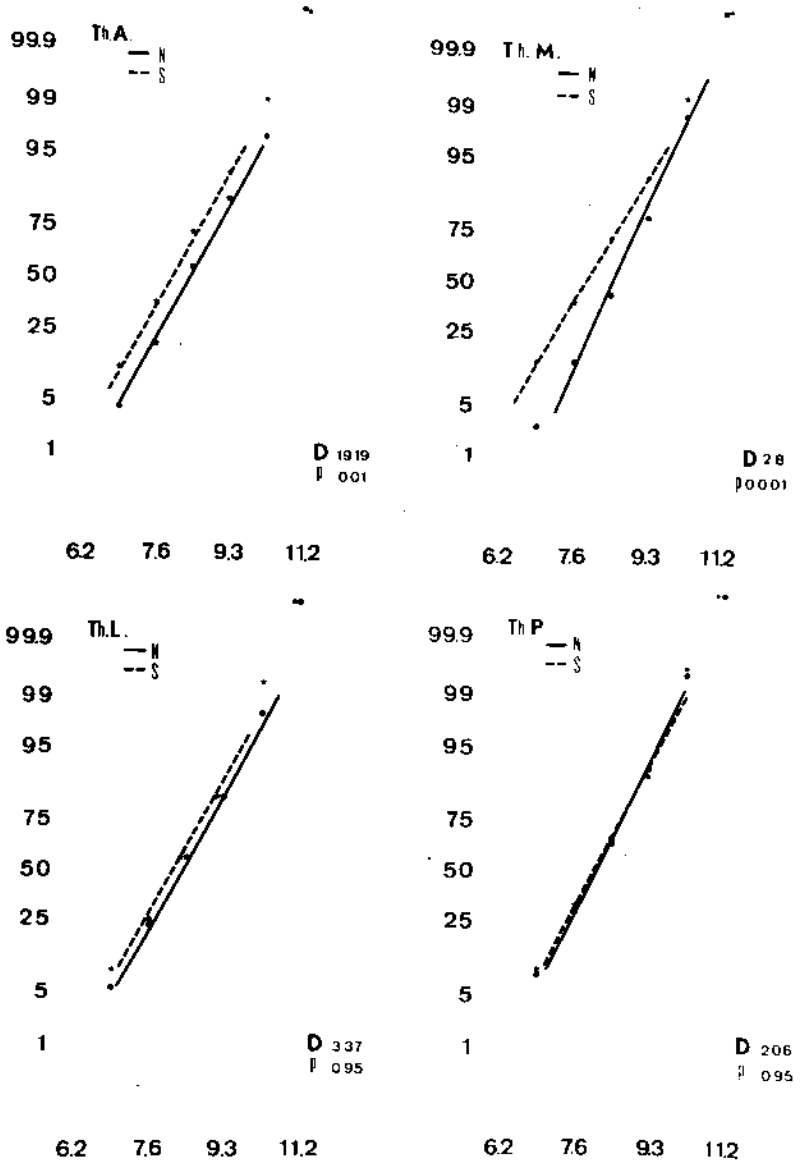



PLATE XXXIX: *Cumulative frequency distribution of microneurons in thalamus anterior, medialis, lateralis and posterior of schizophrenics compared with normals. In ordinate %, in abscise Ø in micra*

In the *thalamus anterior*, the only slight - but statistically insignificant - difference between schizophrenics and controls is a minimal decrease in average diameter of microneurons: 8.39 against 8.79 $\mu$.

The *thalamus medialis* in schizophrenic brains shows some changes: the average diameter of microneurons is smaller than in controls (8.30 $\mu$ against 8.96 $\mu$) and there might be a slight decrease in microneurons: 40 % of the total population against 46.5 % in controls.

In the *thalamus posterior* (pulvinar) of schizophrenic brains, important pathology of the microneurons Golgi type II is apparent: the microneuron density is $\pm$ 40 % smaller than in controls. (This difference is statistically significant: Mann-Withney U-test: u=0; p=0.018). The average diameter of the remaining microneurons, however, is within the normal limits.

Plate XXXVIII shows the average neuronal distribution pattern for schizophrenia compared with the normal distribution in the four thalamic regions: the decrease of microneurons (white bars) in the posterior thalamus of schizophrenics is apparent.

Plate XXXIX shows the cumulative frequency distribution plotted on probability paper of microneurons in schizophrenia (dash-line) against controls (black line): the slight decrease in diameter in anterior and medial thalamus is shown while lateral thalamus and also posterior thalamus have a normal microneuron distribution pattern.

## 3. Summary and discussion of findings in neostriatum and thalamus of catatonic schizophrenics

1. In the *neostriatum* of catatonic schizophrenics, the *macroneuron density* is within normal limits, contrary to what Nagasaka suggested in 1925.

2. The *microneurons Golgi type II* of the *neostriatum* in catatonic schizophrenics are smaller than in control brains: for the caudatum 9.51 $\mu$ $\longleftrightarrow$ 10.38 $\mu$, for the putamen 8.47 $\mu$ $\longleftrightarrow$ 9.13 $\mu$. This finding is in agreement with the qualitative descriptive study by Hopf (1954) of the neostriatum in catatonia: he found less Nissl substance and more lipofuscin in neostriatal neurons, and dwarf cells were more numerous.

The atrophy (or hypotrophy ?) of Golgi type II microneurons of neostriatum in catatonic schizophrenia could be related to the stereotyped motor behavior (Kleist, 1922). These cells indeed

are the postsynaptic cells to the dopaminergic nigrostriatal pathway: excess of dopamine (L-dopa therapy) results in behavioral disturbances (Duvoisin and Yahr, 1972); amphetamine psychosis is produced via dopaminergic mechanisms (Klawans et al., 1972). The hypotrophy of microneurons in catatonia could realize a state of 'hypersensitivity' to normal amounts of dopamine (Van Woert et al., 1972; Cheifetz et al., 1971).

Moreover, the striatum has some function in perception as evidenced by physiological studies (Buser et al., 1974). Therefore, changes in the striatum of schizophrenics could result in distorted perception, as already stated hypothetically by Mettler (1955). The subjective experience of perception disturbances is very important in schizophrenia (Freedman and Madison, 1974).

3. Within the *thalamus* of catatonic schizophrenics, the lateral and anterior nuclei appear to be normal. In the medial thalamus, the macroneuron population appears to be within normal limits as far as numerical density, and average diameter are concerned. This finding, on the basis of objective morphological criteria, weakens the results on a descriptive basis reported by Bäumer (1954), Fünfgeld (1954), Hempel and Treff (1959-1960-1962) in the mediodorsal thalamic nucleus.

The microneuron population of the medial thalamus in catatonia shows normal numerical densities but a slight decrease in average diameter.

In the *posterior thalamus* (pulvinar) of catatonic schizophrenics - never studied quantitatively before -, a definite pathology is found in the *microneuron population:* the numerical density is *40 % decreased* compared with normal brains. In contrast, normal values are obtained for macroneurons. As the average microneuron diameter is unchanged, due to the distribution among cell sizes corresponding completely to normal values and because no reactive glial or mesodermal change is found, it might be postulated that this deficiency in Golgi type II neurons in the pulvinar is *a defect rather than an atrophy.*

The role of this microneuron deficiency in the thalamic pulvinar in the pathophysiology of catatonic schizophrenia could be very important. The pulvinar is an associative nuclear mass with an integrative function in visual and auditory information, having projections to the parietotemporal cortex (Gerebtzoff, 1973). It is a nuclear mass only very developed in higher primates and man.
The Golgi type II neurons are supposed to have an inhibitory function. A *deficiency in inhibition on massive visual and auditory stimuli* could account for *hallucinatory experiences.*

4. While the macroneurons in neostriatum and thalamus of catatonic schizo's appear to be normal, the Golgi type II microneurons, however, in neostriatum are smaller (atrophy-hypotrophy ?) than in control cases and in thalamus posterior they are less numerous (defect, loss ?). If microneurons are GABA-ergic, a *GABA deficiency in catatonic schizophrenia* can be suspected. This is conform with recent experimental studies by Roberts (1972) and Stevens (1974).

5. The relation of the thalamus to psychotic behavior has been pointed out before: tumours or inflammatory processes in the medial and posterior thalamus - if occurring bilaterally - are known to produce schizophrenic-like states (Ajuriaguerra et al., 1954; Martin, 1968).

## Chapter VIII

## IMPORTANCE OF NEOSTRIATAL AND THALAMIC INTERNEURONS

### A. ANATOMICAL EVIDENCES

#### 1. Normal cytoarchitecture of human basal ganglia

Within the group of telencephalic, diencephalic and mesencephalic nuclei in man, the neostriatum and the thalamus are conspicuous because of their size and their neuron population.

The *volume* indeed of neostriatum and thalamus exceeds amply the volume of other nuclei. The total volume of the neostriatum (caudatum + putamen) is ± 10 $cm^3$, the thalamic volume is ± 7.5 $cm^3$. The other nuclei are much smaller: the volume of the pallidum (mediale + laterale) is ± 1.75 $cm^3$; the volume of the nucleus subthalamicus is ± 140 $mm^3$; the volume of the locus niger is ± 750 $mm^3$ (Personal observations: unpublished; von Bonin and Shariff, 1951; Lange and Thörner, 1974).

*The nerve cell density* of the neostriatum and the thalamus is evenso much higher than in the surrounding nuclei. The total nerve cell density (micro + macroneurons) of the neostriatum is ± 23.000/$mm^3$; the total neuron density (micro + macroneurons) of the thalamus anterior, medialis and posterior is ± 10.000/$mm^3$, of the thalamus lateralis ± 5.000/$mm^3$. The neurons are less numerous in other nuclei: the pallidum mediale ± 650/$mm^3$; the pallidum laterale ± 700/$mm^3$; the nucleus subthalamicus pars lateralis ± 3.500/$mm^3$; nucleus subthalamicus pars medialis ± 5.500/$mm^3$; the locus niger ± 800/$mm^3$. (Personal observations: unpublished; Lange and Thörner, 1974; Pakkenberg and Brody, 1963-1965).

*The absalute number* of nerve cells is more important: in the striatum, there are ± 100 million nerve cells (Schröder et al., 1975); in the pallidum laterale and mediale ± 600.000; in the thalamus ± 55 million. For these values shrinking was accounted for.

*The distribution pattern among nerve cell sizes* (neuronal formula) in the neostriatum and the thalamus is very specific: there exist two separate nerve cell populations: the microneuron popu-

lation with an average diameter of ± 9.4 micra in the neostriatum and ± 8.6 micra in the thalamus; the macroneuron population with an average diameter of ± 20 micra in the neostriatum and ± 17 micra in the thalamus. In the surrounding nuclei, only one nerve cell populations exists: in the pallidum mediale with an average diameter of ± 17 micra; in the pallidum laterale ± 15 micra; in the nucleus subthalamicus ± 15 micra. (All these values were obtained with our cytometric technique in the series of normal brains used in this study.)

*The microneuron population* is thus quite characteristic for neostriatum and thalamus and accounts highly for the high nerve cell density: more than 99 % of neurons in the neostriatum are microneurons; in the thalamus, the amount varies between 25 and 60 %.

*The glial cell density*, in contrast to the nerve cell density, does not change so much among different subcortical nuclei. In the neostriatum, the glial density is ± 80.000/mm$^3$; in the thalamus lateralis ± 100.000/mm$^3$; in the thalamus posterior ±95.000/mm$^3$. In the other basal ganglia, slightly higher amounts are noted: pallidum mediale ± 120.000/mm$^3$; pallidum laterale ± 125.000/mm$^3$; nucleus subthalamicus ± 110.000/mm$^3$. These values are obtained with the technique as described by Lange and Thörner (1974).

The values for glial density obtained in our study show that there does not exist a close relationship between nerve cell density and glial density.

*Conclusion:* the high prevalence of microneurons is the main feature, differentiating neostriatal and thalamic cytoarchitectonics from other subcortical nuclei.

## 2. Neostriatum and thalamus in other mammalia

In all mammalian species, neostriatum and thalamus are rather big nuclei.

From lower to higher species, however, considerable changes occur. A detailed description of this evolution is out of scope of this study. Only some points, relevant to the topics discussed, will be stressed.

### *a) Volume*

The division of the neostriatum in caudatum and putamen, evident in human species, is gradually less visible descending to lower species. Almost the same configuration is found in the monkey

as in man. In the dog, the putamen is much less individualized. In the rat, subdividing putamen and caudatum is almost impossible.

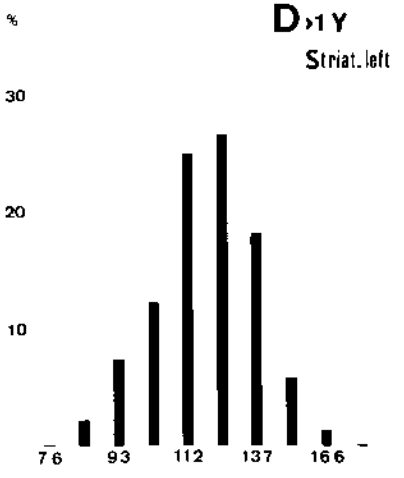
The subdivisions of the thalamus behave differently in the evolution from lower to higher mammals: the anterodorsal nucleus decreases from lower to higher species; the medial nuclei increase considerably in higher species especially in man; the posterior nuclei (pulvinar), non existent in lower species, appear in carnivores as one small nucleus but reach an important size in man; the dorsal and ventral lateral thalamus is well organized in all species; the extralamellar nucleus (reticularis) obtains the same relative volume in all species; the intralaminar (with exception of the nucleus centralis) and the paraventricular nuclei decrease considerably in higher species and man; the geniculate nuclei are important in all mammals.

Summarizing, it might be stressed that with continuing evolution, caudatum and putamen get more individualized and that aspecific thalamic nuclei decrease, specific thalamic nuclei (lateral nuclei - geniculate nuclei) do not change and associative thalamic nuclei (medial and posterior) increase.

*b) Cytology*

In the neostriatum, the clearcut separation of two neuron populations in man does not exist in lower mammals. In the rat, Nissl stain reveals only one population of neurons. Their size lies in between the size of micro- and macroneurons of human neostriatum: average diameter $\pm$ 14 micra (see: plate XXXI). In the dog, a macro- and microneuron population appear but the microneurons are larger than in man: the average diameter is $\pm$ 12 micra. Plate XL shows the percentage microneuron distribution in the adult dog (Beagle). In the monkey, the neuronal morphology of the neostriatum is quite comparable to that in man. The microneurons are equally distributed: average diameter $\pm$ 9 micra. Plate XL depicts the microneuron distribution in putamen and caudatum.

In the thalamus of lower mammals (mouse - rat), no microneurons Golgi type II are found. Carnivores (cat - dog) have thalamic microneurons, but, while the percentage in lateral thalamic nuclei is comparable with the values in man ($\pm$ 20 %), the medial and posterior thalamic nuclei contain only small amounts of microneurons. In the monkey, the thalamic neuron population is almost identical to the human thalamus: plate XLII shows the neuronal formulae in the four thalamic regions of the monkey (Cercopithecus Aethiops). Cercopithecus was chosen because of high encephalisation-index (Bauchot and Stephan, 1966).



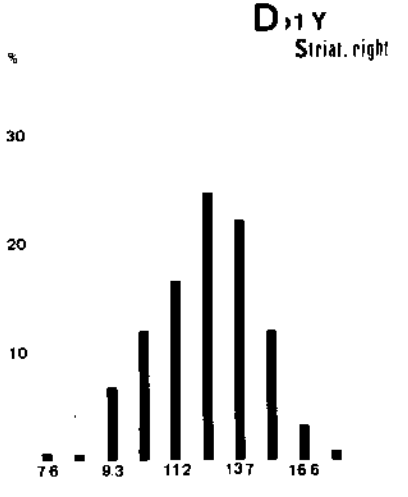
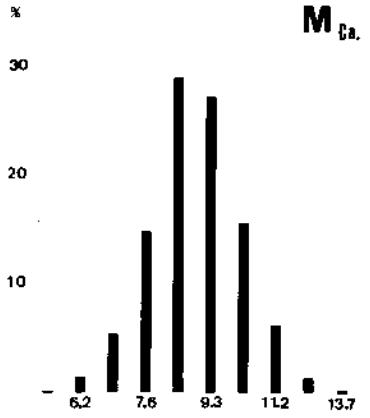




PLATE XL: *Average microneuron distribution in the striatum of the adult dog, left and right*




*Microneuron distribution in the caudatum of the monkey. In ordinate %, in abscise Ø in micra*

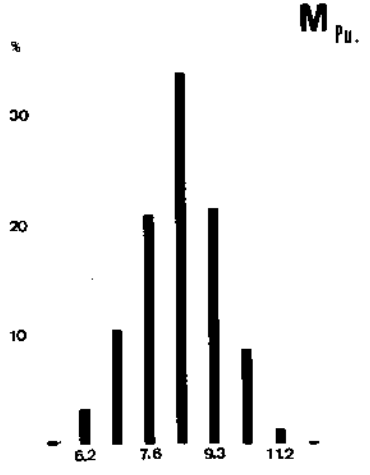



PLATE XLI: *Idem in putamen*

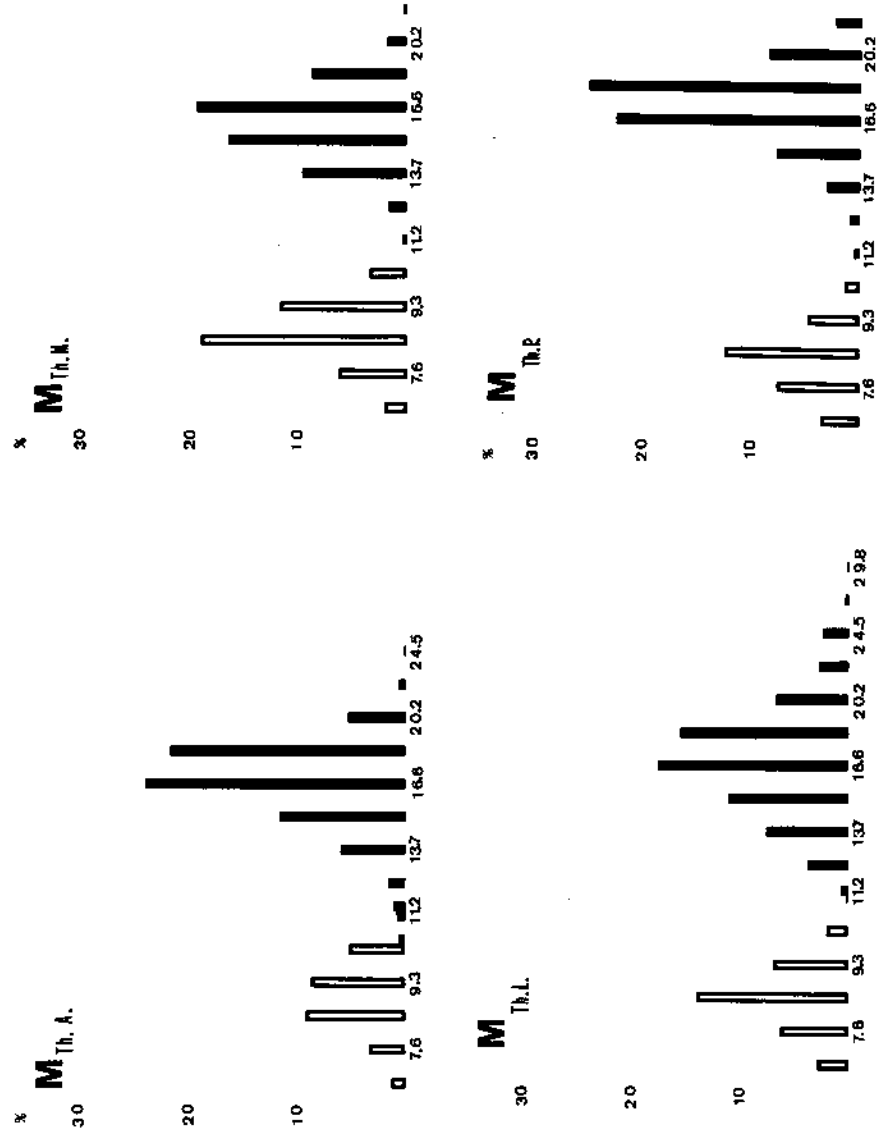



PLATE XLII: *Average micro- (white bars) and macroneuron (black bars) distribution in thalamus anterior, medialis, lateralis and posterior of the monkey. In ordinate %, in absciss Ø in micra*

*Conclusion:* In higher mammals (monkey - man), the neostriatum shows a characteristic subdivision in micro- and macroneuron population. In the thalamus, associative regions increase considerably in size and in those regions, the microneurons - absent in lower species - make up 40 to 60 % of the neuron population.

## B. ROLE IN BASAL GANGLIA FUNCTION

### 1. General remarks

All basal ganglia are highly interrelated structures. It is therefore not feasible to ascribe definite functions to one given nucleus.

Neostriatum and thalamus however can be looked upon as integrative stations in perceptive and motor behavior. The continuous modulation of ongoing information (stimuli) implies a sophisticated neuronal system, sufficiently flexible to cope with all sorts of unexpected events.

The short-axoned Golgi type II interneurons, very numerous within those regions, play an important role in this integration. Indeed, the increasing complexity of behavioral pattern in the evolution from lower to higher mammalian species seems to parallel the increase of microneurons in thalamus and neostriatum. Being interneurons, the function of Golgi type II cells is to modify and select out incoming information in order to produce the most adapted behavioral response.

In this respect, pathological changes to Golgi type II cells - without damage to 'effector' macroneurons - must result in various clinical manifestations, precisely characterized by their irrelevancy to the surrounding world (abnormal movements, bizarre feelings, postural disturbances) rather than by deficiency symptoms.

Changes to the interneurons could be primary or secondary.
Primary changes would be:
- atrophy of this neuron population because of specific vulnerability
- hypotrophy or absence due to developmental abnormalities
- deficiency of certain enzymes involved in neurotransmitter metabolism specific to this neuron type.

Secondary changes are:
- transsynaptic, retro- or anterograde, degeneration (with the primary atrophy in afferent systems)

- inflammatory, tumoral, metabolic disturbances, not limited to certain cell types.

## 2. Situation of microneurons in the pathophysiology of some disorders

*a) Schizophrenia*

The changes to microneurons in neostriatum and thalamus, found in this study, might be primary: in the neostriatum, there exists a hypotrophy of microneurons; in thalamus posterior (pulvinar), there is a defective development of microneurons (chapter VII).

The neostriatal hypotrophy of microneurons could realize a state of 'hypersensitivity' to normal amounts of dopamine, resulting in abnormal motor behavior and postural disturbances. Neuroleptic therapy, by blocking dopamine-receptors, will stop hypersensitivity, but it could never cure the fundamental defect. In stress situations, catecholamine metabolism is profoundly influenced. This could account for the appearance of psychotic behavior.

The low microneuron density in thalamus posterior will diminish the integrating abilities of this structure. Visual and auditory perception could thus be impaired due to inundation with stimuli, with resulting hallucinatory and delirious experiences.

*b) Huntington's Chorea*

The atrophy of microneurons in neostriatum and thalamus lateralis seems primary. The drastic loss of neurons in the neostriatum releases the control on the pallidal efferent system. The pallidal input to ventral lateral thalamus gets evenso influenced because of microneuron atrophy in ventrolateral thalamus.
The atrophy in neostriatum of Huntington's diseased brains seems to spare selectively the 'medium-sized' Golgi type II neurons (chapter V-D), which might have a transmitter substance different from the supposed GABA-ergic 'small-sized' Golgi type II neurons.

The dopaminergic input from the locus niger seems to be normal. The effect of dopamine on the diseased neostriatal neurons might result - due to 'hypersensitivity' or 'transmitter-imbalance' - not only in abnormal movements, but also in abnormal behavior, thus accounting for schizophrenic-like symptomatology in the initial phase of some patients with Huntington's disease.

*c) Parkinson's disease*

The neostriatal microneuron changes, encountered in this study, are probably secondary. The neurons of the locus niger are severely atrophied. This way, the nigro-striatal dopaminergic pathway degenerates. As a result, Golgi type II cells are denervated, but only partially because imput from other nuclei persists. This partial denervation could result in involution of striatal neurons. That corresponds with our findings of decreased microneuron size in caudatum.

This denervation atrophy could account for 'hypersensitivity' to dopamine. This would explain dyskinesia appearing during L-dopa therapy.

## C. EXPERIMENTAL MODELS AND RESEARCH IN INTERNEURONS

The role of human neostriatum and thalamus in behavior and perception is amply evidenced. The numerous Golgi type II neurons in those structures must play a substantial part in their integratory function.

Nevertheless, our knowledge of the morphology of microneurons in physiological and pathological conditions is rather limited. The appearance of thalamic microneuron population is almost unknown in pathological and experimental studies. The differing morphology of the striatum in several species is rarely considered in physiological or biochemical experiments.

A prerequisite for a better understanding of basal ganglia function is a thorough morphological analysis of the structures in man and experimental animals to be compared.

As a result of this study, some suggestions for further research emerge:

1- the animal model, used in behavioral studies, should be preferentially the monkey because of the close similarities in neostriatal and thalamic neuronal cytology to man. In the dog, the neostriatal morphology is quite similar to man but the thalamic cytology differs substantially. The rat is less useful in view of different striatal and thalamic structure.

2- it should be shown that so-called neostriatal Golgi type II neurons are really all short-axoned. There seems to exist two groups of human neostriatal microneurons, 'small' and 'medium' sized ones.

3- the thalamic microneurons should be systematically studied

a) in different animal species
b) in human embryogenesis and development up to 20 yrs of age
c) in different pathological entities.

4- the influence of substances on microneurons in neostriatum and in thalamus, changing behavior and perception, has to be described.

## SUMMARY

Two major nuclei of the human basal ganglia - the neostriatum and the thalamus - were thoroughly analyzed with a quantitative cytometric technique.

Within these structures, special emphasis was placed on the 'interneurons' (Golgi type II microneurons), because of their important function in behavior integration.

The study was performed in brains of normal adults as well as in brains of patients suffering from Huntington's Chorea, Parkinson's disease and catatonic schizophrenia.

1- In *the normal adult human neostriatum* (caudatum + putamen), there exist two distinct neuron populations: the macroneurons and the Golgi type II microneurons. The prevalence of microneurons is evident in these structures: in the caudatum, the ratio large:small neurons is 1:210; in the putamen 1:115.

As the microneuron density in both nuclei is equal, namely $\pm$ 22.000/mm$^3$, the macroneurons appear slightly less numerous in caudatum (125/mm$^3$) than in putamen (195/mm$^3$).

The distribution of microneurons is rather uniform throughout the nuclei, except in caudal regions: because more fibre bundels are passing through,the neurons are less densely arranged.

The average diameter of microneurons decreases with increasing age: in the caudatum below 45 yrs, it is 10.38 µ and above 45 yrs 9.43 µ ; in the putamen below 45 yrs it is 9.13 µ and above 45 yrs 8.72 µ .

The numerical values are equal in the left and right hemispheres.

2- In the *normal adult human thalamus,* two distinct nerve cell populations are also found. In this structure, the macroneurons numerically prevail over the microneurons. The Golgi type II microneurons, however, are very important because of their function as pre- and postsynaptic inhibitory cells. The average size of thalamic microneurons is 8.65 in all thalamic nuclei but their numerical density varies: in Thalamus Anterior 3.450/mm$^3$,

in Thalamus Medialis 4.650/mm$^3$, in Thalamus Lateralis 1.150/mm$^3$, in Thalamus Posterior 4.000/mm$^3$.

The macroneuron numerical density in Thalamus Anterior is 6.750/mm$^3$, in Thalamus Medialis 5.600/mm$^3$, in Thalamus Lateralis 3.350/mm$^3$ and in Thalamus Posterior 6.450/mm$^3$.

It is thus evident that the microneurons in Thalamus Anterior contribute 30 to 35 % of the total neuron population, in Thalamus Medialis 40 to 50 %, in Thalamus Lateralis 20 to 25 % and in Thalamus Posterior 35 to 40 %.

These differential proportions may be related to the function of the thalamic parts: specific nuclei appear to contain less microneurons than associative nuclei.

3- In cases of *Huntington's Chorea*, a severe loss of microneurons appears in the neostriatum and in the Thalamus Lateralis.

In caudatum as well as in putamen, the microneuron loss reaches 90 % in juvenile rigid choreics, 80 % in choreiform cases and 70 % in akinetic adult cases.

In juvenile and choreiform cases, in contrast to akinetic adult cases, the larger sizes of microneurons appear to be better preserved. This finding, corresponding to different clinical symptoms, may suggest that the larger Golgi type II microneurons are different from the more numerous smaller ones. Maybe, the larger Golgi type II cells have another transmitter substance. This would conform with the hypothesis that GABA and acetylcholine play a role in Huntington's Chorea.

While the neurons appear normal in Thalamus Anterior, Medialis and Posterior, the Thalamus Lateralis in Huntington's Chorea shows a distinct 50 % loss of microneurons, atrophy of persisting microneurons and also of macroneurons. This lesion in the ventrolateral thalamus relates certainly to the known input from the striatum via the pallidum.

4- In cases of *Parkinson's disease*, there is no loss of neurons in the neostriatum. The microneurons in the caudatum, however, appear smaller: the average diameter - compared with the normal value - is significantly decreased.

This atrophy may be secondary to the known degeneration of the nigrostriatal fibres in Parkinson's disease. In the thalamus, the posterior and medial nuclei demonstrate a slight atrophy of microneurons. The lateral thalamus, although relay station of motor pathways, is unchanged. The pathology of thalamic and caudatal microneurons in Parkinson's disease may be related to the GABA decrease within basal ganglia in this disease. It is thus

evident, that the disturbance in Parkinson disease is more complicated than dopamine decrease due to neuron loss in Locus Niger.

5- In cases of *catatonic schizophrenia*, there exists a severe microneuron pathology. In the neostriatum, loss of microneurons is not found but the average diameter of microneurons is significantly decreased in caudatum as well as in putamen. The distribution pattern of microneurons favours more an hypotrophy than an atrophy. This hypotrophy of neostriatal Golgi type II neurons may be responsible for the stereotyped motor behavior and perception disturbances in catatonic schizophrenia: the 'hypotrophic' neurons could be 'hypersensitive' to normal amounts of dopamine.

In the Thalamus, the lateral nuclei appear normal. In the associative thalamic nuclei - anterior, medial and posterior nuclei - microneurons are pathological.

In the anterior and medial nuclei there is a slight atrophy without loss of microneurons. In the posterior thalamus (pulvinar), however, Golgi type II neurons are 50 % less numerous than in normals brains. As the microneurons present in schizophrenia are normally distributed among cell sizes and as reactive gliosis does not exist, the low numerical density appears a defect rather than an atrophy. This defect of Golgi type II inhibitory cells in the Pulvinar may be related to hallucinatory experiences.

6- *Neuroleptic drug treatment*, known to influence human behavior, induces distinct changes in the neostriatal neurons of rats. In a short term experiment, low doses of three different neuroleptic drugs (Haloperidol - Chlorpromazine - Pimozide) were administered to rats. Quantitative cytometric evaluation revealed that all neuroleptics caused a decrease in neostriatal neuron size. The decrease in size was more pronounced in rats treated with a neuroleptic, known to give easily extrapyramidal symptoms, and less pronounced in rats treated with a neuroleptic causing fewer extrapyramidal signs. The decrease in size of neostriatal neurons may attribute less available dopamine receptor sites.

7- *Comparative cytometry* of the neostriatum and thalamus of the *rat*, the *dog* and the *monkey* showed that in lower animals the neurocytology is quite different from that in man. In the monkey the neostriatal and thalamic neuron population is well comparable to that in man; this animal, therefore, should be preferentially used in experimental studies of basal ganglia function.

In conclusion of this cytometric morphological study, it may be stated that the pathology of neostriatal and thalamic microneurons is of primary importance in the pathophysiology of basal ganglia and behavioral disorders.

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Further research is needed to know better the development of thalamic microneurons and to discover the influence of substances, changing behavior, on Golgi type II neurons in thalamus and neostriatum.